The evolving epidemiology and emerging biomarkers of cancers in the HIV positive populations

THESIS

presented for the

DEGREE

of

DOCTOR OF PHILOSOPHY

in the Faculty of Population Health Sciences

(Field of study: Epidemiology)

LEAH SHEPHERD

Institute for Global Health

UCL

July 2017

Declaration

I, Leah Shepherd, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Effective and durable HIV treatment and increased longevity of HIV-positive (HIV+) people [1-6] has led to a growing burden of cancers in this population [7-9]. The aims of this thesis were to describe the changing epidemiology of commonly occurring cancers in HIV+ people (with a focus on Europe) and to explore and characterise plasma biomarkers of common cancers in HIV+ people.

Results showed that the incidence of infection unrelated cancers are not declining in HIV+ people, primarily driven by aging of the HIV+ population and higher prevalence of cancer risk factors such as smoking. Smoking presents as one of the few modifiable cancer risk factors which can be targeted to reduce burden. Although smoking cessation quickly reduces the incidence of many smoking related cancers, the incidence of lung cancer remains at a similar level to current smokers ≥5 years after cessation. Risk factors for non-Hodgkin (NHL) and Hodgkin lymphoma (HL) differ, with HL primarily driven by low CD4 cell counts, whereas NHL is driven by a combination of immune deficiency and other HIV-mediated immune dysfunction. Furthermore, markers of immune activation are elevated ≥2 years prior to lymphoma diagnosis and are correlated with high level of HIV viremia. Levels of prostate specific antigen (PSA) were raised and increasing many years prior to prostate cancer diagnosis, however, use of the cut off of PSA >4 ng/mL to identify men at high risk of prostate cancer may not be appropriate in HIV+ men due to lower levels of circulating PSA.

In conclusion, the results of this thesis provide evidence to advise and improve the care of aging HIV+ people at elevated risk for cancers through the identification and characterisation of risk factors for common cancers, investigating possible mechanisms driving cancer genesis, and assessing the usefulness of commonly used diagnostic practices.

Acknowledgements

This page gives me the opportunity to sincerely thank my principal supervisor Professor Amanda Mocroft for all her support, guidance, and encouragement throughout the preparation of this thesis. I really appreciate the time and effort she has put into reading many truly awful drafts, providing constructive feedback, encouragement, motivation, and cookie bribes, when I was ready to pack it in and embark on my new career as a vegan body builder and kale enthusiast. I would also like to thank my secondary supervisor, Professor Jens Lundgren, for all his suggestions, ideas, and clinical input into this thesis, which have been invaluable.

This work would not have been possible without the extensive work of the entire EuroSIDA study group and of course the patients. I am privileged to have had access to such a high quality database. I would like to thank all the investigators and the team from the co-ordinating centre at the Copenhagen HIV Programme. In particular, Dr Ole Kirk, Dr Alvaro Borges, Dr Lene Ryom, and Dr Camilla Hatleberg, for all their advice and support on multiple studies.

In addition, I would like to give my thanks to the HIV Epidemiology and Biostatistics group. In particular, Professor Caroline Sabin, Professor Andrew Phillips, Dr Valentina Cambiano, Dr Colette Smith, and Dr Fiona Lampe for devoting the time to read and comment on specific chapters.

I am also very grateful to Oscar for providing me with caffeine, cups, sleep masks, Sudoku, imported ancho chilli liqueur, protein brownies, edible flowers, jam, cookie monster blue and for putting up with me for the last 4 months. I would also like to thank Valentina, Sophie Jose, Marina Daskalopoulou, and Anna Schultze for dealing with and containing multiple break downs. Finally, I am very grateful to my friends and family for their continued support and encouragement, especially my dad who does not know what a PhD is but was keen for me to get one anyway.

Chapters

Contents

List of figures by chapter

List of tables by chapter

Frequently used abbreviations

95%CI	95% confidence interval
ADC	AIDS defining cancer
aHR	adjusted hazard ratio
AIC	Akaike information criterion
AIDS	Acquired immune deficiency syndrome
aIRR	Adjusted incidence rate ratio
ART	Antiretroviral therapy
AUC	Area under the curve
BHIVA	British HIV association
BMI	Body mass index
BP	Blood pressure
cART	Combination antiretroviral therapy
CDC	Centers for disease control and prevention
CHIP	Center of Excellence for Health, Immunity and Infections
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CVD	Cardiovascular disease
DLBC	Diffuse large B-cell
DRE	Digital rectal exam
EACS	European AIDS clinical society
EBV	Epstein-Barr virus
EC	E-cigarettes
FLC	Free light chain
GEE	Generalised estimating equations
HAART-OC	Highly Active Antiretroviral Therapy Oversight Committee
HBV	Hepatitis B
HCV	Hepatitis C
HHV8	Human herpes virus 8
HIV	Human Immunodeficiency virus
HIV-VL	HIV viral load
HL	Hodgkin lymphoma
HPV	Human papillomavirus
HR	Hazard ratio
HSV	Herpes simplex virus

IARC	International agency for research on smoking
IDU	Injecting drug use
Ig	Immunoglobulin
INSTI	Integrase inhibitor
IQR	Interquartile range
IR	Incidence rate
IRC	Infection related cancer
IRR	Incidence rate ratio
IURC	Infection unrelated cancer
KS	Kaposi's sarcoma
LES	Linear exponential smoothing models
LTFU	Loss to follow-up
NADC	non-AIDS defining cancer
NHL	non-Hodgkin lymphoma
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
OR	Odds ratio
PAF%	Population attributable fraction
PBCNS	Primary brain and central nervous system
PCa	Prostate cancer
PI	Protease inhibitor
PSA	Prostate specific antigen
PYFU	Person years of follow-up
RCT	Randomised control trial
RHR	Ratio of the adjusted hazard ratios
ROC	Receiver operator curve
RS	Reed-Sternberg
SHBG	Sex hormone binding globulin
SIR	Standardised incidence ratio
SIV	Simian immunodeficiency virus
START	Strategic Timing of Antiretroviral Therapy
STI	Sexually transmitted infection
TB	Tuberculosis
WHO	world health organisation

Overview

HIV is a retrovirus that causes immune dysfunction as HIV invades and destroys cells of the immune system, including CD4+ T cells which are a crucial component of the human immune system. HIV inhibits the body's ability to replace CD4 cells, which become depleted after years of viral replication, putting people at risk of opportunistic infections [10, 11]. Early in the HIV epidemic, almost all people with HIV died of an acquired immune deficiency syndrome (AIDS) related cause. However, wide spread availability of combined anti-retroviral therapy (cART) since the 1990s has improved immune-function and reduced the incidence of AIDS defining events [12]. As a result, survival has dramatically improved, with the median age at death projected to reach as high as 75 years [1-6].

Cancers are a significant source of morbidity and mortality and are now the second most common cause of death following AIDS related deaths in HIV-positive (HIV+) people [13]. HIV+ people are at increased risk of many cancers compared to the general population, particularly those with an infection-related cause [8, 14-18]. However, the introduction of cART in 1996 led to restored immune function and a corresponding reduced incidence of infection related cancers (IRC) [19-21]. In contrast, increased overall survival and life expectancy has led to a growing burden of cancers in the HIV+ population and cancers traditionally associated with older age [7-9].

The emergence of cancers as a major source of morbidity and mortality will require changes to ongoing HIV care, as patients who require concurrent services and treatment for oncogenic conditions become more prevalent. As a result, understanding the changing epidemiology of cancers in HIV+ people and the underlying mechanisms will be crucial for future healthcare planning and resource allocation, as well as improving the longevity and quality of life of patients.

Objectives

My PhD has two key objectives

1. To describe the changing epidemiology of commonly occurring cancers in HIV+ people, particularly focusing on specific cancers or groups of cancers that are expected to become a major source of morbidity and mortality as the population ages.
2. To explore and characterise plasma biomarkers of common cancers in HIV+ people in order to better understand the mechanisms leading to cancer development.

These objectives will be investigated through 5 projects each corresponding to a results chapter.

Chapter 3 describes the incidence of infection related (IRC) and infection unrelated cancers (IURC) over time in the aging HIV+ population across Europe, using the EuroSIDA study.

Chapter 4 investigates the association between smoking cessation and cancer risk in HIV+ people, based on data from the D:A:D study.

Chapter 5 compares and contrasts the risk factors for non-Hodgkin (NHL) and Hodgkin lymphoma (HL) development in HIV+ people, based on data from the D:A:D study.

Chapter 6 explores levels of and changes in markers of B-cell activation prior to the development of NHL and HL, using the EuroSIDA study.

Chapter 7 investigates the kinetics of total and free prostate specific antigen (PSA) prior to diagnosis with prostate cancer (PCa) and examines their diagnostic ability, using the EuroSIDA study.

1 Introduction

1.1 Human Immunodeficiency Virus (HIV)

1.1.1 The beginning of the HIV epidemic.

Acquired immune deficiency syndrome (AIDS) was discovered in 1981 when an outbreak of Kaposi's sarcoma (KS, a cancer caused by infection with human herpes virus-8 [HHV8]), and pneumonia (caused by *pneumocystis carinii)*, two rare conditions usually confined to severely immunocompromised individuals, occurred in a group of 26 young and otherwise healthy homosexual men in the USA [22, 23]. Initially AIDS was thought to be perpetuated by an infectious agent linked to homosexual men, however it was not long before the disease was also reported in haemophiliacs [24, 25], transfusion recipients [25, 26], injecting drug users [25], and women [10, 25]. Although immune-deficiency related conditions were not unheard of, AIDS had almost a 100% mortality rate, making it unique [10]. In the years since it was discovered, Human Immunodeficiency Virus (HIV) has progressed from small clusters of infection to a worldwide pandemic [10].

In 1983 the retrovirus responsible for AIDS was simultaneously discovered by two independent institutes. Barre Sinoussi, at The Pasteur institute, France, isolated a novel retrovirus from a lymph node of a patient displaying symptoms often preceding the diagnosis of AIDS and named it lymphadenopathy-associated virus (LAV) [27, 28]. At the same time, Gallo and his team also isolated a novel virus named Human T-lymphotropic retrovirus (HTLV-III) and provided evidence that this virus was the primary cause of AIDS [29-34]. These viruses were determined to be variants of the same virus by 1985 [35] and were renamed as Human Immunodeficiency Virus (HIV) in 1986 [36], in accordance with common nomenclature for retroviruses: beginning with the host species (human), containing a word that describes a characteristic feature (immunodeficiency) and ending in virus [36]. In 1986 it was determined that two separate types of HIV were circulating in the population: HIV-1 and HIV-2. HIV-1 and HIV-2 are only distantly related and show more similarities to other primate specific retroviruses than to each other [10, 37]. HIV-2 tends to exhibit longer latency periods[38], slower disease progression [39], and lower HIV viral load (HIV-VL) [40].

1.1.2 The origin of HIV

The mechanisms by which HIV emerged in humans are widely speculated. Both HIV-1 and HIV-2 are closely related to a group of lentiviruses which circulate in various primate populations,

mainly African apes or old world monkeys, called simian immunodeficiency viruses (SIV). HIV-2 is closely related to SIV which circulates in sooty magnabeys (SIVsm), and has been confirmed as the origin of HIV-2 [10, 37, 41-46]. The origin of HIV-1 is not so clear. Although HIV-1 has long been suspected to have originated from SIV in chimpanzees (SIVcpz), a lack of a reservoir in the wild population shed doubt on this theory [37]. However, in 1999 a group of researchers found a strain of SIVspz present in *Pan troglodytes troglodytes* which was phylogenetically similar to HIV-1 [47, 48] and it is now generally accepted as the probable origin of HIV-1 [10, 37, 47-51].

It is believed HIV-1 and 2 arose from cross-species transmissions of SIV to the human population on several occasions which account for the range of subgroups for both HIV-1 and HIV-2 [37, 42, 52]. Human infection with SIVcpz and SIVsm is thought to arise from exposure to infected blood during the hunting and butchering of chimpanzees and sooty magnabeys for food, bites from pet animals and possible contact with faeces and urine [53, 54]. A disproved [55, 56] alternate theory suggested that HIV-1 was a consequence of an oral polio vaccine (OPV), called CHAT, that was potentially contaminated with SIVcpz from chimpanzee tissue used in the preparation of the vaccine [57-59].

HIV-1 is the primary cause of AIDS worldwide [60, 61]. In most countries it is concentrated within certain risk groups, including men who have sex with men, injecting drug users, sex workers and the sexual partners of these people [62]. Many countries of sub-Saharan Africa have self-sustaining generalised epidemics, although high risk groups still exist [62].

It is estimated that HIV-1 was first introduced into the human population between around 1910 – 1930, most likely originating from central Africa [49, 63-65]. This coincides with urbanisation and the emergence of the first towns in this region [60]. The first reported evidence of AIDS in Africa was in the late 1950's – early 1960's [66, 67]. The earliest confirmed case of HIV was identified from a blood sample of a man from the Democratic Republic of Congo in 1959 [68]. Studies have found that strains had diversified substantially from the original strain that crossed over to humans, indicating that HIV had been circulating in the human population decades previously [63]. HIV-1 was suspected in the a Manchester man in 1959 who died of an AIDS–like illness [69, 70], but this was never confirmed [71].

HIV-1 was present in the USA from around 1967 – 69, more than a decade before the initial identification of AIDS in 1981 [64, 72, 73]. The serological prevalence of HIV in men who have sex with men was retrospectively estimated to be 4.5% in San Francisco [74] and 6.6% in New York City in 1978, suggesting that thousands of people in the USA were already infected by this

time but not identified due to the long asymptomatic phase of HIV [72]. Haiti was proposed as a possible source of the USA epidemic due to the high prevalence of HIV-1 among Haitian immigrants [64, 75-77]. However, the alternative possibility that sex tourism and Haitians returning from the USA introduced HIV-1 to Haiti could not be ruled out [78, 79]. One study found that Haiti had the oldest and most genetically diverse known HIV-1 epidemic outside Sub-Saharan Africa [72], with most of the pandemic clade of subtype B likely originating from a single point migration of the virus out of Haiti between 1966 – 1972 [72].

HIV-1 spread quickly through the pre-established contact networks of high risk groups such as, men who have sex with men and people who inject drugs in the USA and Europe during the early 1980's [10, 72]. The epidemic in Europe emerged 2 to 3 years after the USA [73], and differed in that a high proportion of cases were diagnosed in African immigrants in addition to men who have sex with men [80, 81]. Furthermore, the European epidemic emerged in people who inject drugs later than in men who have sex with men. The introduction of HIV-1 into Europe was thought to be driven by homosexual contact with individuals from the USA, Africa or Haiti; needle sharing in injecting drug users with individuals from the USA; sexual contact with individuals from central Africa; and immigration from central Africa [80-84].

Although specific factors and events that led to the emergence of epidemic HIV in the late 20th century are generally unknown, various contributors have been proposed. The HIV-1 transmission rate prior to this time was slower for an extensive period, possibly circulating in heterosexual populations [72]. The introduction of injectable medications in Africa between 1950 – 1970 acted as a dispersion agent of HIV in the modern era through wide spread unsterile use of needles and syringes [85]. This, in combination with destabilization of social structures [86], rapid growth of cities [63], and an increased prevalence of sexually transmitted diseases, such as genital ulcers [87], has been proposed to have facilitated the adaption and spread of both HIV-1 and HIV-2. The increasingly widespread use of air, land and sea transport networks also enabled rapid dispersion of the virus [88-90].

1.1.3 The HIV virus

Retroviruses are a family of enveloped viruses characterised by the presence of two single stranded RNA genomes and the enzyme reverse transcriptase [10, 91]. The genus lentiviridae (from the Latin lentus meaning 'slow') is characterised by a long interval between initial infection and the onset of serious symptoms (incubation period), a high rate of evolution and mutation, and an ability to integrate into the genome of non-dividing cells [10, 92]. Lentiviruses cause

chronic persistent infections in mammals, including bovines, horses, sheep, felines, and primates [37]. HIV and SIV encompass the primate lentiviruses [37, 91]. SIV covers a diverse group of over 40 different species-specific primate lentiviruses circulating among African non-human primate species [37, 93]. HIV-1 and HIV-2 are the only two lentiviruses known to circulate in human populations [93]. Although it can infect multiple cells in the body, the main target are the CD4 lymphocytes (T-cells or CD4 cells), a crucial part of the immune system [10, 11]. Figure 1.1 gives a graphical illustration of the HIV lifecycle within living cells of the infected host and is explained in detail in the following sections [88].

Figure 1.1 The life cycle of HIV [88].

1.1.3.1 The life cycle of HIV

Viral attachment

The HIV virus attaches itself to the CD4 cells. The pg120 protein on the outer surface of the virus binds to the CD4+ receptor on the cell surface. This then further binds to one or more of a set of coreceptors, notably the CC chemokine receptor CCR5 and CXC chemokine receptor CXCR4 [10, 94]. The specific receptor(s) that are utilised depends on the strain of HIV. T-tropic strains use the CXCR4 receptor have a preference for T-cells and can infect continuous CD4+ T cell lines

and primary CD4+ T cells [94], while M-tropic strains make use of the CCR5 receptor and can infect primary macrophages and primary CD4+T cells[94]. Dual-tropic strains can use both CXCR4 and CCR5 [94]. The binding with the corecepter results in a conformational change in gp120, allowing the gp41 protein to facilitate the fusion of the viral and host cell membranes.

Entry into the cell host.

The HIV virus releases its viral core (or capsid) into the host cell cytoplasm. The viral core is opened, releasing two copies of virus single strand RNA and the essential replication enzymes reverse transcriptase, protease and integrase.

Reverse transcription and integration

A double strand DNA copy, known as the provirus, is transcribed using the virus RNA by the HIV reverse transcriptase enzyme [95]. HIV integrase prepares the HIV DNA for integration into the host genome by cleaving a di-nucleotide from each 3' end of the DNA. It then guides it into the host nucleus and facilitates integration into the host DNA. The HIV DNA is now part of the host cell DNA.

Transcription and replication

Activation of the host cell induces transcription of the HIV DNA into messenger RNA (mRNA) using human enzymes, which contain complete copies of the HIV genetic material. These migrate into the cytoplasm and are used to synthesis new HIV proteins in a process called translation. The viral protease cleaves the long proteins produced by translation into shorter core proteins. HIV replication occurs at a very high rate and mutates extremely rapidly, resulting in immense adaptive potential and high viral genetic diversity within individual hosts, leading to a type of accelerated evolution. This is the reason that HIV is able to develop resistance to individual antiviral drugs within months [10].

Budding

Two new strands of HIV RNA and replication enzymes come together and core proteins assemble around them, forming a new capsid. The capsid then buds (peels off the host cell) taking some of the host membrane and creating a new HIV virus particle. The virus then matures and infects other cells. A single cell can produce thousands of infectious HIV particles. The host CD4 cell dies

shortly after the release of the new virus particles, which is a major reason for the destruction of the immune system [96, 97].

The provirus can lie dormant within a reservoir of latently infected cells, predominantly memory CD4+ T cells, for several years [98-100]. This guarantees lifetime persistence of HIV-1 in the majority of patients, despite effective cART regimens and prevents the development of vaccines for eradication of the virus [24, 101]. Other potential reservoirs include peripheral lymphoid organs and the tissues, bone marrow, the central nervous system and genitourinary tract [100].

1.1.4 HIV progression and clinical staging

The natural history of HIV infection, in the absence of effective treatment, is shown in Figure 1.2 [102].

Figure 1.2 Increase in HIV virus (plasma viremia titer) and the deterioration of the human immune system (CD4 cell count) over the course of an untreated infection [102, 103]

1.1.4.1 Primary infection with HIV

Primary infection (or acute infection) characterises the initial period soon after acquisition of HIV infection [104]. During the first few weeks, viral replication occurs rapidly [102], producing bursts of virus that infect many CD4 cells and involves all lymphatic tissue. This results in high levels of viremia in the blood which can reach over 1 million copies per ml [92] and a subsequent

short term sharp drop in the CD4 cell count occurs within the first 3 months, as shown in Figure 1.2 [92, 102, 104-107]. During this period, the person has a high HIV-VL and is highly infectious, but will be HIV seronegative if tested [105]. During the first few weeks of infection, most people experience seroconversion illness or retroviral syndrome, which represents a detectable humoral and cellular immune response to the virus (within 1 week-3 months) [102, 104, 106, 107]. Symptoms include a glandular fever like illness, rash, headache, feeling generally unwell, aches and pains, mouth ulcers, sore throat, night sweats, weight loss, tiredness, swollen glands, and neurological symptoms like meningitis [104]. The period at which the immune system responds to the virus and HIV antibodies are developed is known as seroconversion. This can take between 8 days to 3 months [102], after which the HIV-VL levels decline significantly (although reduced HIV replication continues in lymph nodes and other reservoirs) and the CD4 cell count is somewhat recovered [102, 104].

1.1.4.2 Chronic infection

Following primary infection, a prolonged period of clinical latency, also known as chronic HIV infection occurs, often lasting 10 – 12 years or more [104, 107-109]. During this time, the patient is usually asymptomatic; the rate of viral replication and HIV-VL levels remain low (although microbially active) and CD4 cell counts gradually decline slowly and continuously, reducing immune function [92, 102, 104, 110-114]. Once the CD4 cell count falls below 500 cells/mm^3, half of the immunological reservoir is destroyed and the patient is at risk of minor infections such as cold sores (herpes simplex), condyloma (warts) and fungal infections, thrush and vaginal candidiasis [92]. It is estimated that CD4 cell counts decline at $5.6x10^6$/L per month until 18 months before AIDS diagnosis [113]. A subset of people are long term non-progressors and are those who have had the virus for >10 years, remain clinically healthy in the absence of treatment, and display anti-HIV immune response [92].

1.1.4.3 Symptomatic HIV infection and clinical AIDS

In the absence of treatment, symptoms begin to manifest a median of 10 years after seroconversion and clinically apparent disease develops. When the CD4 cell count reaches <200 cells/mm^3 the patient becomes highly susceptible to an AIDS defining event, which is the end stage of HIV disease and includes serious opportunistic infections and AIDS defining cancers (ADC) (list of conditions that are considered an AIDS defining event in Table 1.1) [92]. The average survival time after AIDS diagnosis has been estimated to be less than 24 months [102, 103, 113, 115, 116] and varies according to population and type of AIDS defining event [117-119]. In the absence of treatment, time from seroconversion to death varies depending on,

amongst other things, age at seroconversion [120]. Estimates of median survival range from 4 years in those aged 65 at time of seroconversion or over to 12.5 years in those aged 15-24 years [120]. The onset of a second AIDS defining event leads to a 1.5 – 2-fold shorter survival time [121]. Very low CD4 cell counts have been associated with the onset of AIDS and death [122-129]. CD4 cell counts decline over the course of HIV infection and the rate of decline is predictive of disease progression [112, 123, 126, 130, 131] and death [131, 132].

Table 1.1 AIDS-indicator conditions [133, 134].

Candidiasis of bronchi, trachea, or lungs	Lymphoma, Burkitt's (or equivalent term)
Candidiasis, esophageal	Lymphoma, immunoblastic (or equivalent term)
Cervical cancer, invasive, confirmed by biopsy	Lymphoma, primary, of brain (CNS)
Coccidioidomycosis, disseminated or extrapulmonary	Mycobacterium avium complex (MAC) or M. kansasii, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary	Mycobacterium tuberculosis, any site (pulmonary * or extrapulmonary)
Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)	Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Cytomegalovirus disease (other than liver, spleen, or nodes)	Pneumocystis iroveci (formally carinii) pneumonia (PCP)
Cytomegalovirus retinitis (with loss of vision)	Pneumonia, recurrent *
Encephalopathy, HIV-related	Progressive multifocal leukoencephalopathy (PML)
Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis	Salmonella septicemia, recurrent (nontyphoid)
Histoplasmosis, disseminated or extrapulmonary	Toxoplasmosis of brain
Isosporiasis, chronic intestinal (greater than 1 month's duration)	Wasting syndrome due to HIV (involuntary weight loss >10% of baseline body weight) associated with either chronic diarrhoea (two or more loose stools per day for ≥1 month) or chronic weakness and documented fever for ≥1 month)
Kaposi's sarcoma	

1.1.5 Staging of HIV disease

Staging and classification of HIV disease is useful for monitoring the epidemic and for providing important information about HIV clinical care to physicians and patients. There are two main classification systems in use, both of which are explained below.

1.1.5.1 U.S. Centers for Disease Control and Prevention (CDC) classification (1993)

The CDC classification system uses CD4 cell counts and the presence of HIV-specific conditions to classify HIV disease severity (summarised in Table 1.2). The CDC definition of AIDS includes all people with their lowest documented CD4 cell count (also referred to as the CD4 nadir) <200 (or CD4% <14%) and also those previously diagnosed with HIV specific conditions. The classification is based on historic disease, meaning that once categorised, the patient will remain in that category even if they subsequently become asymptomatic. Although the CDC classification system tends to be used more often in epidemiological research than in a clinical setting, nevertheless a working understanding of clinical stages, particularly the AIDS definition, is important [134].

Table 1.2 CDC classification system for HIV-infected adults and adolescents [133, 134].

CD4 cell count categories	Clinical Categories		
	A Asymptomatic, acute HIV or persistent generalised lymphadenopathy	B[1] Symptomatic conditions, not in A or C	C AIDS-indicator conditions (see Table 1.1)
(1) ≥500 cells/μL	A1	B1	C1*
(2)200 - 499 cells/μL	A2	B2	C2*
(3) <200 cells//μL	A3*	B3*	C3*

[1]The Category B Symptomatic Conditions refer to symptomatic conditions which occur in an HIV+ adolescent or adult which are either attributed to HIV infection or indicate a defect in cell-mediated immunity or considered to have a clinical course or management that is complicated by HIV infection [133, 134].* Considered to have AIDS.

1.1.5.2 World Health Organization (WHO) Clinical Staging and Disease Classification system (2007)

The clinical staging and disease classification system developed by WHO, developed in 1990 and revised in 2007, was designed for use in resource limited settings and does not incorporate CD4 cell counts or other laboratory markers which may not be readily available [134]. The clinical staging is classified from stage 1 (early HIV infection) to 4 (advanced HIV infection) and is intended for use on people aged 15 years or older. Clinical staging is classified according to recognisable and treatable clinical symptoms (Table 1.3) and was previously widely used to

determine when to start cART, before the results of the START (Strategic Timing of Antiretroviral Therapy) trial changed treatment guidelines [134-142]. For more details on the START trial, see section 1.1.8.7.1).

Table 1.3 WHO clinical staging and disease classification.

Primary HIV infection	
Asymptomatic	Acute retroviral syndrome
Clinical stage 1	
Asymptomatic	Persistent generalised lymphadenopathy
Clinical stage 2	
Moderate unexplained weight loss (<10% of presumed or measured body weight)	Angular cheilitis
Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis)	Recurrent oral ulceration
Herpes zoster	Papular pruritic eruptions
Fungal nail infections	Seborrheic dermatitis
Clinical stage 3	
Unexplained severe weight loss (>10% of presumed or measured body weight)	Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)
Unexplained chronic diarrhoea for >1 month	Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis
Unexplained persistent fever for >1 month (>37.6ºC, intermittent or constant)	Unexplained anaemia (haemoglobin <8 g/dL)
Persistent oral candidiasis (thrush)	Neutropenia (neutrophils <500 cells/µL)
Oral hairy leukoplakia	Chronic thrombocytopenia (platelets <50,000 cells/µL)
Pulmonary tuberculosis (current)	
Clinical stage 4	
HIV wasting syndrome, as defined by the CDC (see Table 1.1)	Disseminated nontuberculosis mycobacteria infection
Pneumocystis pneumonia	Progressive multifocal leukoencephalopathy
Recurrent severe bacterial pneumonia	Candida of the trachea, bronchi, or lungs
Chronic herpes simplex infection (orolabial,	Chronic cryptosporidiosis (with diarrhoea)

genital, or anorectal site for >1 month or visceral herpes at any site)	
Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)	Chronic isosporiasis
Extrapulmonary tuberculosis	Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis)
Kaposi sarcoma	Recurrent nontyphoidal Salmonella bacteraemia
Cytomegalovirus infection (retinitis or infection of other organs)	Lymphoma (cerebral or B-cell non-Hodgkin)
Central nervous system toxoplasmosis	Invasive cervical carcinoma
HIV encephalopathy	Atypical disseminated leishmaniasis
Cryptococcosis, extrapulmonary (including meningitis)	Symptomatic HIV-associated nephropathy
Symptomatic HIV-associated cardiomyopathy	Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

1.1.6 HIV and the immune system

1.1.6.1 CD4 cell count

The size and composition of the CD4 cell population is regulated by a balance of proliferation of progenitor cells and death of mature progeny[11]. HIV infection disturbs this balance as HIV kills CD4 cells and inhibits the body's ability to replace them. The pool of CD4 cells then become depleted after years of viral replication, putting people at risk of opportunistic infections [10, 11]. A healthy person would usually have a CD4 cell count of between 500 – 1600 CD4+ T cells/mm^3 [92, 143, 144], although it is highly variable [110, 125, 144, 145] and can fluctuate according to time of day [114], recent or acute illness [143, 146], sleep quality [147], psychological factors [146, 148], exercise [149], smoking [143], and drug/pharmaceutical use [145, 146]. Furthermore, the natural daily fluctuation of CD4 cell counts within an individual is large [125, 150].

Women tend to have higher CD4 cell counts than men, which may be further influenced by phase of menstrual cycle and use of the contraceptive pill [151]. However the rate of decline during HIV-infection is similar between the groups [110, 152]. Children have higher CD4 cell counts than adults and CD4 cell counts naturally decline with age [144, 153]. Although a large proportion of the variation observed in CD4 cell count is biological, it is likely that some is due to measurement error and researchers have indicated that short term variations observed over a few days are unlikely to reflect disease progression or response to treatment [150]. Older age at seroconversion is associated with lower CD4 cell count at seroconversion, a steeper CD4 cell count decline [152], and faster rate of HIV progression (independently of CD4 cell count) [130, 152, 154]. Older patients are more likely to develop AIDS than younger people with the same CD4 cell count [154], likely due to increased exposure to opportunistic pathogens that can become reactivated during severe immunosuppression [154].

1.1.6.2 HIV viral load

Assays for quantifying HIV-VL emerged in the early 1990s. There are various techniques to measure HIV-VL, the main ones are reverse transcriptase PCR (RT-PCR), nucleic acid sequence-based amplification (NASBA) and branched DNA (bDNA)[155]. All assays produce fairly similar results, although each technique has advantages and disadvantages, including variations in sensitivity, reproducibility, ease of implementation and cost [155, 156]. Early assays had a lower detection limit of 400-500 copies/ml. This has dramatically improved over time with most current and routinely used assays having a detection limit of 40 – 50 copies/ml [155, 156]. Assays with lower detection limits are important for monitoring of patients on treatment. Highly sensitive assays have been developed with limits as low as 1 copy/ml, but are not routinely available [157, 158].

1.1.6.3 CD4 cell count and HIV viral load as markers of disease progression

Many studies have indicated that both HIV-VL and CD4 are important surrogate markers of HIV-1 disease progression [103, 159, 160]. Risk of disease progression correlates directly with HIV-VL level and inversely with the CD4 cell count [159, 161]. In turn, HIV-VL is inversely correlated with the CD4 cell count [128, 129, 162]. The predictive value of HIV-VL is thought to be higher in the initial stages of infection [131, 163, 164], whereas immunodeficiency, measured by CD4 cell count, becomes a stronger prognostic marker in later disease stages [131, 163-165]. HIV-VL is considered a good long term marker of disease progression, in other words, HIV-VL is predictive of disease progression 5 or 10 years in the future [159, 163, 164, 166-168]. Conversely, CD4 cell counts are better predictors of disease progression in the short term [161, 164, 165, 167]. The

prognosis of HIV+ people is more accurately defined by combined measurement of HIV-VL and CD4 lymphocytes [131, 159].

High HIV-VL strongly predicts an increased rate of CD4 decline [130, 159, 160, 164, 167, 168], and faster progression to AIDS [126-129, 160, 162, 168-171], and death [126, 128]. Furthermore, some studies have shown these associations exist across CD4 cell counts and risk group [131, 159, 160, 166, 169, 172]. In addition, the rate of change in HIV-VL over time (i.e. slope) has been linked to faster disease progression [162, 168]. People with long term asymptomatic disease have low HIV-VL levels [173, 174], further supporting the theory that increasing levels of viremia primarily drives HIV progression. The CD4 cell count is also an important prognostic marker [132], and many therapeutic decisions are based on CD4 cell count in practice. However, it should be kept in mind that people with higher HIV-VL levels can progress rapidly to AIDS and death even when CD4 cell counts are high [103].

1.1.7 Transmission of HIV

The global burden of people living with HIV is increasing, with an estimated 36.7 (34.0–39.8) million people living with HIV in 2015 [175]. This is partly driven by new infections, of which there were 2.1 (1.8–2.4) million in 2015 [175, 176] and a reduced mortality rate due to effective cART [12]. However, the number of new HIV infections is believed to have been declining since 2001 [175, 176].

HIV is a sexually transmitted infection (STI), and can also be transmitted through exposure to bodily fluids [92, 177]. Transmission can occur both vertically from mother to child (through pregnancy or breast feeding) [178] and horizontally through sexual contact (including vaginal, anal, and oral intercourse [179]), reuse of needles and syringes with residue of HIV+ blood [178, 180, 181], and blood transfusions [182, 183]. The risk of HIV transmission per act by exposure type is summarised in Table 1.4. HIV is most infectious in semen [184], blood and possibly cervical secretions, but has also been detected in lymphocytes, cell-free plasma, cerebrospinal fluid, tears, saliva, urine and breast milk in varying concentrations [177]. Risk of HIV infection depends on both the amount of secretions transferred from the HIV+ person and the amount of HIV present in those secretions [184, 185]. Furthermore, transmission risk increases with repeated exposure to HIV [179, 180], and varies by type of sexual contact [179]. HIV has limited survival outside the body. Therefore, HIV is not transmitted through casual or social contact, mosquitoes, lice, bed bugs, swimming pools, sharing of cooking/eating utensils, toilets or airspace with an infected individual[186].

Table 1.4 Estimated Per-Act Probability of Acquiring HIV in untreated populations from an Infected Source, by Exposure Act [187, 188].

1.1.7.1 Sexual transmission

Sexual intercourse (both anal and vaginal) is the most common route of transmission [186, 189]. During sexual contact, dendritic cells, a type of immune cell, come into contact with the virus on the exterior, transport it across mucosal barriers of the vagina, vulva, penis, or rectum, and release the virus directly into lymphatic tissues or lymph node [92]. The virus binds to a CD4 cell, where it is carried to lymphatic tissues and begins the first cycle of infection [92]. The HIV lifecycle is explained in more detail in section 1.1.3.

Although male to male sexual contact drives transmission in central and western Europe [189], most HIV transmissions globally are attributable to heterosexual sex [190]. Risk of sexual transmission is increased by rough sex, later HIV stage, or high HIV-VL of the HIV+ person, and coinfection with other STIs [184, 185]. Condom use, serostatus of chosen sexual partner and sex act performed also impact on risk of transmission [179]. Consistent and correct condom use

reduces the risk of transmission 20-fold, particularly among serodiscordant heterosexual couples [179]. The presence of other STIs can impact on risk by causing genital ulcers (i.e. herpes simplex virus [HSV]), or releasing inflammatory cytokines which increase HIV replication [92]. The risk of transmission is significantly reduced by antiretroviral therapy use by HIV+ people (through the consequent reduction in HIV-VL) and by preexposure prophylaxis in HIV-negative people [179, 191, 192]. There are many other approaches to reducing HIV transmission, some of which are demonstrated in Figure 1.3. Three randomised control trials (RCTs) in African countries have shown male circumcision to reduce the risk of infection through heterosexual sex in men by approximately 60% [92, 185, 193-195] and is recommended by WHO as an intervention to reduce HIV transmission in this group [196]. The role of circumcision has not been fully investigated among men who have sex with men [197]. However, a recent study has shown that circumcision is associated with a reduction in HIV incidence among participants who reported a preference for the insertive role in anal intercourse [198]. There is compelling evidence that male circumcision reduces the risk of transmission for males, however it has no impact on transmission for females and other preventative measures, such as condom use, are still required [48].

Figure 1.3 Changes in risk of transmission based on choices of partner and condom use.

1.1.7.2 Transmission via blood

Transmission of HIV via contact with HIV infected blood or blood products is a highly efficient route of infection [199] and can occur through blood transfusion with infected blood [182, 183], reusing and sharing needles with residue of infected blood [178, 180, 181], or occupational exposure (contact with infected blood) [92, 178, 186, 199]. The most common source of transmission via blood is through the reusing or sharing of syringes and needles among intravenous drug users (IDUs) [181]. Transmission occurs due to residual infected blood which remains on used syringes [178], and risk of transmission depends on a variety of factors including depth of injury, volume of blood, visible blood, location of injury i.e. artery or vein, HIV stage, and HIV-VL [200]. Recipients of blood transfusions with infected blood have the highest risk of infection, with infectivity estimates ranging from 88-100% per transfusion [182, 183, 201]. Blood products were not screened for HIV in the early years of the epidemic, and as a result a number of people became infected through exposure to infected blood products [202]. Blood has been routinely screened for HIV in Europe, USA and Canada, since 1985, and infection via this route is now uncommon where screening is correctly implemented [92].

1.1.7.3 Vertical transmission

A pregnant HIV+ woman can transmit HIV to her baby during pregnancy, labour, delivery, or through breastfeeding [92, 186, 203-206]. In the absence of treatment interventions, the risk of transmission from mother to child ranges from 15-40% [207-210], and is largely related to maternal HIV-VL [211]. In developed countries the rate of mother to child transmissions of HIV has decreased to <2% due to the implementation of universal prenatal HIV testing and counselling, antiretroviral treatment (ART) and combination prophylaxis, elective caesarean delivery, and avoidance of breastfeeding [92, 203, 212]. Coverage of antiretroviral programs for pregnant women increased from 57 in 2011 – 77% in 2015 globally [175].

A disparity in transmission rates exist between high and low-middle income countries, with 90% of children who acquired HIV infection in 2011 living in sub-Saharan Africa [203, 213]. Although this may improve with roll out of option B+ [214]. Rates of vertical transmission remain higher in low-middle income countries [176, 213], however the number of children who acquire HIV perinatally is now approaching the low HIV transmission rates in high-income countries due to increased coverage of preventative ART programmes [176].

1.1.8 HIV treatment

The introduction of combination antiretroviral treatment (cART) in 1996 revolutionised the lives of HIV+ people, and caused a sustained reduction in AIDS defining events and mortality (Figure 1.4)[12]. The mortality rate has reduced from 43.5/100 PYFU in the pre-ART era (1994/5) to 1.15 /100 PYFU in treated populations [12, 215-218]. Studies have shown that with timely diagnosis, access to a variety of drugs and good adherence, people with recently acquired infections can expect to have a life expectancy which is approaching that of HIV-negative individuals [2, 219]. As a result it is expected that non-AIDS defining illnesses, such as cancers, renal and cardiovascular disease, are to become increasing contributors to the burden of disease as the population ages [220, 221].

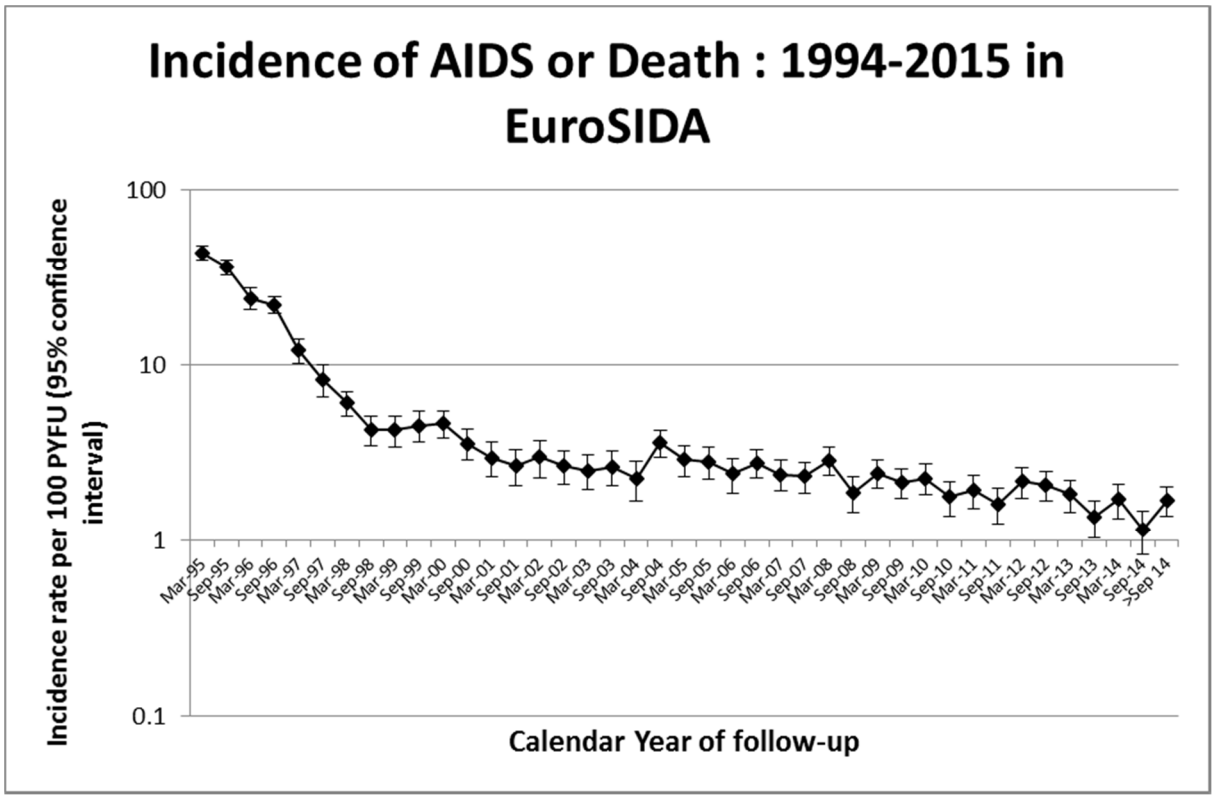


Figure 1.4 Combined incidence of AIDS and death rates in the EuroSIDA cohort, 1994 - 2015.

During the early stages of the epidemic there were no recognised treatments available and death rates were high [222]. In 1986 zidovudine, the first drug that effectively treated HIV, was developed. Initially intended to treat cancer [223], a phase II RCT in HIV+ people found that zidovudine significantly reduced mortality and opportunistic infections in people with AIDS and was terminated after 6 months at which point patients in the control arm were offered zidovudine [223, 224]. However, a very high rate of adverse events was reported in the zidovudine arm [225]. In March 1987 zidovudine became the first licenced drug to treat HIV in

the USA and zidovudine monotherapy (1 drug only) was standard care for HIV+ people [226]. However, HIV with resistance to zidovudine began to appear in patients after 6 months on treatment and treatment only prolonged life by 6 – 18 months [227-230]. There was a clear need for more effective and more tolerable treatments to increase survival [231].

The emergence of additional drugs from the nucleoside reverse transcriptase inhibitors (NRTI) class in 1991 allowed the use of dual therapy (2 drugs in combination) [222, 232]. The introduction of a second drug class (protease inhibitors [PIs]) in 1995 marked the beginning of the cART era, which allowed the use of 3 or more drugs in combination from at least 2 different drug classes (usually 2 NRTIs with one PI, non-nucleoside reverse transcriptase inhibitor [NNRTI] or integrase inhibitor [INSTI]) [222, 232, 233]. The introduction of a third drug class in 1998 (NNRTIs) lead to further options for cART regimens [222, 227, 232]. Each drug class is designed to interfere with different stages of the HIV life cycle (Figure 1.5, for an overview of the HIV lifecycle see section 1.1.3). RCTs have demonstrated that cART use is well tolerated and more effective at reducing HIV-VL and increasing CD4 cell count, as well as reducing the incidence of AIDS and death than dual therapy, and both more effective than monotherapy with lower risk of resistance [226, 234-242].

RCTs are a pivotal part of the process of approving new drugs for general use as well as to identify the best way to use treatment. New HIV drugs are often licenced based on RCTs with a primary endpoint of the proportion of people who achieve HIV-VL less than 50 copies/mL after 48 weeks of treatment rather than through RCTs comparing clinical end points [243] (see section 1.1.6.3 for a discussion of HIV surrogate markers). Before 1997, RCTs used clinical end points, such as HIV disease progression and mortality, to assess drugs for approval [243]. However the introduction of cART, subsequent decline in HIV-related illnesses, and wide spread use of HIV-VL monitoring to assess treatment response, rendered this unfeasible. It was concluded that HIV-VL was an appropriate surrogate end point as treatment-induced decreases in HIV-VL were highly predictive of a clinical benefit within a reasonable time frame [243].

Figure 1.5 Effect of ARVs at various points in the HIV lifecycle [244].

1.1.8.1 Long term cART

As there is currently no cure for HIV and treatment is a lifelong commitment, maximising treatment durability is crucial for successful and sustained outcomes [222]. Current treatment works by inhibiting HIV from integrating with host DNA and reproducing. However, viral reservoirs exist within the body which are beyond the reach of cART, and HIV will resume reproduction if treatment is stopped [100]. As a result, current treatment can only manage HIV, not cure it, and treatment is ongoing [100, 245].

The three main causes of treatment failure are drug toxicity, poor adherence, and development of drug resistance and these three factors are very closely connected [246]. High levels of adherence to cART are required to achieve long term suppression of HIV-VL. Incomplete adherence has been associated with virological rebound, immune decompensation, clinical progression, and HIV drug resistance [247-251]. However, unplanned treatment interruptions or changes may be necessary due to severe drug toxicity, treatment failure illness, surgery that prevents oral administration of medicine, or unavailability of drugs [246, 252].

Drug toxicities are the most frequent reason for discontinuation of a first line regimen in Europe [216, 246]. Toxicities to an individual drug or entire drug class can reduce quality of life of patients, leading to poor adherence to regimens and increasing the risk of developing resistant mutations. In extreme cases toxicities can be fatal, such as mitochondrial toxicity, hepatotoxicity and hypersensitivity reaction [253]. The Division of AIDS (DAIDS) produced the DAIDS adverse

events grading table, which is a shared tool for consistent assessment of the severity of AEs in participants enrolled in RCTs. Toxicities are graded as mild, moderate, severe, or life-threatening based on their severity (Table 1.5) [136, 254]. Clinicians are encouraged to discuss possible side effects due to drugs with their patients prior to treatment initiation to facilitate recognition of symptoms and signs of toxicities.

Table 1.5 Division of AIDS (DAIDS) adverse event grading table [254].

Grade of event	Condition
1: Mild	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated
2: Moderate	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated
3: Severe	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated
4: potentially life threatening and death	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

HIV treatment has developed rapidly over the last 20 years and patient responses have improved due to better adherence as a result of improved toxicity profiles, more convenient regimens (i.e. single tablet, once daily pill regimens) and adherence support [255-258]. CART remains the standard of care for HIV patients internationally. The history of HIV treatment is outlined in Figure 1.6. Since HIV treatment is not the main focus of this thesis, only a brief overview of each class is given here. Information on treatment guidelines and drug toxicity profiles can be found at the British HIV association (BHIVA) [139], AIDSinfo [259], and European AIDS clinical society (EACS) [140]. The current EACS treatment guidelines are outlined in section 1.1.8.7.2.

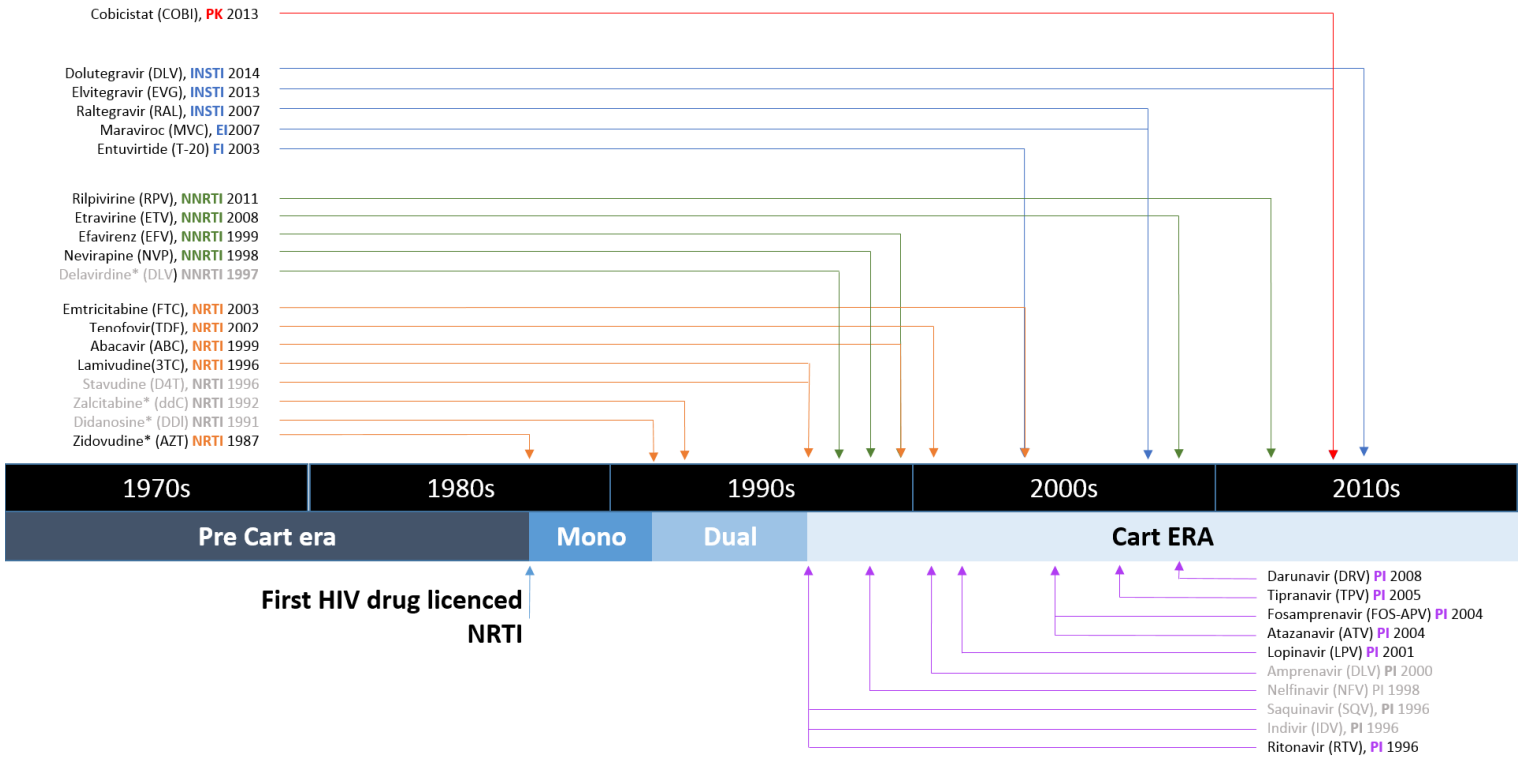


Figure 1.6 HIV treatment timeline history of drugs licenced for use in Europe.
Greyed out drugs are no longer recommended for use**. NRTI** Zidovudine [225, 260], Didanosine (ddl) [261-265], Zalcitabine (ddc) [266-272], Stavudine D4T [273], Lamivudine 3TC [274-280], Abacavir [281-283], Tenofovir [284, 285], Tenofovir-AF [286], Emtricitabine [287-289], **PI** Ritonavir [290], Indinavir [237, 238, 291-293], Saquinavir [234, 236, 294-296], Nelfinavir [297], Amprenavir [298, 299], Lopinavir/r [300-302], Atazanavir [303-308], Fosamprenavir [309-312], Tipranavir [313, 314], Darunavir [315-322], **NNRTI** Delavirdine [323-326], Nevirapine [241, 242, 327, 328], Efavirenz [329, 330], Etravirine [331, 332], Rilpivirine [333, 334], **Fusion/Entry/INSTI** Enfuvirtide [335-337], Maraviroc [338-341], Raltegravir [342-348], Elvitegravir [349-352], Dolutegravir [353-360]. **PK**: Cobicistat [361, 362].

1.1.8.2 Nucleoside reverse transcriptase inhibitors

The NRTI class was the first antiretroviral drug class approved for the treatment of HIV. NRTIs are “structural analogues” of nucleosides, which are converted into nucleotide analogues via cellular kinases – the molecular building blocks necessary for HIV reproduction [232, 363]. The nucleotide analogues interrupt the reverse transcription phase of the HIV lifecycle, in which the HIV enzyme reverse transcriptase converts single strand HIV RNA into double strand DNA [244]. When an analogue is used during reverse transcription instead of a viral nucleoside, the reverse transcriptase enzyme is prevented from creating the required chemical bonds to allow further addition of natural nucleotides [363]. This terminates the growing of the viral DNA chain and HIV replication within the cell is halted [364-366]. It should be noted that nucleotide analogue reverse-transcriptase inhibitors (NtRTIs) follow the same function as NRTIs, however they are analogues of nucleotide as opposed to nucleosides, which allows for the usual conversion of nucleoside to nucleotide to be skipped [363]. NRTIs licenced in Europe are summarised in Figure 1.6.

As previously mentioned, zidovudine was the first licenced drug to treat HIV [226], shortly followed by the emergence of didanosine and zalcitabine in 1991 and 1992 respectively [261, 262, 265]. Many other NRTIs have been developed over the years. The current EACS and BHIVA guidelines recommend tenofovir/emtricitabine or tenofovir-AF/emtricitabine as first line with abacavir/lamivudine as an alternate therapy, depending on third drug class used [139, 140]. Use of other NRTIs in naïve patients is no longer recommended due to mitochondrial toxicities associated with extended NRTI use including liver damage (manifesting as either lactic acidosis along with hepatic steatosis), myopathy, cardiotoxicity and peripheral neuropathy [225, 367, 368]. Both abacavir and zidovudine have been linked with cardiotoxicities and tenofovir with renal disease [367, 369-373]. Although zidovudine use is no longer recommended, it is still administered in some situations, such as pregnancy [368]. Zalcitabine and stavudine use is associated with significant mitochondrial and hepatic toxicities [368]. Zalcitabine was withdrawn from the market for adverse event concerns at the end of 2006 and stavudine is not recommended due to the long-term irreversible side effects [374]. In Europe, stavudine use has been progressively reduced during the past decade although it has not been yet completely phased out [375]. Newer NRTIs generally have fewer side effects and are more tolerable than older NRTIs [367].

1.1.8.3 Protease inhibitors

PIs disrupt the HIV lifecycle by binding to the protease enzyme and blocking the breakdown (cleavage) of large viral polyproteins into smaller functional subunits used to make new HIV particles [232, 364, 365, 376]. PIs do not stop HIV replication all together, however virions produced in the presence of PIs are immature and are not capable of infecting new cells [364]. Various RCTs have demonstrated the effectiveness of PIs in HIV+ people [371]. PIs approved for use in Europe are summarized in Figure 1.6.

First generation PIs had limited clinical utility due to high pill burden and low bioavailability, which lead to poor adherence and antiretroviral efficacy [233]. The first PIs to be developed were saquinavir [234-236] followed by indinavir, both licensed for use in Europe in 1996. Use of saquinavir boosted with ritonavir is no longer recommended due to high pill burden and possible association with QT interval prolongation [368, 377], and despite RCTs demonstrating favourable efficacy of indinavir containing cART, adverse renal toxicity and strict dietary intake recommendations had a negative impact on long term success [238, 291].

Ritonavir was found to be very potent against HIV, but was poorly tolerated due to toxicities and severe gastrointestinal symptoms [233, 290, 378-382]. However, it was found to increase concentrations of other PIs when coadministerd in low doses, a phenomenon known as boosting [383]. Ritonavir inhibits the primary enzyme involved in the metabolism of most PIs (the CYP3A4 isozyme located in the intestinal track and liver) leading to higher and more consistent concentration in the blood stream [383, 384]. Use of ritonavir as a booster for other PIs was found to be safe, generally well tolerated, with less frequent dosing, and better antiretroviral efficacy properties in the short term than a non-boosted PI regimen [294, 295, 301, 302, 383, 384]. An alternative drug used as a booster is cobicistat, which is classed as a pharmacokinetic enhancer and is licenced for use with atazanavir and darunavir [361, 362].

Darunavir boosted with either ritonavir or cobicistat is the only PI which is a currently recommended option for first-line cART, in combination with two NRTIs. Boosted atazanavir with either ritonavir or cobicistat and lopinavir boosted with ritonavir are listed as suitable alternate therapy options [139, 140]. PIs have been associated with gastrointestinal disorders, dysfunctions of lipid and glucose metabolism, sexual dysfunction, hepatic toxicity and increased risk of bleeding [385].

1.1.8.4 Non-nucleosides reverse transcript inhibitors

The first drug from the NNRTI class, nevirapine, was introduced with in 1998 and lead to further cART options [226, 227]. Like NRTIs, NNRTIs interrupt the HIV lifecycle by preventing conversion of HIV-RNA to HIV-DNA during reverse transcriptase. The difference between NRTI and NNRTIs is how the drugs interact with the reverse transcriptase enzyme [232, 363, 364]. Rather than acting as faulty nucleotides, NNRTIs bind to a hydrophobic pocket near the polymerase active site of the reverse transcriptase enzyme [232, 363, 364]. This changes the shape of the binding site making it incompatible with HIV-RNA, and thus interrupting the conversion of HIV RNA to DNA [232, 363, 364]. NNRTIs licensed for use in Europe are summarised in Figure 1.6. NNRTIs are only effective against HIV-1 [386].

Several RCTs demonstrated the superior efficacy of NNRTI use in combination with two NRTIs compared to dual or monotherapy [239-242]. NNRTI based regimens are generally well tolerated, with common adverse events including rash which can be treated with steroids and antihistamines. Efavirenz has been associated with central nervous system (CNS)-related side effects, including insomnia and vivid dreams [387]. Current guidelines recommend rilpivirine for first line treatment of antiretroviral naïve patients, with efavirenz as an alternative [139, 140]. Rilpivirine has less adverse events, a better lipid profile and similar rates of virological suppression than efavirenz, but higher rates of virological failure and resistance [333, 334, 388]. Effectiveness of efavirenz has been demonstrated across multiple RCTs, however the associated CNS-side effects make it less tolerable than other regimens [136, 368].

1.1.8.5 Integrase inhibitors

INSTIs are a recently developed drug class which target the HIV integrase enzyme in order to prevent the integration of HIV-DNA into the hosts DNA and in doing so inhibit replication [232, 389]. Raltegravir was the first INSTI to be approved in Europe in 2009 and there are currently three drugs from this class licenced for use in Europe: raltegravir, dolutegravir, and elvitegravir (Figure 1.6) [232].

Regimens containing raltegravir, dolutegravir, or elvitegravir have been shown to be non-inferior to other preferred antiretroviral regimens for antiretroviral naïve patients [351, 352, 358], and are well tolerated in both naïve and experienced patients [232, 390]. Elvitegravir boosted with cobicistat, dolutegravir and raltegravir are all approved as components of antiretroviral treatment options [136, 368]. Elvitegravir is a promising new drug, however, studies indicate a potentially high degree of cross resistance with raltegravir [391].

Raltegravir, dolutegravir, and elvitegravir are currently recommended to be included in first line cART regimens in combination with two NRTIs [139, 140]. Common adverse events are gastrointestinal effects, hyperbilirubinemia, and low eGFR. Dolutegravir has been associated with systemic hypersensitivity syndrome in <1% of users [140].

1.1.8.6 Entry and fusion inhibitors

Entry inhibitors prevent HIV from entering CD4 cells, by interfering with the ability of HIV to fuse with the cellular membrane of CD4 cells [392, 393]. The three stages of entry which are targeted include attachment of the viral gp120 to the CD4 T-cell receptor, binding of the GP120 to CCR5 or CDCR4 co-receptors, and fusion of the viral and cellular membranes (see section 1.1.3 for a detailed description of the HIV lifecycle) [392, 393]. There are currently only two drugs licenced from this class, maraviroc and enfuvirtide (previously known as T-20). Enfuvirtide was the first drug from this class to be licensed in Europe in 2003 [393]. It was developed for use in antiretroviral experienced people, and it has been found to be effective in in this group and in those with multidrug resistant HIV [337, 394, 395].

Maraviroc is the only licenced drug of the CCR5 receptor antagonist class and is used people with R5 HIV infection [396]. Maraviroc prevents HIV from entering CD4 cells by binding with the CCR5 receptor and as a result blocks gp120 interaction with the CCR5 receptor [397]. Maraviroc is not licenced in Europe for initial ART use, but can be used as salvage therapy in antiretroviral experienced people with multidrug resistant HIV [368].

1.1.8.7 Optimal treatment

1.1.8.7.1 Initiation of treatment

Several RCTs have investigated treatment strategy. The START study was a RCT which assessed the benefits and risks of earlier antiretroviral treatment. Antiretroviral naïve patients with CD4 cell counts > 500 cell/mm^3 were randomised to either initiate treatment immediately, or to initiate after CD4 cell count dropped below 350 cells/mm^3 (deferred treatment) [141, 142]. The trial demonstrated that those who initiated treatment immediately had a 57% reduction in the risk of serious AIDS defining event, serious non–AIDS defining event, or death from any cause [141].

The WHO, EACS, and BHIVA guidelines changed to recommend that all HIV+ people initiate cART regardless of CD4 cell count (from a previous cut-off of below 350 cells/mm^3) [135-140]. In

addition, the EACS guidelines recommend assessing the person's readiness to start and remain on cART prior to initiation [140]. A number of physiological and psychosocial factors, such as cognitive function, alcohol and drug use, high risk sex behaviours and depression also impact on the use of available treatments and resources by affecting drug uptake and adherence [104, 136]. Genotypic resistance testing is recommended prior to initiation of treatment [136]. It is recommended that the HIV-VL level and CD4 cell count should be measured before starting treatment to obtain a baseline to assess subsequent treatment response [136].

1.1.8.7.2 Current treatment guidelines and optimal ART regimen

Advancements in HIV treatment have transformed HIV infection from a fatal condition to a manageable chronic disease [245]. There are currently 29 antiretroviral drugs approved for use in Europe, which allows for a vast array of treatment options and regimens. Success rates are highest with initial therapy and diminish with subsequent regimens [398]. As a result, it is important that optimal treatment regimens are identified so that people commencing treatment can maximise the benefit and adherence to treatment regimes. Due to many different reasons there is still some debate over which choice of first-line treatment is optimal, one of the most important factors being long term durability. Use of fixed dose combinations, where two or more drugs are combined in a single dose, are thought to improve compliance [399]. For example, atripla is a fixed dose combination of efavirenz, emtricitabine, and tenofovir.

It is currently recommended that initial regimens use a combination of two NRTIs and third active drug of either a ritonavir boosted PI, NNRTI or INSTI as outlined in Table 1.6 [139, 140]. A drug from column A should be combined with the drugs from column B. Alternative treatment regimens are given in the lower part of Table 1.6. The third active drug should be individualised based on a variety patient factors including potential side effects, dosing requirements, dosing convenience, individual preference, co-morbidities, drug interactions and cost [400].

Although cART use is highly cost effective, it remains a major contributing factor to the cost of managing HIV [400]. Generic drugs are becoming available and stand to dramatically reduce the cost of some regimens. Currently, the EACS guidelines recommend the use of generics to replace the same non-generic drug, as long as they are available as part of a fixed dose combination where applicable [140]. However, it is possible that antiretroviral use may shift towards more inconvenient regimens, with higher pill burden and twice daily regimens, in place of more expensive yet convenient regiments [400, 401].

Table 1.6 EACS Initial recommended regimen for antiretroviral naïve HIV+ adults [140].

A	B
INSTI	**NRTI backbone**
Recommended regimens	
DTG	ABC/3TC or TDF/FTC or TAF/FTC
EVG/c	TDF/FTC or TAF/FTC
RAL	TDF/FTC or TAF/FTC
NNRTI	**NRTI backbone**
RPV	TDF/FTC or TAF/FTC
Boosted PI	**NRTI backbone**
DRV/r	TDF/FTC or TAF/FTC
DRV/c	TDF/FTC or TAF/FTC
Alternative regimens	
INSTI	**NRTI backbone**
RAL	ABC/3TC
NNRTI	**NRTI backbone**
EFV	ABC/3TC or TDF/FTC
Boosted PI	**NRTI backbone**
ATV/r	ABC/3TC or TDF/FTC or TAF/FTC
ATV/c	ABC/3TC or TDF/FTC or TAF/FTC
DRV/r	ABC/3TC
DRV/c	ABC/3TC
LPV/r	TDF/FTC or TAF/FTC
Dual combinations	
3TC	LPV/r
RAL	DRV/c or DRV/r

PI/r: boosted with ritonavir, PI/C: boosted with cobicistat

1.1.9 Testing for HIV

In December 2013, the UNAIDS programme set a goal to reach the following target by 2020: 90% of people living with HIV will know their HIV status, 90% of all people diagnosed with HIV will receive sustained antiretroviral therapy, and 90% of all people receiving antiretroviral therapy will have viral suppression [402]. This would result in 73% of HIV+ people worldwide to be virally suppressed [402]. The HIV cascade of care in Europe in 2014 is shown in Figure 1.7. It has been estimated that 76% of people who have HIV are tested and diagnosed, 78% of those

diagnosed are on treatment, and 88% of those on treatment are virally suppressed [403]. Overall, 53% of all HIV+ people (diagnosed and undiagnosed) are virally suppressed [403]. Similar estimates have been produced for the USA [203]. In addition, the CDC has estimated that in the USA up to 31% of people who test positive for HIV do not return to collect their test results and are therefore unaware of their status [404]. Optimising the availability of timely HIV testing facilitates linkage to care and initiation of treatment, and thus reduces new transmissions.

Figure 1.7 Cross-country continuum for 16 countries of Europe and Central Asia EU and EEA countries in 2014.
EU and EEA countries included: Austria, Bulgaria, Denmark, France, Germany, Luxembourg, the Netherlands, Romania, Spain, Sweden and the UK Non-EEA countries included: Armenia, Azerbaijan, Georgia and Serbia [403]

1.1.10 The global epidemic

There were estimated to be 36.7 (34.0–39.8) million people living with HIV in 2015, two thirds of which live in sub-Saharan Africa where women make up more than half of the HIV+ population [175]. The rate of new HIV infections per 100,000 population has declined by 38% from 3.4 (3.1 – 3.7) million in 2001 to 2.1 (1.8 – 2.4) to in 2015 (Figure 1.8), with a simultaneous decline in AIDS deaths from 2.3 (2.1 – 2.6) in 2005 to 1.1 (0.9 – 1.3) in 2015 [175, 405]. However, the number of people living with HIV is increasing (Figure 1.9) as more people have access to effective antiretroviral therapy which has had a dramatic improvement on the life expectancy [1-3, 175]. There is substantial variation in the HIV epidemic by region (Figure 1.10 and Figure 1.11) [175]. Sub-Saharan Africa is the region most affected by the HIV epidemic, with nearly 1 in every 20 adults (4.9%) living with HIV [175, 176, 213].

Figure 1.8 Estimated total number of new infections globally by calendar time [175].

Figure 1.9 Estimated total people living with HIV [175].

Figure 1.10 Estimated number of new HIV infections by region in 2015 [406, 407].

Figure 1.11 Estimated number of people living with HIV by region in 2015 [406, 407].

1.1.11 The European epidemic

The HIV epidemic in Europe remains a public health issue, and is heterogeneous across countries. It is mainly concentrated in key populations (although these vary by country) such as men who have sex with men, injecting drug users, migrants, and people originating from countries with generalised epidemics, such as Sub-Sahara African counties [408].

There were 153,407 new diagnoses of HIV reported in 50/53 countries of the WHO European region in 2015 (defined in the footnote of Table 1.7), corresponding to a diagnosis rate [IR] of 17.6 per 100,000 population [408]. Almost two thirds of new HIV diagnoses in the European region in 2015 occurred in from Russia (98,177, 64%), corresponding to a rate of 67/100,000 population [408]. There were an additional 55,230 diagnoses in 2015 in the rest of the region, of which 41% (22,911 cases) were in east, 49% (27,022 cases) in west, and 10% (5,297) in central Europe. Countries with the highest rates of HIV diagnosis were Russia (67 per 100,000 population), Ukraine (30), Belarus (24), Estonia (20) and Moldova (20), Latvia (20), and Georgia (17). The breakdown of new HIV diagnoses in 2015 by region and in key groups are shown in Table 1.7 [408].

Despite significant investment and efforts to control and prevention of HIV infection, the overall incidence continues to increase over the last decade, with a slight decline in recent years (Figure 1.12). Since 2006, the incidence of new HIV diagnoses per 100,000 population has increased by 59% from 11.1 in 2006 to 17.6 in 2015 per 100,000 population (including Russia), driven mainly by increases in eastern Europe, which represents a 108% increase from 22.8 in 2006 to 47.5 in

2015 (Figure 1.13) [408]. An increase in incidence was observed in central Europe (133 %, from 1.1 to 2.8 from 2006 to 2015). In western Europe incidence declined by 20%, from 7.9 to 6.3 in 2006 and 20105 respectively (Figure 1.13) [408].

Table 1.7 HIV diagnoses in the WHO European Region 2015 [408].

Russian data are included in the numbers in parentheses for the European Region and the East.
* No data received from Bosnia and Herzegovina, Russia, Turkmenistan, Uzbekistan. All data presented were reported to ECDC/WHO through the European Surveillance System (TESSy), except for data for Russia which were obtained through the Russian Federal Scientific and Methodological Center for Prevention and Control of AIDS
** EU/EEA rate is adjusted for reporting delay, the corresponding estimated number of new diagnoses adjusted for reporting delay is 32483.
The who region consists of: Western Europe : Andorra, Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Israel, Italy, Luxembourg, Malta, Monaco, the Netherlands, Norway, Portugal, San Marino, Spain, Sweden, Switzerland and the United Kingdom; Central Europe: Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, the Czech Republic, Hungary, Poland, Romania, Serbia and Montenegro, Slovakia, Slovenia, The former Yugoslav Republic of Macedonia and Turkey; Central Asian republics : Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan; Eastern Europe: Baltic states (Estonia, Latvia and Lithuania), Belarus, the Caucasus republics (Armenia, Azerbaijan and Georgia), the central Asian republics mentioned above, the Republic of Moldova, the Russian Federation and Ukraine;

Figure 1.12 Rate of new HIV diagnoses in the WHO European Region and European Union and European economic area (EU/EAA) 1986 – 2015 [408].

Figure 1.13 Incidence of HIV diagnoses over time within the WHO European Region by geographical area 2006 - 2015 [408].

1.1.11.1 Eastern Europe

The Eastern European epidemic began in 1995 in Ukraine, and spread to Belarus, the Republic of Moldova and the Russian Federation in 1996, Latvia in 1998, Estonia in 2000, Lithuania in 2002 and central Asia in 2003 [409, 410]. Eastern Europe is a major public health challenge due to the rising incidence of new HIV diagnosis, high incidence of AIDS defining events, and AIDS related mortality [408]. In 2015, almost three quarters of all AIDS diagnoses in Europe occurred in the east and increased by 80% between 2006 and 2015, however this has slowed since 2011 [408]. The high rate of AIDS diagnoses likely reflects a delay in HIV testing and diagnosis, late diagnosis, poor retention in care and barriers to accessing cART in this region [409, 410].

There are various barriers to the effective management of HIV in Eastern Europe, including structural barriers, treatment barriers, and social stigma [411]. Transmission of HIV in this region is primarily driven by IDU and needle sharing, however, heterosexual sex has also emerged as major contributor [409]. Eastern Europe has one of the largest IDU epidemics in the world a high prevalence of hepatitis C (HCV) coinfection [412]. HCV and HIV are interlinked with higher incidence of tuberculosis (TB), multidrug resistant TB (MDR-TB), end stage liver disease, and death [413-415]. Although needle substitution programs (NSP) and opioid substitution therapy (OST) programs are available in many countries, coverage of NSP is generally low and OST programs are very limited with only 1% of people who use injecting drugs receiving OST [416]. Lack of access to cART and low cART use in eastern Europe is driven by a lack of integration of health care services (such as HIV, TB, and OST), an expectation that HIV treatment is dependent on treated drug use (which is highly limited), and complex administrative procedures [417].

Social stigma and discrimination play a key part in the ongoing transmission of HIV, fuelling fear of testing positive, disclosing their HIV status to family and sexual partners, seeking out care, support, and treatment [411]. Stigma surrounding HIV is compounded in already stigmatised groups, such as such as people who inject drugs, Men who have sex with men, sex workers and migrants [411].

1.2 Cancers in the context of HIV

The availability of effective cART (see section 1.1.8 for an overview of HIV treatment) and improvements in the efficacy and tolerability of treatment regimens has led to increased immune-function and reduced incidence of AIDS defining events in HIV+ people [12]. As a result, survival has dramatically improved, with the median age at death projected to be as high as 75 years [1-6] and traditionally age associated conditions such as many cancers, cardiovascular events, and liver disease, are becoming key components of HIV care [418, 419]. Cancers are a significant source of morbidity and mortality and are now the second most common cause of death in HIV+ people following AIDS defining deaths (Figure 1.14) [13, 220]. It is estimated that 30 – 40% of HIV+ people are likely to develop cancer during the duration of their HIV infection [418]. Increased risk could be partially explained by high prevalence of traditional cancer risk factors such as smoking [420, 421], alcohol use [422, 423], and high risk sexual behaviour (associated with increased risk of some oncogenic viruses) [9, 424, 425] in the HIV+ population. However, coinfection with other oncogenic viruses [426], immunodeficiency [15, 427], activated inflammation and coagulation [428], direct pro-oncogenic effect of HIV [429] and cART toxicity [18] may also contribute.

As it has only been 20 years since the introduction of cART and many carcinogenic processes have long latency periods, evidence of an increased risk of cancers may emerge in the near future in addition to than those discussed in this section.

Figure 1.14 Most common causes of death in people with HIV [220].

1.2.1 AIDS-defining and non-AIDS defining cancers

Due to the historical classification of illnesses into AIDS (defined in section 1.1.4) and non-AIDS defining events, it has become custom to also classify cancers in this way. AIDS defining cancers (ADC) are Hodgkin lymphoma (HL), KS, and cervical cancer [133]. All other cancers are considered non-AIDS defining cancers (NADC) [133].

1.2.2 Common cancers in HIV-positive people

In the late cART era (2002 – present), the most commonly occurring cancers in the HIV+ population include: Non-Hodgkin lymphoma (NHL) (IR: 8 – 25 /10,000 PYFU), KS (6 – 23 /10,000 PYFU), HL (3 – 6 /10,000 PYFU), anal cancers (3 – 13/10,000 PYFU), prostate cancers (6 – 10/10,000 PYFU), head and neck cancers (2/10,000 PYFU), colorectal cancers (3-4/10,000 PYFU) and lung cancers (4 – 10/10,000 PYFU) [430-436]. Cervical cancers are also highly common in women (5 - 13/ 10,000 PYFU) [433, 436].

1.2.2.1 Non-Hodgkin Lymphoma

NHL remains the most common ADC in HIV+ people [430, 437]. Effective cART has led to a significant decline in the incidence of NHL [16, 20, 21, 438-442]. This decline has been observed for all subtypes (although mixed results have been reported for Burkitt's lymphomas) [440, 441, 443]. Despite this, NHL account for approximately half of all ADCs [444] and incidence currently remains around 10-fold higher [445] in HIV+ compared to negative people, albeit a decline from earlier estimates of 10 to 200-fold higher [15, 442, 446-448]. The lifetime risk of NHL has been estimated as 1 in 25 in HIV+ people in recent years [449].

NHL can be grouped into primary central nervous system (PBCNS) lymphoma, and the systemic lymphomas: Burkitt's, immunoblastic, diffuse large B cell and other subtypes [442, 450, 451]. The pathology and relationship between immunodeficiency and lymphoma development vary by subtype in HIV+ people [452]. For example, PBCNS lymphoma generally occur at advanced disease stages at very low CD4 cell counts [451], whereas systemic lymphomas can also occur in people with higher CD4, with almost half occurring in people with current CD4 >200 cells/mm^3 (however an increased risk increases with lower CD4 is still observed) [450]. NHL is discussed in more detail in chapters 5 and 6.

1.2.2.2 Kaposi's sarcoma

KS is the second most common ADC in HIV+ people in the late cART era [436, 453]. KS is a vascular tumour which commonly manifests as multiple lesions of the skin and mouth, but can also affect the lungs, liver, stomach and intestines [454]. KS is a rare type of cancer in the general population, however it occurs at an extremely high rate in immunocompromised populations, with a 112 – 3640-fold higher incidence in HIV+ people than in the general population [8, 15, 16, 442]. The lifetime risk of KS in HIV+ people has been estimated to be approximately 1 in 25 in recent years [449]. KS incidence highly elevated in those with very low CD4 cell counts (most occur with CD4 cell count < 150 cells/mm^3) [16, 455, 456], and multiple studies have demonstrated a notable decline in risk after cART initiation [21, 216, 457-460].

1.2.2.3 Cervical cancer

Cervical cancers are considered an ADC [133] and almost all cervical cancers are caused by Human papillomavirus (HPV) [461] the majority of which are caused by genotypes 16 (50%) and 18 (10-15%) and the remainder by other subtypes [461-463]. Risk of cervical cancer is elevated in HIV+ women, with highly variable estimated incidences of between 4 - 10 fold higher in HIV+ relative to HIV-negative women [15, 21, 436, 438, 444, 464, 465]. Early studies showed no decline in cervical cancers since the introduction of cART [20, 21, 438], however more recent studies have shown a decline of 12% per year [430, 436, 464]. A less prominent decline in cervical cancer incidence is also evident in the general population [7, 436, 464].

1.2.2.4 Hodgkin lymphoma

HL is characterised by the presence of Reed-Sternberg (RS) cells, the malignant cell population of this cancer type, within the cancer tissue [466]. HL is one of the most common NADCs that occur in HIV+ people. The incidence has remained stable [19] or even increased [21, 430, 467]

in HIV+ people since the introduction of cART [15, 20, 21, 438, 444, 468], and is estimated to be 11-fold higher (with estimates ranging from 5 – 19) than in the general population [8, 14-16, 438, 469]. This may in part be linked to a higher HL incidence associated with NNRTI use [467] or it may be more strongly linked to prior immunodeficiency duration.

The incidence of HL has been linked to lower CD4 cell count [427] where incidence highest at low - moderate CD4 cell counts [427, 470, 471]. The lifetime risk of HL in HIV+ people has been estimated as 1 in 125 in recent years [449]. HL is discussed in more detail in chapters 5 and 6.

1.2.2.5 Head and neck cancer

Some of the head and neck cancers are related to HPV infection [472-474]. HPV related head and neck cancers are one of the more frequently occurring NADCs, however the incidence is similar to that of the background population [14, 430, 437]. There is no evidence of a decline in the incidence of head and neck cancers since the introduction of cART [7, 467, 475]. The lifetime risk of oral cavity/pharyngeal cancers in HIV+ people has been estimated to be 1 in 100 in recent years [449].

1.2.2.6 Anal cancer

Anal cancers are related to HPV infection [476, 477] not considered an ADC, although the incidence is between 25 – over 100-fold higher in the HIV+ people (in both men and women, but for men who have sex with men in particular) compared to the general population and have a higher relative incidence than invasive cervical cancer, which is considered an ADC [14-16, 19, 430, 437, 438, 478-480]. Some studies have shown an association between anal cancers and lower nadir CD4 cell count [438, 481] and duration of immune suppression [433, 482], however no decline in incidence since the introduction of cART has been observed, and some studies have reported an increase [14, 19, 430, 432, 437, 438, 467, 479-481]. The lifetime risk of anal cancers in HIV+ people has been estimated to be 1 in 59 in recent years [449].

1.2.2.7 Liver cancer

The incidence of liver cancer is between 2 – 6-fold higher in HIV+ people compared to HIV-negative people [7, 15, 16, 20, 430, 437, 483] and are almost exclusively caused by Hepatitis B (HBV) and HCV [474, 484]. Coinfection with HCV and/or HBV and HIV is common due to shared transmission pathways, with up to 9% of HIV+ people from developed countries coinfected with

HBV [485]. The lifetime risk in of liver cancer in HIV+ people has been estimated to be 1 in 72 in recent years [449].

1.2.2.8 Prostate cancer

Prostate cancer (PCa) is the most common solid neoplasm in the older European man [1]. The incidence of PCa in HIV+ men has been estimated to be 27 (95% confidence interval [95%CI]: 43 – 8)% lower than HIV-negative men after adjusting for differences in PCa screening practices; a result that is consistent in many studies in the cART era [15, 16, 21, 431, 438, 469, 486-488]. Prostate cancer is explored in more detail in chapter 7.

1.2.2.9 Lung cancer

Lung cancer incidence in HIV+ people has been estimated to be 2 – 4-fold higher than in HIV-negative people [430, 433, 437], although the relative contribution of life-style related risk factors, such as smoking, towards elevated cancer risk, is not clear. The prevalence of smoking is high in HIV+ people with around 40 – 70% estimated to be current smokers, which is 2 – 3-fold higher than the general population [20, 489-496]. The lifetime risk in of liver cancer in HIV+ people has been estimated to be 1 in 25 in recent years [449]. Lung cancer is investigated in more detail in chapter 4.

1.2.3 Infection-related and unrelated cancers

In the general population it is estimated that between 15 – 20% of all incident cancers are associated with a viral infection [442]. HIV+ people are known to have higher rates of cancers with an infection-related cause (IRC) due to increased immune deficiency [8, 14-18, 427]. The classification of cancers into ADC and NADC, as described in section 1.2.1, is arbitrary and less relevant to clinical practice in the late cART era. A more natural classification is as IRC and infection-unrelated cancers (IURC), which are the terms to be used throughout this thesis. The three ADCs (NHL, KS, and cervical cancers) each have some degree of causal link with viruses and are therefore considered IRC:

- Epstein-Barr virus (EBV) has been linked to 30-100% of all NHL in HIV+ people [71, 72]
- human herpesvirus 8 (HHV8) infection is required for KS in both HIV+ and HIV-negative people [474, 497, 498]
- Almost all cervical cancers are caused by HPV [461].

In addition, the following NADC also have some degree of causal link with viruses and are therefore considered IRC:

- EBV causes 80-100% of Hodgkin's lymphoma (HL) [474, 499, 500].
- Liver cancers are almost exclusively caused by HCV and HBV [474, 484].
- Helicobacter pylori (*H.Pylori*) is associated with stomach cancer [474, 501]. However a small proportion of gastric cancers have also been linked with EBV (10%) [502].
- HPV causes some cancers of the head and neck, anus, penis, vulva and vagina[472-474]

Chapter 3 contains a more detailed discussion of IURC and IRC.

1.2.4 HIV and aging

The proportion of HIV+ adults aged over 50 years has increased in all regions of Europe since 2007, particularly in western and central Europe and North America (Figure 1.15) [503]. This is primarily driven by increased survival of HIV+ people due to effective treatment and a decline in incidence among younger adults (resulting in a lower prevalence of HIV in these age groups) [405, 503]. In addition, people aged over 50 years continue to be sexually active and have similar risk factors for HIV acquisition to younger people [405, 503]. This raises multiple issues with regard to future HIV care for older HIV+ people, for instance the increased complexity due to co-management and treatment of age-associated conditions, such as cardiovascular disease, diabetes and cancers [504].

Figure 1.15 The percentage of all people living with HIV aged over 50 years between 1995 and 2012 by region [503].

In addition, it has been suggested that HIV+ people suffer from premature "aging" of the immune system or immune aging ("inflamm-ageing") [505-508]. This theory is primarily driven by the following observations. First, HIV treatment does not fully restore health and HIV+ people

continue to have a shorter life expectancy than HIV-negative people despite long term treatment [219, 509-513]. Second, HIV+ people also have increased risk of traditionally "age related" complications, including heart disease, cancer, liver disease, kidney disease, bone disease, neurocognitive decline, and cataracts [505, 506, 514, 515]. Finally, HIV-associated inflammation and immunosenescence have been implicated as causally related to the premature onset of cancers, and end stage organ diseases, which occur despite effective treatment [505, 506, 516-520].

1.2.5 Known risk factors for common cancers

Cancers are a heterogeneous collection of disease, and risk factors vary significantly both within and between different cancer types. Table 1.8 shows some commonly known risk factors for various cancers in HIV+ people.

Table 1.8 Common risk factors for cancers in HIV-positive people.

Demographic factors	
Age	Older age has been linked to KS, NHL, cervical, lung and liver cancers [433].
Gender	Males have higher risk NHL, HL, lung, anal and liver cancers [433].
HIV Transmission group	Men who have sex with men have higher risk of KS, NHL, and anal cancers compared to other risk groups. Injecting drug users have higher risk of lung and liver cancers, however the association with liver cancers is due to the high prevalence of HCV in this group [433].
HIV-associated and other health factors	
CD4	Lower CD4 cell count has been linked to many cancers, including NHL, cervical cancers, KS, head and neck cancers, lung, colorectal, and liver cancers [16, 17, 433]. Lower CD4 cell count has been linked with lower HL risk, however risk is highest in people with CD4 49 – 99 cells/mm^3 [17]. Longer duration of CD4 < 200 cells/mm^3 has been linked with higher risk of anal cancer [433, 482].
HIV-VL	Higher current HIV-VL has been linked to higher risk of NHL, KS, cervical and head and neck cancers [16, 433]. Duration of time with HIV-VL < 200 copies/mL has been linked to lower high risk of anal cancers [433, 482].
HIV treatment	HIV treatment for 6 months or more is associated with lower risk of NHL, KS, and cervical cancers [433, 482].
HCV and HBV coinfection	HCV and HBV coinfection is strongly associated with liver cancers [433, 477, 482, 521].

Oncogenic viruses	High prevalence of oncogenic viruses in HIV+ people has led to higher incidence of IRCs, as discussed in section 1.2.3 [521].
Chronic inflammation	HIV-associated and otherwise [522].
Life style factors	
Smoking	A strong risk factor for lung cancers, as well as other cancers such as head and neck cancers, cervical cancers, and leukaemia [433, 482, 521].
Alcohol use	A strong risk factor for liver cancer [433], has also been implicated in head and neck cancers, colorectal, breast cancer [482, 521].
High risk sexual behaviours	Have been linked to a higher prevalence of HPV, and therefore HPV associated cancers [433].
Obesity	Absence of excess body fat reduces the risk of breast cancers, colon and rectal, oesophagus, kidney, gastric, gallbladder, pancreas, ovary, thyroid, multiple myeloma [521, 523].
Other	
Cancer causing substances	Includes work place and house hold exposures, such as asbestos and radon with lung cancer [524].
Sunlight	Sunlight has been linked to incidence of skin cancers [525].
Radiation	Radiation has been conclusively linked to lung, bone, and liver cancer. Some evidence of an association with leukaemia, and cancers of the pancreas and prostate also exist [526].
Pollution	Long term exposure to ambient fine particles is associated with elevated lung cancer risk [527].
Hormones	Such as breast cancer risk following combined hormone therapy (oestrogen and progesterone) [528].

KS: Kaposi's sarcoma, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, HIV-VL: HIV viral load, HCV: hepatitis C, HBV: hepatitis B, IRC: infection related cancer, HPV: human papillomavirus.

1.2.6 Treatment and prognosis in HIV-positive people with cancers

Clinical care of HIV+ people diagnosed with cancers is not straightforward and requires the co-operation of oncologists, haematologists, and HIV physicians [529]. The topic of cancer treatment in HIV+ people is broad and complex. Treatment strategies vary according to type and stage of cancer, drug interaction and toxicities with HIV treatment, and existing HIV-associated immune suppression [7, 530]. There is some evidence that HIV+ people present with more

aggressive and advanced disease at diagnosis [7, 530]. Poorer outcomes in HIV+ people have historically been reported [7, 531]. However, cancer survival has improved in recent years due to advancements in HIV and cancer therapies and some studies have shown survival for specific cancers to be similar to the general population [532, 533]. Five year relative survival for some cancers in a cohort of HIV+ Italian people and for the Italian general population are summarised in Table 1.9. 5 year survival is best for ADC and lowest for NADC [534]. One recent study showed that cancer related mortality within 5 years of diagnosis was higher among HIV+ compared with HIV-negative individuals for prostate and lung cancers, but not HL, anal cancer, or colorectal cancer [535]. Decreased survival following cancer diagnosis has been associated with lower CD4 cell count and injecting drug use. Use of cART has been shown to improve short – medium term survival of people with ADC [534, 536, 537]. Use of cART has been shown to improve survival for HL [538], but not other NADCs [534].

Table 1.9 Five year relative survival following cancer diagnosis in people with HIV in an Italian cohort [534].

Observational-Time	**1998-2012**	**2000-2004**
Cancer Type or site (ICD-10)	**5-years survival in the Master Cohort of HIV+ people (standardized for age and gender)**	**5-years survival in the Italian general population (AIRTUM) (standardized for age and gender)**
AIDS-defining cancer		
Kaposi sarcoma	80 (73-85)	87 (83-90)
Non-Hodgkin lymphoma	55 (48-62)	60 (59-60)
Cervical cancer	88 (70-95)	68 (66-69)
Non AIDS-defining cancer		
Liver cancer	32(20-45)	15 (15-16)
Hodgkin lymphoma	58(42-71)	83 (81-84)
Lung cancer	30 (15-47)	14(14-14)
Breast cancer	79 (55-91)	85(85-85)

Note: the five-year relative survival (age and gender-standardized) in the Italian general population for the cancers diagnosed between 2000 and 2004, and the 5-years relative survival in the Master cohort for the cancers diagnosed in the period 1998-2012**.**

Treatment recommendations for HIV-associated cancers in HIV+ people are given by BHIVA [139, 529], however a lack of RCT data means no gold standard exists for many cancers. Various drug interactions and overlapping toxicities for cancer treatments and antiviral drugs have been

described, including interactions between cancer treatments metabolized by P450 Enzymes with PIs (and ritonavir in particular) [529], and overlapping toxicities between zidovudine and cytotoxic chemotherapy (both cause myelosuppression and anaemia) [529]. Raltegravir does not use CYP pathway and is thought to be a potential cART option to reduce the risk of drug interactions [539]. The guidelines state that potential interactions and overlapping toxicities between cART, opportunistic infection prophylaxis, and cancer therapy should be considered when making treatment decisions [529, 530].

It is recommended that all HIV+ people diagnosed with ADCs should start cART. Similarly, HIV+ people starting chemoradiotherapy for NADCs should also start cART, unless contraindicated [529]. Furthermore, all HIV+ people diagnosed with cancer should be referred to centers that have expertise in the management of both diseases and that handle large volume of patients [529]. Several studies have shown that HIV+ people with cancer and /or HIV attending larger specialist centers have better patient outcomes [540-545]. Current treatment for cancer in HIV+ people is the same as for HIV-negative people.

1.3 Justification and objectives

At the commencement of this thesis, cancer had emerged as a major area of research in HIV+ people and was gathering a lot of interest, however, there were several research gaps that needed to be addressed. First, the majority of the research in cancer incidence and cancer burden in HIV+ people was focused on insured people within the USA. The changing epidemiology of cancers in the European population and the impact of aging on cancer incidence HIV+ people needed to be better characterised. Second, despite the well characterised harms of smoking in HIV+ people, the clinical benefits of smoking cessation on cancer risk had not been addressed in a well controlled, adequately powered study. Third, although both NHL and HL are common cancers in HIV+ people, research into the risk factors for NHL and HL within the same population was limited [433] and no studies had attempted to directly compare the two. Fourth, the mechanisms of the genesis of lymphomas in HIV+ people were not clear and whether B-cell activation prior to lymphoma development was driven by HIV facilitated B cell activation and proliferation (and also promoted EBV expansion) or other factors such as ongoing EBV replication was unspecified. Fifth, PCa is a highly common cancer in HIV-negative men and PSA testing is used to identify men at high risk for PCa in the general population to undergo further testing. There was limited data available on variations in PSA testing practices in HIV+ men. Most studies were USA-based, where PSA testing has been widely used since 1980, and may not be generalizable to European settings [431, 546]. No studies had evaluated levels of PSA to prior to

PCa diagnosis in HIV+ men or verified the use of PSA > 4 ng/mL to identify men at high risk for PCas.

My PhD has two key objectives

1. To describe the changing epidemiology of commonly occurring cancers in HIV+ people, particularly focusing on specific cancers or groups of cancers that are expected to become a major source of morbidity and mortality as the population ages.
2. To explore and characterise plasma biomarkers of common cancers in HIV+ people in order to better understand the mechanisms leading to cancer development.

These objectives will be investigated through 5 projects each corresponding to a results chapter.

Chapter 3 describes the changing incidence of infection-related and infection-unrelated cancers that occur as the HIV+ population ages across Europe, using the EuroSIDA study.

Chapter 4 investigates the impact of smoking cessation on cancer risk in HIV+ people, based on data from the D:A:D study.

Chapter 5 compares and contrasts the risk factors for non-Hodgkin (NHL) and Hodgkin lymphoma (HL) development in HIV+ people, based on data from the D:A:D study.

Chapter 6 explores markers of B-cell dysfunction and activation prior to the development of NHL and HL, using the EuroSIDA study.

Chapter 7 investigates the kinetics and diagnostic ability of total and free prostate specific antigen (PSA) associated with prostate cancer (PCa) risk prior to diagnosis, using the EuroSIDA study.

2 Data and methodology

2.1 Data

2.1.1 EuroSIDA

2.1.1.1 Overview

The studies presented in chapters 3, 6, and 7 are based on data from the EuroSIDA cohort, which is one of the largest prospective observational cohort studies of HIV-positive (HIV+) people in 35 European countries, as well as Israel and Argentina (Figure 2.1). EuroSIDA was established in May 1994 as the successor of the retrospective AIDS in Europe cohort study [547] and currently follows in excess of 22,000 people under care in a network of 115 hospitals. The main objective of EuroSIDA is to assess the impact of antiretroviral drugs on the long term prognosis for the general population of HIV+ people living in Europe and to follow the long term clinical prognosis of these people. However future objectives also included looking at AIDS (ADC) and non-AIDS (NADC) defining cancers, organ specific end stage diseases, long term and rare toxicities of combination antiretroviral therapy (cART), the HIV epidemic in Eastern Europe, Hepatitis B (HBV) and C (HCV) coinfection and HIV drug resistance.

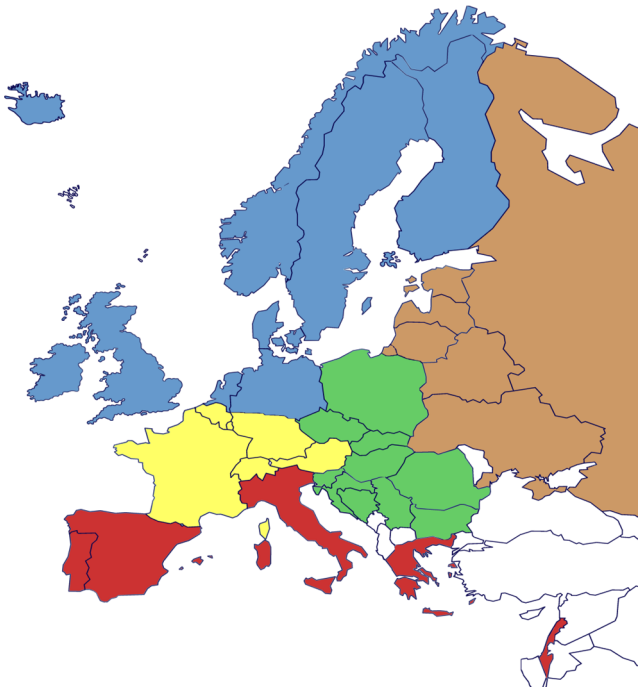
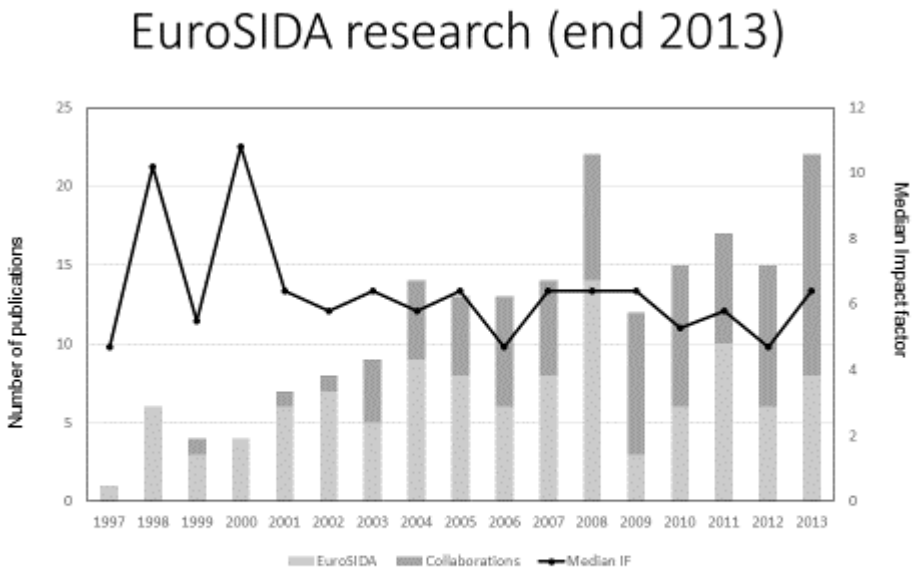


Figure 2.1 The European countries involved in EuroSIDA (Israel and Argentina are non-European participants).

EuroSIDA has enrolled over 22,000 HIV+ people over the 20 years the study has been running. This makes it particularly useful for looking at the natural history of disease, effects of different treatment strategies and long term side effects, and long term patient outcomes such as cancers and other non-AIDS defining events associated with aging. Furthermore, EuroSIDA has been able to track the course of the HIV epidemic across Europe, the effects of the introduction of cART, the use of cART in different regions of Europe, and shifts in the epidemic over time.

EuroSIDA is involved in or initiated several international cohort collaborative studies, which allow research questions which are not answerable in the individual cohorts to be addressed. These include the ART-Cohort Collaboration (ART-CC) and the Data Collection on Adverse Events (D:A:D) study, The Pursuing Later Treatment Options (PLATO) collaboration, and Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study. EuroSIDA has contributed significantly to the understanding of the HIV epidemic, including the impact of cART on the combined AIDS and death rate in HIV+ people, and has informed treatment guidelines across Europe. Production of scientific papers is the primary objective of the study. As of April 2017 EuroSIDA has had over 259 publications and presents original research at most major HIV related conferences. A summary of EuroSIDA publications over time is given in Figure 2.2 and full details of publications and presentations can be found at http://www.chip.dk/Ongoing-Studies/EuroSIDA/About.



Summary	All	EuroSIDA	Collaborations
Publications	196	110	86
Median IF	6.4	5.8	6.4
H-index	57	38	31
Citations	13817	6188	7629

Figure 2.2 Summary of EuroSIDA and collaborative publications as of 2013.

2.1.1.2 Enrolment of people

People aged 16 years or older were enrolled over 10 periods of enrolment (each of which is referred to as a cohort) from May 1994 with the most recent cohort (Cohort X) enrolled from May 2014. Each cohort commenced and continued until a predefined number of people had been enrolled. The target number of enrolments for each center is primarily determined by the total number of people under followed-up at the center and the ability of the center to provide follow-up data. However, maintenance of overall regional representativeness of the entire cohort is also taken into account.

The first cohort to be enrolled (cohort I and initially referred to as EuroSIDA I) began in May 1994 and enrolled 3,115 people. The second, cohort II, began in November 1995 and recruited 1,363 people. The third, cohort III, from February 1997 and recruited 2837 people. Since then, a further 7 cohorts have been added (Table 2.1). Recruitment is ongoing to ensure people in EuroSIDA are periodically updated to reflect the current HIV+ population, and also to replace people who have died or are lost to follow-up.

Table 2.1 10 Enrolment within EuroSIDA by cohort.

Cohort	N of people	Enrolment date
I	3115	Spring 1994
II	1363	Winter 1995
III	2837	Spring 1997
IV	1225	Spring 1999
V	1223	Autumn 2001
VI	2119	Spring 2004
VII	2458	Winter 2005
VIII	2259	Summer 2008
IX	2469	Winter 2012
X	3985	Spring 2014

People who are under follow-up during each period of enrolment are recruited in such a way that ensures a random selection of people currently under care in each clinic. People were required to have a booked routine outpatient appointment to be eligible for recruitment. This ensures people are already in routine care and provides as close to an unbiased and representative selection of people as possible from each clinic. The eligibility criteria for enrolment have changed over time. People were initially required to have a CD4 count below 500 cells/mm^3 within 4 months prior to the date of enrolment for cohorts I – III. However, this

restriction was relaxed for cohorts IV and later as CD4 cell counts improved due to the generally widespread availability of cART across Europe [548]. From cohort V onwards, recruitment included people from Eastern Europe as research in this region was a priority due to the severity and variation in the driving factors of the HIV epidemic [549]. Cohort X was the first EuroSIDA cohort composed entirely of HCV/HIV coinfected people. There has been increasing interest in HCV/HIV coinfected people in recent years. An entirely HCV/HIV coinfected cohort will facilitate the monitoring of these people over time and also follow the roll out and impact of second generation HCV drugs.

Analysis on EuroSIDA is often divided into 4 – 6 demographic regions, depending on the research question to be answered and the number of people available for analysis. The regions are southern, west central, north, east and central eastern Europe, and Argentina and are shown in Table 2.2. In analyses using Cohorts I - VI, the east and east central regions were combined due to small numbers, however these have been increasingly been treated as separate regions from cohort VII onwards due to increased recruitment of people from eastern Europe.

Table 2.2 EuroSIDA regions by country and center.

Region	Countries	Centers
Southern Europe	Spain, Portugal, Italy, Greece, Serbia, Montenegro, Israel, and Argentina[1]	26
West central	France, Belgium, South Germany, Luxemburg, Switzerland, Austria	26
North	United Kingdom, Ireland, Netherlands, North Germany, Denmark, Finland, Sweden, Norway, Iceland	24
East[2]	Belarus, Estonia, Latvia, Lithuania, Russia, Georgia, and Ukraine	20
East Central Europe[2]	Poland, Czech Republic, Slovenia, Slovakia, Hungary, Romania, Bulgaria, Croatia, Bosnia & Herzegovina, and Serbia.	18
Argentina	Argentina	1

[1]If numbers are small, Argentina is combined with Southern Europe region.

2.1.1.3 The EuroSIDA organization

The structure of the EuroSIDA organisation is shown in Figure 2.3. EuroSIDA is governed by a steering committee made up of 14 European scientists and physicians and the current chair of the steering committee is Professor Jürgen Rockstroh (Bonn, Germany), election to the steering committee is for a period of 2 years and is renewed by election every 4-5 years. A list of the steering committee members of the EuroSIDA study group are shown in Appendix I. Approximately every two months, the steering committee hold teleconferences to discuss the progress of current projects, approval of new project proposals, and the overall running of EuroSIDA, funding and sponsorship. In addition, face to face meetings are held at most major European HIV conferences (such as the International Drug Congress in HIV Infection and the European AIDS clinical society conferences [EACS]). Separately to the steering committee, a core group of clinicians and statisticians meet 3-4 times a year to discuss future projects, work priorities, and provide general feedback. This active collaboration between clinicians, statisticians, epidemiologists, and HIV virologists, hepatologists and pharmacologists is crucial for the running and ongoing development of the study.

Every potential research project within EuroSIDA must first be approved by the steering committee and goes through a rigorous review process. Briefly, the proposing researcher must put together a study proposal which summarised the background, objectives, significance, feasibility, possible limitations, study design, data, statistical analysis, timelines, potential conferences and budget. The proposal is then reviewed by two members of the steering committee and any amendments are incorporated. The proposal is introduced and discussed at the next scheduled steering committee meeting. If there are no further comments or recommendations the project is approved. The template for new study proposals can be found at http://www.chip.dk/Ongoing-Studies/EuroSIDA/Study-documents.

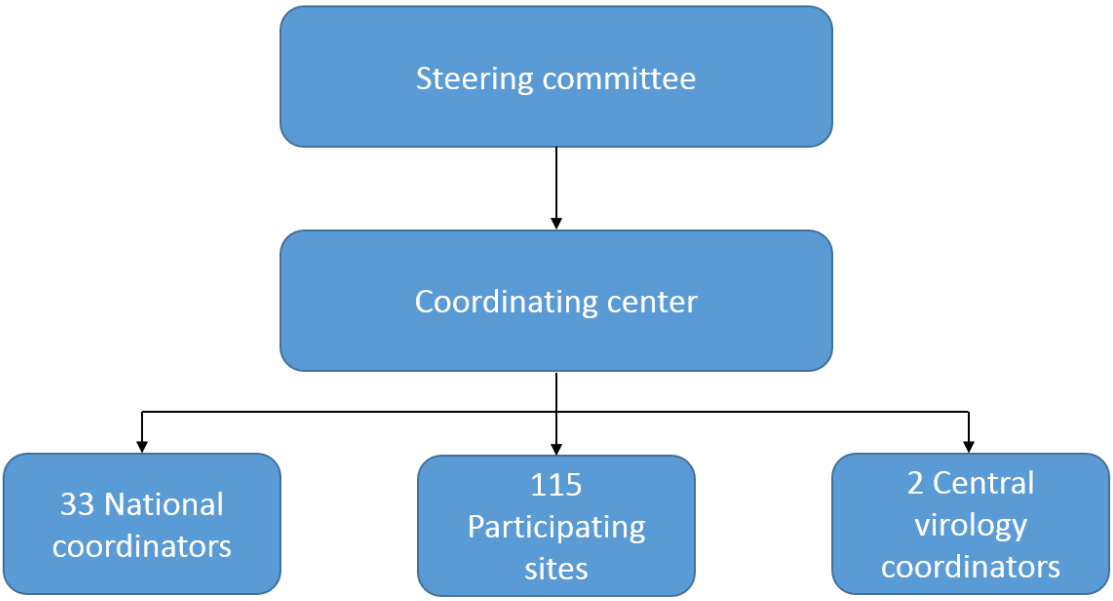


Figure 2.3 Structure of the EuroSIDA organisation.

The Center of Excellence for Health, Immunity and Infections (CHIP) is a research department, headed by Professor Jens Lundgren. CHIP is responsible for the overall coordination of the EuroSIDA study (among other research activities) and for the management of the EuroSIDA database (http://www.chip.dk/Ongoing-Studies/EuroSIDA/About). The statistical support is provided by the statistical center which is based at the university College London (UCL) medical School, Royal Free Campus, and London, UK. There are 3 working groups which conduct focused research in the areas of biomarkers, Hepatitis, and virology.

2.1.1.4 The epidemiology of resistance to antivirals group

The Epidemiology of resistance to antivirals group in EuroSIDA has historically led research projects in the field of HIV drug resistance. The main objective is to evaluate associations between genotypic resistance test results and virological, immunological and clinical outcomes. EuroSIDA maintains an additional database on HIV resistance tests performed locally in clinics which are reported to the central database. In addition, plasma samples are also prospectively collected for patients enrolled in a subset of centres approximately every 6 months. Retrospective testing of these samples is possible as long as funding is secured for specific projects. The collection and shipping guidelines for these samples follows those outlined for the plasma repository in the section 2.1.1.9. The group originally included two virological laboratories: the ICVC international clinical virology center, based in London, UK and the

IrsiCaixa Foundation, Badalona, Spain. The collaboration with ICVC ended in 2004, since then all virological analyses of the samples are performed at the IrsiCaixa Foundation Centre.

2.1.1.5 The hepatitis group

Hepatitis coinfection is common in HIV+ people and has become an area of increasing interest. The main objective of this group is to assess the natural history of chronic HBV and HCV in HIV+ people, how they impact on the tolerability and efficacy of HIV treatment, the roll out of hepatitis treatments and associated patient outcomes, as well as regional differences in these parameters. The EuroSIDA steering committee decided in 2014 to enrol an entirely HCV/HIV coinfected cohort (cohort X) with a target of 4,000 people in order to increase the ability to focus on the impact of the new oral direct-acting antiviral hepatitis C drugs as they are gradually introduced across Europe. EuroSIDA contains a large number of HCV/HIV coinfected people, with approximately 35% of all people in EuroSIDA being anti-HCV IgG positive. The EuroSIDA hepatitis group is headed by Jürgen Rockstroh and Lars Peters.

2.1.1.6 Biomarkers group

The biomarkers group conducts research into the roles of circulating biomarkers in HIV+ people. The main objective is to identify and evaluate the use of serum biomarkers for various conditions, with a particular focus on cancers, in HIV+ people. The biomarkers group is comprised of members from both the statistical and coordinating centers. Projects undertaken by this group are varied and address a broad range of conditions. A group of disease specific clinicians act as an advisory board to provide disease specific clinical advice throughout the design, analysis and interpretation of each study. The studies presented in chapters 6 and 7 involve biomarkers.

2.1.1.7 Data collection

The EuroSIDA data has been collected using electronic forms through the REDCAP system since the beginning of 2017. A standardized electronic data collection form is completed by the sites at the time of enrolment and every 12 months thereafter. Historically, EuroSIDA data was collected using standardized paper forms every 6 months from 1994 to 2015. A process of switching from paper to electronic forms using the REDCAP system started in 2015 and in 2017 it was decided to move from a 6 monthly to a 12 monthly data collection. Some but not all sites provide an electronic download of data in HICDEP format [550]. Basic demographic, clinical and laboratory data are collected, including all CD4 cell counts and HIV viral loads (HIV-VL) measured

since last follow-up, starting and stopping dates of all antiretroviral drugs, smoking status, dates of AIDS defining diagnoses (using the 1993 CDC clinical definition[133], includes ADCs) and of non-AIDS defining diagnoses (prospectively recorded and included in quality assurance since 1 January 2001, includes NADCs) [221]. The data collected in EuroSIDA are summarised in Table 2.3. Those interested in more detail regarding the electronic forms and the wide range of data collected can contact the staff at the coordinating center to gain access to the REDCAP data system for a test person.

EuroSIDA collects information on both ADCs and NADCs in section G1 of the follow up form. The question 'Any new AIDS defining malignancies?' is used to identify whether any new ADCs have been diagnosed since the last follow-up form was completed. If no, then the option "No" is selected with an X. If "Yes" then they are prompted to complete the additional questions detailing date of diagnosis, method of diagnosis (definitive, presumptive, autopsy), and location (selected from a list of common cancers or as free text). Prior to 2001, NADCs were non-routinely collected using a passive reporting system. Subsequently, the standardised forms were updated to collect data on all new diagnoses of NADCs in 2001 in a similar fashion to ADCs [221].

Table 2.3 Summary of data items collected in EuroSIDA.

Demographics
Cohort
Region of Europe
Country
Center ID
Date of birth
Gender
Origin
Mode of HIV infection
Race

Basic clinical information
Dates of clinic visits
Height
Weight
Blood pressure
Smoking
Family history of MI
Pregnancy in women
Active injecting drug use
Alcohol abuse
Hospitalisations

AIDS defining cancers (ADC)
Dates and diagnosis (definitive, presumptive, autopsy)

Non-AIDS defining cancers (NADC)
Dates and diagnosis (definitive, presumptive, autopsy)

AIDS defining events
Date of AIDS onset

Other clinical events
Diagnosed since last follow-up (with date of diagnosis):
Cardiovascular events
Diabetes
severe hepatic encephalopathy (stage III or IV)
Pancreatitis
Renal disease
Lactic acidosis
Avascular Necrosis of the femural head
Bone fractures

Laboratory values (and dates of measurement)
Serum total and HDL cholesterol
Serum triglycerides
Plasma glucose
S-creatinine
Haemoglobin
Platelet count
ALT and AST
INR
Bilirubin
S-lactate (not LDH)
S-amylase
CD4 cell counts
HIV viral load
HIV subtyping
Resistance testing
Prostate specific antigen
parathyroid hormone
25 hydroxy-vitamin D
Toxoplasma antibody
CMV antibody
Proteinuria
Diastolic and systolic blood pressure

Antiretroviral treatment
History of antiretrovirals taken:
Starting and stopping dates
If discontinued, reason for discontinuation
Adherence rating

Hepatitis virology/serology results and dates*:
HBV antibody
HBsAg
HBV DNA
HCV antibody
HCV RNA
HCV genotype

Liver fibrosis parameters
Liver biopsy
Fibroscan® elastography
Hyaluronic acid
APRI (calculation)
FIB-4 (calculation)

Treatment for HBV and HCV infection:
Start and stop dates
Adherence rating

Treatment against infections
Drugs to prevent or treat opportunistic infection:
Start and stop dates

Treatment related to risk of cardiovascular disease
Medication related to risk of cardiovascular disease:
Starting and stopping dates

Severe opportunistic infections
Dates and diagnosis (definitive, presumptive, autopsy)

Other severe infections
Dates and diagnosis (definitive, presumptive, autopsy)

Influenza
Dates and diagnosis (definitive, presumptive, autopsy)
Treatment
Hospitalisations

For people who died
Date of death
Presumed cause
CoDe case report form including autopsy report [551, 552]

2.1.1.8 Data quality

Staff from the EuroSIDA coordinating center periodically visit all sites participating in EuroSIDA to ensure high data quality is maintained and to ensure correct person inclusion according to the established criteria. All reported ADC and NADC (since 2001) have been source verified against case notes at the sites by members of the coordinating office to ensure data accuracy, as well as for all other major clinical events and a random sample of 10% of all other people. To date, EuroSIDA has collected a relatively large number of NADCs (and other non-AIDS defining events) due to the large size and long follow-up time. This allows for research questions focussing on NADCs to be investigated which might not be viable in other cohorts. Occasionally, monitoring is used to obtain extra information from case notes that were not included in the original data submission.

2.1.1.9 Plasma sample repository

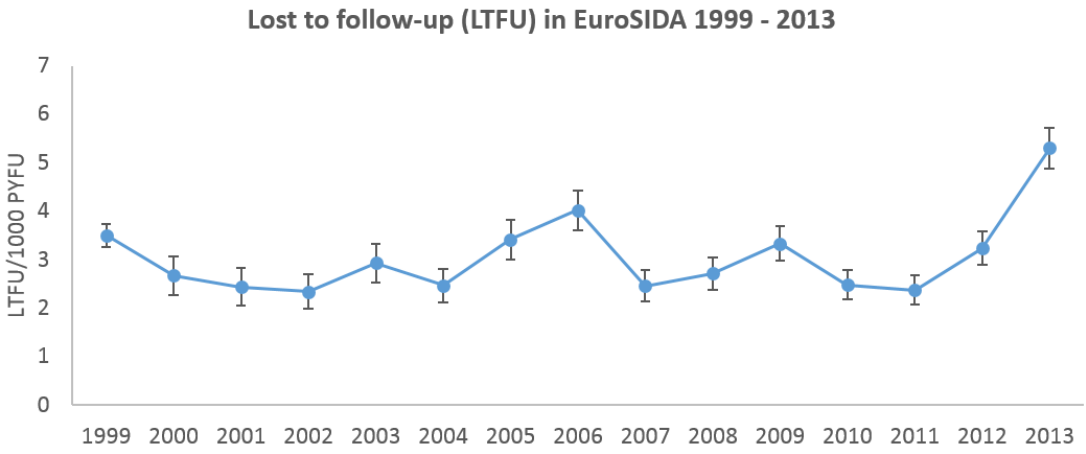
The plasma sample repository was established in 1997. The repository contains a large number of prospectively collected plasma samples for approximately 13,563 EuroSIDA participants, stored at six-month intervals. It currently holds approximately 105,747 unique samples (or 171,1023 samples including duplicates). For each sample, between 3-5 ml of EDTA blood is collected, separated by centrifugation (e.g. 1.500 g, 15 min.), stored in 2 x 1 ml aliquots in 1,8 ml screw top cryovials (e.g. Nunc 377267 or similar). Sites are recommended to store samples locally at a – 70° Celsius or liquid nitrogen within 4-6 hours of venesection. If - 70° Celsius or liquid nitrogen is not available, site are advised to use - 20° Celsius freezer. If plasma have been stored in freezers with temperatures above - 50° Celsius, or if more than 6 hours have passed before plasma has been frozen, then the sample labelled accordingly. Samples are periodically shipped in batches to the central repository located at the coordinating center. The coordinating center maintains a list of people detailing which have had samples collected, the date of collection, as well as whether sample has been used for a previous analysis. When required for a specific analysis, the sample id's can be requested, extracted from the freezers and analysed. Since samples are collected prospectively and independently of clinical events, they make EuroSIDA an ideal cohort to investigate potential biomarkers for various conditions within nested case-control studies. The plasma samples have been used to identify genotypic resistance mutations for projects on HIV resistance mutations [553], to extract HCV antibodies and HCV RNA viral loads to classify the status of HCV infection for projects on HCV coinfected individuals [554], and to measure lab values not reported in the main EuroSIDA database, such as vitamin D level prior to event in HIV+ people [555, 556].

2.1.1.10 Loss to follow-up

EuroSIDA has a low lost to follow up rate of 3.72 per 100 person years of follow-up (PYFU) and has been consistent over time [557], ranging between 2 and 8 per 100 PYFU [557] (Figure 2.4). The total number of loss to follow-up in each calendar year is shown in the data table under the graph. Older patients, those with higher CD4 count, and those who have started cART had lower incidence of loss to follow-up and loss to follow-up has been found to be higher in those from East Europe.

2.1.1.11 Ethical approval and funding

Clinical centres participating in EuroSIDA are all required to obtain ethics approval from their local authority in order to contribute to the study. Copies of the ethics approval form for each center are kept at the coordinating centre, as required by the previous funders (1994-2015), the European commission.



N LTFU	837	171	152	167	208	197	265	376	227	266	351	251	231	354	594

Figure 2.4 Loss to follow-up (LTFU) in EuroSIDA 1998 – 2013.

EuroSIDA was supported 2010-2015 by the European Union's Seventh Framework Programme for research, technological development and demonstration under EuroCoord grant agreement n° 260694. Current support includes unrestricted grants by Bristol-Myers Squibb, Gilead, GlaxoSmithKline LLC, Janssen R&D, Merck and Co. Inc., Pfizer Inc. The participation of centres from Switzerland was supported by The Swiss National Science Foundation (Grant 108787). The study is also supported by a grant [grant number DNRF126] from the Danish National Research Foundation.

2.1.1.12 Summary of key characteristics

The key characteristics of people under follow-up in EuroSIDA and the D:A:D Study (to be discussed in section 2.1.2) on the 1 January 2015 are given in Table 2.4. There were 11,266 people under follow-up, with a median of 9 (Inter quartile range (IQR]: 3-16) years of follow-up per person. The median age was 49 (IQR: 41 – 56) years. Approximately two thirds the cohort were from south, west-central and north Europe, and the remaining one third from east central, east, and Argentina. Just under three quarters were male (72%). The main mode of HIV transmission was sex between men (38%), followed by heterosexual contact (32%) and injecting drug use (23%). Of those under follow-up, 28% had had an AIDS defining event, 10% a non-AIDS defining event, 32% had HCV and 5% had HBV. 81% were on cART, 87% had a HIV-VL ≤ 500 copies/ml. The median CD4 cell count was 590 (IQR: 418, 801) cells/mm^3.

Table 2.4 Key characteristics of the EuroSIDA cohort and the D:A:D Study for people under follow-up on the 1 January 2015.

	EuroSIDA	D:A:D
	N(%) or median (IQR)	*N(%) or median (IQR)*
Total	11,266 (100)	28,785 (100)
Age (years)		
16 - 34	1,233 (10.9)	1,733 (6.0)
35 - 44	2,731 (24.2)	6,509 (22.6)
45 - 54	4,227 (37.5)	11,897 (41.3)
55 - 64	2,141 (19.0)	5,971 (20.7)
65+	924 (8.2)	2,673 (9.3)
Missing	10 (0.1)	2 (0.0)
Median (IQR)	49.2 (41.1,55.7)	50.3 (44.0,56.6)
gender		
Male	8,133 (72.2)	21,270 (73.9)
Female	3,129 (27.8)	7,514 (26.1)
Missing	4 (0.0)	1 (0.0)
Region of Europe		
South	2,451 (21.8)	-
West central	2,572 (22.8)	-
North	2,509 (22.3)	-
East central	1,737 (15.4)	-
East	1,532 (13.6)	-
Argentina	465 (4.1)	-
HIV mode of transmission		
Sex between men	4,297 (38.1)	13,750 (47.8)
IDU	2,531 (22.5)	3,128 (10.9)
Heterosexual	3,600 (32.0)	10,197 (35.4)
Other or Unknown	838 (7.4)	1,710 (5.9)
HBV[1]	551 (4.9)	919 (3.2)
HCV[2]	3,629 (32.2)	5,782 (20.1)
Previous AIDS defining event	3,187 (28.3)	8,741 (30.4)
Previous non-AIDS defining event	1,142 (10.1)	-
On cART[3]	9,103 (80.8)	22,842 (79.4)
HIV-VL (copies/mL)		
≤ 500	9,801 (87.0)	26,567 (92.3)
>500	694 (6.2)	2,204 (7.7)
Unknown	771 (6.8)	14 (0.0)
Median (IQR)	36.0 (<19,<40)	50.0 (50.0,50.0)
CD4 cell count (cells/mm^3)		
<200	453 (4.0)	1,310 (4.6)
200-350	1,094 (9.7)	2,050 (7.1)
351-500	1,806 (16.0)	4,424 (15.4)
>500	5,918 (52.5)	17,995 (62.5)
Missing	1,995 (17.7)	3,006 (10.4)
Median (IQR)	590 (418, 801)	634 (462, 835)
Median follow-up per person (years, IQR)	8.9 (2.8, 15.6)	12.3 (8.8, 15.2)

IQR: Interquartile range, HBV: Hepatitis B, HCV: Hepatitis C, cART: combination antiretroviral therapy, HIV-VL: HIV viral load.

[1] Positive HBV status was defined by a prior positive HBsAG surface antigen test or presence of detectable HBV DNA,

[2] Positive HCV status was defined as having a prior positive HCV surface antibody test

[3] currently on ≥ 3 antiretroviral drugs from any drug class

2.1.2 The Data collection on adverse events of Anti-HIV Drugs (D:A:D) study

2.1.2.1 Overview

Chapters 4 and 5 utilise data from The D:A:D Study, which was a large, observational, and multinational multi-cohort study of HIV+ people set up in 1999 as part of a European Medicines Agency (EMA) regulatory initiative. The collaboration was founded to address clinical questions which could not readily be answered within the individual participating cohorts, and the main objective was to assess the potential associations between exposure to cART and cardiovascular disease (CVD) in HIV+ people. Additionally, the D:A:D Study has investigated potential associations between cART and other serious non-AIDS events, such as end stage liver and renal disease, cancer and death as well as risk factors for these events. In 2017, the regulatory requirements formally ended and the funding of the D:A:D Study was concluded.

2.1.2.2 Participating Cohorts

The D:A:D Study incorporated data from 11 individual cohorts of HIV+ people including follow-up on more than 49,000 people from 212 clinics in 33 countries in Europe, USA and Australia (Figure 2.5). The study had accrued 467,477 person years of follow-up as of 1 February 2016. The study population consisted of both ART-naive and experienced people, enrolled during three enrolment cohorts (I: 1999-2001, II: 2001-2004 III: 2004-2009). The original study population of 23,441 people were enrolled December 1999 - April 2001, and was referred to as D:A:D Cohort I. An additional 12,900 were enrolled in D:A:D Cohort II throughout the spring 2004, and more than 16,000 were enrolled in D:A:D Cohort III in 2010.

As shown in Figure 2.5, EuroSIDA was one of the largest cohorts that contributed data to the D:A:D Study. As of December 2016, EuroSIDA had contributed 12,000 people and 110,032 person years of follow-up, accounting for 24.1% of all people and 23.5% of person years of follow-up included the D:A:D Study.

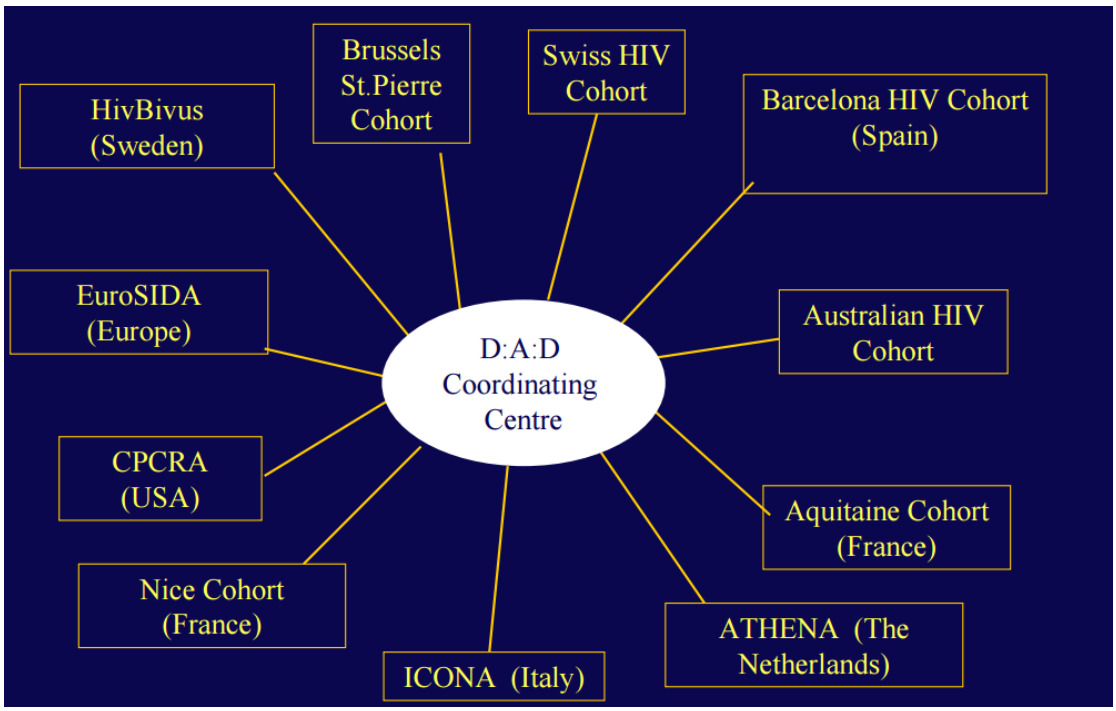


Figure 2.5 Individual cohorts contributing to the D:A:D study.

2.1.2.3 The D:A:D organizational structure

The D:A:D Study was headed by the D:A:D Steering Committee, consisting of the study chair, Professor Jens Lundgren, principal investigators for each of the contributing cohorts, members of the statistical department and representatives from the Highly Active Antiretroviral Therapy Oversight Committee (HAART-OC) (see Appendix II). The HAART-OC was a collaborative committee with representation from academic institutions, the EMA, The US Food and Drug Administration, the patient community and all pharmaceutical companies with licensed antiretroviral drugs in the European Union.

The D:A:D coordinating office was located at CHIP, Denmark. The coordinating office was responsible for collecting data in real-time from the contributing cohorts, continuous data queries and cleaning and an annual data merger process, data cleaning as well as event adjudication, review of events with external experts, annual monitoring of the D:A:D Study sites, organisation of meetings and development and maintenance of study reports and manuals. The statistical center was located at the Royal Free Campus of UCL medical school, UK, and provided statistical support and support for further cleaning of data.

2.1.2.4 Data collection

Data is stored in a central database at the D:A:D coordinating office at CHIP and collected in in two ways. First, each participating cohort gathers and computerises its own data which is collected bi-annually electronically using the using the HICDEP data exchange protocol [550]. This data includes information on basic socio-demographics, use of ARVs, various HIV and laboratory markers, opportunistic infections and CVD risk factors such as familiar dispositions, diabetes, hypertension and smoking status. Second, clinical events are reported to D:A:D in real time, using designated case report forms, as defined by the D:A:D Study Manual of Operations (MOOP, for more details see http://www.chip.dk/Studies/DAD/Study-Documents).

The D:A:D Study data collection on centrally validated events included cardiovascular disease (CVD), including Myocardial Infarctions (MIs) (primary endpoint), Strokes, Diabetes, Invasive Coronary Procedures (ICPs) and causes of death from 1999. The D:A:D Study data collection was expanded in 2004 to include NADC, End-Stage Liver Disease (ESLD), End-Stage Renal Disease (ESRD) and causes of death according to CoDe (Coding causes of Death in HIV); and AIDS-Defining Cancer (ADC) [133] from 2013 (collected electronically from 1999). Since the participating cohorts had systematically collected NADCs from 2004 or earlier, prospectively collected NADCs diagnosed after 1 January 2004 were obtained from each cohort, except for the Swiss cohort where all events were collected prospectively from 2008. For NADCs, detailed information included date of diagnosis, type/location of cancer, stage of disease and histology/cytology report or other applied diagnostic methods (imaging, laboratory markers or clinical evaluation) (for details see http://www.chip.dk/Studies/DAD/Study-Documents).

2.1.2.5 Data quality

One of the most important strengths of the D:A:D Study was that all reported clinical events were centrally validated at the D:A:D coordinating center by a medical doctor and difficult cases were additionally reviewed by an expert in the relevant field (cardiology, nephrology and oncology). Events were also monitored at the D:A:D Study sites annually to ensure that data were accurate. Additionally, continuous and rigorous data querying of study sites for extra information by the D:A:D coordination centre throughout the year contributed to a high level of data quality. The validation of NADCs and ADCs were conducted as follows: All reported cancers were evaluated and classified as definite, probable or possible cancers. This classification reflects the degree of certainty of the NADC diagnosis (degree of strength in descending order): *definite*: based on supportive histology/cytology reports or a detailed summary of histopathological findings/cancer diagnosis in a hospital discharge

summary/consultation note; *probable:* strong suspicion of cancer supported by (i) evidence from radiological or other imaging technique or (ii) biochemical assay; *possible*: strong suspicion of cancer by visual inspection (e.g. skin metastasis, suspected malignant melanoma, tissue growth resembling cancer visualized during endoscopy/anoscopy) not explained by other known conditions. Events not reaching any of these categories of classification were classified as null events and not included in analyses

2.1.2.6 Loss to follow-up

The annual loss to follow-up rates in the individual participating cohorts as well as overall in the D:A:D Study was very low (<3%), and had been since the study was initiated.

2.1.2.7 Ethics approval and funding

The D:A:D Study had appropriate ethical committee approval from each participating country. Support for the study was given by HAART-OC and a number of pharmaceutical companies producing antiretroviral drugs contribute financially. The funding of the D:A:D Study was provided by the HAART-OC and in addition each cohort received additional funding in a national level (for details see Appendix II).

2.1.2.8 Summary of key characteristics

The key characteristics of people under follow-up in the D:A:D Study on the 1 January 2015 are given in Table 2.4. There were 28,785 people under follow-up, with a median of 12 (IQR: 9 – 15) years of follow-up per person. The median age was 50 (IQR: 44 – 57) years. Just under three quarters were male (74%). The main HIV transmission mode was sex between men (48%), followed by heterosexual contact (35%) and injecting drug use (11%). Of those under follow-up, 30% had had an AIDS defining event, 20% had HCV and 3% had HBV. 79% were on cART, 92% had a HIV-VL ≤ 500 copies/ml. The median CD4 cell count was 634 (IQR: 462, 835) cells/mm^3.

2.2 Statistical methods

This section provides a detailed description of the statistical methods used throughout this thesis. A brief snap shot of the data source, version and items used for each analysis as well as the statistical methods used is shown in Table 2.5. The methods section of each results chapter also summarizes the data and methods used, however greater detail is given here.

Table 2.5 Summary of data source and methods for each chapter.

Chapter	Data	Study Design	N included	Inclusion criterion	Primary Endpoint	Statistical analysis
3	EuroSIDA D36	Prospective cohort study	15,648	All people with follow-up after 1 /1/2004	Diagnosis of infection related or unrelated cancers	Poisson regression and Linear exponential smoothing models
4	D:A:D (Feb 2016 merger)	Collaboration of 11 prospective cohorts	35,442	All people with follow-up after 1 /1/2004 with no history of any cancer	Primary diagnosis of lung cancer, other smoking related cancer (excluding lung) and smoking unrelated cancers	Poisson regression
5	D:A:D (Feb 2015 merger)	Collaboration of 11 prospective cohorts	41,420	All people with follow-up after 1 January 2004, with ≥1 CD4 cell count, and no history of NHL or HL	Primary diagnosis of NHL and HL	Cox regression and Marginal cox models
6	EuroSIDA D39	Matched 1-2 Nested case-control study	**Cases:** 73 **Controls:** 143	**Cases**: Men with a primary NHL or HL diagnosis after 1 /1/2001 with ≥1 plasma sample available prior to the diagnosis date. **Controls**: Men with no history of NHL or HL and ≥1 plasma sample available at the diagnosis date of each case	Primary diagnosis of NHL and HL	Conditional logistic regression and random effects models
7	EuroSIDA D38	1.Prospective cohort study	4482	All men with follow-up after 1 January 2008 who attended clinics who performed PSA tests on >5% of men annually.	PSA testing	Poisson regression
		2.Matched 1-2 Nested case-control study	**Cases:** 21 **controls:**40	**Cases**: Men with a primary PCa diagnosis after 1 /1/2001 with ≥1 plasma sample available prior to the date of diagnosis. **Controls**: Men with no history of PCa and ≥1 plasma sample available at the date of diagnosis of each case	Primary diagnosis of prostate cancer	Conditional logistic regression, random effects models, Sensitivity, specificity and ROC analysis

PCa: Prostate cancer, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma

2.2.1 Summary statistics.

The first step when analysing data is to produce summary statistics. This serves several purposes:

- to provide a description of the characteristics of the population being studied
- to compare the study population with other populations i.e. for example other studies in the literature
- identify possible data errors or inconsistencies
- provide insight into the quality of the data.

Cohort and case-control studies are observational studies, meaning that the investigator does not intervene and rather simply "observes" and assesses the strength of the relationship between an exposure and disease variable. Unlike randomised control trials (RCTs), patients are not randomised, therefore there are likely to be differences between the exposure groups. For example, in a cohort study looking at the association between body mass index (BMI) and cancer risk, those with a higher BMI are also likely to be older than those with a lower BMI. Summary statistics are required to identify any such unbalances between the exposure groups under investigation. The types of summary statistics used throughout this thesis are outlined below.

2.2.1.1 Categorical data

Categorical variables were described using the frequency (N) and percentage (%) within each group. Differences across group were compared using Pearson's chi-squared tests when there were ≥5 and Fisher exact tests when there were <5 people in any level. Chapters 6 and 7 in this thesis involve nested matched case-control studies, for which differences across groups were compared using conditional logistic regression.

2.2.1.2 Numerical data

Continuous variables were described using the median and the IQR. The median is a valid measure of central tendency when the data has a symmetrical or skewed distribution and is less susceptible to outliers. Another common approach is to present the mean and standard deviation (SD), which is valid for symmetrically distributed data but not skewed data, and is more susceptible to outliers (see Figure 2.6). For this reason, Median and IQR were used throughout this thesis.

To compare numerical variables across two groups of people, the Wilcoxon rank sum test was used. This is a non-parametric test and is valid in both normally and non-normally distributed data, as there is no underlying assumption about the data distribution. The kruskal-wallis test (an extension of the Wilcoxon rank sum test) was used when comparing 3 or more groups. The signed rank test was used to compare a change in a numerical variable across two groups of people, when the change between two measurements on the same person was of interest.

Figure 2.6 Examples of normal and skewed distributions [558].

Other commonly used tests include parametric unpaired t-tests (used to compare 2 groups of people and is the parametric equivalent of the Wilcoxon ranked sum test) and F-tests (used to compare >2 groups of people and is the parametric equivalent of the kruskal-wallis test), and paired t-tests (parametric equivalent of the signed rank test) when comparing values within the same person. These tests are only appropriate when the data is normal (an underlying assumption of these test).

All statistical tests presented in this thesis were two sided with a type I error rate of 5%, i.e. a P-value <0.05 was considered statistically significant, meaning there was sufficient evidence to reject the null hypothesis (usually no difference between the groups or independence) in favour of the alternate (usually there is a difference between the groups or dependence). When interpreting data, it is important to also consider the clinical importance of your results. For this reason, importance was also given to the effect size and width of the confidence intervals (wider indicates less certainty). All statistical analyses were performed using SAS 9.4 (Statistical Analysis Software, Cary NC, USA).

2.2.2 Statistical models

Statistical models are a class of mathematical models which represent a simplification of the complex real world processes which gave rise to the data observed. Statistical models allow us to quantify the relationship between a dependent variable (sometimes referred to as the outcome or response) and a set of independent variables (referred to as predictors, explanatory variables or covariates). For example: describing how the incidence of cancer increases with older age. They also incorporate a random element to account for differences between the predicted model and the observed data. Furthermore, they can also be used as a method to adjust for known confounding variables.

Alternatively, they can be used to predict the chance of an outcome of interest based on a set of observed variables. For example: what is the chance that a given person develops a non-Hodgkin lymphoma based on their age, gender and CD4 cell count.

2.2.2.1 Confounding

A Confounder is a variable whose presence distorts the association between the variables being studied so that the results do not reflect the actual relationship (Figure 2.7) [559]. Confounding is a real effect, not a bias, that may lead to errors in the conclusion of a study. To be a confounder, a variable must satisfy all of the following criteria (Figure 2.8) [559]:

- be associated with the exposure
- be an independent risk factor of the outcome
- and not lie on the causal pathway between exposure and outcome (mediator)

For example, older individuals are at higher risk for cancers, however, older individuals also tend to earn more than younger individuals, who have spent less time in the work force. If age was not adjusted for in the analysis then we may come to the conclusion that people with higher incomes are more likely to develop cancers than people with lower incomes. In this scenario we say that age is confounding the relationship between income and development of a cancer.

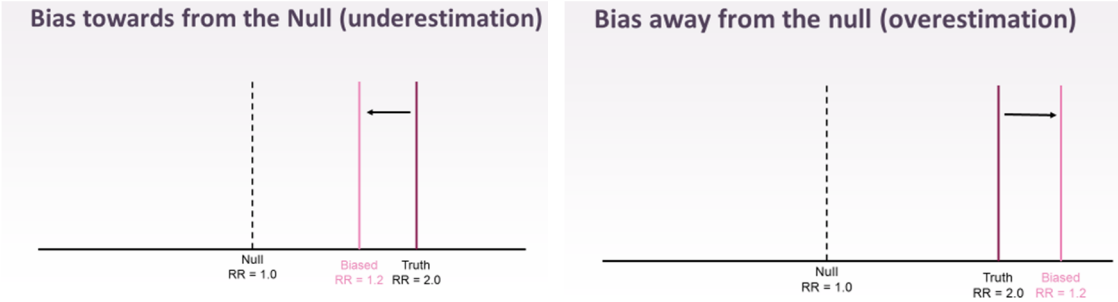


Figure 2.7 Effect of confounding on the estimate of an effect.

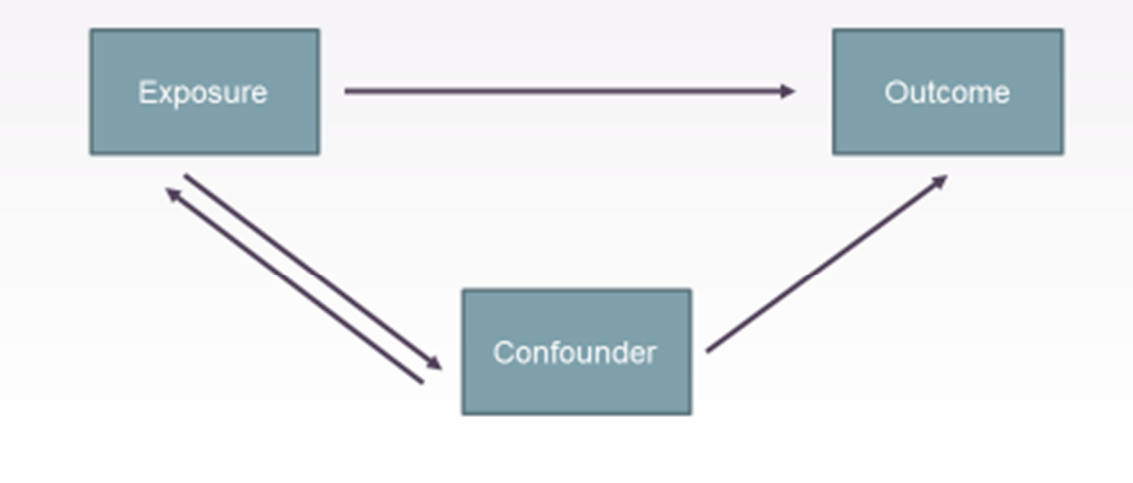


Figure 2.8 Definition of a counfounder.

The effects of confounding can be excluded or controlled for by design by using RCTs or matching; or during analysis using restriction, stratification, or statistical modelling (if the confounding variables are known).

Confounding is removed in the design phase of RCTs, as people are randomly assigned to each intervention arm. Randomisation means that characteristics of the people included are likely to be balanced between them. However, confounding is a major issue in the analysis of observational data as people are not randomised and there are often significant imbalances between groups of interest in the study population. Multivariate models can be used to control for confounding due to known confounders that have been collected.

Fitting a statistical model including only one predictor (the variable of interest) will produce an "unadjusted" estimate of the association with the outcome of interest, which does not take into account the possible effects of confounding. Statistical models including more than one predictor are called multivariate models. By including potential confounders (as additional predictors) in a multivariate model, we are able to produce "adjusted" estimates of the

association, which accounts for the effects of confounding variables. These estimates effectively show the association between the variable of interest and the outcome, while holding all other variables in the model constant, therefore removing their effect.

The magnitude of the effect of confounding can be assessed by observing the difference between the unadjusted and adjusted estimates of the association. However, it is impossible to completely remove the effects of confounding when analysing observational studies. Confounding due to unmeasured and unknown variables cannot be controlled for through statistical modelling and the risk of residual confounding remains in any cohort study, however well designed. Residual confounding can be due to insufficiently detailed information, improper classification or over simplification of information, measurement error and miss-specified relationships between variables and outcomes.

A related concept is effect modification (also known as interaction), which refers to when the association between the variable of interest and the outcome varies across the levels of a third factor. Effect modification is a genuine finding and should be explored and presented rather than adjusted away. For example, smoking has been shown to increase the persistence of oral HPV infection (independently of sexual behaviours). Furthermore, it has been suggested that the persistence of oral HPV infection in women who smoke is significantly longer than men who smoke (where the effect is only marginal). In this example there is an interaction between smoking status and gender. Multivariate models can include interaction terms, however it is not common to test for all possible interaction terms, as the large number of tests increases the risk of a false positive result. Therefore, the inclusion of interaction terms are usually decided a priori based on scientific interest or of biological importance.

Throughout this thesis, both explanatory and predictive models have been used. The models used for explanatory purposes include logistic regression models, conditional logistic regression models and Poisson regression models, generalised estimating equations (GEEs), and random effects models. Linear exponential smoothing (LES) models, a very basic method of predictive modelling, was used and is described in chapter 3.

2.2.2.2 Logistic regression

Logistic regression models are appropriate for when the outcome of interest is binary or dichotomous, such as success and failure or presence or absence of a characteristic of interest. For example, say we are interested in whether people do or do not have lung cancer, where the

proportion of people who have the outcome of interest is p and the proportion of people who do not is p-1. A very simple analysis would be to compare the proportion between the two groups: the proportion of people who have lung cancer in males and females. If we wanted to take into account several possible confounding variables, we need to fit a multivariate model. The simplest model we could fit would be to fit the linear probability model, this is where the proportion is assumed to be a linear function of the predictors (x_i) (Equation 2.1).

$$p = \beta_o + \beta_1 x_1 + \beta_2 X_2 + \cdots + \beta_i x_i$$

Equation 2.1 Linear probability model.

$$Logit(p) = \log\left(\frac{p}{1-p}\right) = \beta_o + \beta_1 x_1 + \beta_2 X_2 + \cdots + \beta_i x_i$$

Equation 2.2 Logistic regression model using logit link.

The problem with fitting this model is the proportion (p_i) can range from 0 – 1, however the linear function of predictors can take on any real value from $-\infty$ to ∞ (as shown by the red line in Figure 2.9). As a result, a transformation is applied to the proportion, which ensures that the predicted values will lie within the valid range (as shown by the blue line in Figure 2.9) [560-562]. To do this, the logarithm of the odds is modelled instead of the proportion, where the odds is defined as the proportion of those who have the event divided by the proportion of those who do not ($odds = \frac{p}{1-p}$). This transformation is called the log odds or logit and is referred to as a "link" function (Equation 2.2).

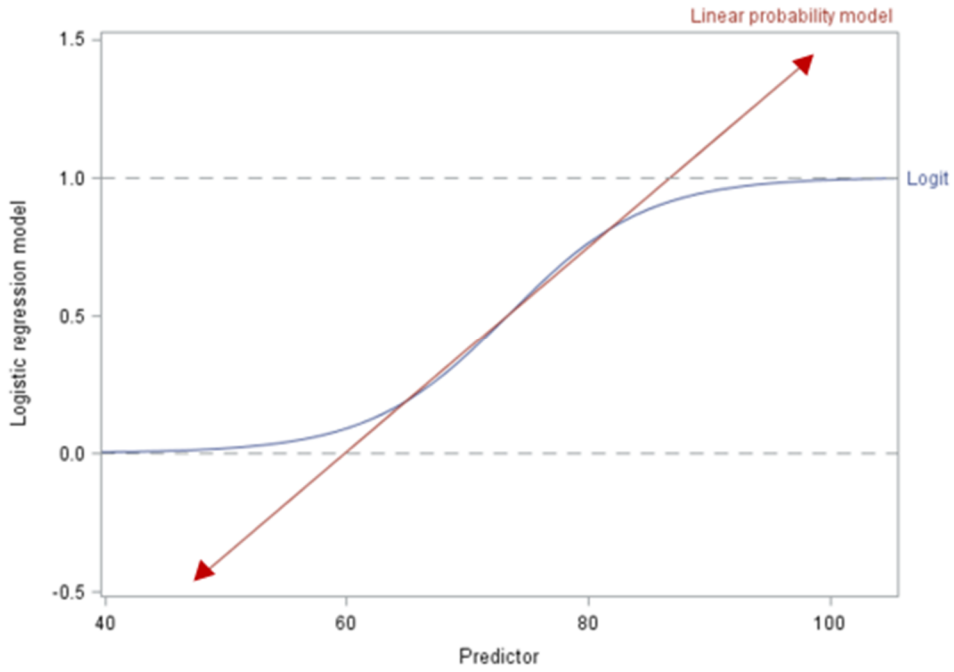


Figure 2.9 Comparison of logistic vs linear probability model.

The output from the logistic regression model is straightforward to interpret. The logit function is the log of the odds of an event, which is the number of successes divided by the number of fails. Therefore, the back transformed (or exponentiated) estimates of the regression coefficients β_i (and 95% confidence intervals [95%CI]) represent the odds ratio (and 95%CI) for the association between each predictor (x_i) and the outcome [560-562]. Two examples of how to interpret the back transformed coefficients are given below for the case where the outcome is lung cancer

Categorical explanatory variable: Gender

The odds ratio tells us whether the odds of lung cancer was higher or lower for females compared to males. An odds ratio of >1 indicates that the odds of lung cancer was higher in females, whereas an odds ratio of <1 indicates that the odds was lower in females, relative to males. Furthermore, if the 95%CI does not contain the value 1, this is analogous to a p-value of <0.05 and a statistically significant result.

Numerical explanatory variable: age

The odds ratio tells us whether the odds of lung cancer was higher or lower for a 1 year increase in age. An odds ratio of >1 indicates that the odds of lung cancer was higher for a 1 year older age, whereas an odds ratio of <1 indicates that the odds was lower for a 1 year older age. Furthermore, if the 95%CI does not contain the value 1, this is analogous to a p-value of <0.05 and a statistically significant result.

Assumptions:

- The outcome has a binomial distribution
- The logit of the proportion is a linear function of the predictors
- Observations are independent
- Independent variables are not linear combinations of each other
- Equal follow-up time

2.2.2.3 Conditional Logistic regression

The studies presented in chapters 6 and 7 use the matched nested case-control study design to answer the respective research questions [560]. Matching is sometimes used in studies with binary outcomes where the people included are “matched” or “paired” in some way. Each case (person with outcome of interest) is “matched” with one or more controls (person or people without the outcome of interest) where they are chosen to be the same or similar for major confounding variables. Each case and its respective matched controls are referred to as a “matched set”. For example, controls might be selected to be a similar age (within 10 years). Standard logistic regression can produce bias results in the presence of matching, and a variant called “conditional logistic regression” should be used [560]. Parameter estimates are estimated by considering possible combinations of exposures, conditional on the observed exposures within each matched set. Results from the conditional logistic regression are interpreted in the same way as classical logistic regression [560].

2.2.2.4 Time to event data

Poisson and Cox regression analysis are commonly used when dealing with time to an event data. A great advantage of these methods is that they allow us to deal with “censored data”, meaning that the “time to event” is unknown as the event of interest occurs outside of the period of observation. There are three types of censoring that can occur:

- right censored: the event of interest occurs after the period of observation
- left censored: the event of interest occurs before the period of observation
- interval censored: the event of interest is known to occur within a given time interval, however the exact date is unknown [563].

This should not be confused with truncation:

- left truncation: occurs when the subjects have been at risk before entering the study.
- right truncation: occurs when the entire study population have already experienced the event of interest before entering the study.

In most epidemiological studies, including many analyses in EuroSIDA and the D:A:D Study, the most common issues are right censoring and left truncation. Often participants have historical data available prior to recruitment, however, analyses are almost always left truncated at the date of enrolment (or relevant date). Properly accounting for censoring is an important element of time to event analysis as it allows us to include incomplete data. If only individuals with complete data were included in analyses (i.e. those with a known baseline and last visit date), then a large amount of information would be lost and we would potentially produce biased results.

2.2.2.4.1 Poisson regression models

Poisson regression is used for the analysis of count data or rates. The structure of the Poisson regression is similar to the logistic regression model, except that it is used when we have observed events over time and the outcome of interest is the incidence rate of an event rather than the odds of event. It is used to estimate incidence rate ratios between groups in the same way that logistic regression is used to estimate odds ratios between groups. The incidence rate of an event is defined in Equation 2.3.

$$Incidence\ rate\ (\lambda) = \frac{Number\ of\ events}{Total\ person\ years\ follow\ up}$$

Equation 2.3 Incidence rate.

The incidence rate can range from 0 to ∞, however the linear function of predictors can take on any real value from $-\infty$ to ∞. As done with the logistic regression model we use a logarithmic transformation to map the range to $-\infty$ to ∞, referred to as the log link function.

The form of the Poisson regression model is shown in Equation 2.4, where λ is the incidence rate, β_i are the estimated linear regression coefficients and x_i are the predictors of interest.

$$Log(\lambda) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_i x_i$$

Equation 2.4 Poisson regression model.

The output from the Poisson regression model is straightforward to interpret. The back transformed estimates of the regression coefficients β_i (and 95%CI) represent the incidence rate ratio (and 95%CI) for the association between each predictor (x_i) and the outcome [560-562]. Two examples of how to interpret the back transformed coefficients are given below for the case where the outcome is lung cancer.

Categorical predictor: Gender

The incidence rate ratio tells us whether the incidence of lung cancer was higher or lower for females compared to males. An incidence rate ratio of >1 indicates that the incidence of lung cancer was higher in females, whereas an incidence rate ratio of <1 indicates that the incidence was lower in females, relative to males. Furthermore, if the confidence interval does not contain the value 1, this is analogous to a p-value of <0.05 and a statistically significant result.

Numerical predictor: age

The incidence rate ratio tells us whether the incidence rate of lung cancer was higher or lower for a 1 year increase in age. An incidence rate ratio of >1 indicates that the incidence of lung cancer was higher for a 1 year older age, whereas an incidence rate ratio of <1 indicates that the incidence was lower for a 1 year older age. Furthermore, if the 95%CI does not contain the value 1, this is analogous to a p-value of <0.05 and a statistically significant result.

Assumptions:

- The outcome follows a Poisson distribution
- The log of the incidence rate is a linear function of the predictors
- Observations are independent
- Independent variables are not linear combinations of each other

2.2.2.4.2 Proportional Cox regression

One of the most commonly used methods to analysis time to event data is Cox proportional hazards regression. The form of the model (Equation 2.5) is very similar to that of logistic regression and Poisson regression, except that the outcome is defined as the hazard function [560, 561, 564]. The hazard function is shown in Equation 2.6 where $f(t)$ is the density function of survival times and $S(t)$ is the survivor function [560, 561, 564]. The survivor function, shown in Equation 2.7, is a summary measure of survival times, and displays the probability of survival at time t. $F(t)$ is the cumulative probability of failure at time t [560, 561, 564]. The terms β_n are the estimated linear regression coefficients and x_n are the predictors. The $\log(h_0(t))$ term represents the baseline hazard function.

$$Log(h(t)) = \log(h_0(t)) + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_i x_i$$

Equation 2.5 The proportional cox model.

$$h(t) = \frac{f(t)}{S(t)}$$

Equation 2.6 The hazard function.

$$S(t) = 1 - F(t)$$

Equation 2.7 The survivor function.

As suggested by the name, a key assumption of this model is that the ratio of the hazards when comparing predictors is constant over time.

The back transformed estimates of the coefficients β_i (and 95%CIs) are straight forward to interpret, and represent the estimated hazard ratio (and 95%CI) for the association between each predictor (x_i) and the outcome. Two examples of how to interpret the back transformed coefficients are given below for the case where the outcome is lung cancer.

Categorical predictor: Gender

The hazard ratio tells us whether the risk of lung cancer was higher or lower for females compared to males. A hazard ratio of >1 risk that the hazard rate of lung cancer was higher in females, whereas a hazard ratio of <1 indicates that the hazard rate was lower in females, relative to males. Furthermore, if the 95%CI does not contain the value 1, this is analogous to a p-value of <0.05 and a statistically significant result.

Numerical predictor: age

The hazard ratio tells us whether the risk of lung cancer was higher or lower for a 1 year increase in age. A hazard ratio of >1 indicates that the hazard rate of lung cancer was higher for a 1 year older age, whereas a hazard ratio of <1 indicates that the hazard rate was lower for a 1 year older age. Furthermore, if the 95%CI does not contain the value 1, this is analogous to a p-value of <0.05 and a statistically significant result.

Assumptions

- Non-informative censoring: The mechanisms that give rise to censored individuals are not related to the probability of an event occurring.
- Proportional hazards assumption: Ratio of the hazards comparing different predictors is constant over time.

2.2.2.4.3 Comparison of Poisson regression and Cox proportional hazards models

Poisson regression and Cox proportional hazard models have many similarities and are often used in similar circumstances, producing similar results. The key difference is that the outcome for Poisson regression is a count or a rate over specific follow-up period, whereas for Cox regression the outcome is time to event. Furthermore, the cox model also allows for censoring. The Cox proportional hazard models are essentially extensions of Poisson regression models where no assumptions are made about the baseline hazard rate.

2.2.2.4.4 Marginal Cox models

The Cox proportional hazards model described above allows for only one event per person, however this can be extended to the situation where each subject can experience several events, or multiple events data. Multiple events data can be analysed by a method proposed by Wei, Lin, and Weissfeld based on the marginal Cox models [565]. This is sometimes referred to

as the Wei, Lin and Weissfeld test, and allows for two or more dependent outcomes to be jointly modelled and compared.

2.2.2.5 Generalised estimating equations (GEE)

Both logistic and Poisson regression assume observations are independent. This assumption is violated if the data is clustered (i.e. if people within a cluster are more similar to each other than those that are not). A special type of clustering is repeated measures in longitudinal data, which is essentially clustering within individuals. Repeated measures within the same individual are going to be more similar to each other than observations on different subjects.

For example, subjects within EuroSIDA develop several different types of cancers during their follow-up, meaning each person may develop more than one cancer during follow-up. If clustering is not dealt with during the analysis, the standard errors will be underestimated, leading to overly narrow confidence intervals and small p-values and results may potentially be biased.

GEEs are one method that can be used to take account of within individual variability when each individual may contribute multiple endpoints [566]. They modify both parameter and standard error estimates to take into account clustering.

2.2.2.6 Random effects models

Random effects models are another method of analysing data with repeated measurements within individuals or clustering. They are also referred to as multi-level models because of the hierarchical structure of the data: individuals are clustered within a higher level for example i.e. observations clustered within people, people clustered within clinics, clinics clustered within countries.

Random effect models explicitly model both between cluster and within cluster variation. The simplest model incorporates a similar structure to that of a classical linear model (such as logistic regression explained in the section 2.2.2.2) with the addition of a term that shifts the linear predictor (the fixed effects) by an amount that randomly varies between individuals or clusters (the random effect) (Equation 2.8). This is often referred to as a “random intercepts model”, as the “intercept” term is shifted up or down depending on the cluster.

$$Y_i = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 x_1} + \boldsymbol{\beta_2 x_2} + \boldsymbol{\beta_3 x_3} + \cdots + \boldsymbol{\beta_i x_i} + \boldsymbol{u_i}$$

Fixed effects Random effects

Equation 2.8 Random intercepts model.
The green component represents the fixed linear predictor and the red component reflects the additional random effect on the predicted value due to being in cluster i.

The random effects (u_i) vary randomly between the clusters and are assumed to explain the clustering within each individual, such that after allowing for random effects, observations within the same cluster are now independent.

The full random effects model is shown in Equation 2.9. Where y is a vector of observations for each individual, β is a vector of coefficients for fixed effects, u is a vector of random effects, ϵ is a vector of random error terms and X and Z are matrices of fixed and random predictors.

$$y = \boldsymbol{\beta X} + \boldsymbol{uZ} + \epsilon$$

Equation 2.9 Random effects model.

A fixed effect refers to a variable which is assumed to have the same effect across all individuals, regardless of cluster [567]. For example, in longitudinal studies where observations are clustered within people, age and gender are commonly considered to be fixed effects, as the effect of these variables tend to be constant between people. The inclusion of random effects, which vary for each individual, allows for the modelling of individual curves, or profiles, for each individual. For example, if a random intercept term is included in the model it allows the intercept to vary for each individual. Whereas additionally including a random slope variable allows completely separate curves to be modelled for each individual, with different intercepts and slopes. The model then combines these individual curves to estimate an overall effect for each variable [567]. This is illustrated in Figure 2.10.

Figure 2.10 A comparison of a standard linear model, a random intercepts model and a random slopes model [568].

As previously mentioned, observations within individuals or clusters are more similar than those between individuals. For example, if levels of a plasma biomarker, such as prostate specific antigen, were measured multiple times on the same individual, then a high measurement would be more likely to be followed by another high measurement. We can take this account by specifying a covariance structure. Various correlation structures are shown in Table 2.6.

Table 2.6 Covariance structures for multilevel modelling.

Unstructured	UN	The UN structure is the most flexible as no restrictions are placed on the covariance terms.	$\sigma_{ij} = \sigma_{ij}$
autoregressive structure	AR(1)	Assumes that observations which are taken close together are more similar than those taken further apart. Assumes that all measurements are evenly spaced.	σ $^{\lvert I-J\rvert}$
Compound symmetry	CS	Assumes there is a correlation between two separate measurements, but it is assumed that the correlation is constant regardless of how far apart the measurements are	σ
Spatial power	SP(POW)	This is the AR(1) structure generalised to the situation where the distance between measurements i and j are not consistent. I.e. time is considered continuous.	σ $^{-t_j}$

2.2.2.7 Modelling strategy

Each chapter includes a brief methods section which gives a summary of the methods used to analyse the data. A description of the predictors considered is provided. Most of the variables investigated are well known in HIV and cancer research. However, detailed explanations are provided were new or novel approaches are used.

For all analyses, both baseline and time updated variables were used. These are also detailed in each chapter. However generally, baseline variables included non-varying variables such as ethnicity, sex and HIV transmission mode. Time-updating variables included variables that are routinely collected by EuroSIDA at regular intervals, such as CD4 cell counts and HIV-VL measurements. Time-updating variables determine the short term association with an outcome of interest.

The modelling approach used here involved univariate analysis followed by multivariate analysis, which included variables which were statistically significant in univariate analyses at the 10% level (p-value <0.1). However, in some analyses, variables included in analysis were selected *a priori*, based on expert clinical knowledge and review of the current literature. Each chapter will specify the model building process used.

Sensitivity analyses were performed to test the assumptions of the study design and definitions used. Further details are given in each subsequent chapter in the methodology and discussion sections.

2.2.3 Handling missing data

Incomplete and missing data were included in two ways.

1. **Complete case-method**: This approach restricts the analysis to people who had valid values of key variables. This method was only used for key variables that were considered to be crucial. This included date of birth, gender, and CD4 at first visit or entry. This method has been showed to be unbiased when the missing data is not missing as a function of either the outcome of interest or the model error term [569]. However, when many data are missing the complete-case method is highly inefficient as it leads to a large number of cases being excluded.

2. **Include a missing category**: This methods was used when moderate amounts of data were missing or incompletes. This approach is straight forward and retains maximal

individuals in the analysis but is known to produce biased estimates in some circumstances [569].

2.3 Summary

This chapter provides a brief overview of the two key data sources and statistical methods used throughout this thesis. However, each chapter involved the use of specific methodology tailored to the research question of interest. Although care has been taken to minimise the effect of inherent bias, each analysis will have its own strengths and weaknesses which are addressed in detail in each chapter. A detailed explanation of the methods for each chapter is included in the methods section of each chapter. The relative strengths and weaknesses of each piece of work are discussed in the discussion sections as well as chapter 8 of this thesis.

3 Infection related and unrelated cancers, HIV, and the aging population

3.1 Introduction

An increased risk of infection related cancers (IRCs), particularly Kaposi's sarcoma (KS) and non-Hodgkin lymphoma (NHL) has been observed since the beginning of the HIV epidemic [8, 14-18, 22, 23, 427, 570]. However, increased risk of some non-infection related cancers (IURCS), such as lung cancers, has also been reported in recent years [14, 15]. The exact mechanisms for increased cancer risk in HIV-positive (HIV+) people are complex, poorly understood, and involve multiple processes [18]. The excess risk is, in part, explained by HIV associated immune dysfunction [8, 14-18, 427, 571], and activated inflammation and coagulation pathways [572-574]. Furthermore the wide spread availability of combination antiretroviral therapy (cART) has improved survival of HIV+ people [1-6, 475, 575, 576]. This has led to an "aging" of the HIV+ population in high income countries and an increasing risk of traditionally age-associated cancers. Increased survival has also increased the prevalence of prolonged exposure to cART toxicities [18, 577-579] and exposure to the possible pro-oncogenic effects of HIV itself [580-582], both of which have also been linked with increased cancer risk. Finally, HIV+ people have a higher prevalence of many established risk factors, for example: smoking [420, 421], alcohol use [422, 423] and sexual behaviours and oncogenic viruses (for example, a higher number of sexual partners has been associated with HPV [human papillomavirus] infection) [9, 424, 425, 583]. Despite improvements in HIV treatment and patient outcomes, cancer continues to be a major contributor to morbidity and mortality in HIV+ people [7, 584], and is the second most common cause of death after AIDS defining deaths [220].

A broad overview of cancers in HIV+ people is given in chapter 1 section 1.2, including justification of the classification of cancers into IRC and IURC, a brief epidemiological summary of common cancers in HIV+ people, and a brief discussion on HIV and aging. Here, I summarise the history of cancers in HIV+ people.

3.1.1 HIV, immune deficiency, and cancer

The immune system uses two processes to prevent cancer development including the clearance and suppression of oncogenic viruses, and the process of "immune surveillance", during which the innate and adaptive immune systems work together to identify and destroy cancer cells [18, 475, 585]. HIV+ people are more susceptible to oncogenic viruses due to HIV associated immune deficiency and dysfunction, and as a result, have a higher risk of associated IRCs than HIV-

negative people [16, 17, 20, 427, 432, 441, 459, 465, 468, 470, 578, 586]. Before the availability of effective cART, the incidence of many IRCs were extremely elevated in HIV+ people relative to the HIV-negative population. This includes the AIDS defining cancers (ADCs): KS (Rates up of around 1000-fold higher than the general population have been estimated), NHL (with rates ranging from 10 to 200-fold higher), and invasive cervical cancer (3-12 fold higher) [15, 430, 438, 442, 446-448, 465, 469]. The elevated incidence of NHL and KS was strongly associated with to the high prevalence of immune deficiency [475]. While there is some evidence of an association between invasive cervical cancer and immune deficiency [475], this is also linked with increased acquisition of HPV in HIV+ women compared to HIV-negative [587]. HIV+ women have increased HPV cervical viral load and reduced HPV clearance than HIV-negative women, both of which are associated with low CD4 cell count [587, 588]. Other IRCs are also at elevated risk in HIV+ people included Hodgkin lymphoma (HL, SIR: 8 – 16 times higher than the general population), anal cancers (SIR: 26 – 37), liver cancers (SIR: 2 – 7), head and neck cancers (SIR: 1.6 – 4.3), and stomach cancers (SIR: 1.5 – 2) [430, 469, 589-591]. Interestingly, some IURCs have also been linked with immune deficiency [16, 427, 433, 592, 593] which may reflect other the effect of HIV on other components of immune function such as reduced immune surveillance.

3.1.2 The introduction of combination antiretroviral therapy and cancer risk

The introduction of cART in 1996 had a major impact on the epidemiology of cancer in HIV+ people. Since the introduction of cART, a clear decline over time has been consistently reported in KS and NHL [7, 17-19, 21, 437, 438, 441, 444, 451, 459, 465, 470, 483], likely due to restored immune function from effective cART [20, 459, 594]. Early studies showed no decline in cervical cancers since the introduction of cART [20, 21, 438], however more recent studies have shown a decline of 12% per year [430, 436, 464]. No declines have been reported for HL [19, 21, 430, 467], anal cancers [14, 19, 430, 432, 436-438, 465, 467, 479-481], HPV associated head and neck cancers [437], liver cancers [436, 464], or stomach cancers [437].

3.1.3 The impact of improved survival, reduced mortality, and age associated cancers

Availability of effective cART and improvements in the efficacy and tolerability of cART regimens has led to increased immune function and reduced incidence of AIDS defining events in HIV+ people [12]. As a result, survival has dramatically improved, with the median age at death projected to be as high as 75 years in high income countries [1-6]. Briefly, it is estimated that approximately one third of HIV+ people in high income countries are aged over 50 years, and

this proportion is increasing [405, 503]. Not surprisingly, a tangible increase in the incidence of IURCs has been observed in HIV+ people as a result of longer survival, and will increasingly contribute to morbidity and mortality as the older population grows [419]. Several IURCs have been identified as having excess risk in HIV+ people relative to the general population, including lung cancer (SIR: 2.7, see Chapter 1 section 1.2.2.9), kidney cancer (SIR: 1.5-1.7), brain cancer (SIR: 1.8 - 2.2), cancer of the testes (SIR: 1.3- 1.4), melanoma (SIR: 1.2), multiple myeloma (SIR: 2.6 - 2.7), and leukaemia (SIR: 2.6 - 3.2) [15, 475].

3.1.4 Future of HIV care

As the HIV+ population continues to grow and the proportion aged over 50 years continues to rise, the burden from IURC, which are predominantly age associated cancers (including prostate [PCa], breast and lung cancer [7, 587]), will continue to increase. This raises multiple issues in regard to future HIV care for older HIV+ people, such as the increased complexity due to the detection, diagnosis, comanagement and treatment of such cancers [504]. In addition, older HIV+ people face a number of distinct clinical challenges, including slower immunologic recovery when treated with cART than in younger people, and complicated treatment histories in those with longer HIV duration due to exposure to toxic drugs earlier in the epidemic [504]. It has also been suggested that people aged over 50 may be less likely to be tested for HIV and more likely to be diagnosed later in the course of HIV infection [419, 504, 595]. This is a growing concern for future healthcare planning and resource allocation as well as possible integration of healthcare services. The changing epidemiology of cancers in Europe and the impact of aging on cancer burden in HIV+ people needs to be better characterised.

3.2 Aims

There were two aims of this chapter

1. To describe the association between age and the incidence of IRC and IURC within EuroSIDA, as well as to identify other risk factors that may vary over time
2. To estimate the change in incidence of IRC and IURC in HIV+ people in recent years and to forecast the expected incidence for the next 5-10 years.

3.3 Methods

This study was performed within the EuroSIDA cohort, which is detailed in chapter 2 section 2.1.1, using the D36 release of the EuroSIDA cohort (included information on 18,791 people).

Non-AIDS defining cancers (NADCs) have been routinely and prospectively collected and included in quality assurance in the EuroSIDA study since 1 January 2001. Baseline was defined as the later of either first visit or 1 January 2001. People were followed until the later of last visit or death.

3.3.1 Inclusion criteria

Of the 18,791 people enrolled in the D36 release of EuroSIDA, I included all people with prospective follow-up after 1 January 2001 and aged over 16 (N=15,648). The selection of participants is detailed in Figure 3.1. I compared the characteristics of those excluded to those included at their first visit date (which is not the same as the baseline date). At first visit, those who were excluded were of a similar age (median age 36 in those excluded vs 37 years in those included). Of those excluded, a lower proportion were female (18% vs 27%), were almost exclusively from south, west central and northern Europe (99% vs 66%), and a higher proportion acquired their HIV through sex between men (51% vs 39%). The median CD4 cell count in those excluded was lower than those included at first visit (115 vs 350 cells/mm^3). Use of cART in those excluded was lower at first visit (16% vs 50%), and three quarters had no recorded HIV viral load (HIV-VL) measurement (76% vs 23%).

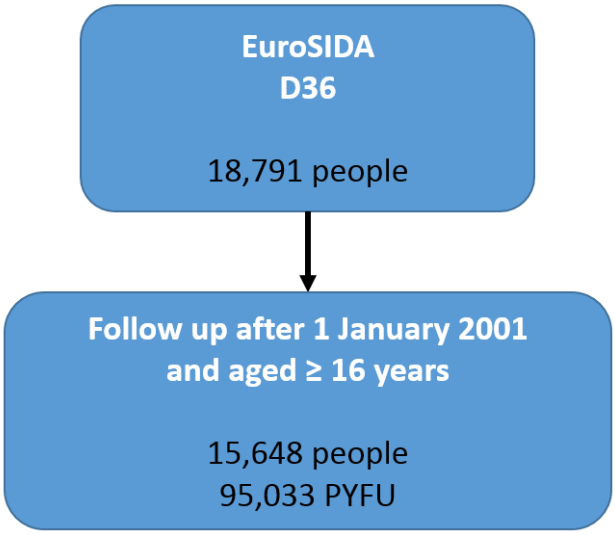


Figure 3.1 Flow chart for inclusion of EuroSIDA participants in analysis.

3.3.2 Outcomes

The primary outcomes for this study were the incidence of IRC and IURC diagnosis during follow-up. All cancers diagnosed after 1 January 2001 were included. IRCs were defined as all cancers with a probably infectious cause: KS (caused by human herpesvirus 8 [HHV8]), NHL and HL (Epstein-Barr virus [EBV]), invasive cervical cancers, cancers of the head and neck, anus, penis, vulva and vagina (HPV), liver cancer (Hepatitis B [HBV] and C [HCV]) and stomach cancer (*H. Pylori*]) [474], as detailed in Table 3.1. These groupings were derived from the published literature [474] and reviewed by clinicians. All remaining cancers were defined as IURCs (Table 3.1).

Multiple diagnoses per person were allowed, however metastatic, secondary and repeated diagnoses of the same cancer type, pre-cancers and non-melanoma skin cancers (not routinely collected) were not included as outcomes. It was assumed that each cancer event within the same person was unrelated to previous cancers (as metastatic, secondary and repeated cancers were not counted). All cancers diagnosed during the follow-up period were included and classified using the International Classification of Diseases and Related Health Problems, 10th edition code classification system [596]. The ICD10 codes for each cancer type are shown in Table 3.1. Furthermore, free text fields for cancer diagnoses were also used to identify additional diagnoses and coded to the appropriate ICD10 code.

Table 3.1 Definitions and classification of cancers according to infection related (IRC) and infection unrelated (IURC) in EuroSIDA.

Cancer group	Cancer type	Icd10 codes
Total		
Infection related cancers (IRC)	**Total**	
Epstein-Barr virus (EBV)	HL	C81
	NHL	C82,C83,C84,C85,C96
Hepatitis B or C (HBV/HCV)	Liver	C22
Human herpesvirus 8 (HHV8)	KS	C46
Human Papilloma virus (HPV)	Anal	C21
	Cervix	C53
	Oral or Pharynx	C01, C04, C09, C10, C11, C12, C13, C14
	Penis	C60
	Vulva or Vagina	C51, C52
Helicobacter Pylori (*H.Pylori*)	Stomach	C16
Infection unrelated cancers (IURC)	**Total**	
	Accessory sinuses	C31
	Bladder	C67
	Bone and articular cartilage	C41
	Brain	C71
	Breast	C50
	Bronchus and lung	C34
	Colon and Rectal	C18, C20
	Connective and soft tissue	
	Corpus uteri	C54
	Endocrine glands and related structures	C75
	Eye	C69
	Gallbladder	C23
	Kidney, except renal pelvis	C64
	Larynx	C32
	Leukaemia	C95
	Lymphoid leukaemia	C91
	Melanoma	C43
	Meninges	C70
	Mouth	C06
	Multiple myeloma and malignant plasma cell neoplasms	C90
	Myeloid leukaemia	C92
	Nasal cavity and middle ear	C30
	Oesophagus	C15
	Other urinary organs	C68
	Other female genital organs	C57
	Ovarian	C56
	Pancreas	C25
	Parotid gland	C07
	Prostate	C61
	Retroperitoneum and peritoneum	C48
	Testis	C62
	Thyroid gland	C73
	Unknown site	C80, C87

3.3.3 Variables included in analyses

Both baseline and time updated variables from the EuroSIDA database were included in this analysis, as detailed in Table 3.2. Current HIV-VL and CD4 cell count were Log_{10} and log_2 transformed respectively, due to the right skewed nature of the data, and also for interpretive reasons i.e. a one unit increase in log_{10} Current HIV-VL is equivalent to a 10-fold increase, and a one unit increase in log_2 CD4 cell count is equivalent to a 2-fold increase.

Table 3.2 Summary of baseline and time updated variables.

Variable	Time updated	Levels	Definitions and comments
Age (years)	Yes	Continuous (per 10 years older) and categorised into ≤35, 36 – 40, 41 – 50, ≥ 51 years	
Calendar year	Yes	Continuous (per year) and Categorised by year	
Region of Europe		East, east central, south, west central, north Europe, and Argentina	See chapter 2 section 2.1.1.2
Ethnicity		White, Non-white and other (includes Asian, black and unknown race)	
Gender specific HIV mode of transmission		Sex between men, heterosexual (male), heterosexual (female), IDU (male), IDU (female), other or unknown (male), other or unknown(female)	Unknown gender was assumed to be male
HIV mode of transmission (forecasts only)		Sex between men, heterosexual, IDU, other or unknown	
BMI (kg/m^2)		Under weight (<18), normal weight (18 – 25), Over weight (25 – 30), obese(30+)	Classified according to the WHO standard [597]
Smoking status	Yes	Non-smokers, current smokers, previous smokers, unknown	
Current CD4 cell count ($cells/mm^3$)	Yes	Continuous (per 2-fold higher) and <200, 200 – 349, 350 – 499, ≥500 $cells/mm^3$, unknown	Within 6 months prior to date of interest
Nadir CD4 cell count ($cells/ mm^3$)		Continuous. Was not included in models due to correlation with current CD4 cell count.	Lowest recorded CD4 cell count measurement prior to date of interest
Current HIV-VL (copies/mL)	Yes	Continuous (per 10-fold higher) and <400, ≥ 400 copies/mL, unknown	Within 6 months prior to date of interest
Prior ADC diagnosis	Yes	Yes , No	Classified according to the 1993 CDC clinical definition [133]
Prior AIDS defining event (excluding ADC)	Yes	Yes , No	Classified according to the 1993 CDC clinical definition [133]
Prior NADC diagnosis	Yes	Yes , No	
Prior non-AIDS defining event (excluding NADC)	Yes	Yes , No	Pancreatitis, grade 3 or 4 hepatic encephalopathy or liver-related death, myocardial infarction, stroke, coronary artery bypass graft, coronary angioplasty, carotid endarterectomy, and end-stage renal disease [221]
HBV coinfection	Yes	Positive, negative, or unknown	Most recent positive HBsAG surface antigen test or presence of detectable HBV DNA

HCV coinfection	Yes	Positive, negative, or unknown	A prior positive HCV surface antibody test
Antiretroviral use	Yes	Yes, no	Ever received ≥ 1 antiretroviral drug
Protease inhibitor (PI) experienced	Yes	Yes, no	Ever received ≥ 1 PI
Non-nucleoside reverse transcriptase inhibitor (NNRTI) experienced	Yes	Yes, no	Ever received ≥ 1 NNRTI

BMI: Body mass index, IDU: through injecting drug use, HIV-VL: HIV viral load, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, HBV: Hepatitis B, HCV; Hepatitis C, cART combination antiretroviral therapy.

3.3.4 Statistical methods

3.3.4.1 Characteristics at baseline and at time of first cancer diagnosis.

The characteristics at baseline of all people included in this analysis are presented using numbers and percentages for categorical variables and medians with interquartile ranges (IQR) for numeric variables. Those who developed an IRC and an IURC were compared at time of first cancer diagnosis. All bivariate associations were tested using chi squared tests for categorical variables and Kruskal-Wallis tests for numerical variables.

3.3.4.2 Crude incidence of infection related and infection unrelated cancer

The incidence of IRC and IURC was calculated per 1000 person years of follow-up (PYFU). Follow-up began at the latter of first visit or 1 January 2001 and individuals were censored at last visit or death. Incidence was stratified by time updated calendar year of follow-up, age group, and current CD4 cell count category. Incidence rates were also calculated for cancers grouped by oncogenic virus: EBV, HHV8, HPV, HCV and HBV, and *H.Pylori* and as well as selected IURC cancers of interest where numbers allowed (It was decided a priori to use N≥20). Sex specific rates were calculated for HPV associated cancers combined, as well as invasive cervical cancer, prostate, and breast cancer.

3.3.4.3 Factors associated with infection related and infection unrelated cancer incidence

Poisson regression with generalised estimating equations assuming auto-regressive (AR1) correlation were used to estimate the association between age and other factors and IRC and IURC incidence. The AR(1) correlation structure was selected in order to assume that correlation

diminished with increasing calendar time between observations. Variables included in the model were identified a priori based on expert clinical input, availability of data, and the published literature.

Models were adjusted for various baseline and time updated variables (as listed in Table 3.2). It was decided a priori to stratify associations between IRC and IURC with CD4 cell count and smoking status by age (<50 and ≥50 years) due to the strong associations with both age and cancer risk. Interactions between age and all other variables were also investigated.

3.3.4.4 Adjusted population attributable fractions (PAF%)

Adjusted population attributable fractions (PAF%) of IRC and IURC were calculated for each statistically significant modifiable risk factor as well as age in the adjusted models. The PAF% indicates the proportion of excess cancers within the cohort attributable to each risk factor. In order to calculate the PAF%, I used the case based approach detailed in Equation 3.1 [598, 599]. Where RR_i represents the relative risk of those in category *i* relative to the reference category and CF_i represents the case fraction, or in other words, the proportion of people with a cancer exposed to the risk factor out of all those who developed a cancer. There were two reasons why I chose to use the case based formula (Equation 3.1) instead of the commonly used population based approach (Equation 3.2, where PF_i represents the proportion of people exposed in the population). First, it can be used for exposures with three or more categories (the population based approach in Equation 3.2 overestimates the PAF when used for non binary exposures). Second, the case based approach in Equation 3.1 produces an internally valid estimate of the PAF in the presence of confounding (use of the population based approach in Equation 3.2 produces spurious estimates) [598, 599].

$$Case\ based\ PAF_i\% = CF_i \times \frac{RR_i - 1}{RR_i}$$

Equation 3.1 formula for PAF using the case based approach as detailed in [599, 600]

$$Population\ based\ PAF_i\% = PF_i \times \frac{(RR_i - 1) \times PF_i}{1 + (RR_i - 1)PF_i}$$

Equation 3.2 formula for PAF using the case based approach as detailed in [599, 600]

For example, if 100 people included in my study developed an IRC of which 60 were current smokers and the adjusted relative risk of IRC in current smoking relative to never smokers was 1.9. The PAF% based on Equation 3.1 would be

$$\frac{60}{100} \times \frac{1.9 - 1}{1.9} = 0.28 \; or \; 28\%$$

3.3.4.5 Forecasting future incidence

Future crude IRC and IURC biannual incidence in people enrolled before 1 January 2001 was forecast using linear exponential smoothing (LES) models. LES are simple models which smooth time series data and extrapolate current trends into the short term future. They work on the assumption that the time series is locally stationary and vary slowly over time. They break the time series into two components: level and trend, and use these estimates to forecast into the near future. LES models were used to project the current incidence forwards, using log incidence as the outcome and time as the dependant variable. Forecasts were restricted to those enrolled prior to 2001. This ensures a stable population over time (i.e. no new recruitments), however people were allowed to leave the cohort i.e. death or loss to follow-up. No further adjustments for covariates were made.

LES were fit to the data overall, and stratified by key characteristics, including baseline age group (<50, ≥50 years), CD4 cell count (<350, ≥350 cells/mm^3), HIV mode of transmission, and smoking status. These variables were selected as they represented populations that may be at higher risk of cancer that could be targeted for screening and prevention, i.e. older age, CD4 cell count < 350 cells/mm^3, those who acquired their HIV through sex between men, those who acquired their HIV through injecting drug use (IDU), and current smokers.

All statistical tests were two sided with a type I error rate of 5%. All statistical analyses were performed using SAS 9.3 (Statistical Analysis Software, Cary NC, USA).

3.4 Results

15,648 persons contributed 95,033 PYFU with a median follow-up of 6.0 (Interquartile range [IQR]:2.5-10.7) years per person. 610 people (3.9%) developed at least one cancer (accounting for 643 cancer events), 374 people developed an IRC (N=388 IRC events) and 247 people developed an IURC (N=255 IURC events). Please note, a small number of people developed both an IRC and an IURC (N=11).

3.4.1 Characteristics at the patient population at baseline

Baseline characteristics of the cohort are shown in Table 3.3. At baseline, 16.0% were aged 50 or older, 72.6% of the population were male, 88.3% were of white ethnicity and 38.7% attained HIV through sex between men. Approximately one third of people were current smokers and one third had never smoked. At baseline, less than 5% had had a prior ADC diagnosis and 24% an AIDS defining event (other than ADC). Less than 2% had a prior NADC and less than 2% had a prior non-AIDS defining event (other than NADC). Close to three quarters were cART experienced. The median CD4 cell count at baseline was 410 (IQR: 265, 588) cells/mm^3 with 14.8% having $\leq$ 200 cells/mm^3 and the median HIV-VL was 123 (IQR: ≤50, 5200) copies/mL with 54.5% having HIV-VL$\leq$ 400 copies/mL. 23.0% and 5.6% were HCV coinfected and HBV coinfected respectively.

Table 3.3 Baseline[1] characteristics of people included in analysis.

Characteristics		N (%)/Median(IQR)
Overall		15,648 (100.0)
N (%)		
Age (years)		
	≤35	5,419 (34.63)
	36 – 40	3,337 (21.33)
	41 – 50	4,390 (28.05)
	51 +	2,502 (15.99)
Baseline year		
	2001 – 2005	9,583 (61.24)
	2006 – 2009	4,004 (25.59)
	2010 – 2012	2,061 (13.17)
Region		
	Argentina	597 (3.82)
	East	2,733 (17.47)
	East central	2,041 (13.04)
	North	3,220 (20.58)
	West central	3,332 (21.29)
	South	3,725 (23.80)
Gender		
	Female	4,292 (27.43)
	Male	11,356 (72.57)
White ethnicity		13,821 (88.32)
Gender specific HIV transmission group		
	Sex between men	6,051 (38.67)
	IDU (Male)	2,290 (14.63)
	IDU (Female)	1,091 (6.97)
	Heterosexual (Male)	2,178 (13.92)
	Heterosexual (Female)	2,830 (18.09)
	Other (Male)	910 (5.82)
	Other (Female)	298 (1.90)
BMI		
	Under weight	360 (2.30)
	Normal weight	7,422 (47.43)
	Over weight	2,326 (14.86)
	Obese	483 (3.09)
	Unknown	5,057 (32.32)
Smoking status		
	Current	5,393 (34.46)
	Previous	61 (0.39)
	Never	5,030 (32.14)
	Unknown	5,164 (33.00)
Prior ADC diagnosis		734 (4.69)
Prior AIDS defining event (excluding ADC)		3,811 (24.35)
Prior NADC diagnosis		222 (1.42)
Prior non-AIDS defining event (excluding NADC)		297 (1.90)
HBV coinfection		868 (5.55)
HCV coinfection		3,607 (23.05)
History of HIV treatment		
	None	2,921 (18.67)
	ART	781 (4.99)
	cART	11,946 (76.34)

Baseline CD4 cell count (cells/mm^3)		
	Unknown	380 (2.43)
	≤200	2,319 (14.82)
	200 – 349	3,693 (23.60)
	350 – 499	3,754 (23.99)
	500 +	5,502 (35.16)
Baseline HIV-VL (copies/mL)		
	< 400	8,526 (54.49)
	≥ 400	5,624 (35.94)
	Unknown	1,498 (9.6%)
Median (IQR)		
Age (years)		39 (33,46)
CD4 cell count (cells/mm^3)		410 (265,588)
Nadir CD4 cell count (cells/mm^3)		182 (76,303)
log10 HIV-VL (copies/ml)		123 (<50,5200)
calendar year of baseline		2003 (2001,2008)

IDU: injecting drug use, BMI: body mass index, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, HBV: hepatitis B, HCV: Hepatitis C, ART: antiretroviral therapy, cART: combination antiretroviral therapy, HIV-VL: HIV viral load.

[1] Baseline was defined as the latest of first visit or 01 January 2001.

3.4.2 Characteristics at date of first cancer in those who developed IRC compared to IURC at first cancer diagnosis

Characteristics at date of first cancer are shown in Table 3.4. At diagnosis, those who developed IURCs relative to IRC were older (Median age: [IURC] 54 IQR: 46, 61 vs [IRC] 46 IQR: 39, 52 years), had a higher CD4 cell count (446 IQR: 295, 608 vs 342 IQR: 182, 546 cells/mm^3) and a higher proportion had a suppressed HIV-VL (85.0 vs 59.4% with HIV-VL <400 copies/mL). A higher proportion of those who developed NADC relative to ADCs were currently on or had previously been on cART (87.0 vs 81.3%), a lower proportion were HCV coinfected (17.4 vs 21.9%) and the proportion of HBV coinfected (8.9 vs 11.0%) was similar. Proportions of current smokers in IURCS (45.8%) and IRCs (44.7%) were similar. At diagnosis of IURC, 7.7% had a prior ADC diagnosis, 28.7% had a prior AIDS defining event (other than ADC), 7.3% had a prior NADC diagnosis and 15.0% had a prior non-AIDS defining event. Of those who developed IRC, 9.1% had been previously diagnosed with an ADC, 36.9% had a prior AIDS defining event (other than ADC), 4.0% had a prior NADC diagnosis and 9.6% had a prior non AIDS defining event (other than NADC).

Table 3.4 Characteristics of people at diagnosis of first infection related cancer (IRC) and first infection unrelated cancer (IURC) during follow-up.

Total	First IRC diagnosis	First IURC diagnosis
Overall	**374[2] (100.0)**	**247[2] (100.0)**
N (%)		
Age (years)		
≤35	53 (14.17)	4 (1.62)
36 – 40	50 (13.37)	13 (5.26)
41 – 50	150 (40.11)	73 (29.55)
≥51	121 (32.35)	157 (63.56)
Calendar year of diagnosis		
2001 – 2005	161 (43.05)	84 (34.01)
2006 – 2009	142 (37.97)	105 (42.51)
2010 – 2012	71 (18.98)	58 (23.48)
Region		
Argentina	13 (3.48)	6 (2.43)
East	14 (3.74)	6 (2.43)
East central	40 (10.70)	25 (10.12)
North	86 (22.99)	63 (25.51)
West central	97 (25.94)	71 (28.74)
South	124 (33.16)	76 (30.77)
Gender		
Female	79 (21.12)	53 (21.46)
Male	295 (78.88)	194 (78.54)
White ethnicity	331 (88.50)	228 (92.31)
Gender specific HIV mode of transmission		
Sex between men	191 (51.07)	123 (49.80)
IDU (Male)	49 (13.10)	24 (9.72)
IDU (Female)	32 (8.56)	10 (4.05)
Heterosexual (Male)	35 (9.36)	32 (12.96)
Heterosexual (Female)	43 (11.50)	36 (14.57)
Other (Male)	20 (5.35)	16 (6.48)
Other (Female)	4 (1.07)	6 (2.43)
BMI		
Under weight	13 (3.48)	7 (2.83)
Normal weight	207 (55.35)	126 (51.01)
Over weight	50 (13.37)	43 (17.41)
Obese	6 (1.60)	6 (2.43)
Unknown	98 (26.20)	65 (26.32)
Smoking status		
Current	167 (44.65)	113 (45.75)
Previous	24 (6.42)	27 (10.93)
Never	130 (34.76)	85 (34.41)
Unknown	53 (14.17)	22 (8.91)
Prior ADC diagnosis[2]	34 (9.09)	19 (7.69)
Prior AIDS defining events (excluding ADC)[2]	138 (36.90)	71 (28.74)
Prior NADC diagnosis	15 (4.01)	18 (7.29)
Prior non-AIDS defining events (excluding NADC)[3]	36 (9.63)	37 (14.98)
HBV coinfection	41 (10.96)	22 (8.91)
HCV coinfection	82 (21.93)	43 (17.41)

HIV treatment		
None	46 (12.30)	17 (6.88)
ART	24 (6.42)	15 (6.07)
cART	304 (81.28)	215 (87.04)
Current CD4 cell count (cells/mm^3)		
Unknown	2 (0.53)	0 (0.00)
<200	99 (26.47)	31 (12.55)
200 – 349	89 (23.80)	49 (19.84)
350 – 499	75 (20.05)	63 (25.51)
≥500	109 (29.14)	104 (42.11)
HIV-VL (copies/mL)		
< 400	222 (59.36)	210 (85.02)
≥ 400	152 (40.64)	37 (14.98)
Median (IQR)		
Age (years)	46 (39,52)	54 (46,61)
CD4 cell count (cells/mm^3)	342 (182,546)	446 (295,608)
Nadir CD4 cell count (cells/mm^3)	124.5 (47,250)	160 (70,255)
log10 HIV-VL (copies/mL)	61 (<50,20002)	<50 (<50,84)
Calendar year	2006 (2003,2009)	2007 (2005,2009)

IRC: infection related cancer, IURC: infection unrelated cancer, IDU: injecting drug use, BMI: body mass index, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, BMI: Body mass index, HCV: hepatitis C, HBV: hepatitis B, ART: antiretroviral therapy, cART: combination antiretroviral therapy, HIV-VL: HIV viral load.

[1] Baseline was defined as the latest of first visit or 01 January 2001.

[2] 11 people developed both an IRC and an IURC during follow-up and are included in both columns.

3.4.3 Description of cancers

3.4.3.1 Overall

610 people developed 643 cancers. Of the 643 cancers, 388 (60.3%) were IRC and the remaining 255 (39.7%) were IURC (Figure 3.2). Among all cancers, the most common were NHL (N=116), anal (N=83), KS (N=62), and lung (N=55). The most common IRCs were NHL, anal, KS and HL (N=43). Cancers of the lung, prostate (N=28), colorectal (N=23) and breast (N=26) were common IURCs. Of the IRC, almost 90% were related to EBV, HPV or HHV8.

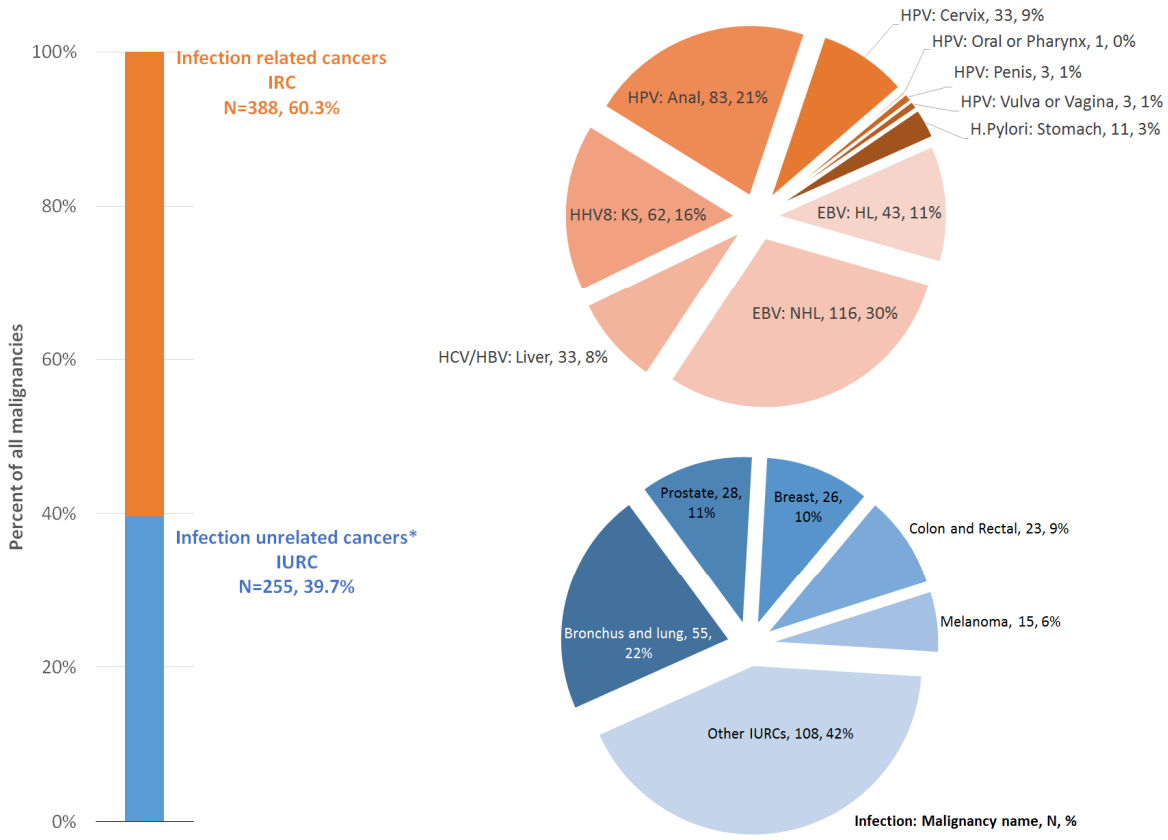


Figure 3.2 Distribution of IRC and IURC. The labels for each cancer include the cancer site, frequency, and the percent.

* Only the 5 highest frequency IURCs are displayed in the graph. A list of all cancers included are listed in Table 3.1

IRC: infection related cancer, IURC: infection unrelated cancer, HPV: human papillomavirus, EBV: Epstein-Barr virus, HCV: hepatitis C, HBV: hepatitis B, HHV8: human herpesvirus 8, KS: Kaposi's sarcoma, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma.

3.4.3.2 Frequencies of cancers in different age groups

In those aged under 50 years (N=353 cancers), the most frequently occurring cancers were exclusively IRC: NHL (N=79), anal cancers (N=53), KS (N=45), HL (N=32) and cervical cancers (N=29) (Figure 3.3). In those aged over 50 years (N=290 cancer), the most frequently occurring cancers were those of the lung (N=37), NHL (N=37), anal (N=30), prostate (N=27) and KS (N=17) (Figure 3.4). The distribution of cancers in those aged over 50 was more heterogeneous than those in younger people. The 5 most frequently occurring cancers in those aged < 50 years accounted for 93% of all cancers, whereas the 5 most frequently occurring cancers accounted for 58% of the all cancers observed and the remaining 42% explained by various other IURCs.

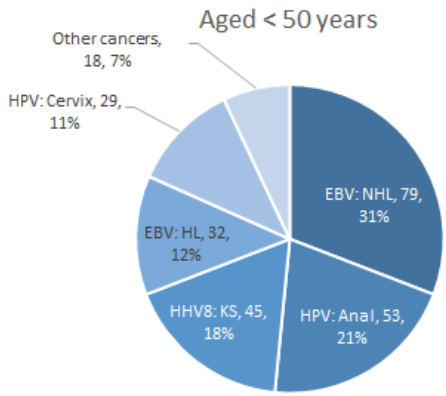


Figure 3.3 Distribution of cancers in HIV+ people aged < 50 years.

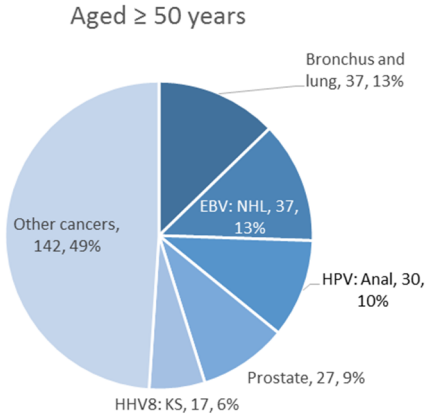


Figure 3.4 Distribution of cancers in HIV+ people aged ≥ 50 years.
IURC: infection unrelated cancer, HPV: human papillomavirus, EBV: Epstein-Barr virus, HCV: hepatitis C, HBV: hepatitis B, HHV8: human herpesvirus 8, KS: Kaposi's sarcoma, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma.

3.4.4 Unadjusted incidence of infection related and infection unrelated cancer

3.4.4.1 Overall

The incidences of IRC and IURC by calendar time are shown in Figure 3.5 and Figure 3.6 respectively. The unadjusted incidence of IRC declined by 4.9 (95%CI: 1.7, 7.9) % /year from 6.1 (95%CI: 4.4, 8.3)/1000 PYFU in 2001 to 3.6 (95%CI: 2.7, 4.9) in 2011/12. The unadjusted incidence of IURC increased by 3.4 (95%CI: -0.4, 7.3) % /year from 1.8 (95%CI: 1.0, 3.2)/1000 PYFU in 2001 to 2.9 (95%CI: 2.0, 4.0) in 2011/12, although this increase was non-significant in univariate analysis (P=0.08). The shift in distribution of cancers over time from IRC towards IURC are summarised in Figure 3.7. The proportion of all cancers that were IURCs in 2001 almost doubled from 22.4% in 2001 to 44.2% in 2011/12, driven by the decline in IRC incidence and slight increase in IURC incidence per year.

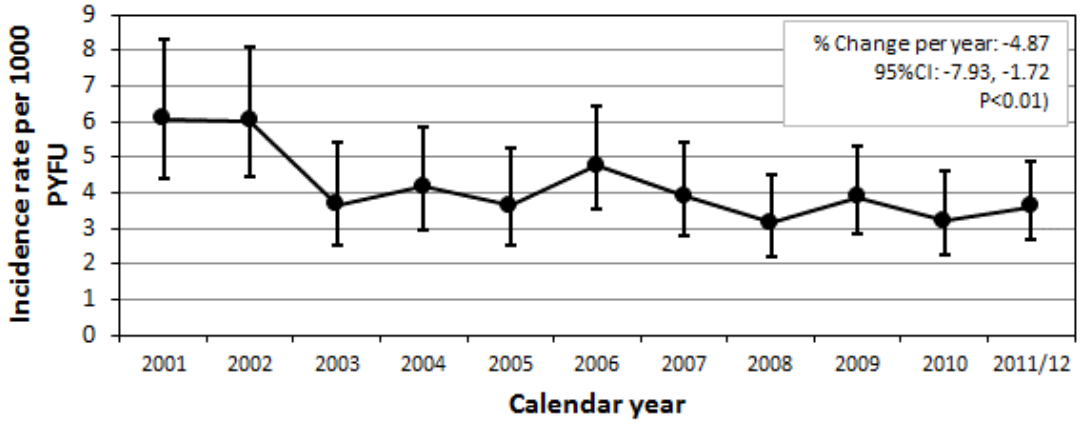


Figure 3.5 Crude incidence of IRC over time from 2001 to 2011/12.
The percentage change in the crude incidence is given in the insert.

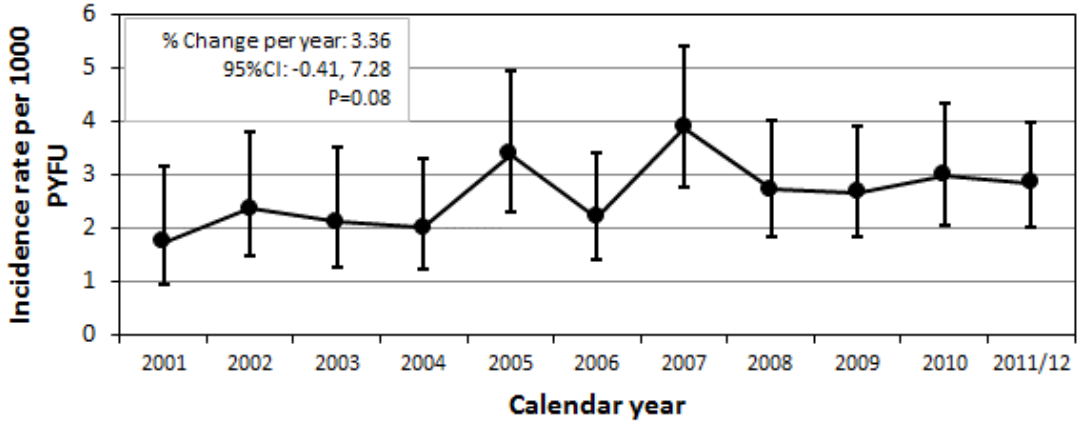


Figure 3.6 Crude incidence of IURC over time from 2001 to 2011/12.
The percentage change in the crude incidence is given in the insert.

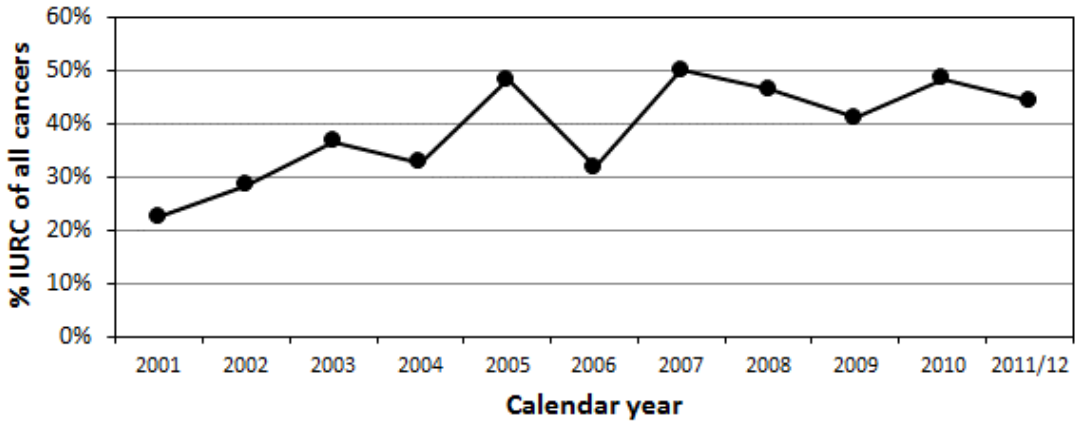


Figure 3.7 Percent of all cancers diagnosed that were IURC per calendar year.

3.4.4.2 Incidence of individual cancers

The overall crude incidence as well as change in incidence over time of selected individual cancers is shown in Table 3.5. The unadjusted incidence of both HL and NHL (both EBV related), and KS declined over time by 10.6 (95%CI: 1.6, 18.8)%, 11.4 (95%CI: 6.0, 16.4)% and 8.8 (95%CI: 1.3, 15.8)% respectively. The only significantly increasing incidence observed was for liver cancers, which was increasing by 16.5 (95%CI: 3.2, 31.6)% per year. There was no significant change over time in other IRCs or individual IURCs.

Table 3.5 Crude incidence of infection related (IRC) and infection unrelated (IURC) cancers and percentage change over time in crude incidence per year since 2001.

Cancers		N	Crude IR (95% CI) /1000 PYFU	% change per year	P-value
IRC	Overall	388	4.08 (3.69, 4.52)	-4.87 (-7.93,-1.72)	<0.01
EBV	Overall	159	1.67 (1.43, 1.95)	-11.2 (-15.5,-6.64)	<0.01
	HL	43	0.45 (0.34, 0.61)	-10.6 (-18.8,-1.63)	0.02
	NHL	116	1.23 (1.02, 1.47)	-11.4 (-16.4,-6.02)	<0.01
HPV	Male	81	1.16 (0.93, 1.44)	1.64 (-5.20, 8.96)	0.65
	Female	42	1.68 (1.24, 2.28)	-0.41 (-9.68, 9.81)	0.93
	Invasive cervical cancer (females)	33	1.33 (0.94, 1.87)	0.48 (-10.0,12.23)	0.93
	Anal cancer	83	0.88 (0.71, 1.09)	0.15 (-6.49, 7.27)	0.97
HHV8	Kaposi's Sarcoma	62	0.65 (0.51, 0.84)	-8.82 (-15.8,-1.30)	0.02
HBV/HCV	Liver	33	0.35 (0.25, 0.49)	16.53 (3.22,31.57)	0.01
H.Pylori	Stomach	11	0.12 (0.06, 0.21)	-8.63 (-24.3,10.28)	0.35
IURC	Overall	255	2.68 (2.37, 3.04)	3.36 (-0.41, 7.28)	0.08
	Lung	55	0.58 (0.44, 0.75)	4.98 (-3.69,14.43)	0.27
	Prostate (males)	28	0.40 (0.28, 0.58)	8.40 (-4.09,22.51)	0.20
	Breast (females)	26	0.27 (0.19, 0.40)	8.55 (-4.50,23.39)	0.21
	Colon and rectal	23	0.24 (0.16, 0.36)	-7.67 (-18.9, 5.13)	0.23
	Other (all cancers with <20 events, combined)	123	1.29 (1.08, 1.54)	3.28 (-2.33, 9.23)	0.26

IRC: infection related cancer, IURC: infection unrelated cancer, HPV: human papillomavirus, EBV: Epstein-Barr virus, HCV: hepatitis C, HBV: hepatitis B, HHV8: human herpesvirus 8, KS: Kaposi's sarcoma, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma.

3.4.5 Risk factors for infection related and infection unrelated cancer

The unadjusted (IRR) and adjusted (aIRR) incidence rate ratios for IRC and IURC for various risk factors are given in Table 3.6 and Table 3.7.

3.4.5.1 Adjusted results for infection related cancer

Age was associated with higher IRC incidence after adjustment (Table 3.6). Those aged older than 50 years had a 1.62 (95%CI: 1.14, 2.31)-fold higher incidence of IRC cancer incidence than those aged 36–40 years, equivalent to a 17% increase in incidence for a 10 year increase in age (95%CI:1.05, 1.32). As expected, factors most strongly related to a higher incidence of IRC were predominantly HIV associated. For example, IRC incidence increased with decreasing current CD4 cell count category in a dose response like manner, where CD4 cell count < 200 cells/mm3 (compared to ≥500 cells/mm3) was associated with a more than 3-fold higher IRC incidence (aIRR: 3.77; 95%CI: 2.59, 5.51). Furthermore, higher IRC incidence was also associated with current HIV-VL ≥ 400 copies/mL (aIRR: 1.84; 95%CI: 1.39, 2.43) and having had a previous ADC diagnosis (aIRR: 1.41; 95%CI: 1.02, 1.96). Higher IRC incidence was associated with HBV coinfection (aIRR: 1.70; 95%CI: 1.24, 2.32) and incidence was approximately halved in both males (aIRR: 0.54; 95%CI: 0.38, 0.78) and females (aIRR: 0.57; 95%CI: 0.40, 0.80) who acquired their HIV through heterosexual sex (relative to through sex between men). IRC incidence was lower in east compared to west central Europe (aIRR: 0.28; 95%CI: 0.14, 0.53).

3.4.5.2 Adjusted results for infection unrelated cancer

A dramatic rise in IURC incidence was observed with older age, with those aged 41–50 and 51 or older having 2.37 (95%CI: 1.31, 4.27) and 7.33 (95%CI: 4.07, 13.21)-fold higher incidence of IURC than those aged 36 – 40 years, after adjustment for other factors (Table 3.7). This corresponds to approximately a 2-fold increase in risk for a 10 year increase in age (aIRR: 2.07; 95%CI: 1.84, 2.32 P<0.01). IURC incidence was also elevated in those with a prior NADC diagnosis (aIRR: 2.13; 95%CI: 1.42, 3.20), current smokers relative to non-smokers (aIRR: 1.56; 95%CI: 1.17, 2.08), and those with HBV coinfection (aIRR: 1.73; 95%CI: 1.17, 2.55). IURC incidence was elevated in those with a current CD4 cell count < 200 relative to ≥500 cells/mm^3 (aIRR: 1.99; 95%CI: 1.26, 3.17), but was similar across other CD4 cell count categories.

Table 3.6 Unadjusted (IRR) and adjusted incidence rate ratios (aIRR) of infection unrelated cancers (IRC).

	Univariate		Multivariate	
Variable	**IRR(95%CI)**	**P**	**aIRR(95%CI)**	**P**
Age (years)				
≤35	1.04(0.71,1.54)	0.84	1.34(0.90,2.01)	0.15
36 – 40	ref		ref	
41 – 50	1.32(0.96,1.81)	0.09	1.34(0.97,1.85)	0.07
≥51	1.44(1.03,2.00)	0.03	1.62(1.14,2.31)	<.01
Calendar year (per year)	0.95 (0.92, 0.98)	<.01	1.01(0.97,1.05)	0.55
Gender specific HIV transmission group				
Sex between men	ref		ref	
IDU (males)	0.76(0.56,1.04)	0.09	0.83(0.57,1.20)	0.32
IDU (females)	1.04(0.71,1.51)	0.85	1.21(0.79,1.86)	0.39
Heterosexual sex (Males)	0.55(0.39,0.79)	<.01	0.54(0.38,0.78)	<.01
Heterosexual sex (females)	0.52(0.37,0.72)	<.01	0.57(0.40,0.80)	<.01
Other (Males)	0.82(0.51,1.31)	0.40	0.79(0.49,1.26)	0.31
Other (Females)	0.44(0.17,1.18)	0.10	0.55(0.21,1.48)	0.24
Region of Europe				
South	1.17(0.90,1.53)	0.24	1.31(0.99,1.74)	0.06
North	0.90(0.67,1.21)	0.50	0.90(0.68,1.20)	0.48
West central	ref		ref	
East central	0.80(0.55,1.16)	0.24	0.89(0.60,1.32)	0.56
East	0.34(0.20,0.60)	<.01	0.28(0.14,0.53)	<.01
Argentina	0.97(0.54,1.72)	0.90	1.02(0.57,1.83)	0.94
Current CD4 cell count (cells/mm^3)				
<200	4.70(3.60,6.14)	<.01	3.77(2.59,5.51)	<.01
200 – 349	2.03(1.53,2.69)	<.01	1.83(1.35,2.48)	<.01
350 – 499	1.31(0.98,1.76)	0.07	1.24(0.92,1.67)	0.16
≥500	ref		ref	
Current HIV-VL ≥400 (copies/mL)	2.27(1.84,2.78)	<.01	1.84(1.39,2.43)	<.01
Prior ADC diagnosis	1.76(1.28,2.41)	<.01	1.41(1.02,1.96)	0.04
Prior AIDS defining event (excl. ADC)	1.51(1.22,1.86)	<.01	1.25(1.00,1.57)	0.05
Prior NADC diagnosis			N/A	.
prior non-AIDS defining event (excl. NADC)			N/A	.
HBV coinfected	1.92(1.41,2.59)	<.01	1.70(1.24,2.32)	<.01
HCV coinfected	0.89(0.69,1.13)	0.33	0.77(0.56,1.06)	0.11
BMI				
Under weight	1.34(0.77,2.33)	0.29	1.16(0.66,2.04)	0.61
Normal weight	ref		ref	
Over weight	0.73(0.54,1.00)	0.05	0.73(0.54,1.00)	0.05
Obese	0.48(0.22,1.08)	0.08	0.55(0.25,1.22)	0.14
Unknown	0.93(0.73,1.19)	0.58	0.85(0.66,1.09)	0.2
Non-white Ethnicity	0.91(0.66,1.26)	0.58	0.98(0.71,1.37)	0.92
cART naïve	1.02(0.73,1.43)	0.92	1.36(0.80,2.29)	0.25
PI experienced	1.36(1.05,1.76)	0.02	1.14(0.79,1.64)	0.48
NNRTI experienced	1.00(0.81,1.24)	0.98	0.90(0.70,1.16)	0.4
Smoking status				
Never	ref		ref	
Previous	0.79(0.52,1.19)	0.26	0.78(0.51,1.19)	0.25
Current	1.20(0.95,1.51)	0.12	1.15(0.91,1.46)	0.24
Unknown	1.14(0.83,1.58)	0.41	0.98(0.69,1.39)	0.9

IRR: incidence rate ratio, aIRR: adjusted incidence rate ratio, IDU: injecting drug use, HIV-VL: HIV viral load, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, HCV: hepatitis C, HBV: hepatitis B, BMI: body mass index, cART: combination antiretroviral therapy, PI: protease inhibitor, NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor.

Table 3.7 Unadjusted (IRR) and adjusted incidence rate ratios (aIRR) of infection unrelated cancers (IURC).

	Univariate		Multivariate	
Variable	**IRR(95%CI)**	**P**	**aIRR(95%CI)**	**P**
Age (years)				
≤35	0.30(0.10,0.93)	0.04	0.33(0.12,0.96)	0.04
36 – 40	ref		ref	
41 – 50	2.39(1.32,4.30)	<.01	2.37(1.31,4.27)	<.01
≥51	7.43(4.22,13.08)	<.01	7.33(4.07,13.21)	<.01
Calendar year (per year)	1.03(1.00,1.07)	0.08	1.00(0.96,1.04)	0.95
Gender specific HIV transmission group				
Sex between men	ref		ref	
IDU (males)	0.59(0.38,0.92)	0.02	0.97(0.57,1.64)	0.91
IDU (females)	0.48(0.25,0.90)	0.02	0.91(0.46,1.80)	0.78
Heterosexual sex (Males)	0.84(0.57,1.24)	0.38	0.91(0.61,1.36)	0.64
Heterosexual sex (females)	0.68(0.47,0.99)	0.04	1.25(0.86,1.83)	0.24
Other (Males)	0.93(0.55,1.56)	0.78	0.93(0.55,1.57)	0.79
Other (Females)	1.04(0.46,2.33)	0.93	1.59(0.71,3.54)	0.26
Region of Europe				
South	1.05(0.76,1.45)	0.78	1.34(0.97,1.86)	0.07
North	0.95(0.67,1.33)	0.76	1.06(0.75,1.51)	0.74
West central	ref		ref	
East central	0.72(0.46,1.13)	0.15	1.16(0.73,1.85)	0.54
East	0.21(0.09,0.48)	<.01	0.61(0.27,1.37)	0.23
Argentina	0.64(0.28,1.46)	0.29	0.91(0.39,2.08)	0.82
Current CD4 cell count (cells/mm^3)				
<200	1.46(0.98,2.16)	0.06	1.99(1.26,3.17)	<.01
200 – 349	1.14(0.81,1.61)	0.44	1.30(0.89,1.88)	0.17
350 – 499	1.19(0.88,1.62)	0.26	1.29(0.95,1.75)	0.11
≥500	ref		ref	
Current HIV-VL ≥400 (copies/mL)	0.56(0.40,0.79)	<.01	0.91(0.62,1.35)	0.66
Prior ADC diagnosis	1.26(0.79,2.03)	0.33	0.92(0.57,1.49)	0.74
Prior AIDS defining event (excl. ADC)	1.01(0.77,1.34)	0.93	0.85(0.64,1.14)	0.29
Prior NADC diagnosis	3.66(2.47,5.44)	<.01	2.13(1.42,3.20)	<.01
prior non-AIDS defining event (excl. NADC)	2.50(1.63,3.85)	<.01	1.36(0.87,2.10)	0.17
HBV coinfected	1.73(1.16,2.57)	0.01	1.73(1.17,2.55)	<.01
HCV coinfected	0.66(0.47,0.92)	0.01	0.90(0.60,1.37)	0.62
Body mass index				
Under weight	1.19(0.56,2.53)	0.65	1.24(0.59,2.61)	0.56
Normal weight	ref		ref	
Overweight weight	1.04(0.74,1.47)	0.82	0.91(0.64,1.29)	0.6
Obese	0.80(0.35,1.79)	0.58	0.65(0.29,1.47)	0.3
Unknown	1.01(0.75,1.37)	0.94	1.01(0.75,1.36)	0.96
Non-white ethnicity	0.55(0.34,0.87)	0.01	0.62(0.38,1.01)	0.06
cART naïve	0.97(0.58,1.65)	0.69	1.07(0.51,2.29)	0.85
PI experienced	1.73(1.21,2.47)	<.01	1.05(0.69,1.60)	0.81
NNRTI experienced	1.45(1.10,1.92)	0.01	1.13(0.84,1.52)	0.43
Smoking status				
Never	ref		ref	
Previous	1.27(0.82,1.96)	0.29	1.24(0.80,1.91)	0.34
Current	1.19(0.90,1.58)	0.23	1.56(1.17,2.08)	<.01
Unknown	0.70(0.44,1.11)	0.13	0.87(0.53,1.42)	0.57

IRR: incidence rate ratio, aIRR: adjusted incidence rate ratio, IDU: injecting drug use, HIV-VL: HIV viral load, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, HCV: hepatitis C, HBV: hepatitis B, BMI: body mass index, cART: combination antiretroviral therapy, PI: protease inhibitor, NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor.

3.4.6 Population attributable fractions (PAF%)

The PAF% was calculated for variables that were significant modifiable risk factors for IRC and IURC as well as age in multivariate analyses (section 3.4.5) and are summarised in Table 3.8. For IRC, the highest proportion of excess IRCs was associated with HIV associated factors, such as a current CD4 cell count <200 cells/mm^3 and HIV-VL > 400 copies/mL, which accounted for 20.5% and 19.3% of excess IRCs respectively. Just over 12% of excess IRCs were attributed to being aged over 50 years (relative to 36 – 40 years). Although HBV coinfection was associated with a significantly higher IRC incidence, this accounted for only 5.3% of excess IRCs overall.

The highest proportion of excess IURCs was attributable to being aged over 50 years (relative to 36 – 40 years), with 55.9% of excess IURCs attributed to this group. The second largest contributor was smoking status, 16.4 of all excess IURCs attributed to this behaviour. Having a CD4 cell count <200 cells/mm^3 also contributed to excess risk, however the percent attributed was low at 6.3%. Prior HBV coinfection was also associated with higher risk of IURC, however again only 5% of excess risk was attributed to this group.

Table 3.8 Population attributable fractions (PAF%) for factors associated with the incidence of infection related cancer (IRC) and infection unrelated cancer (IURC).

	IRC		IURC	
Variable	**PAF% (%)**	**P<0.05 in multivariate analysis (Table 3.6)**	**PAF% (%)**	**P<0.05 in multivariate analysis (Table 3.7)**
Age (years)				
≤35	3.6(-1.6,7.0)		-3.1(-11.9,-0.1)	
36 –40	Ref		Ref	
41 – 50	10.4(-1.2,18.7)		16.5(6.9,21.9)	
≥51	**12.3(3.9,18.2)**	*	**55.9(48.8,59.8)**	*
Current CD4 cell count (cells/mm^3)				
<200	**20.5(17.1,22.8)**	*	**6.3(2.6,8.6)**	*
200 – 349	**10.6(6.1,14.0)**	*	4.4(-2.3,9.0)	
350 – 499	3.8(-1.7,7.9)		5.8(-1.5,11.1)	
≥500	Ref		Ref	
Current HIV-VL ≥400 copies/mL	**19.3(11.8,24.9)**	*	-1.4(-9.5,4.0)	
HBV coinfection	**5.3(2.5,7.3)**	*	**5.0(1.7,7.2)**	*
HCV coinfection	-6.5(-17.2,1.2)		-1.9(-11.7,4.6)	
cART naïve	2.5(-2.3,5.4)		0.3(-3.8,2.2)	
Smoking status				
Never	Ref		Ref	
Previous	-1.9(-6.3,1.1)		2.1(-2.8,5.2)	
Smoker	**6.0(-4.6,14.3)**	*	**16.4(6.8,23.7)**	*
Unknown	-0.3(-6.3,3.9)		-1.3(-7.8,2.6)	

* P<0.05 for aIRR in multivariate model. Where the PAF% is blue and bolded are statistically significant.
PAF%: Population attributable fraction, IUC: infection related cancer, IURC: infection unrelated cancer, HIV-VL: HIV viral load, HBV: hepatitis B, HCV: hepatitis C, cART: combination antiretroviral therapy.
Models were adjusted for the following baseline variables: age, calendar year, ethnicity, region, gender specific mode of HIV transmission, body mass index, smoking status, current HIV viral load, CD4 cell count, current hepatitis B and C coinfection, prior ADC diagnosis, prior AIDS-defining event diagnoses (excluding ADCs), prior NADCs, prior non-AIDS-defining events, cART naïve, and regimen.

3.4.7 Interaction between immune deficiency, age, and smoking status

The association between current CD4 cell count and smoking status and IRC and IURC incidence stratified by those aged <50 and ≥50 years is shown in Figure 3.8 **-** Figure 3.11**.** As mentioned in the methods, it was decided to investigate this a priori.

Figure 3.8 shows the association between CD4 cell count and IRC incidence in those aged <50 years (left) and in those aged ≥50 years (right). The incidence of IRC significantly increased with decreasing CD4 cell count at a similar rate in those aged under <50 and ≥50 years (P-value for interaction = 0.82). There was no association between smoking status and IRC incidence in multivariate analysis, however for consistency and comparison with IURC, I have provided the stratified analysis. As expected, there was no difference in the association between smoking status and IRC incidence in those aged <50 or ≥50 years (P- for interaction = 0.31, Figure 3.9).

Figure 3.10 shows the association between CD4 cell count and IURC incidence in those aged <50 years (left) in those aged ≥50 years (right). It is clear that the incidence of IURC was highest in those with CD4 cell count < 200 cells/mm^3 in those aged <50 years (aIRR 2.52; 95% CI 1.40, 4.54; P=0.01, left), but there was no association between IURC incidence and CD4 cell count in those aged ≥50 (right), although, the P-value of the interaction term did not reach statistical significance (P=0.09). Figure 3.11 shows the association between smoking status and IURC incidence in those aged <50 years (left) in those aged ≥50 years (right). In those aged ≥50 there was a higher incidence of IURC in current smokers (aIRR: 1.75; 95%CI: 1.23, 4.49; P<0.01; right), however there was no significant association between smoking status and IURC incidence in those aged <50 (aIRR: 1.12; 95%CI 0.71, 1.77; P=0.51, left), although the test for interaction was non-significant (P-value for interaction = 0.32). All other interactions with age were non-significant for both outcomes.

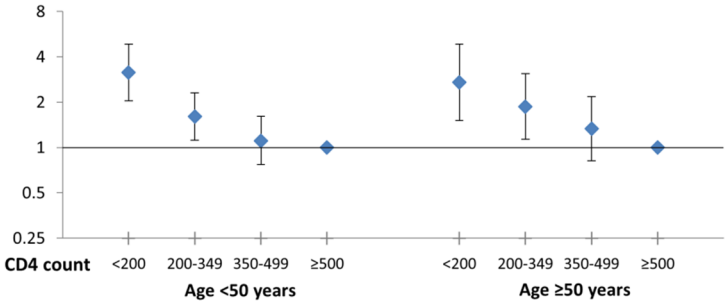


Figure 3.8 Adjusted incidence rate ratios (aIRR) of infection related cancer (IRC) for current CD4 cell count in those aged <50 and ≥50 years.

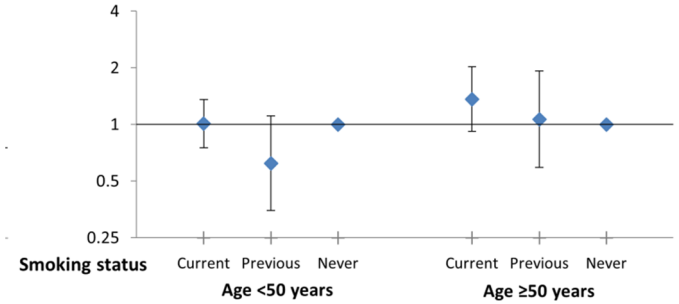


Figure 3.9 Adjusted incidence rate ratios (aIRR) of infection related cancer (IRC) for smoking status in those aged <50 and ≥50 years.

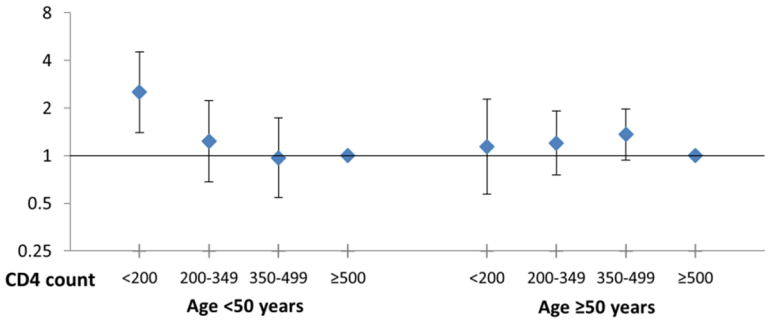


Figure 3.10 Adjusted incidence rate ratios (aIRR) of infection unrelated cancer (IURC) for current CD4 cell count in those aged <50 and ≥50 years.

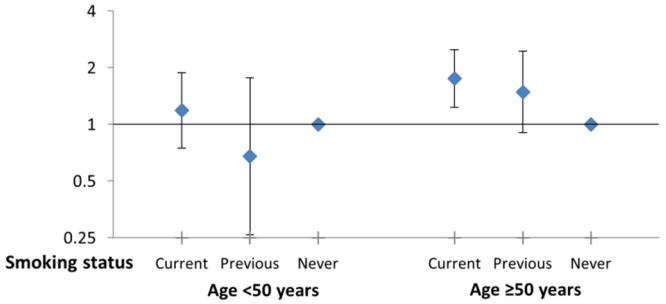


Figure 3.11 Adjusted incidence rate ratios (aIRR) of infection unrelated cancer (IURC for smoking status in those aged <50 and ≥50 years.

Models were adjusted for the following baseline variables: age, calendar year, ethnicity, region, gender specific mode of HIV transmission, body mass index, smoking status, current HIV viral load, current CD4 cell count, current hepatitis B and C coinfection, prior AIDS defining cancer (ADC), diagnosis, prior AIDS-defining event diagnoses (excluding ADCs), prior non-AIDS defining cancer (NADCs), prior non-AIDS-defining events (excluding NADCs), cART naive and regimen.

3.4.8 Association between calendar year and age, HIV related factors, and demographic variables on cancer

Table 3.9 shows the change in aIRR estimates controlling for calendar year and age (model A), then adding HIV related factors (model B) and finally adding demographic and lifestyle variables (Model C). This stepwise building of models shows the extent to which, for example, the association between age and calendar year and IRC is further modified by the addition of other variables. The incidence for IRC was estimated to be 16% higher for a 10 year increase in age and decline by almost half for a 10 year increase in calendar time (model A). This decrease over time was no longer significant after adjustment for HIV-specific variables, which indicates that changes in these variables over time explain some of the decrease in IRC over time that was observed. For IURC, the incidence was 2-fold higher for a 10 year increase in age and only a 10% increase per 10 additional years of follow-up (although not significant). Neither of these estimates changed after adjustment for HIV related factors (B) and demographic and lifestyle factors (C).

Table 3.9 Change in adjusted incidence rate ratios (aIRR) for infection related cancer (IRC) and infection unrelated cancer (IURC) when sequentially adjusting for variables.

	aIRR (95%CI) of IRC			aIRR (95%CI) of IURC		
	Model A	**Model B**	**Model C**	**Model A**	**Model B**	**Model C**
Age (10 years older)	1.16(1.05,1.27)*	1.25(1.14,1.38)*	1.24(1.09,1.42)*	2.05(1.87,2.25)*	2.05(1.86,2.26)*	1.96(1.7,2.26)*
Calendar year (10 years longer)	0.52(0.36,0.76)*	1.43(0.91,2.26)	1.43(0.9,2.25)	1.09(0.73,1.63)	0.99(0.56,1.75)	0.87(0.48,1.58)
Log $_2$ CD4 cell count		0.77(0.7,0.84)*	0.79(0.71,0.88)*		0.81(0.7,0.93)*	0.88(0.73,1.06)
Log $_{10}$ HIV-VL		1.27(1.14,1.4)*	1.32(1.18,1.48)*		0.99(0.84,1.16)	1.04(0.87,1.24)
Prior ADC		1.62(1.17,2.24)*	1.68(1.15,2.45)*		0.95(0.58,1.54)	1.13(0.66,1.91)
Prior AIDS event (excl. ADC)		1.12(0.89,1.41)	1.47(1.14,1.9)*		0.89(0.66,1.2)	0.83(0.59,1.17)
Prior NADC			-			2.2(1.38,3.5)*
Prior non-AIDS event (excl. NADC)			-			0.85(0.47,1.51)
Current smoker			1.3(0.98,1.72)			1.79(1.27,2.52)*
Previous smoker			1.04(0.65,1.64)			1.16(0.69,1.96)
Never smoker			1			1

* P< 0.05

IRC: infection related cancer, IURC: infection unrelated factors, ADC: AIDS defining cancers, NADC: non-AIDS defining cancer, HIV-VL: HIV viral load, HBV: hepatitis B, HCV: hepatitis C, cART: combination antiretroviral therapy

Model A contains age and calendar year

Model B contains variables in model A, CD4 cell count, current HIV-VL, prior ADC and prior AIDS defining event (excluding ADCs)

Model C contains variables in A and B and ethnicity, region of Europe, gender specific mode of HIV transmission mode, BMI, smoking status, current HIV-VL, CD4 cell count, current HBV/HCV coinfection, prior ADC diagnosis, prior AIDS-defining event diagnoses (excluding ADCs), prior NADCs, prior non-AIDS defining events (excluding NADCs), cART naive and regimen.

3.4.9 The future burden of infection related and infection unrelated cancer

Assuming current trends continue, the crude IRC incidence for those recruited to EuroSIDA before 2001 was forecast to decline (Figure 3.12), from an incidence of 3.1 (95%CI:1.5,5.9)/1000 PYFU in Jul-Dec 2011 to 2.2 (95%CI: 0.9, 4.3) after 5 and 1.6 (95%CI: 0.6, 3.4) after 10 years. Forecasted crude IURC incidence increased from 4.1 (95%CI: 2.2, 7.2) Jul-Dec 2011 to 5.9 (95%CI: 3.2, 10.2) and 7.8 (95%CI: 4.3, 13.5) after 5 and 10 years respectivley (Figure 3.13).

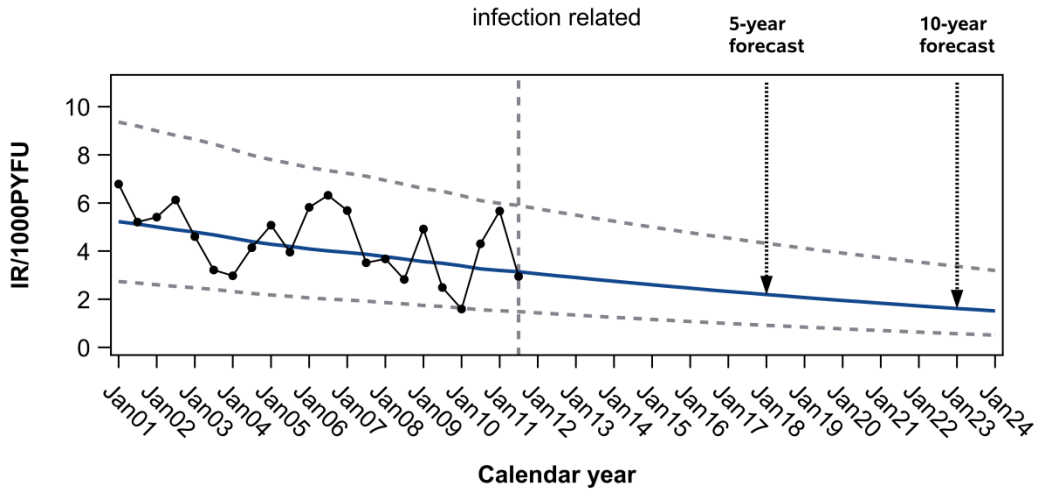


Figure 3.12 Semi-annual crude incidence rate of infection related cancer (IRC)/1000 PYFU for those recruited before 2001 with 5 and 10 year forecast.

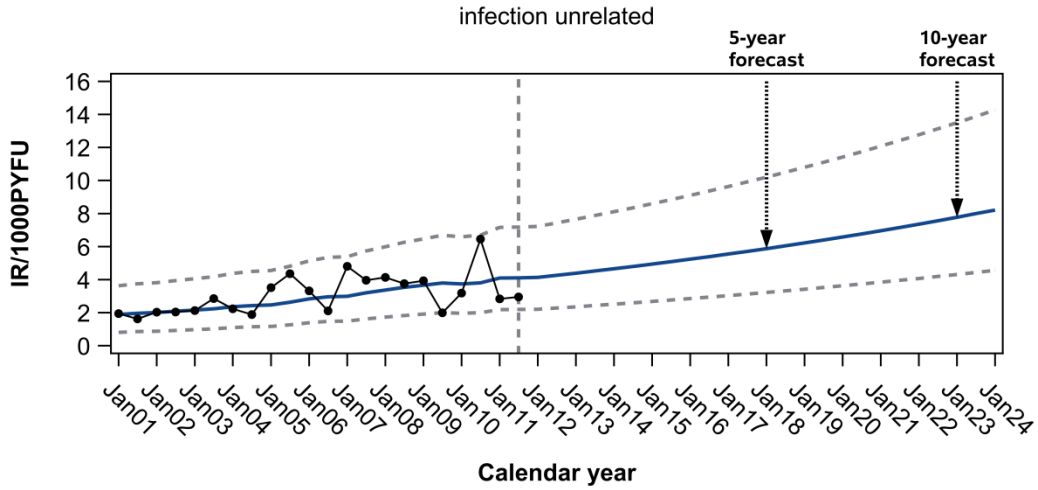


Figure 3.13 Semi-annual crude incidence rate of infection unrelated cancer (IURC)/1000 PYFU for those recruited before 2001 with 5 and 10 year forecast.
IRC: infection related cancer, IURC: infection unrelated cancer, PYFU: person years of follow-up, IR: incidence rate, PYFU: person years of follow up.

Table 3.10 shows the forecasted incidence of IRC and IURC at 5 years, overall and within different subgroups. IRC incidence was overall forecast to decline across all strata, with the exception of people who aquired HIV through IDU, in which the incidence remained stable at 4.9 /1000 PYFU.

The largest relative declines were seen in those who were aged ≥50 years with a decline of 61.1% from 1.8 in Jul-Dec 2011 to 0.7 /1000 PYFU after 5 years, and those who acquired HIV through sex between men, with a decline of 48.4% from 3.1 in Jul-Dec 2011 to 1.6 /1000 PYFU after 5 years. The groups with the highest IRC incidence after 5 years were those who acquired HIV through IDU (IR: 4.9/1000 PYFU), those who acquired HIV through heterosexual sex (3.3/1000 PYFU) and persons who had ever smoked (3.3/1000 PYFU).

Conversely, overall IURC incidence was predicted to increase in all strata, with the exception of those who had never smoked for whom the IURC incidence was forecast to decrease from 1.7 /1000 PYFU in Jul-Dec 2011 to 0.84 /1000 PYFU after 5 years. The largest relative increase in IURC incidence was for those who acquired HIV through IDU, which more than doubled from 3.4 in Jul-Dec 2011 to 7.0 /1000 PYFU after 5 years, and those who have ever smoked with a relative increase of 76% from 4.5 in Jul-Dec 2011 to 7.95 /1000 PYFU after 5 years. The groups with the highest IRC incidence after 5 years were those aged older than 50 years (IR: 15.3/1000 PYFU), those had whoever smoked (7.95/1000 PYFU) and those who acquired HIV through IDU (7.0/1000 PYFU), however numbers were small and estimates uncertain, as reflected by the wide confidence intervals for these estimates.

Table 3.10 Forecasted 5 year incidence of crude IRC and IURC for those recruited to EuroSIDA before 2001 within strata.

Subgroups	Incidence of IRC /1000 PYFU		Incidence of IURC /1000 PYFU	
	Jul – Dec 2011	Forecast at 5 years	Jul – Dec 2011	Forecast at 5 years
Overall	3.1 (1.5, 5.9)	2.2 (0.9, 4.3)	4.1 (2.2, 7.2)	5.9 (3.2, 10.2)
baseline age				
< 50 years	3.3 (1.8, 5.83)	2.4 (1.1, 4.35)	2.8 (1.0, 6.0)	4.4 (1.9, 9.2)
≥50 years	1.8 (0, 11.6)	0.74 (0, 7.04)	10.4 (3.4, 28.8)	15.3 (5.1, 42.7)
Baseline CD4				
<350 cells/mm^3	4.3 (1.5, 10.2)	2.9 (0.8, 7.4)	4.1 (0.6, 15.5)	5.6 (1.0, 20.5)
≥ 350 cells/mm^3	2.7(1.0, 5.7)	2.0 (0.6, 4.6)	3.6 (1.0, 9.5)	5.2 (1.6, 13.5)
HIV transmission mode				
Sex between men	3.1 (1.0, 7.0)	1.6 (0.3, 4.2)	4.2(1.5, 9.9)	5.0 (1.8, 12.1)
Heterosexual	3.8 (0.6, 13.7)	3.3(0.2, 14.1)	2.6 (0.0, 11.7)	3.3 (0.2, 14.1)
IDU	4.9 (1.0, 16.9)	4.9 (0.9, 17.1)	3.4 (0.0, 22.1)	7.0 (0.5, 42.4)
Baseline smoking status				
Ever smokers	4.2 (0.7, 14.8)	3.3 (0.39, 12.34)	4.5 (0.3, 21.5)	7.9 (1.1, 36.4)
Non- smokers	3.5 (0.65, 11.4)	2.6 (0.3, 9.2)	1.7 (0.0, 10.6)	0.84 (0.0, 7.01)

IRC: infection related cancer, IURC: infection unrelated cancer

3.5 Discussion

This study identifies that IRC and IURC have different risk factors. Older age was the largest contributor to IURC incidence, whereas IRC was primarily driven by HIV related risk factors such as higher HIV-VL and lower CD4 cell counts. Through this study, I was able to demonstrate an expected decline in IRC incidence over time, likely due to control of HIV related factors through effective cART. Simultaneously, IURC incidence was expected to rise, primarily due to aging of the HIV+ population. The burden of cancers is expected to shift towards IURC due to the non-declining IURC incidence, the increasing proportion of HIV+ people aged over 50 years, as well as higher prevalence of known cancer risk factors, such as smoking, and low prevalence of untreated HIV infection [405, 503]. The future of HIV care will need to address the increasingly complexity of management of the older HIV+ patient. Integration of HIV and oncological services for the detection, diagnosis, comanagement and treatment of complex comorbidities, such as cancers, may be needed in order to optimise care [504]. At the time of this study, this was one of the largest studies in Europe to investigate the changing incidence of IRCs and IURCs over time and to investigate associated risk factors, including demographic, HIV and lifestyle factors, in the late cART era. This study contained a comparably large number of prospectively collected IRC and IURC events over a long follow-up time of up to 12 years. Several other studies had performed similar analyses at the time of this study [16, 20, 432, 433, 592, 601, 602], however most were performed within HIV+ people in the USA, where the patient demographics and health care structure is considerably different to those in Europe (and may not be generalizable) [16, 432, 601] or other non-EU populations [602]. Furthermore, many compared populations or groups, for example: HIV+ vs HIV-negative or HIV+ over time, rather than investigating individual level risk factors in HIV+ people [8, 20]. Ecological studies present associations between risk factors and outcomes for populations overall rather than the individual, and therefore, associations may not accurately reflect those at the individual level [603].

3.5.1 Change in incidence of infection related and infection unrelated cancer over time

The crude incidence of IRC has declined since 2001, with a clear decrease between 2001 and 2003 and stabilized thereafter. The change in IRC over time was driven by the control of HIV related factors, such as controlling HIV viremia leading to CD4 recovery, likely due to improvements in treatment regimes, treatment uptake and adherence [594]. Furthermore, the proportion of all cancers that were IURCs almost doubled from 22.4% in 2001 to 44.2% in 2011/12, driven by the decline in IRC incidence and slight increase in IURC incidence per year. This demonstrates a shift in cancer burden towards IURCs in recent years.

The decline in IRC incidence was driven by the decline in EBV and HHV8 associated cancers, consistent with previous studies [7, 8, 16-21, 430, 438, 439, 441, 442, 444, 450, 451, 459, 465, 470, 478, 483, 604]. In this study, HL incidence was decreasing by 10% per year, which is not consistent with most previous research [8, 19, 21, 430, 467]. A study from the Swiss HIV cohort study showed increasing rates of HL between the pre and early HARRT periods, but were fairly stable thereafter [430]. There are several potential explanations for this difference. First, my study covers the period from 2001-2011/12, whereas many other studies cover a much earlier period and include a large number of people with advanced HIV infection. Second, the potential benefits of cART for reducing HL incidence through improved immune function and EBV control may take a long time to be visible at the population level [605-608]. For more detail on the pathogenesis and risk factors of NHL and HL, see chapters 5 and 6. The decline in IRC incidence was not consistent across individual cancers. Invasive cervical and anal cancers were both stable over time in this study. Non declining cervical and anal cancer rates have been commonly reported in the literature [14, 19, 430, 432, 436-438, 465, 467, 479-481, 609], however, a decline in cervical cancer has been observed in recent years [436, 464]. In my study, liver cancers were increasing which is consistent with previous studies [436, 437, 609]. However, the increase presented here of 16.5% per year was higher than expected, with an increase of 8.5% per year in the USA and 11% per year in Europe reported previously [610]. The increase in liver cancers in EuroSIDA may in part be described by changes in EuroSIDA recruitment patterns over time, such as the increased focus on eastern Europe (which have a very high prevalence of IDU and HCV coinfection [412] and alcohol use [611], as well as the intensification of case finding processes.

3.5.2 Forecasts of infection related and infection unrelated incidence

The incidence of IRCs was forecast to continue to decline in the near future. This was consistent in all strata except IDUs who may be less likely to start and adhere to treatment and have generally poorer clinical outcomes [612]. The incidence of IURCs was forecast to increase in the near future across all strata with exception of non-smokers, driven by lower lung cancer rates, and supports the need for smoking prevention initiatives and smoking cessation programs. If the declining IRC and non-declining IURC incidence continue in the coming years, the burden of cancers is expected to shift towards IURC due to the growing HIV+ population and the increasing proportion of aged over 50 years [175, 405, 503]. Such a shift has been demonstrated in HIV+ people with AIDS in the USA, where the number of NADCs has exceeding that of ADCs since 2003 and the total cancer burden has increased since the early 2000s (following a steep decline

following the introduction of cART) due to the growth and aging HIV+ population (Figure 3.14) [7].

Figure 3.14 Total number of cancer cases (i.e. cancer burden) among people with AIDS in the United States, 1990–2005 [7].

3.5.3 Risk factors

3.5.3.1 HIV Associated factors: HIV viral load and CD4 cell count

The strongest predictors for IRC were low current CD4 cell count and high current HV-VL. This was not surprising given the strong link between reduced immune function and loss of control of oncogenic viruses, particularly EBV (explored further in chapters 5 and 6) and HHV8 [453, 606-608]. Many previous studies have found a strong association between immune deficiency and individual IRCs, including KS, NHL, HL, and liver cancer [17, 20, 475, 487]. Although a French study demonstrated and protective association with higher $\log_2$ CD4 cell count [433], a clear link between CD4 cell count and cervical cancers has not been established in most cohort studies [17, 487]. However, low CD4 cell counts have been linked to higher prevalence of HPV infection (the cause of cervical cancer), and lower rates of HPV clearance in HIV+ women [613-615]. A link between anal cancers and duration of immune suppression [433], nadir CD4 [438, 481], and CD4 cell count [16, 479] has previously been reported. There is growing evidence that HIV-VL is

associated with incidence of some IRCs (mainly NHL and KS) independently of CD4 cell count [16, 429, 433, 616].

IURC incidence was modestly associated with current CD4 cell count, however this only accounted for a small percentage (6.3%) of excess IURC cases. This association appeared to be limited to those aged <50 years although the interaction between age and CD4 cell count was non-significant, possibly due to limited power. In the START trial (summarised in chapter 1 section 1.1.8.7.1), those in the immediate cART had a 51 (21 – 115)% lower risk of IURC relative to differed cART arm but did not reach statistical significance (P=0.1). However, high baseline CD8 cell count was associated with increased risk, indicating that immune dysfunction may play a role in IURC development [616].

3.5.3.2 Age

Elevated IRC incidence was (although less prominently than for IURC) associated with higher age. This probably reflects longer exposure to oncogenic viruses, such as replicating EBV, due to longer survival. The effects of aging on the immune system are thought to occur at an accelerated rate in HIV+ people, and may also explain this result (for more detail on HIV and aging see chapter 1 section 1.2.4) [3, 617]. A similar association between IRC and age was also identified by the START study [616]. The proportion of excess IRCs that were attributable to being aged 51 year or older (PAF%: 12.3%), HIV-VL>400 copies/mL (19.3%) and CD4 cell count <200 cells/mm^3 (20.5%) were comparable, despite the strongest predictors of IRC being current CD4 cell counts and HIV-VL.

Higher IURC incidence was strongly associated with age, and linked with more than 50% of the excess of IURC incidence in EuroSIDA. The 2-fold higher IURC incidence for a 10 year increase in age is similar to findings of the SMART study [594], START study [616] and to data published online by the European cancer observatory, which showed a 1.9-fold increase per 10 years older in incidence of all cancers in the general population [618]. Similar results were found for the UK (Figure 3.15).

Figure 3.15 All cancers (C00-C97 Excl. C44) average number of new cases per year and age-specific incidence rates, UK, 2011-2014 [619].

3.5.3.3 Current smoking status

No association between smoking and IRCs was identified. This was surprising given the established link between HPV associated head and neck cancers and tobacco use [620, 621]. This may suggest an alternative pathophysiological mechanism in the setting of HIV infection, however, this is more likely due to the small number of head and neck cancers, the high proportion of people with unknown smoking status, and the lack of detailed smoking information, such as duration and intensity, in EuroSIDA (see chapter 2 section 2.1.1.7 for a list of data items collected in EuroSIDA).

Current smoking was strongly associated with IURC incidence, driven by the causal link between lung cancer and tobacco use [594, 620-622], and accounted for 16.4% of excess cancers in this group. When stratified by age, current smoking was associated with higher IURC incidence in those aged over 50 years only. This is probably in part due to the correlation between age and the two strongest risk factors for lung cancer in the general population: smoking duration and age at smoking cessation [622]. The strong association between lung cancer and older age has been repeatedly shown in both HIV+ [617, 623] and the general population [622, 623], however, it is difficult to assess this association without detailed smoking information. In addition, some

studies have shown a higher prevalence of smoking status in the older age groups in HIV+ people which may also explain the result [421, 489]. I have explored the association between smoking and cancer in Chapter 4.

3.5.3.4 Hepatitis C and Hepatitis B coinfection

HBV coinfection was associated with high IRC incidence, driven by liver cancer, as expected [474, 624, 625]. Surprisingly, HBV coinfection was also associated with higher overall IURC incidence, although due to small numbers I could not identify if this was driven by one cancer type. Other studies have found a link between HBV and non-liver related outcomes, such as an excess of non-liver cancer deaths in two studies in Asia in those with chronic HBV [626, 627], and several studies have found a link with elevated B cell NHL risk [464, 477]. Misclassification of metastatic liver cancer as other primary cancers is also a possible explanation. Unfortunately, due to insufficient data, I was unable to explore this further. Although the association between HBV and both IRC and IURC incidence was relatively strong, HBV coinfection only accounted for approximately 5% of excess IRC and IURC due to the small proportion of people with IRC that were HBV coinfected. No association between IRC or IURC and HCV coinfection was identified, despite the established link with liver cancers (although liver cancers made up a relatively small proportion of the IRCs included) [474]. Liver cancers tend to develop several decades after initial hepatitis infection [628, 629] and are likely to become a source of burden in the aging HIV/hepatitis coinfected population.

3.5.3.5 Other factors

Incidence of IRC varied by region with lower incidence in eastern Europe, as well as by mode of HIV transmission, with lower incidence in males and females who acquired HIV through heterosexual sex. This could reflect a competing risk of death in these groups (i.e. they died before they could develop an IRC), although, I did perform a competing risks analysis and the results were unchanged. Alternatively, the results could be influenced by unmeasured confounding across regions or mode of HIV transmission groups, such as differences in treatment quality or availability, cancer screening and detection rates, or socio-demographic differences.

IRC incidence was higher in those who had a prior ADC or AIDS defining event. This could indicate additional immune dysfunction in advanced HIV infection that is not captured by low CD4 cell counts alone and uncontrolled replication of oncogenic viruses (such as EBV) [607]. Furthermore, some studies have shown increased risk of NHL following diagnosis of KS, and vice

versa [435, 630], thought to be due to shared risk factors and simultaneous infection with EBV and HHV8 in HIV+ people [607]. Alternatively, it could just reflect increased monitoring of HIV+ people following an AIDS defining event. After adjustment, IRC incidence was not associated with ethnicity, cART use, cART regimen, or BMI.

IURC incidence was associated with a prior NADC. Studies in the general population have identified previous cancer as a risk factor for subsequent cancers [631]. For example, breast cancer is 8-fold higher in HL survivors, particularly those who received radiotherapy rather than chemotherapy[632], and 14% higher in melanoma survivors [633]. Colorectal cancer survivors are also at higher risk of cancers of the digestive system [631]. The increase may also be explained by shared risk factors, such as smoking, that facilitate the development of multiple cancers simultaneously (See chapter 1 section 1.2.5 for a list of common cancer risk factors). Furthermore, increased cancer surveillance and clinic visits in cancer survivors for the first 2 - 3 years following diagnosis may also contribute [634], although, doctor visits are higher for up to 10 years following diagnosis, which may increase the chance a cancer is detected [635]. Alternatively, my result could reflect misclassified of metastatic cancers or recurrence of primary cancers as second primary cancers in EuroSIDA. IURC incidence was not associated with gender specific HIV transmission mode category, region, ethnicity, previous ADC or AIDS-defining diagnosis, previous non-AIDS defining event (excluding NADC), HCV coinfection, cART use, cART regimen, or BMI.

3.5.4 Strengths of these analyses

EuroSIDA is a large heterogeneous cohort of HIV+ people with a relatively large number of prospectively collected cancers. All reported diagnoses of cancers are validated and undergo a rigorous quality assurance process. Furthermore, EuroSIDA collects a range of longitudinal demographic, HIV related, laboratory and treatment variables on each patient, which allows for rich patient level analyses to explore individual level variations. Many existing studies compare populations of HIV+ individuals over time, or HIV+ and HIV-negative individuals to identify differences in risk. These studies give an extremely important overview of changes in the HIV+ population overall, however they are limited in terms of identifying patient relevant factors to improve patient outcomes. This study identifies a list of patient based risk factors for IRC and IURC in HIV+ populations, and demonstrates the shift in the cancers occurring in HIV+ people over time.

3.5.5 Limitations of these analyses

The limitations of this study should be noted. EuroSIDA has a relatively large number of prospectively collected cancers, but despite this, the frequencies of individual cancers were small and could not be investigated individually. EuroSIDA is an observational study, and although analyses were adjusted for various potential confounders, I cannot rule out the effects of confounding that remains due to unmeasured or unknown factors. Furthermore, several known risk factors for cancer are either not collected or collected in an over simplified manor. For example: the lack of detailed life time smoking and alcohol use information, such as duration or intensity. Although detailed information on CD4 cell count and HIV-VL is available, alternative measures of immune dysfunction (such as CD8) were not available. Another significant limitation is the lack of other medication or treatment used in people under follow-up in EuroSIDA, such as cancer treatments. As a result, I was not able to investigate mortality and survival following cancer diagnosis adequately.

Linear exponential smoothing models are a simple method of forecasting which assume the continuation of previous population trends. These methods can depict how the incidence may change over time on a broad level, but cannot formally identify the reasons i.e. due to continuing changes in the underlying population such as aging or improved treatment uptake and efficacy. However, I could speculate based on the risk factor analysis. A limited amount of historical data was available for forecasting, contributing to large uncertainty around forecasts. HIV-specific population projections were not available at the time of this analysis, which prevented the use of more advanced methodologies, such as age-period-cohort models.

3.6 Conclusions

This chapter shows that the incidence of IURC is expected to increase due to aging of the HIV+ population. Conversely, IRC incidence is expected to decline as a result of the low prevalence of advanced and untreated HIV infection and severe immunodeficiency. Reducing the incidence of IURCs should therefore be a priority in the coming years as the proportion of HIV+ people aged over 50 years continues to increase. *S*tudies evaluating cost-benefit of screening in the HIV+ population and targeted preventive interventions, such as cessation programs for smoking and alcohol use, and vaccinations for oncogenic viruses such as HBV and HPV should be considered to reduce the burden of avoidable cancers.

There is a need for research into the risk factors, diagnostic markers, and pathological mechanisms of common cancers in HIV+ people. Smoking prevalence tends to be higher in HIV+ people and the burden of lung cancer is expected to increase as the population ages. The benefits of smoking cessation on cancer risk is explored in chapter 4. Furthermore, EBV associated cancers (NHL) and (HL) account for the highest number of cancer diagnoses in this population, and although declining, incidence is still elevated in HIV+ people and contribute significantly to cancer burden. Risk factors for NHL and HL are identified and compared in chapter 5, and markers of B cell activation preceding lymphoma development are addressed in chapter 6. Finally, prostate cancers (PCa) are the most common cancer in older European men in the general population and are strongly associated with age. The burden of PCa is expected to increase in HIV+ people as the population age. Prostate specific antigen (PSA) is a diagnostic marker used in the general population and its use in HIV+ men is assessed in chapter 7.

3.7 Publications

This chapter was published in HIV Medicine in 2016 [636] and is included in Appendix III. This chapter was presented at the European AIDS clinical society (EACS) conference in 2013 and the slides are included in Appendix IV.

4 Cessation of cigarette smoking and the impact on cancer incidence in the D:A:D Study

4.1 Introduction

As outlined in chapter 3, in the context of available and effective HIV treatment, cancers are a major source of morbidity and mortality in HIV-positive (HIV+) people, due to a combination of longer life expectancy, reduced immune function, and life style related factors [220, 637]. However, the relative contribution of life style related risk factors, such as smoking, towards elevated cancer risk, is not clear [638]. Smoking is a known risk factor for many cancers in the general population, and therefore, smoking cessation presents as an important modifiable factor to reduce cancer risk [639].

4.1.1 Smoking, cancer, and other morbidity in the general population

In the general population, tobacco smoking is an established risk factor for many cancers and the leading causes of cancer worldwide [622, 640]. It is estimated that more than 1.1 billion people worldwide were smokers in 2015 with a prevalence of 27.3% in Europe [641]. In addition, lung cancers are now the most common cancers with an estimated 1.2 million new cases per year and are one of the most common causes of death globally [642, 643]. Incidence of lung cancer is highest in regions where the smoking epidemic is oldest such as the USA and Europe [642]. Therefore, smoking is currently one of most important modifiable factors for cancer risk prevention [622, 644, 645].

Incidence of smoking related cancer and mortality has declined in high income countries in recent years due to a combination of increasing rates of smoking cessation as well as the proportion of people who never start smoking [646]. However, smoking cessation remains uncommon in low to middle income countries and the burden of smoking related morbidity and mortality in these regions are expected to rise if smoking cessation rates do not increase [646].

4.1.1.1 The harms of smoking

Current smokers are on average 2-3 times more likely to die from any cause than those who have never smoked mainly due to smoking related diseases in high income countries [647-649]. In addition, studies from the UK and USA have estimated that smokers loose approximately a decade of life compared with non-smokers in the general population [647, 648, 650, 651].

Unfortunately, it is difficult to properly measure the harms of smoking at a population level, as the full impacts may not be evident for up to 50 years after smoking enters a population [647].

It was established that smoking causes lung cancer in the 1950's [622, 647, 651-653], and it has been estimated that half of the excess mortality in current smokers is due to lung cancer [647]. The strongest risk factor for lung cancer in the general population is longer smoking duration [622, 654], followed by smoking intensity (i.e. the daily number of cigarettes smoked) [622, 647]. All histological types of cancer have been causally linked to cigarette smoking, however, stronger associations for squamous cell and small cell carcinomas than for large cell carcinomas and adenocarcinomas have been reported [655, 656]. Smoking has also been causally linked with many other cancers, which are listed in Table 4.1 [622, 640]. Risk of many other conditions have been linked to smoking, including cardiovascular disease (CVD), respiratory diseases (such as chronic obstructive pulmonary disease [COPD], pneumonia, and tuberculosis), diabetes, rheumatoid arthritis, adverse effects on infant and child health, reduced fertility, pregnancy complications, still births and low birth weight [622, 657]. In addition, smoking also causes systemic inflammation and impaired immune function [657].

4.1.1.2 The benefits of cessation

The benefits of smoking cessation in the general population are well established and include reduced mortality (restored longevity) and reduced incidence of lung cancer relative to those who continue smoking [654, 658, 659]. However, estimates of the benefit in the general population are highly variable and there is uncertainty about the size and timing of these effects and how they vary according to age at cessation, smoking history, histology of lung cancer, and the presence of other smoking related diseases [654, 660]. Nonetheless, most epidemiological studies show a greater benefit of cessation in those with shorter smoking duration, lower intensity of smoking, and younger age at cessation [647, 651]. Although smoking cessation can reverse much of the carcinogenic effects of smoking, the risk will never return to that of never smokers due to residual genetic damage [654].

It has been estimated that the benefits of cessation on the incidence of lung cancer take between 5 – 9 years to become apparent, and increase with longer time since cessation [654, 661-663]. Following smoking cessation, the time for the excess lung cancer risk due to smoking to reduce by half (or the "half-life") is estimated to be 10 years [661]. Similar to lung cancer incidence, risk of lung cancer mortality is also reduced following cessation and declines with increasing time since cessation [654]. Reductions in risks after cessation have also been

observed for many other cancers (although less pronounced than for lung cancers) [654] including cancers of the head and neck, oesophagus, stomach, bladder cancer, pancreatic, and cervix [654, 663]. Although the incidence of liver cancer is lower in former smokers than current smokers, there is insufficient data on time since cessation, and conflicting results also exist for myeloid leukaemia [654]. There is substantial variation in estimates from epidemiological studies published in the literature of the effect of cessation on cancer incidence partially due to differences between studies in smoking duration, smoking intensity, age, age at cessation, gender and other factors [654].

4.1.1.3 The introduction of E-cigarettes

E-cigarettes (EC) have had rapid uptake in the general population since their introduction in 2007 [664] and are thought to help with achieving successful smoking cessation or at least reduce smoking intensity [665-667]. A Cochrane review showed that people who used ECs were more than 2-fold more likely to cease smoking for at least 6 months relative to those who did not [668]. Furthermore, ECs do not involve combustion and prevent exposure to associated harmful chemicals such as tar and carbon monoxide [665]. This has numerous health benefits including potentially reduced incidence and progression of many cancers [665, 669]. ECs are thought to be safer for long term health than traditional cigarettes [670], however, it is important to remember that ECs are not risk free [665, 671]. ECs involve their own mix of potentially harmful chemicals, including nitrosamines, formaldehyde, and heavy metals, which are potential carcinogens, and have not been available long enough to assess the long term health impacts [665, 670, 672-677]. This is a concern for conditions with long latency periods, such as cancers, which may take up to several decades for an elevated risk to emerge [665]. Furthermore, it is difficult to tease out the health impact of ECs as many people use them in conjunction with conventional cigarettes [665].

4.1.2 Smoking and cancer in HIV-positive people.

4.1.2.1 Prevalence of smoking in HIV-positive people

Studies of HIV+ people often find higher smoking prevalence (between 40 – 84%) than the general population (European countries: 13 – 45%, USA: 17%) [20, 421, 489-496, 641, 678-683], with prevalence estimated to be 2-3-fold higher in HIV+ populations [491, 493, 679, 682, 684], however estimates are highly variable between countries [685]. One French study demonstrated variation in prevalence estimates according to age, gender, HIV transmission group, country, and ethnic origin, and ranged from 9% in women from sub-Saharan Africa to 81% in people who

acquired HIV through injecting drug use (IDU) [492]. Demographic factors may contribute to higher smoking prevalence among HIV+ people and previous studies have found the following factors to be associated with a higher prevalence of smoking: older age, male gender, higher body mass index (BMI), alcohol use, white ethnicity, living in a smoking environment, lower income, less social support, illicit drug use (including IDU), and depressive symptoms [493, 678-681, 686, 687]. Smoking in HIV+ people is also thought to be linked with elevated stress and reduced quality of life due to living with a HIV+ diagnosis and associated treatment [492]. In addition, HIV+ smokers are thought to have high nicotine dependence with an average of 16 – 23 cigarettes smoked per day [420, 491].

4.1.2.2 Harms of smoking in HIV-positive people

Similar to the general population, HIV+ people also suffer from adverse health outcomes due to smoking, including increased mortality (reduced longevity), morbidity, cancer risk, and reduced quality of life [20, 621, 682, 684, 688-690]. In those on combination antiretroviral therapy (cART), HIV+ smokers lose more life years due to smoking rather than due to HIV infection [496, 691]. Furthermore, the risk of death from any cause in HIV+ smokers was found to be 2 – 4-fold higher than never smokers in those on cART [496, 691].

Smoking increases the risks of various diseases that are of concern in HIV+ people. These include a range of pulmonary complications such as lower respiratory tract infections (tuberculosis and bacterial pneumonia), COPD, and lung and other cancers [433, 638, 682, 688, 690, 692-709]. Other comorbidities associated with smoking include CVD [682, 710, 711], perinatal mortality [712], periodontal disease [713], accelerated bone loss, fractures [714-716], and a decreased quality of life [688, 690].

It has been suggested that the lungs of HIV+ people may be especially susceptible to the harms of smoking [689] and studies have been shown that HIV+ smokers have higher rates of morbidity and mortality than HIV-negative smokers controlling for other demographic factors [688, 691]. Smoking is thought to have detrimental effects on immune function and to increase activation and inflammation in the lung in the context of HIV [696, 717]. This may put HIV+ smokers at higher risk of several pulmonary diseases (which are known risk factors for lung cancer) relative to HIV+ people who have never smoked [696]. This is particularly worrying in the context of HIV due to existing immune dysfunction in this population and elevated risk of many pulmonary disorders in the absence of smoking [507, 718-721].

4.1.2.2.1 Lung cancer

Lung cancers are one of the most common non-AIDS defining cancers (NADC) in HIV+ people lung cancer related deaths are the leading cause of NADC related death in HIV+ people in high income countries [7, 21, 444, 722, 723]. Many studies have shown an excess incidence of lung cancer in HIV+ people relative to the general population [14, 15, 433, 638, 705-707, 724-728], however, it is hard to disentangle how much of this excess is due to the effects of HIV infection or the higher prevalence of smoking in HIV+ people [638]. One study from the USA showed that the incidence of lung cancer was elevated in HIV+ relative to HIV-negative people largely due to differences in smoking behaviour. However, lung cancer incidence was still 2.5-fold higher after adjustment for differences in smoking behaviour [725] (a result that has been replicated in other studies [706, 728]) and suggests that HIV plays either a direct or indirect role in the development of lung cancers in HIV+ people. Another recent study demonstrated that HIV+ people were not at excess risk of lung cancer relative to HIV-negative people after accounting for differences in cancer risk factors and history of pneumonia [729]. It remains unclear whether smoking has a greater impact on lung cancer incidence in HIV+ people and no evidence of a synergistic relationship between smoking and HIV have been formally identified [728]. Some studies have found that HIV+ people present with lung cancer at a younger age [535, 584, 623, 727, 730], a later stage [727, 731], and after less exposure to smoking than HIV-negative people [730]. However, data from larger studies have found no evidence of a later stage of lung cancer at diagnosis in HIV+ people relative to the background population [535, 706, 732],. This also suggests that the higher lung cancer risk observed in HIV+ relative to HIV-negative people is not due to differences in cancer surveillance by clinicians [706].

The prognosis of HIV+ people following a lung cancer diagnosis is poor compared to the HIV-negative population in the cART era (5 year survival estimates in HIV+ and HIV-negative people following diagnosis of 10% and 19% respectively) [531, 535, 707, 726-728]. The reason for this disparity is largely attributed to differences in lung cancer treatment in HIV+ and HIV-negative people [733, 734], however, lower treatment tolerance, interaction between cancer treatment and cART, cART toxicities, or competing risk of death due to HIV related factors may also contribute [707, 734]. However one recent study has shown that lung cancer outcomes have converged in HIV+ and HIV-negative people in recent years [735].

4.1.2.2.2 Other smoking related cancers

Evidence of elevated incidence of other smoking related cancers (as defined Table 4.1) in HIV+ people is mixed. Consistently higher standardised incidence ratios (SIR) of cervical cancers [15, 20, 436], some head and neck cancers [15, 20, 521], and liver cancers [14, 15, 436, 521] have been demonstrated in HIV+ people. Conversely, some studies report higher incidence of colon and rectal cancers [15, 19], while others demonstrate comparable incidence to the general population [19, 436, 521]. Similar conflicting results for cancer of the oesophagus (elevated: [15], similar: [521]), stomach (Elevated: [15], similar: [521]), leukaemia (Elevated: [15], similar: [521]), and kidney (elevated: [14] similar: [521]) have been reported. There is no evidence of elevated incidence of ovarian cancers [15] or pancreatic cancer [19, 521]. However, many smoking related cancers are also associated with oncogenic viruses and, therefore, incidence is often increased in the context of immune suppression [8, 14-18, 427], as outlined in chapter 1 section 1.2.3 and addressed in chapter 3. A Danish study found excess risk of infection related cancers (IRCs) and smoking related cancers (including lung) in HIV+ people relative to controls, but not other cancers [621]. Never smokers had increased risk for IRCs but not smoking related cancers [621].

4.1.2.3 Smoking cessation

The incidence of most cancers, including lung, increase with older age [736, 737]. Therefore, as the HIV+ population ages [405, 503] and the burden of these cancers increase [7, 449, 584], smoking presents as a critically important evidence-based modifiable risk factor [639]. HIV+ people have regular contact with various healthcare facilities, and therefore, there are multiple opportunities for smoking cessation interventions [738]. However, HIV+ people who smoke have additional barriers to cessation that are less prominent in HIV-negative people. For example, smoking, drug, and alcohol use are closely intertwined in the context of HIV [492, 686] and the likelihood of smoking is higher when also using drugs or alcohol. Furthermore, multiple substance use is a marker for smoking cessation failure [420, 421, 491, 739-741] and additional psychological stress and depression in HIV+ people make cessation more difficult and reduce readiness to quit [420, 491, 686, 739-741]. For example, it has been reported that HIV+ people use smoking to cope with the stress of living with HIV and to relieve the side effects of HIV treatment [420, 492]. Previous studies have reported that between 30 – 60% of HIV+ smokers have attempted to quit since their HIV diagnosis [493, 686]. However, the motivation to quit in HIV+ people is mixed [421, 493, 686], with studies reporting variable estimates of the proportion of HIV+ people interested in quitting as well as readiness to quit [421, 493, 686, 742]. In addition,

studies have shown that HIV+ people are less likely to quit than the general population [743, 744].

4.1.3 Aims and objectives

Despite the well characterised harms of smoking in HIV+ people, the clinical benefits of smoking cessation on cancer risk have not, to my knowledge, been reported in large epidemiological studies. The aim of this study was to estimate cancer rates after smoking cessation in HIV+ people from the D:A:D study.

4.2 Methods

4.2.1 Study design and participants

This study was conducted within the Data Collection on Adverse events of Anti-HIV Drugs Study (D:A:D) collaboration using the 17th merger the D:A:D database (which included follow-up on 49,706 people). AIDS defining cancers (ADC), including Kaposi's sarcoma (KS), invasive cervical cancers, and non-Hodgkin lymphoma (NHL) have been collected in D:A:D as such since 1999. NADCs, including lung, anal, and Hodgkin lymphoma (HL), have been routinely collected and validated since 1 January 2004. The D:A:D study is described in detail in chapter 2 section 2.1.2.

4.2.2 Outcomes

Four main outcomes were assessed: 1. the first diagnosis of any cancer during prospective follow-up (referred to as all cancers). Cancers were further divided into the categories: (2) lung cancer, (3) any smoking related cancer (excluding lung), including cancers of the head and neck, oesophagus, stomach, pancreas, liver, kidney and urinary, colon and rectal, cervical, ovary, acute myeloid leukaemia and chronic myeloid leukaemia [477]. Finally, all remaining cancers not included in (2) and (3) were considered to be smoking unrelated. The cancers considered to be smoking related were selected based on the IARC Monographs on the Evaluation of Carcinogenic Risks to Humans [477]. These cancers have been causally linked with smoking. A list of cancers included in each group are listed in Table 4.1.

Table 4.1 The classification of cancers into lung cancers, smoking related cancers (excluding lung), and smoking unrelated cancers in D:A:D.

Lung cancer	Smoking related cancers (excluding lung)[1]	Smoking unrelated cancers
lung	acute myeloid leukaemia (AML)	anal
	chronic myeloid leukaemia (CML)	bladder
	cervical	bone
	colon and rectal	breast
	head and neck	connective tissue
	kidney and urinary	gallbladder
	liver	Hodgkin lymphoma (HL)
	oesophagus	Kaposi's sarcoma (KS)
	ovary	acute lymphoid leukaemia
	pancreas	unspecified leukaemia
	stomach	lip
		malignant melanoma
		multiple myeloma
		non-Hodgkin lymphoma (NHL)
		penis
		prostate
		testicular
		uterus
		other, unspecified or unknown

[1] Smoking related cancers were selected in accordance with the IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

4.2.3 Inclusion criteria

Baseline was defined as the latest of date of study entry or 1st January 2004, and people were followed from baseline until the earliest of their first cancer diagnosis, last visit plus 6 months, death, or 1st February 2016.

There were 42,334 D:A:D people with follow-up after the 1 January 2004. I excluded a total of 6,892 people, where 2,422 people had a prior cancer diagnosis (including 16 had a first diagnosis of metastatic cancer), 4,463 had no smoking information during follow-up, and 7 had no recorded gender. This resulted in 35,442 people available for analysis. Those excluded were older, and a higher proportion were male compared to those included. A flow chart for the inclusion criteria is shown in Figure 4.1.

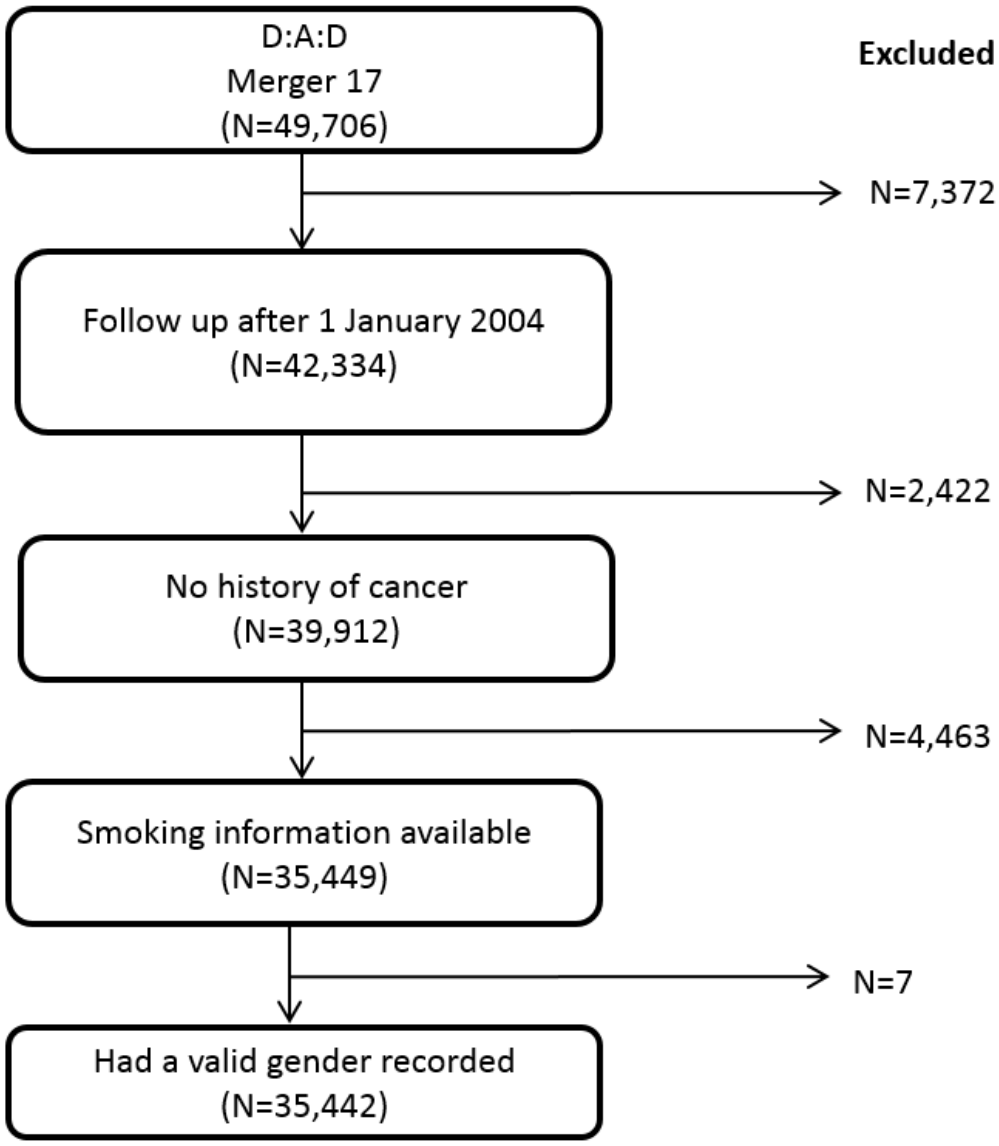


Figure 4.1 Flow chart for inclusion of D:A:D participants in analysis

4.2.4 Current smoking status and time since cessation

Smoking status was collected by the individual cohorts and reported to D:A:D study electronically using the using the HICDEP data exchange protocol on a bi-annual basis [550] (as mentioned in chapter 2 section 2.1.2.4). Smoking status was reported as current smoker (yes/no) and ever smoker (yes/no) at each visit based on physician inquiry at each center. In this chapter, smoking status was considered as a time updated variable. The categorisation of the variable is shown in Figure 4.2 and was defined as: never smokers, current smokers, ex smokers at baseline, and ex smokers who stopped smoking during prospective follow-up. The ex smokers were split in this way as the date of stopping smoking was not available in those who stopped prior to baseline, and therefore, time since cessation could not be determined. For those who stopped smoking during follow-up, time since smoking cessation was further categorised into

those who stopped <1 year, 1 – 1.9 years, 2 – 2.9 years, 3 – 3.9 years, 4 – 4.9 years, and ≥5 years prior. For example, at a specific point during follow-up, a person who quit smoking 1.5 years prior but during prospective follow-up would be in the ex smokers: 1 – 1.9 years category. If they restarted smoking, they would then move from the ex smokers category to the current smokers category. If they ceased smoking again, they would move back to the ex smokers category and their time since cessation would restart at zero i.e. the ex smokers: < 1 year category. Change in smoking behaviour was attributed to the midpoint of respective visits. If smoking status was missing at any visit, the most recently recorded previous smoking status was carried forward. Duration of smoking in D:A:D was calculated as the cumulative number of years spent as a current smoker during active D:A:D follow-up.

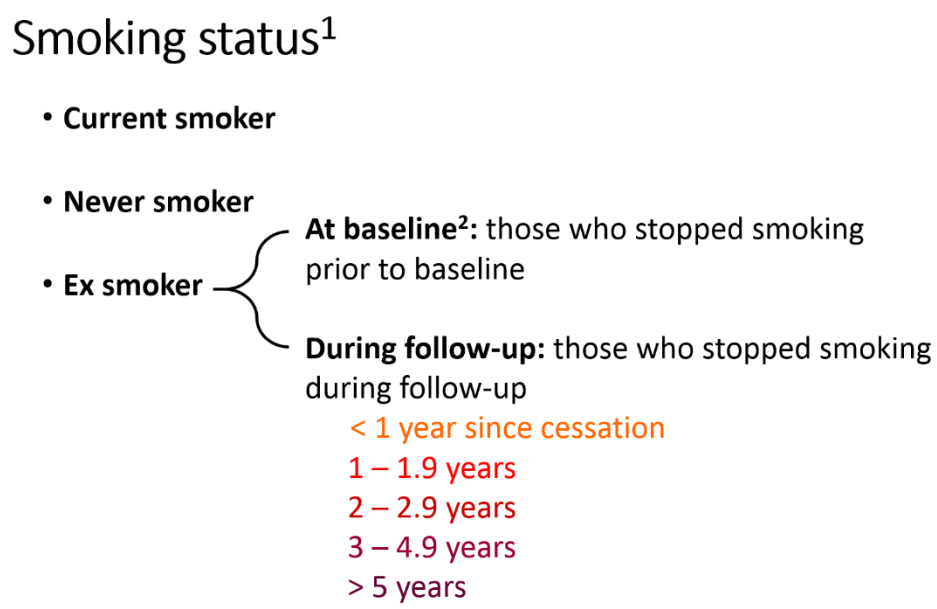


Figure 4.2 definition of current smoking status.
Baseline was defined as latest of date of study entry or 1st January 2004.
[1] Smoking status represents current smoking behaviour and is time updated
[2] Information on the date of stopping was not available and therefore time since stopping for previous smokers at baseline could not be determined

4.2.5 Variables included in analyses

Both baseline and time updated variables from the D:A:D database were included in this analysis, as detailed in Table 4.2. Person years of follow-up (PYFU) were calculated per month of additional follow-up.

Table 4.2 Summary of baseline and time updated variables.

Variable	Time updated	Levels	Definitions and comments
Age (years)	Yes	Continuous (per 10 years older) and categorised into 16 – 39, 40 – 49, ≥ 50 years	
Calendar year of baseline		Continuous (per year) and categorised by year (2004 – 2005, 2006 – 2007, 2008 – 2009, 2010 – 2011, 2012 – 2016)	
Calendar year of follow-up	Yes	Continuous (per year)	
Ethnicity		white, black or other, or unknown	For multivariate analyses the categories "black or other", and "unknown" were combined into one category called "non-white" due to low numbers.
Gender		Male, female	
HIV mode of transmission		Sex between men, heterosexual, IDU, other or unknown	
BMI (kg/m^2)	Yes	Under weight (<18), normal weight (18 – 25), Over weight (25 – 30), obese(30+)	Classified according to the WHO standard [597]
Smoking status	Yes	Never smokers, current smokers, ex smokers at baseline, ex smokers during follow-up: < 1, 1 – 2 smokers, 2 – 3, 3 – 5, > 5 years since cessation	See Figure 4.2
Current CD4 cell count (cells/mm^3)	Yes	Continuous (per 2-fold higher) and <200, 200 – 349, 350 – 499, ≥500 cells/mm^3, unknown	
Nadir CD4 cell count (cells/ mm^3)	Yes	Continuous and included in baseline tables only	Lowest recorded CD4 cell count measurement prior to date
Current HIV-VL (copies/mL)	Yes	Continuous (per 10-fold higher) and <400, ≥ 400 copies/mL, unknown	
Prior AIDS defining event (excluding ADC)	Yes	Yes , no	Classified according to the 1993 CDC clinical definition [133]
Prior cardiovascular disease (CVD)	Yes	Yes , no	Defined as whether the person ever had a diagnosis since entry into D:A:D.
Hypertension	Yes	Yes, no	Defined as ever having Systolic BP >140, Diastolic BP >90 mm Hg or receiving any antihypertensive drugs or ACE inhibitors since entry into D:A:D
Diabetes	Yes	Yes, no	Defined as a reported diabetes diagnosis on a case-report form or use of anti-diabetic medication since entry into D:A:D
Severe anaemia	Yes	Yes, no	Haemoglobin < 8 g/dl since entry into D:A:D

HBV status	Yes	Positive, prior, negative, or unknown	Positive HBV status was defined by a prior positive HBsAG surface antigen test or presence of detectable HBV DNA, Prior HBV status was defined by a prior positive HBsAG surface antigen test or presence of detectable HBV DNA, with a negative latest HBsAG surface antigen test and/or undetectable HBV DNA.
HCV status	Yes	Positive, negative, or unknown	Positive HCV status was defined as having a prior positive HCV surface antibody test.
cART use	Yes	On cART, not on cART, unknown	Defined as being on at least 1 protease inhibitor [PI] or non-nucleoside reverse transcriptase inhibitor [NNRTI].
Cumulative smoking duration	Yes	Continuous (per year longer)	Accumulated time as a current smoker since entry into D:A:D.

IDU: injecting drug use, BMI: Body mass index, HIV-VL: HIV viral load, ADC: AIDS defining cancer, HBV: Hepatitis B, HCV; Hepatitis C, cART combination antiretroviral therapy.

4.2.6 Statistical analysis

4.2.6.1 Characteristics at baseline

The characteristics of all people included in this analysis and those who experienced each of the four cancer outcomes during follow-up were described using numbers and percentages for categorical variables and median with interquartile range (IQR) for numerical variables at baseline. All bivariate associations were tested using chi squared tests for categorical variables and Kruskal-Wallis tests for numerical variables.

4.2.6.2 Incidence rates of cancer

Incidence rates per 1000 PYFU of each cancer outcome were calculated according to key variables of interest. These included current calendar year of follow-up and smoking status.

4.2.6.3 The association between smoking status and cancer

Poisson regression models were used to explore the association between time-updated smoking status and each outcome separately. The main exposure of interest was smoking status with a focus on time since cessation. Models were adjusted for baseline and time varying factors listed in Table 4.2. All variables included in the models were identified a priori based on expert clinical input, availability of data, and the published literature. A priori, I was additionally interested in interactions between smoking status and gender, HIV mode of transmission, age and CD4 cell count. Sensitivity analyses were also performed omitting all periods of follow-up where smoking status was missing or not updated for more than 2 years, excluding those with unknown or

unspecified cancer type, and adjusting for baseline calendar year instead of current calendar year.

All statistical tests were two sided and a type I error rate of 5%. All statistical analyses were performed using SAS 9.4 (Statistical Analysis Software, Cary NC, USA).

4.3 Results

There were 35,442 people included in the analysis who contributed 309,803 PYFU with a median follow-up of 9.9 (IQR: 6.7, 12.0) years per person. Of the 35,442 people included, 2,183 people developed a cancer during follow-up and the distribution of cancers are shown in Figure 4.3. Of these, 271 (12%) were lung cancers, 577 (26%) were other smoking related cancers (excluding lung), and 1,335 (61%) were smoking unrelated cancers. The most common smoking related cancers (excluding lung) were head and neck cancers (N=139, 24%), liver cancer (N= 136, 24%), cervical cancers (N= 57, 10%) and colon and rectal cancers (N= 47, 8%). The most common smoking unrelated cancers were NHL (N=301, 23%), KS (N=276, 21%), anal cancers (N=158, 12%) and Prostate cancers (N=131, 10%).

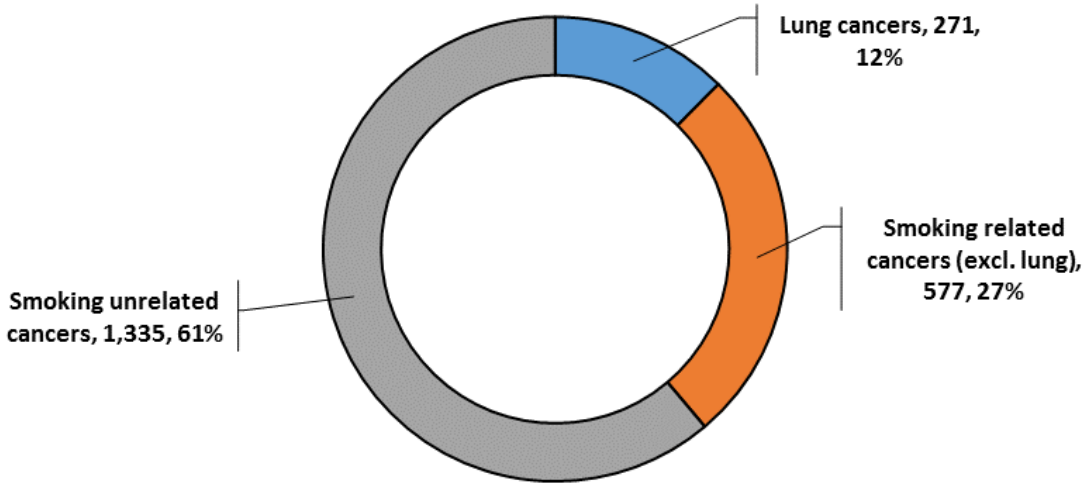


Figure 4.3 The distribution of lung cancers, smoking related cancers (excluding lung), and smoking unrelated cancers in D:A:D.
[1] Smoking related cancers were selected in accordance with the IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

4.3.1 Baseline characteristics

Baseline characteristics of the study population, as well as those who developed cancer during follow are shown in Table 4.3. Overall, 16.6% of people were aged ≥ 50 years at baseline with a median age of 40 (IQR 34, 46) years, 72.6% were of male gender, 53.2% were of white ethnicity

(37.1% were missing ethnicity information), and 41.9% acquired their HIV through sex between men. Just under three quarters had a baseline date in 2004 or 2005. Approximately two thirds of people had a BMI within the healthy range: 18 – 26 and 1 in 5 people had a previous AIDS defining event (excluding ADCs). 18.3% of people had ever had hepatitis B (HBV) and 21.0% had ever had hepatitis C (HCV). A history of CVD, diabetes, hypertension, or severe anaemia was reported in 2.1%, 2.5%, 14.4% and 19.3% of the study population, respectively. Just over half were on cART (52.0%) at baseline, 40.6% had a HIV viral load (HIV-VL) of ≤ 50 copies/mL and a median CD4 cell count of 444 (294,632) cells/mm^3.

Table 4.3 Baseline[1] characteristics of D:A:D participants included in the analysis

Factors	All included persons	People who developed a cancer during follow-up			
		All cancers	Lung cancer	Other smoking related cancers (excluding lung)	Smoking unrelated cancers
N (%)	35,442 (100)	2,183 (100)	271 (100)	577 (100)	1,335 (100)
Age (years)					
<30	5,063 (14.3)	109 (5.0)	1 (0.4)	18 (3.1)	90 (6.7)
30 - 39	12,955 (36.6)	518 (23.7)	37 (13.7)	108 (18.7)	373 (27.9)
40 - 49	11,530 (32.5)	829 (38.0)	105 (38.7)	258 (44.7)	466 (34.9)
50 - 59	4,265 (12.0)	486 (22.3)	92 (33.9)	126 (21.8)	268 (20.1)
≥60	1,629 (4.6)	241 (11.0)	36 (13.3)	67 (11.6)	138 (10.3)
Male	25,717 (72.6)	1,723 (78.9)	217 (80.1)	410 (71.1)	1,096 (82.1)
HIV risk group					
Homosexual	14,845 (41.9)	1,033 (47.3)	118 (43.5)	180 (31.2)	735 (55.1)
IDU	5,753 (16.2)	378 (17.3)	59 (21.8)	164 (28.4)	155 (11.6)
Heterosexual	12,796 (36.1)	632 (29.0)	77 (28.4)	194 (33.6)	361 (27.0)
Unknown	2,048 (5.8)	140 (6.4)	17 (6.3)	39 (6.8)	84 (6.3)
Ethnicity					
White	18,872 (53.2)	1,071 (49.1)	135 (49.8)	291 (50.4)	645 (48.3)
Black, other	3,437 (9.7)	100 (4.6)	4 (1.5)	32 (5.5)	64 (4.8)
Unknown	13,133 (37.1)	1,012 (46.4)	132 (48.7)	254 (44.0)	626 (46.9)
BMI (kg/m^2)					
<18	1,210 (3.4)	100 (4.6)	22 (8.1)	32 (5.5)	46 (3.4)
18 - 26	24,150 (68.1)	1,541 (70.6)	195 (72.0)	406 (70.4)	940 (70.4)
27 - 30	4,630 (13.1)	268 (12.3)	22 (8.1)	74 (12.8)	172 (12.9)
>30	1,575 (4.4)	87 (4.0)	9 (3.3)	21 (3.6)	57 (4.3)
unknown	3,877 (10.9)	187 (8.6)	23 (8.5)	44 (7.6)	120 (9.0)
Baseline Year					
2004 - 2005	25,790 (72.8)	1,766 (80.9)	232 (85.6)	509 (88.2)	1,025 (76.8)
2006 - 2007	4,980 (14.1)	255 (11.7)	24 (8.9)	46 (8.0)	185 (13.9)
2008 - 2009	4,453 (12.6)	158 (7.2)	14 (5.2)	21 (3.6)	123 (9.2)
2010 - 2011	111 (0.3)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
2012 - 2015	108 (0.3)	3 (0.1)	1 (0.4)	1 (0.2)	1 (0.1)

HIV-VL (copies/mL)					
≤ 50	14,405 (40.6)	892 (40.9)	143 (52.8)	300 (52.0)	449 (33.6)
51 - 1,000	5,165 (14.6)	291 (13.3)	40 (14.8)	95 (16.5)	156 (11.7)
1,001 - 10,000	4,135 (11.7)	240 (11.0)	25 (9.2)	54 (9.4)	161 (12.1)
10,001 - 100,000	6,682 (18.9)	454 (20.8)	41 (15.1)	72 (12.5)	341 (25.5)
>100,001	2,627 (7.4)	212 (9.7)	13 (4.8)	41 (7.1)	158 (11.8)
Unknown	2,428 (6.9)	94 (4.3)	9 (3.3)	15 (2.6)	70 (5.2)
CD4 cell count (cells/mm^3)					
<100	1,551 (4.4)	148 (6.8)	12 (4.4)	41 (7.1)	95 (7.1)
100 - 199	2,666 (7.5)	216 (9.9)	33 (12.2)	58 (10.1)	125 (9.4)
200 - 299	4,420 (12.5)	298 (13.7)	43 (15.9)	80 (13.9)	175 (13.1)
300 - 399	5,699 (16.1)	323 (14.8)	35 (12.9)	77 (13.3)	211 (15.8)
400 - 499	5,387 (15.2)	299 (13.7)	38 (14.0)	79 (13.7)	182 (13.6)
≥ 500	14,117 (39.8)	823 (37.7)	106 (39.1)	227 (39.3)	490 (36.7)
Unknown	1,602 (4.5)	76 (3.5)	4 (1.5)	15 (2.6)	57 (4.3)
Previous AIDS defining event (excluding ADC)	6,536 (18.4)	500 (22.9)	74 (27.3)	178 (30.8)	248 (18.6)
HBV					
Negative	24,858 (70.1)	1,481 (67.8)	174 (64.2)	358 (62.0)	949 (71.1)
Positive	1,618 (4.6)	130 (6.0)	20 (7.4)	54 (9.4)	56 (4.2)
Prior	4,867 (13.7)	349 (16.0)	49 (18.1)	106 (18.4)	194 (14.5)
Unknown	4,099 (11.6)	223 (10.2)	28 (10.3)	59 (10.2)	136 (10.2)
HCV					
Negative	23,139 (65.3)	1,430 (65.5)	171 (63.1)	315 (54.6)	944 (70.7)
Positive	7,434 (21.0)	493 (22.6)	74 (27.3)	204 (35.4)	215 (16.1)
Unknown	4,869 (13.7)	260 (11.9)	26 (9.6)	58 (10.1)	176 (13.2)
Prior CVD	740 (2.1)	83 (3.8)	17 (6.3)	21 (3.6)	45 (3.4)
Diabetes	874 (2.5)	92 (4.2)	12 (4.4)	25 (4.3)	55 (4.1)
Prior Hypertension	5,114 (14.4)	454 (20.8)	56 (20.7)	137 (23.7)	261 (19.6)
Severe anaemia	6,842 (19.3)	403 (18.5)	36 (13.3)	137 (23.7)	230 (17.2)
On cART					
No	6,495 (18.3)	454 (20.8)	52 (19.2)	129 (22.4)	273 (20.4)
Yes	18,447 (52.0)	1,165 (53.4)	169 (62.4)	370 (64.1)	626 (46.9)
Unknown	10,500 (29.6)	564 (25.8)	50 (18.5)	78 (13.5)	436 (32.7)

	N	Median(IQR)	N	Median(IQR)	N	Median(IQR)	N	Median(IQR)	N	Median(IQR)
Age (years)	35442	39.8 (33.8,46.2)	2183	44.7 (39.2,53.5)	271	49.6 (42.8,55.8)	577	45.4 (40.8,53.5)	1335	43.5 (37.9,52.6)
CD4 cell count (cells/mm^3)	33840	444 (294,632)	2107	420 (255,616)	267	420 (250,672)	562	420 (250,620)	1278	420 (259,605)
Nadir CD4 cell count (cells/mm^3)	33840	247 (120,400)	2107	212 (90,359)	267	190 (86,309)	562	185 (70,313)	1278	234 (102,390)
HIV-VL (copies/mL)	33014	194 (<50,15685)	2089	224 (<50,23100)	262	<50 (<50,3000)	562	<50 (<50,3100)	1265	1870 (<50,39800)

BMI: body mass index, ADC: AIDS defining cancer, HBV: Hepatitis B, HCV: hepatitis C, cART: combination antiretroviral therapy, CVD: cardiovascular disease, HIV-VL: HIV viral load, IQR: interquartile range.

[1] Baseline defined as the latest of 1 January 2004 or entry into D:A:D

The distribution of smoking status at baseline is shown Figure 4.4. Of the 35,442 people included in the analysis, 49% were reported to be current smokers at baseline, 21% were previous smokers and 30% were never smokers. A similar distribution in smoking behaviour is demonstrated in those who went on to develop any cancer, smoking related cancers (excluding lung) and smoking unrelated cancers during follow-up. However, those who went on to develop lung cancer had a much higher proportion of people reported to be current smokers at baseline (72%) and a much lower proportion were reported as never smokers (6%).

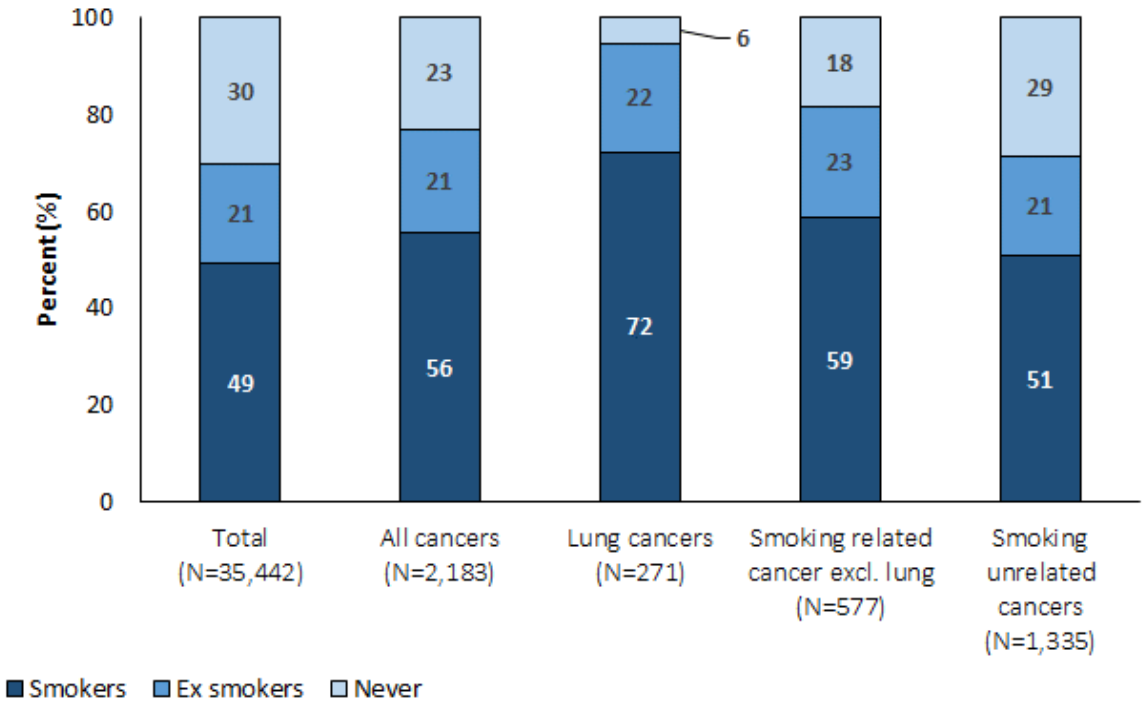


Figure 4.4 Smoking status at baseline[1]

[1]Baseline defined as the latest of 1 January 2004 or entry into D:A:D

4.3.2 Crude incidence of cancer

The overall crude incidence of all cancers combined was 7.05 (95%CI: 6.76, 7.35) events per 1000 PYFU. The crude incidence of lung cancers was 0.88 (95%CI: 0.78, 0.99) events per 1000 PYFU, smoking related cancers (excluding lung) was 1.86 (95%CI: 1.72, 2.02) events per 1000 PYFU, and smoking unrelated cancers was 4.31 (95%CI: 4.08, 4.55) events per 1000 PYFU.

4.3.2.1 Crude incidence of cancer by calendar year

The crude incidence by calendar year of all four cancer outcomes is shown in Figure 4.5. The incidence of all cancers was stable over time (% change per year: -0.6%, 95%CI: -1.9, 0.6%, unadjusted P for trend = 0.32). Crude incidence of lung cancer was also stable over time (%

change per year: 0.0%, 95%CI: -3.7, 3.5%, unadjusted P for trend = 0.94). The crude incidence of smoking related cancers (excluding lung) was increasing over time by 4.8% (95%CI: 2.2, 7.4%, unadjusted P for trend <0.01) per year from 1.17 (0.79, 1.71) events per 1000 PYFU in 2004 to 1.86 (1.37, 2.54) events per 1000 PYFU in 2015/16. Conversely, the incidence of smoking unrelated cancers declined by 3.0% (95%CI: 1.4, 4.5%, unadjusted P for trend <0.01) events per 1000 PYFU from 3.90 (95%CI: 3.16, 4.82) events per 1000 PYFU in 2004 to 2.38 (95%CI: 1.81, 3.13) events per 1000 PYFU in 2015/16.

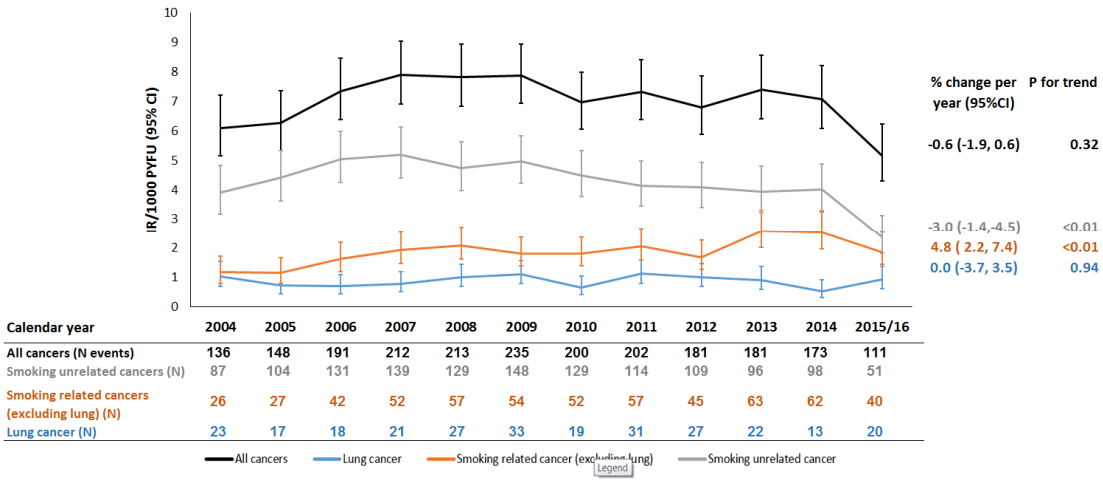


Figure 4.5 Crude incidence rates of each cancer by calendar year
IR: incidence rate, PYFU: person years of follow-up.
The unadjusted percentage change per year (% change per year) incidence of each cancer outcome per more recent calendar year is shown to the right of the graph.

4.3.2.2 Crude incidence of cancer by smoking status

The crude incidence of each outcome by current smoking status is shown in Figure 4.6 to Figure 4.9. It should be noted that the graphs are on different scales in order to clearly display the associations. The corresponding frequencies and crude incidence rates for each cancer group are shown in Table 4.4. The incidence of all cancers was highest within the first year of cessation (Figure 4.6), after which the incidence declined to a similar level to current smokers and remained stable thereafter (Unadjusted P for trend <0.01). Lung cancers incidence was negligible in those who never smoked (Figure 4.7), with <1 event per 10,000 PYFU, but was highly elevated in current smokers. Incidence was highest within the first year of cessation after which the incidence declined to a similar level to current smokers and remained stable thereafter (unadjusted P for trend =0.02). Incidence of smoking related cancers (excluding lung) was highest in the first year after cessation, however incidence declined and thereafter (P for trend = 0.34, Figure 4.8). Incidence of smoking unrelated cancers was stable for the first 3 years following cessation, with a slight decline in incidence thereafter (for trend = 0.01, Figure 4.9).

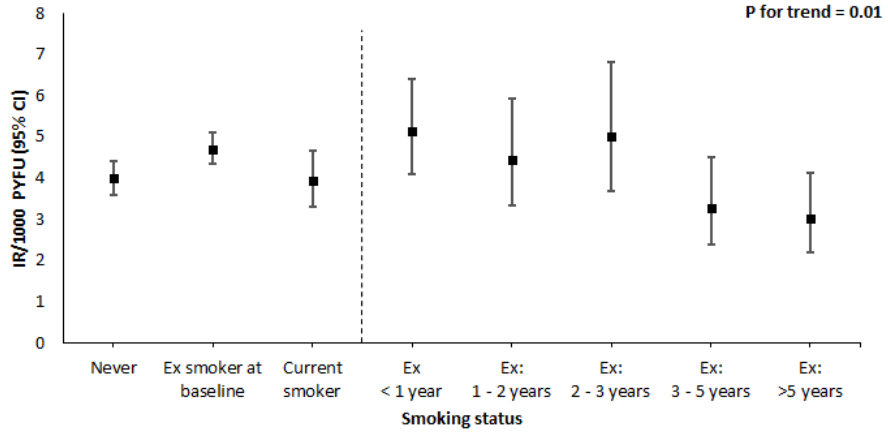


Figure 4.6 Crude incidence rate (IR) of all cancers by smoking status[1]

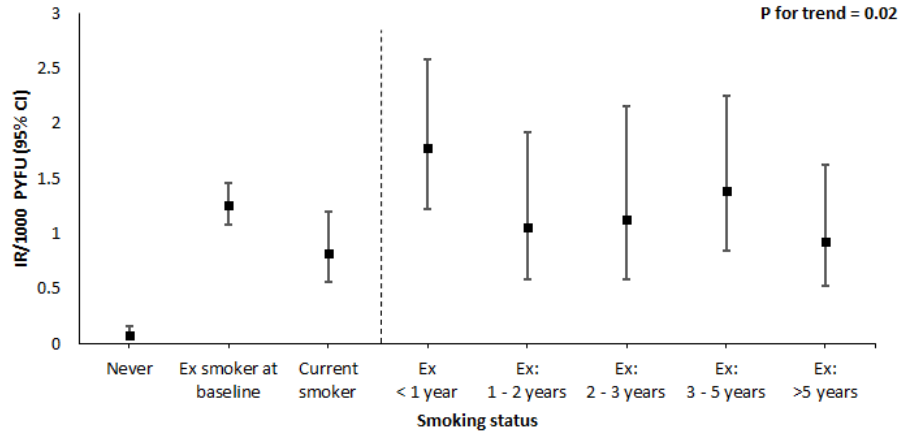


Figure 4.7 Crude incidence rate (IR) of lung cancer by smoking status[1]

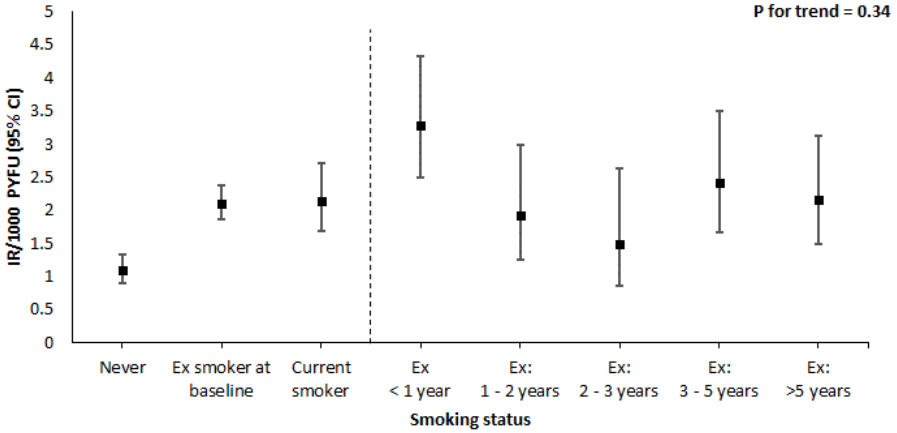


Figure 4.8 Crude incidence rate (IR) of smoking related cancers (excl. lung) by smoking status[1]

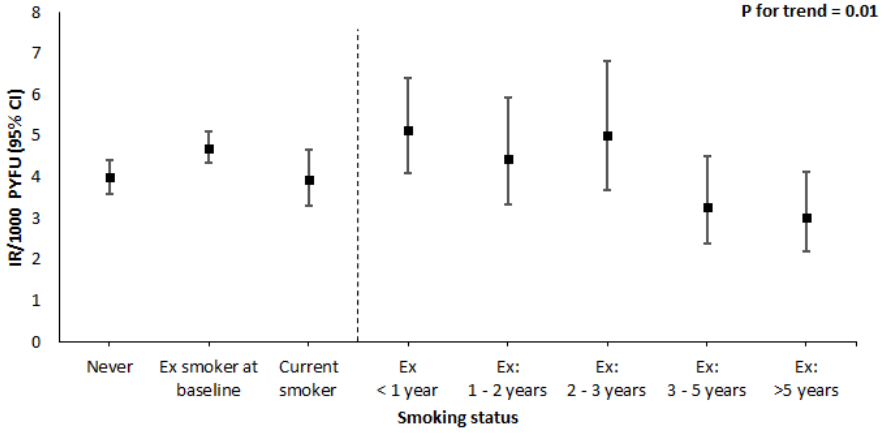


Figure 4.9 Crude incidence rate (IR) of smoking unrelated cancers by smoking status[1]

IR: incidence rate, PYFU: person years of follow-up.

[1]The left hand side of the graph shows the incidence in those who reported to be never smokers, current smokers and those who reported to be previous smokers prior to baseline. The right hand side shows the crude incidence of cancer with increasing time since cessation in those who quit during active follow-up.

Table 4.4 Crude incidence rate (IR) and unadjusted incidence rate ratio (IRR) of each cancer group

Smoking status	PYFU	All cancers				Lung cancers			
		N	IR (95%CI)/1000 PYFU	IRR (95%CI)	P	N	IR (95%CI)/1000 PYFU	IRR (95%CI)	P
Total	**309802.67**	**2183**	**7.05 (6.76, 7.35)**			**271**	**0.88 (0.78, 0.99)**		
Never	89818.48	463	5.16 (4.71, 5.65)	Reference		7	0.08 (0.04, 0.16)	Reference	
Current smoker	31887.46	219	8.06 (7.59, 8.56)	1.56 (1.40,1.74)	<0.01	163	1.26 (1.08, 1.46)	16.10 (7.55,34.30)	<.001
previous smoker at baseline	129924.38	1047	6.87 (6.02, 7.84)	1.33 (1.13,1.56)	<0.01	26	0.82 (0.56, 1.20)	10.46 (4.54,24.10)	<.001
Ex: < 1 year	15222.8	155	10.18 (8.70,11.92)	1.98 (1.65,2.37)	<0.01	27	1.77 (1.22, 2.59)	22.76 (9.91,52.26)	<.001
Ex: 1 - 2 years	10372.76	77	7.42 (5.94, 9.28)	1.44 (1.13,1.83)	<0.01	11	1.06 (0.59, 1.92)	13.61 (5.28,35.10)	<.001
Ex: 2 - 3 years	8009.84	61	7.62 (5.93, 9.79)	1.48 (1.13,1.93)	<0.01	9	1.12 (0.59, 2.16)	14.42 (5.37,38.71)	<.001
Ex: 3 - 5 years	11581.43	82	7.08 (5.70, 8.79)	1.37 (1.09,1.74)	0.01	16	1.38 (0.85, 2.26)	17.73 (7.29,43.08)	<.001
Ex: >5 years	12985.52	79	6.08 (4.88, 7.59)	1.18 (0.93,1.50)	0.17	12	0.92 (0.53, 1.63)	11.86 (4.67,30.13)	<.001
Smoking status	**PYFU**	**Smoking related cancers (excl. lung)**				**Smoking unrelated cancers**			
		N	IR (95%CI)/1000 PYFU	IRR (95%CI)	P	N	IR (95%CI)/1000 PYFU	IRR (95%CI)	P
Total	**309802.67**	**577**	**1.86 (1.72, 2.02)**			**1335**	**4.31 (4.08, 4.55)**		
Never	89818.48	98	1.09 (0.90, 1.33)	Reference		358	3.99 (3.59, 4.42)	Reference	
Current smoker	31887.46	273	2.10 (1.87, 2.37)	1.93 (1.53,2.43)	<0.01	611	4.70 (4.34, 5.09)	1.18 (1.04,1.34)	0.01
previous smoker at baseline	129924.38	68	2.13 (1.68, 2.71)	1.95 (1.44,2.66)	<0.01	125	3.92 (3.29, 4.67)	0.98 (0.80,1.21)	0.87
Ex: < 1 year	15222.8	50	3.29 (2.49, 4.33)	3.01 (2.14,4.23)	<0.01	78	5.12 (4.10, 6.40)	1.29 (1.01,1.64)	0.05
Ex: 1 - 2 years	10372.76	20	1.93 (1.24, 2.99)	1.77 (1.09,2.86)	0.02	46	4.44 (3.32, 5.92)	1.11 (0.82,1.51)	0.5
Ex: 2 - 3 years	8009.84	12	1.50 (0.85, 2.64)	1.37 (0.75,2.50)	0.30	40	4.99 (3.66, 6.81)	1.25 (0.90,1.74)	0.18
Ex: 3 - 5 years	11581.43	28	2.42 (1.67, 3.50)	2.22 (1.46,3.37)	<0.01	38	3.28 (2.39, 4.51)	0.82 (0.59,1.15)	0.25
Ex: >5 years	12985.52	28	2.16 (1.49, 3.12)	1.98 (1.30,3.01)	0.00	39	3.00 (2.19, 4.11)	0.75 (0.54,1.05)	0.09

IR: incidence rate, IRR: unadjusted incidence rate ratio, PYFU: person years of follow-up.

4.3.3 The association between smoking cessation and cancer

The unadjusted IRRs of each cancer outcome for smoking status are shown in Table 4.4. The adjusted IRRs (aIRR) are shown in Table 4.5. In order to give a visual representation for the change in incidence with time since cessation, the adjusted associations are also displayed in Figure 4.10, however, the graphs are on different scales in order to clearly display each association. Of note, the scale for the aIRR of lung cancer spans from 0 to 32, whereas the scales do not exceed 4 for all other cancers outcomes. Patterns of association were similar before and after adjustment.

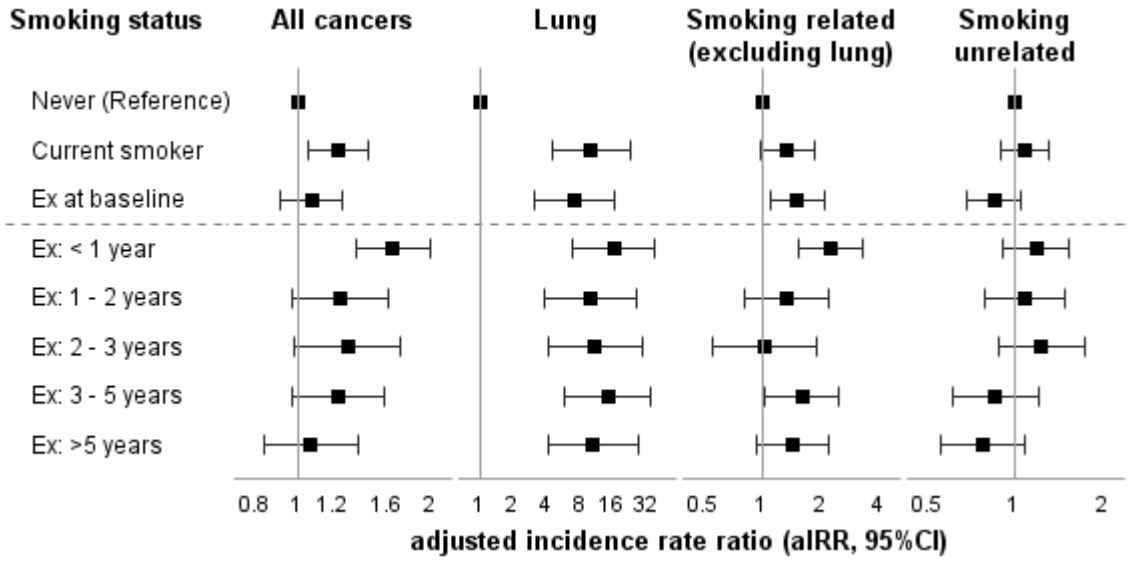


Figure 4.10 Adjusted incidence rate ratio (aIRR) according to smoking status with never smokers as the reference category

Models were adjusted for age, calendar year, gender, ethnicity, HIV mode of transmission, body mass index, current CD4 cell count, current HIV viral load, AIDS defining events (excluding AIDS defining cancers), Hepatitis C and B coinfection, hypertension, anaemia, diabetes, cardiovascular disease, combination antiretroviral therapy use, cumulative smoking duration during D:A:D follow-up.

After adjustment (Table 4.5), the incidence of all cancer combined was 1.24 (95%CI: 1.06, 1.45)-fold higher in current smokers relative to never smokers. A similarly elevated incidence was observed within one year of cessation relative to non-smokers, which declined with increasing time since cessation (P for trend=0.03), to a level comparable to non-smokers after 1 - 2 years of smoking cessation (aIRR: 1.25; 59%CI: 0.97, 1.61).

Table 4.5 Adjusted incidence rate ratio (aIRR) according to smoking status with never smokers as the reference category

Smoking status	All cancer			Lung cancer			Smoking related cancers (excl. lung)			Smoking unrelated cancers		
	aIRR[1] (95%CI)	P	P for trend[1]	aIRR[1] (95%CI)	P	P for trend[1]	aIRR[1] (95%CI)	P	P for trend[1]	aIRR (95%CI) [1]	P	P for trend[1]
Never	reference			reference			reference			reference		
Current smoker	1.24 (1.06,1.45)	0.01		10.16 (4.46,23.12)	<0.01		1.34 (0.97,1.85)	0.07		1.08 (0.89,1.31)	0.44	
previous smoker at baseline	1.07 (0.91,1.27)	0.40		7.16 (3.10,16.56)	<0.01		1.51 (1.10,2.08)	0.01		0.84 (0.68,1.04)	0.11	
Ex: < 1 year	1.64 (1.35,2.00)	<0.01	0.03	16.25 (6.86,38.49)	<0.01	0.02	2.23 (1.52,3.27)	<0.01	0.41	1.18 (0.91,1.52)	0.22	0.23
Ex: 1 - 2 years	1.25 (0.97,1.61)	0.08		10.25 (3.91,26.83)	<0.01		1.34 (0.81,2.21)	0.25		1.07 (0.78,1.47)	0.69	
Ex: 2 - 3 years	1.29 (0.98,1.70)	0.07		11.13 (4.10,30.20)	<0.01		1.02 (0.55,1.89)	0.94		1.23 (0.87,1.72)	0.24	
Ex: 3 - 5 years	1.24 (0.97,1.58)	0.08		14.44 (5.85,35.67)	<0.01		1.60 (1.03,2.50)	0.04		0.85 (0.60,1.19)	0.35	
Ex: >5 years	1.07 (0.84,1.37)	0.59		10.65 (4.11,27.59)	<0.01		1.44 (0.93,2.22)	0.10		0.77 (0.55,1.08)	0.13	

[1] Models were adjusted for age, calendar year, gender, ethnicity, HIV mode of transmission, BMI, current CD4 cell count, current HIV-VL, AIDS defining events (excluding ADC), HCV coinfection, HBV coinfection, hypertension, anaemia, diabetes, CVD, cART use, cumulative smoking duration during D:A:D follow-up.

Lung cancer incidence was more than 10-fold higher in current smokers relative to never smokers (aIRR: 10.16; 95%CI: 4.46, 23.12, Table 4.5). In those who ceased smoking during follow-up, the incidence was more than 16-fold higher in the first year following cessation, however, the incidence then quickly returned to approximately 10-fold higher after 1 - 2 years of cessation and remained stable thereafter. I also looked at the aIRR using current smoking as the reference category (see Table 4.6) and the incidence of lung cancer was not different to current smokers after the first year, and even after >5 years (aIRR: 1.05 95%CI: 0.52, 2.10). Although the P for trend was significant (P=0.03), this was driven by the increase in the first year and there was no evidence of a decline thereafter.

Table 4.6 Adjusted incidence rate ratio (aIRR) of lung cancer according to smoking status with current smokers as the reference category

Smoking status	aIRR (95%CI) [1]	P
Never	0.1(0.04, 0.22)	<0.01
Current smoker	Reference	
previous smoker at baseline	0.71(0.43, 1.15)	0.16
Ex: < 1 year	1.60(1.05, 2.44)	0.03
Ex: 1 - 2 years	1.01(0.53, 1.91)	0.98
Ex: 2 - 3 years	1.10(0.54, 2.21)	0.78
Ex: 3 - 5 years	1.42(0.79, 2.54)	0.24
Ex: >5 years	1.05(0.52, 2.10)	0.89

[1] Models were adjusted for age, calendar year, gender, ethnicity, HIV mode of transmission, BMI, current CD4 cell count, current HIV-VL, AIDS defining events (excluding ADC), HCV coinfection, HBV coinfection, hypertension, anaemia, diabetes, CVD, cART use, cumulative smoking duration during D:A:D follow-up.

Incidence of smoking related cancers (excluding lung) was highest within the first year of smoking cessation (aIRR: 2.23 95%CI: 1.52, 3.73, Table 4.5) and returned to a level similar to never smokers after 1-2 year of smoking cessation (aIRR: 1.34 95%CI: 0.81, 2.21). However it should be noted that the magnitude of association in this group was similar to that of current smokers (aIRR: 1.34 95%CI: 0.97, 1.85), and the magnitude was even higher in those who quit more than 3 years prior. Incidence of smoking unrelated cancers did not vary according to smoking status (global P=0.13) and there was no change with increasing time since cessation (P for trend = 0.23).

4.3.3.1 Smoking cessation and cancer incidence by gender, age, and CD4 cell count

The association between current smoking status and all cancers combined differed between genders (P for interaction <0.01), however this was not detectable for individual cancer outcomes. The association according to gender is shown in Figure 4.11. In females, incidence of

all cancers was 1.88 (95%CI: 1.40, 2.52)-fold higher in current smokers relative to never smokers, and remained similarly elevated for 2 years after cessation, after which incidence declined to a level similar to never smokers. In males, incidence was not elevated in current smokers but was 1.55 (95%CI: 1.21, 2.0)-fold higher in in the first year of smoking cessation and returned to a level similar to never smokers thereafter (1.05 95%CI: 0.77, 1.44). The association between smoking status and all cancers combined did not vary according to age, HIV mode of transmission, or CD4 cell count (all P for interaction > 0.05). There was no significant interactions between age, HIV mode of transmission, and CD4 cell count and smoking status for lung cancers, smoking related cancers (excluding lung) and smoking unrelated cancers (all P for interaction> 0.05).

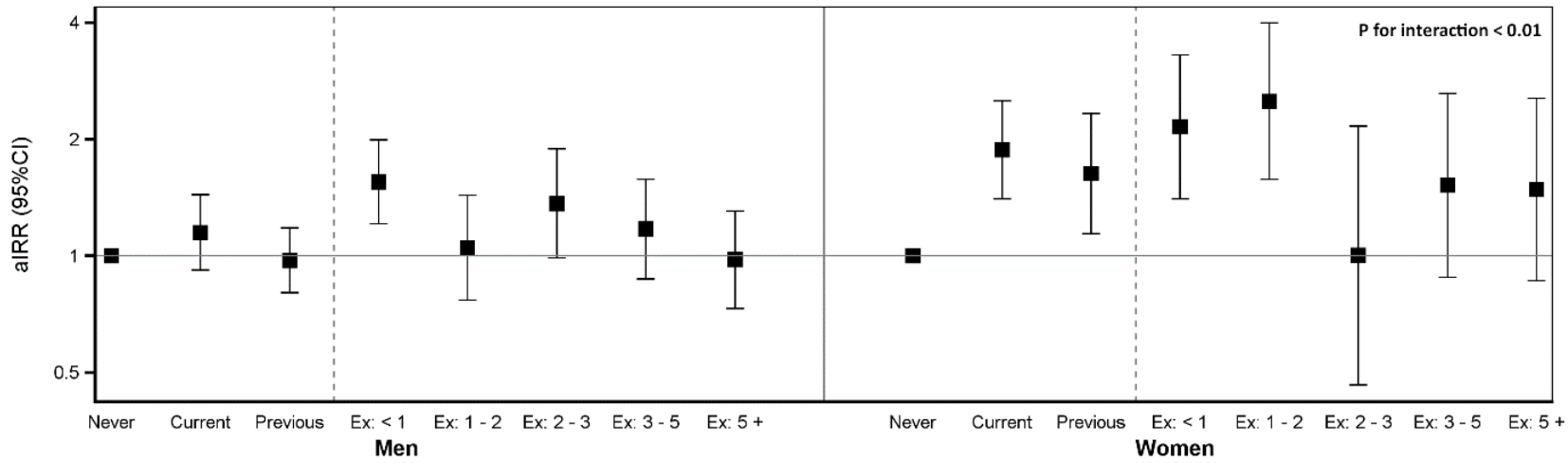


Figure 4.11 aIRR of lung cancer according to smoking status by gender

aIRR: Adjusted incidence rate ratio.

[1] Models were adjusted for Age, calendar year, gender, ethnicity, HIV mode of transmission, body mass index, current CD4 cell count, current HIV-VL, AIDS defining events (excluding AIDS defining cancers), Hepatitis B and C coinfection, hypertension, anaemia, diabetes, cardiovascular disease, combination antiretroviral therapy use, cumulative smoking duration during D:A:D follow-up

4.3.4 Other associations with cancer incidence

The aIRR for each outcome across various risk factors is shown in Table 4.7 to Table 4.10. It should be noted that these tables display the other associations from the same model used for the associations with smoking status shown in Table 4.5 and Figure 4.10.

4.3.4.1.1 All cancers combined

After adjustment, incidence of all cancers combined (Table 4.7) increased with older age, and was lower in females relative to males. Higher incidence was also observed for lower CD4 cell count, in those with a BMI < 18, current HIV-VL > 1,000 copies/mL, a prior AIDS defining diagnosis (excluding ADC), a history of diabetes or hypertension, and in those with severe anaemia. Longer cumulative smoking duration during D:A:D follow-up was also associated with higher cancer risk. Incidence was lower in those who acquired HIV through heterosexual sex (relative to sex between men). There was no association with ethnicity, cART use, HBV or HCV coinfection, or a history of CVD.

Table 4.7 Adjusted incidence rate ratios (aIRR) for all cancers combined

Factor		aIRR[1] (95%CI)	P	Factor		aIRR[1] (95%CI)	P
age (years)				**Current HIV-VL (copies/mL)**			
	<40	reference			≤50	reference	
	40 - 49	1.82 (1.59,2.08)	<.001		51 - 1,000	1.07 (0.94,1.22)	0.322
	≥ 50	3.64 (3.18,4.17)	<.001		>1,000	1.66 (1.45,1.90)	<.001
Calendar year		0.98 (0.97,1.00)	0.023		Unknown	0.90 (0.48,1.68)	0.734
Female		0.83 (0.72,0.94)	0.005	**HCV coinfection**			
Non-white ethnicity		1.08 (0.98,1.18)	0.128		Positive	0.95 (0.82,1.10)	0.494
HIV mode of transmission					Negative	reference	
	Sex between men	reference			Unknown	0.74 (0.59,0.94)	0.012
	IDU	0.94 (0.78,1.12)	0.489	**HBV coinfection**			
	Heterosexual	0.77 (0.69,0.87)	<.001		Positive/prior	1.00 (0.90,1.12)	0.938
	Unknown	0.97 (0.80,1.17)	0.724		Negative	reference	
BMI (kg/m^2)					Unknown	1.04 (0.85,1.28)	0.714
	<18	1.45 (1.21,1.73)	<.001	**Hypertension**		1.16 (1.06,1.28)	0.002
	18 - 26	reference		**Anaemia**			
	27 - 30	0.77 (0.68,0.88)	<.001		severe anaemia	1.87 (1.67,2.10)	<.001
	>30	0.74 (0.61,0.89)	0.002		mild anaemia/normal	reference	
	unknown	0.85 (0.68,1.06)	0.144		Unknown	1.23 (1.07,1.40)	0.003
Current CD4 cell count (cells/mm^3)				**cART use**			
	<100	4.09 (3.38,4.96)	<.001		No	0.92 (0.81,1.05)	0.233
	100 - 199	2.23 (1.87,2.66)	<.001		Yes	reference	
	200 - 299	1.86 (1.61,2.15)	<.001		Unknown	1.15 (0.95,1.39)	0.145
	300 - 399	1.62 (1.43,1.84)	<.001	**AIDS defining event (excl. ADC)**		1.15 (1.05,1.27)	0.004
	400 - 499	1.37 (1.21,1.56)	<.001	**Diabetes**		1.38 (1.19,1.60)	<.001
	≥ 500	reference		**CVD**		1.12 (0.95,1.33)	0.188
	Unknown	4.26 (1.86,9.75)	<.001	**Cumulative smoking duration in D:A:D**		1.03 (1.01,1.05)	<.001

aIRR: adjusted incidence rate ratio, IDU: injecting drug use, BMI: Body mass index, HIV-VL: HIV viral load, HCV: hepatitis C, HBV: hepatitis B, cART: combination antiretroviral therapy, ADC: AIDS defining cancer, CVD: cardiovascular disease.

[1]Adjusted for all variables in the table and smoking status

4.3.4.1.2 Lung cancers

After adjustment, Incidence of lung cancers (Table 4.8) increased markedly with older age and was higher in those with a BMI < 18. Higher incidence was also observed for lower CD4 cell count, with elevated incidence in those with CD4 < 300 cells/mm^3 relative to CD4 ≥ 500 cells/mm^3. Incidence increased by 7 (95%CI: 3, 12)% with each additional year of cumulative smoking duration during follow-up in D:A:D. There was no association with gender, ethnicity, HIV mode of transmission, current HIV-VL, HCV or HBV coinfection, prior AIDS defining events (excluding ADC), hypertension, diabetes, anaemia, CVD, or cART use.

Table 4.8 Adjusted incidence rate ratios (aIRR) for lung cancers

Factor		aIRR[1] (95%CI)	P	Factor		aIRR[1] (95%CI)	P
age (years)				**Current HIV-VL (copies/mL)**			
	<40	reference			≤50	reference	
	40 - 49	9.23 (3.94,21.63)	<.001		51 - 1,000	0.96 (0.66,1.40)	0.837
	≥ 50	29.35 (12.62,68.24)	<.001		>1,000	0.96 (0.61,1.51)	0.861
Calendar year		0.92 (0.88,0.96)	<.001		Unknown	0.44 (0.12,1.56)	0.202
Female		1.01 (0.70,1.46)	0.938	**HCV coinfection**			
Non-white ethnicity		1.14 (0.87,1.48)	0.343		Positive	0.86 (0.57,1.31)	0.494
HIV mode of transmission					Negative	reference	
	Sex between men	reference			Unknown	0.38 (0.16,0.88)	0.024
	IDU	1.17 (0.69,1.98)	0.567	**HBV coinfection**			
	Heterosexual	1.01 (0.72,1.41)	0.953		Positive/prior	1.07 (0.78,1.45)	0.68
	Unknown	1.16 (0.69,1.97)	0.571		Negative	reference	
BMI (kg/m^2)					Unknown	1.57 (0.89,2.75)	0.117
	<18	2.42 (1.61,3.63)	<.001	**Hypertension**		1.14 (0.86,1.50)	0.369
	18 - 26	reference		**Anaemia**			
	27 - 30	0.77 (0.54,1.12)	0.17		severe anaemia	1.26 (0.90,1.78)	0.179
	>30	0.57 (0.29,1.11)	0.099		mild anaemia/normal	reference	
	unknown	1.56 (0.91,2.67)	0.103		Unknown	1.23 (0.85,1.77)	0.267
Current CD4 cell count (cells/mm^3)				**cART use**			
	<100	2.60 (1.23,5.49)	0.012		No	1.03 (0.71,1.49)	0.872
	100 - 199	2.34 (1.41,3.90)	0.001		Yes	reference	
	200 - 299	2.69 (1.86,3.91)	<.001		Unknown	1.27 (0.69,2.35)	0.445
	300 - 399	1.92 (1.34,2.75)	<.001	**AIDS defining event (excl. ADC)**		1.26 (0.97,1.64)	0.082
	400 - 499	1.69 (1.20,2.39)	0.003	**Diabetes**		1.29 (0.85,1.95)	0.228
	≥ 500	reference		**CVD**		1.24 (0.80,1.90)	0.339
	Unknown	10.73 (3.18,36.23)	<.001	**Cumulative smoking duration in D:A:D**		1.07 (1.03,1.12)	0.001

aIRR: adjusted incidence rate ratio, IDU: injecting drug use, BMI: Body mass index, HIV-VL: HIV viral load, HCV: hepatitis C, HBV: hepatitis B, cART: combination antiretroviral therapy, ADC: AIDS defining cancer, CVD: cardiovascular disease.

[1]Adjusted for all variables in the table and smoking status

4.3.4.1.3 Smoking related cancers (excluding lung)

After adjustment, Incidence of smoking related cancers (excluding lung) (Table 4.9) increased with older age, was higher in those with a BMI < 18, and in those who acquired HIV through IDU or heterosexual sex (relative to sex between men). Incidence also declined with lower CD4 cell count, with the highest incidence in those with a CD4 cell count of 100 – 199 cells/mm^3. Incidence was elevated in those who were HCV coinfected, had a history of hypertension, diabetes, or severe anaemia, or a prior AIDS defining event (excluding ADC). Incidence increased by 4 (95%CI: 1, 7) % per year for each additional year of cumulative smoking duration. Incidence was not associated with gender, ethnicity, Current HIV-VL, HBV coinfection, a history of CVD, or cART use.

Table 4.9 Adjusted incidence rate ratios (aIRR) for smoking related cancers (excluding lung)

Factor		aIRR[1] (95%CI)	P	Factor		aIRR[1] (95%CI)	P
age (years)				**Current HIV-VL (copies/mL)**			
	<40	reference			≤50	reference	
	40 - 49	3.63 (2.53,5.21)	<.001		51 - 1,000	0.93 (0.72,1.20)	0.577
	≥ 50	7.69 (5.36,11.03)	<.001		>1,000	1.04 (0.77,1.41)	0.796
Calendar year		0.99 (0.97,1.02)	0.627		Unknown	0.64 (0.18,2.35)	0.505
Female		1.05 (0.84,1.31)	0.662	**HCV coinfection**			
Non-white ethnicity		1.18 (0.99,1.41)	0.071		Positive	1.37 (1.04,1.81)	0.023
HIV mode of transmission					Negative	reference	
	Sex between men	reference			Unknown	0.92 (0.57,1.48)	0.726
	IDU	1.65 (1.19,2.31)	0.003	**HBV coinfection**			
	Heterosexual	1.26 (1.00,1.59)	0.053		Positive/prior	1.14 (0.93,1.40)	0.212
	Unknown	1.36 (0.95,1.96)	0.096		Negative	reference	
BMI (kg/m^2)					Unknown	0.78 (0.49,1.24)	0.302
	<18	1.52 (1.10,2.09)	0.011	**Hypertension**		1.44 (1.20,1.73)	<.001
	18 - 26	reference		**Anaemia**			
	27 - 30	0.82 (0.65,1.05)	0.113		severe anaemia	2.00 (1.62,2.46)	<.001
	>30	0.74 (0.51,1.06)	0.101		mild anaemia/normal	reference	
	unknown	0.87 (0.55,1.37)	0.539		Unknown	0.98 (0.73,1.32)	0.898
Current CD4 cell count (cells/mm^3)				**cART use**			
	<100	1.97 (1.22,3.17)	0.005		No	0.80 (0.62,1.04)	0.102
	100 - 199	2.22 (1.63,3.03)	<.001		Yes	reference	
	200 - 299	1.62 (1.23,2.13)	<.001		Unknown	1.04 (0.65,1.66)	0.861
	300 - 399	1.40 (1.09,1.80)	0.009	**AIDS defining event (excl. ADC)**		1.43 (1.20,1.70)	<.001
	400 - 499	1.01 (0.77,1.31)	0.968	**Diabetes**		1.61 (1.24,2.07)	<.001
	≥ 500	reference		**CVD**		1.17 (0.87,1.58)	0.296
	Unknown	6.75 (1.55,29.39)	0.011	**Cumulative smoking duration in D:A:D**		1.04 (1.01,1.07)	0.006

aIRR: adjusted incidence rate ratio, IDU: injecting drug use, BMI: Body mass index, HIV-VL: HIV viral load, HCV: hepatitis C, HBV: hepatitis B, cART: combination antiretroviral therapy, ADC: AIDS defining cancer, CVD: cardiovascular disease.

[1]Adjusted for all variables in the table and smoking status

4.3.4.1.4 Smoking unrelated cancers

After adjustment, Incidence of smoking unrelated cancers (Table 4.10). Incidence was increased with older age, however the association was much more modest than for lung cancers and smoking related cancers (excluding lung). Incidence was lower in females, those who acquired HIV through IDU or heterosexual sex (relative to sex between men), those with a BMI of 27 or higher, and HCV coinfected people. Incidence increased strongly with lower CD4 cell count, and higher HIV-VL, and a history of severe anaemia or diabetes. Incidence was not associated with ethnicity, HBV coinfection, a prior AIDS defining event, a history of hypertension or CVD, cART use, or cumulative duration of smoking while under follow-up in D:A:D.

Table 4.10 Adjusted incidence rate ratios (aIRR) for smoking unrelated cancers

Factor		aIRR[1] (95%CI)	P	Factor		aIRR[1] (95%CI)	P
age (years)				**Current HIV-VL (copies/mL)**			
	<40	reference			≤50	reference	
	40 - 49	1.41 (1.21,1.64)	<.001		51 - 1,000	1.19 (1.01,1.42)	0.042
	≥ 50	2.48 (2.11,2.90)	<.001		>1,000	2.12 (1.79,2.51)	<.001
Calendar year		0.99 (0.97,1.01)	0.324		Unknown	1.26 (0.59,2.66)	0.549
Female		0.69 (0.57,0.82)	<.001	**HCV coinfection**			
Non-white ethnicity		1.01 (0.89,1.14)	0.864		Positive	0.80 (0.65,0.97)	0.026
HIV mode of transmission					Negative	reference	
	Sex between men	reference			Unknown	0.76 (0.58,1.01)	0.055
	IDU	0.66 (0.51,0.85)	0.001	**HBV coinfection**			
	Heterosexual	0.63 (0.54,0.73)	<.001		Positive/prior	0.92 (0.80,1.07)	0.291
	Unknown	0.85 (0.67,1.08)	0.182		Negative	reference	
BMI (kg/m^2)					Unknown	1.05 (0.81,1.36)	0.701
	<18	1.12 (0.86,1.47)	0.392	**Hypertension**		1.05 (0.93,1.20)	0.429
	18 - 26	reference		**Anaemia**			
	27 - 30	0.76 (0.65,0.90)	0.001		severe anaemia	2.00 (1.72,2.32)	<.001
	>30	0.78 (0.61,1.00)	0.047		mild anaemia/normal	reference	
	unknown	0.75 (0.57,1.00)	0.047		Unknown	1.31 (1.11,1.54)	0.001
Current CD4 cell count (cells/mm^3)				**cART use**			
	<100	5.63 (4.50,7.03)	<.001		No	0.96 (0.81,1.13)	0.594
	100 - 199	2.23 (1.76,2.83)	<.001		Yes	reference	
	200 - 299	1.84 (1.52,2.22)	<.001		Unknown	1.07 (0.86,1.34)	0.534
	300 - 399	1.68 (1.43,1.98)	<.001	**AIDS defining event (excl. ADC)**		1.00 (0.88,1.14)	0.985
	400 - 499	1.49 (1.27,1.74)	<.001	**Diabetes**		1.29 (1.05,1.59)	0.015
	≥ 500	reference		**CVD**		1.06 (0.83,1.34)	0.652
	Unknown	3.39 (1.18,9.73)	0.023	**Cumulative smoking duration in D:A:D**		1.01 (0.99,1.03)	0.285

aIRR: adjusted incidence rate ratio, IDU: injecting drug use, BMI: Body mass index, HIV-VL: HIV viral load, HCV: hepatitis C, HBV: hepatitis B, cART: combination antiretroviral therapy, ADC: AIDS defining cancer, CVD: cardiovascular disease.

[1]Adjusted for all variables in the table and smoking status

4.3.5 Sensitivity analyses

Results were consistent after removing follow-up with missing smoking status or > 2 years since last reported smoking status, excluding those with unknown or unspecified cancer type, and adjusting for baseline calendar year instead of current calendar year.

4.4 Discussion

To my knowledge, the results presented in this chapter are the first to show the impact of smoking cessation on cancer incidence in the HIV+ population, in a large observational study with a relatively large number of prospectively collected cancers. In this chapter, I have shown that lung cancer incidence remains highly elevated in HIV+ people at a level similar to current smokers >5 years after cessation. Lung cancer incidence fell after the first year of cessation, however, no decline was evident thereafter and incidence remained more than 10-fold higher than never smokers. In contrast, incidence of other smoking related cancers (excluding lung) declined after 1 year of smoking cessation, however the magnitude of associations were similar to current and previous smokers at baseline thereafter (although, not statistically significant with the exception of 3 – 5 years post quitting,), possibly indicating ongoing elevated risk. As expected, no association between smoking behaviour and smoking unrelated cancer was shown. These results support the need for research into and implementation of smoking cessation efforts in HIV+ people while also highlighting that risk does not vanish after smoking cessation, warranting ongoing monitoring and awareness of lung cancer.

4.4.1 Impact of cessation on cancer incidence

4.4.1.1 Lung cancer

Lung cancer incidence remained highly elevated in the first 5 years after cessation, with a peak in the first year, after which levels remained at a similar level to current smokers. One study in the US population showed elevated lung cancer mortality in the first 2 years since cessation which declined thereafter [745]. Most studies in the general population have shown that the incidence and mortality of lung cancer to be either similar or lower those who recently quit compared to current smokers, but do not demonstrate a peak [654, 662, 663]. However, it is difficult to compare as most studies have highly heterogeneous results, longer follow-up time and use broader categorisation of time since cessation (I.e. < 3 or <5 years rather than < 1 year). the peak in the first year following cessation observed in my study is likely driven by existing

disease [654]. This includes people who quit smoking because they feel unwell either because of their undiagnosed (non-clinical) cancer or because of another condition (such as an AIDS defining diagnosis or treatment failure), which may lead to cancer diagnosis due to increased intensity of medical surveillance [727]. It is possible that the increase in cancer incidence in the first year following cessation may reflect a larger impact of reverse causality in HIV+ people, possibly due to higher frequency of contact with care and elevated risk of other comorbidities [746].

The lack of decline in the subsequent years is concerning as it is in contrast to similar studies in the general population that show a clear decline in lung cancer risk within 5 – 9 years of cessation, and a 50% reduction in the harms of smoking after 10 years [654, 661]. One USA study by Sigel *et al* [747] compared lung cancer incidence in people with AIDS who ceased smoking by <1 and >1 year prior. In that study, compared to never smokers, lung cancer incidence was 10-fold higher in those who quit within the last year and declined to 5 in those who quit more than 1 year prior [747]. The reported RR for lung cancer in current smokers by Sigel *et al* [747] was 5-fold higher than in never smokers, and was lower than that reported in D:A:D, and with a more rapid decline in incidence following cessation. However, this study did find a similar peak for recent quitters. The study by Sigel *et al* [747] was in people with AIDS, and therefore, the elevated incidence in the first year and the subsequent decline is probably driven by existing disease. Furthermore, differences in smoking prevalence and intensity in European countries and the USA (prevalence: 27.3% and 17.2% respectively) may also explain differences [641, 748].

Research in the HIV-negative population has indicated that the full effects of smoking are not reflected in national cancer rates until regular smoking has been entrenched in that population for at least 50 years [622, 654]. Therefore, it is likely that our study underestimates the harms of smoking and the benefits of cessation, as the HIV+ population is relatively young and the smoking epidemic in HIV+ people has not yet matured [477, 622, 654]. For example, the benefit of smoking cessation in the general population is most apparent when the full effects of long-term smoking are established, such as among men in the United States, United Kingdom, and Eastern European countries [654]. The long-term effects of smoking will become more apparent in HIV+ people as they age.

4.4.1.1.1 Mechanisms

The mechanisms for the elevated incidence of lung cancer incidence and persistence of risk in HIV+ people are largely unknown [727], however several possible explanations exist. It is well

known that smoking rates are often higher in HIV+ compared to HIV-negative people, and some studies also indicate that lung cancer may develop with less smoking exposure [728] which could lead to a more prominent residual effect of long term tobacco use. In addition to this, HIV may play either a direct or indirect role in lung cancer genesis [294, 706, 707]. HIV infection may contribute to increased susceptibility of lung cancer through reduced immune surveillance (due to HIV mediated immune dysfunction), CD8 cell dysfunction and activity, as well as increased systemic and pulmonary inflammation. Pulmonary infections and COPD are have been associated with increased inflammation in the lung and higher lung cancer incidence in the general population [699, 727, 749-753]. HIV+ people are particularly susceptible to pulmonary infections and COPD which may explain the increased risk [688, 707, 719, 725, 726, 729, 754-756]. It has also been suggested that HIV infection is associated with antioxidant deficiencies which increase oxidative stress of the lung and promote carcinogenesis [654, 757]. In addition, it is possible that HIV viral proteins may have some direct oncogenic activity [758]. Some studies have noted a higher than expected number of adenocarcinomas in HIV+ people [726]. Adenocarcinomas develop after less exposure to genetic damage than other cancers of the lung and a link between genomic instability and lung cancer in HIV+ people has been observed [726, 759, 760]. Increased survival of people living with HIV may prolong exposure to genomic instability which increases risk of adenocarcinoma of the lung [726, 760].

Alternatively, studies have suggested that the elevated lung cancer risk in HIV+ people could simply reflect more intensive medical evaluations or screening bias in people with HIV, particularly after an AIDS defining event [617, 727]. Furthermore, It is also possible that the slow decline in the HIV+ population is due to differences in the intensity and duration of historical smoking or younger age at smoking initiation in HIV + people compared to HIV-negative people [725]. In addition, the presence of other lung cancer risk factors, such as use of inhaled illicit drugs, passive smoking, as well as socio economic factors [492] that may not change, or may even intensify, with changes in smoking status could even mask benefits of smoking cessation.

4.4.1.2 Smoking related cancers (excluding lung)

In this chapter, the incidence of smoking related cancers (excluding lung) following smoking cessation was elevated in recent quitters and returned to a level similar to that of never smokers after one year. The peak in smoking related cancers (excluding lung) within the first year following cessation is likely driven by existing disease, which is discussed in section 4.4.1.1. Although there was statistically little evidence of a difference in incidence of smoking related cancers (excluding lung) between never smokers and ex smokers after 1 year, the magnitude of

association in this group is similar to that of current smokers (and even higher in those who quit more than 3 years prior), which may be evidence of elevated risk. Although the benefits of cessation are well documented, the harms of smoking cannot be entirely reversed [654]. There is very little evidence to suggest that smoking cessation can reverse the transformation of cancer cells, however, cessation can slow the progression of cancer development [654]. In addition, it has been shown in the general population that younger age, shorter smoking duration, and lower smoking intensity at time of cessation can result in a cancer risk which approaches that of never smokers [654].

4.4.1.3 Smoking unrelated cancers.

The risk of smoking unrelated cancers was similar to that of never smokers regardless of time since smoking cessation. There was a slight but non-significant decline in incidence over time, driven by the lower incidence in those who had stopped smoking for more than 3 years. This could reflect other lifestyle changes, such as improvements to diet and exercise, in people who choose to stop smoking which further reduce their cancer risk [761, 762].

4.4.2 Smoking prevention and cessation in HIV-positive people

Smoking prevention and cessation are the most effective ways to prevent lung cancers. Cessation tools, including behavioural interventions and pharmacologic agents (i.e. nicotine replacement therapy), have been shown to be effective for smoking cessation in HIV+ smokers [763-767]. The observed declines in smoking related cancers (excluding lung) supports the need for smoking cessation efforts, however, the remaining long term elevated risk of lung cancer indicates that smoking prevention in addition to cessation efforts is needed in HIV+ people. In addition, it is important to keep in mind that the benefits of smoking cessation are not restricted to cancers, but can have beneficial all smoking related diseases and also lengthen life [477, 647]. This is particularly important in HIV+ people who are known to have higher risk of many diseases which can be exacerbated by smoking, including CVD, renal complications, bone fractures and metabolic bone diseases, COPD, pulmonary infections, and pneumonia [420, 682, 711]. Studies in HIV+ people have shown a lower risk of AIDS defining events, CVD, non-AIDS defining malignancies, and pneumonia in those who cease smoking [682, 708, 711].

4.4.3 Associations with other factors

4.4.3.1 Age

Older age was strongly associated with lung cancer risk, which is repeatedly shown in both the HIV+ and HIV-negative literature [617, 622, 654, 725]. This could reflect longer exposure to inflammation which is also associated with aging [707], or prolong exposure to genomic instability which increases risk of adenocarcinoma of the lung [726, 760]. Alternatively, the association with age could simply reflect a residual effect of lifetime duration of smoking [622, 654]. I did adjust for cumulative duration of smoking while under follow-up in D:A:D, however many people will have a history of smoking long before entry into D:A:D. A less strong association with age was seen for smoking related cancers (excluding lung) and smoking unrelated cancers. These groups of cancers contains a mix of IRC and infection unrelated cancers (IURC), many of which are associated with older age (see chapter 3).

4.4.3.2 Current CD4 cell count and HIV viral load

Lower current CD4 cell count was associated with lung cancer diagnosis, and is consistent with many published studies [16, 427, 433, 609, 705, 728, 768]. Lower CD4 cell counts may reflect an indirect role of HIV in lung cancer development, through reduced immune surveillance and increased susceptibility of pulmonary infections, both of which increase lung cancer risk. A recent publication demonstrated no association between CD4 and lung cancer incidence after adjustment for cancer risk factors and history of pneumonia [729]. A similar association was seen between CD4 cell count and infection related cancers (excluding lung). Neither lung cancer nor other smoking related cancers (excluding lung) were associated with either current or AUC of HIV-VL. This has been observed in other studies [638, 705]. It has also been proposed that the lung may serve as a compartment in which HIV activity may not reflect systemic viral suppression [707, 758, 769-771]. Smoking unrelated cancer was strongly linked to lower current CD4 cell counts and higher current HIV-VL, probably driven by the high proportion of IRCs in this group (primarily NHL and KS). This is explored in depth in for all IRCs in chapter 3 and for NHL and HL specifically in chapter 5. In addition, I found that the interaction between current CD4 and each cancer outcome was not significant. I.e. the association between smoking status and each cancer outcome was similar in those with higher and lower CD4 cell counts.

4.4.3.3 Gender

Incidence of lung cancer and smoking related cancers (excluding lung) did not vary by gender. Elevated incidence of smoking unrelated cancers was linked to male gender, likely driven by NHL and HL (see chapter 5). There was a difference in the effect of smoking cessation on the incidence of all cancer according to gender, where the association between current smoking and cancer risk was stronger and remain elevated for longer after cessation, although this association was not evident within individual cancer outcomes. This may reflect a difference in approach to smoking cessation in men and women, where men may be more likely to quit due to poor health or existing disease as indicated by the elevated incidence within the first year. I found this result surprising given that prevalence and intensity of smoking tends to be lower in women [641].

4.4.3.4 HIV mode of transmission

Incidence of lung cancer did not vary by HIV mode of transmission. However, incidence of infection related cancers (excluding lung) was higher in those who acquired HIV through routes other than sex between men. This is probably driven by the higher proportion of HCV coinfected people in these groups, and the established association between HCV and HBV and liver cancers [474, 624, 625] (see chapter 3). Furthermore, incidence of smoking unrelated cancers was lower according to those who acquired their HIV through means other than sex between men, likely reflecting competing risks of death in this group.

4.4.3.5 Comorbidities

Those who developed smoking related cancers (excluding lung) were more likely to have a history of comorbidities, including hypertension, anaemia, AIDS defining events (excluding ADC), and diabetes. Similarly, smoking unrelated cancers was associated with anaemia and diabetes. The incidence of lung cancer was not associated with a history of any of the comorbid conditions investigated. The associations with anaemia are not surprising as anaemia is common in people diagnosed with many types of cancer, particularly leukaemia and lymphomas [772, 773]. The link between diabetes and smoking related cancers (excluding lung) and smoking unrelated cancers is likely in part due to sharing several common lifestyle risk factors, including poor diet, alcohol use, and obesity [774]. In addition, a meta analysis in the general population found elevated levels of liver, pancreas, endometrium, colon and rectum, breast, and bladder cancer in those with diabetes [774], which may also explain the association.

4.4.4 Strengths of these analyses

The major strengths of the data presented here include the use of the D:A:D database, which contains data from a large, multi-cohort study spanning 3 continents with relatively large numbers of prospectively collected cancers and access to individual level patient and clinical information. All NADCs are centrally validated and require histological evidence of diagnosis, which minimises the chances of misclassification of cancer events. This is one of the few studies in HIV+ people with detailed smoking information over a long period of time and on a large scale.

4.4.5 Limitations of these analyses

These analyses have a number of limitations which need to be kept in mind when interpreting these data. This is an observational study and therefore we cannot establish causality or rule out the effects of unmeasured or residual confounding. Another significant limitation is that smoking information in D:A:D is updated at the clinic visit, therefore, exact start and stop dates of smoking episodes, intensity, and lifetime duration were not available. Furthermore, smoking information is collected according to physician inquiry and smoking (and possibly restarting of smoking after quitting) may be underreported, due to social desirability bias, concerns about the effect on health care, or lack of inquiry by physicians (who for example may assume smoking has not been restarted). All adjusted analyses included cumulative duration of smoking as reported in D:A:D and did not impact on the associations presented. However, it is possible that people may be misclassified as previous smokers who restart smoking between visits, which would lead to an underestimation of the benefits of smoking cessation [654]. To reduce the impact of this, the midpoint between clinic visits was used to define smoking behaviours during follow-up and results were consistent after removing follow-up with missing smoking status or > 2 years since last reported smoking status. Furthermore, a previous study in D:A:D found a decline in CVD following cessation to that of never smokers after 3 years [711], which are similar to results from the general population. This provides some evidence of validity of the smoking information in D:A:D.

At baseline, the proportion of people on cART was low (Table 4.3). This was due to a number of participants with no antiretroviral drug information available at baseline (categorised as unknown). However, this proportion increased over follow-up to approximately 80% as information became available.

The interpretation of analyses looking at time since cessation are complex. This is because previous smokers with a long time since cessation often quit at a younger age or after a shorter smoking duration than those with shorter times since cessation [654]. Age at cessation and duration of smoking are strong risk factors for lung cancer and therefore, disentangling the effects of duration of cessation, age at cessation, and duration of past smoking is not straight forward. Furthermore, there are several potential confounders which were not collected within D:A:D (for example: physical activity level, diet, recreational drug use, and socioeconomic status).

These analyses had a relatively short follow-up period of a median of 10 years (relative to studies in the HIV-negative population with follow-up of 30 years or longer), and therefore the long-term benefits of smoking cessation could not be assessed. Short term benefits of smoking cessation are more difficult to establish than the long term benefits because many people cease smoking due to pre-existing cancers or other health problems which can inflate estimates of cancer risk and bias results [477, 654]. Most studies in the HIV-negative population do not conclusively establish the benefit of cessation on lung cancer risk within the first 5 years of cessation [654]. In addition, this study does not take into account that various factors are associated with cessation attempts and success (for example age, income, education) and the relationship of these factors to cessation attempts may also be different from the relationship with cessation success [775]. For example, younger people are more likely to attempt cessation [775], however older people are more likely to achieve long term cessation [654, 776].

4.5 Conclusion

In conclusion, incidence of lung cancer incidence in HIV+ people appears to remain more than 10-fold higher several years after cessation, at a similar level to current smokers, and with no evidence of a decline after the first year. This suggests that the oncogenic potential for smoking is not reduced for lung cancer in the time frame that we have investigated. This is in contrast with similar studies in HIV-negative people, which show a consistent decline in lung cancer incidence with increasing time since cessation. Although incidence of smoking related cancers (excluding lung) was similar to never smokers after 1 year following cessations, the magnitude of effect was similar to current smokers and may indicate remaining risk. Deterring uptake of smoking and smoking cessation efforts should be a priority to reduce the risk of cancer, however, monitoring and awareness of lung cancer should continue in those who stop smoking. These results strongly support efforts into smoking cessation and prevention. Studies following HIV+

people throughout their lifetimes are needed to determine when the benefit of cessation will be seen.

4.6 Publications

This chapter was awarded a young investigator scholarship and presented at CROI 2017 as an oral presentation slides are in Appendix V. A manuscript has been drafted with the intention to submit to JAMA.

5 Differences in virological and immunological risk factors for non-Hodgkin and Hodgkin lymphoma in the D:A:D study

5.1 Introduction

5.1.1 Non-Hodgkin and Hodgkin lymphoma in the general population

Malignant lymphomas are a heterogeneous group of cancers, which vary in terms of clinical and biological features [607, 777]. In the general population, non-Hodgkin lymphomas (NHL) account for around 90% of all malignant lymphomas; Hodgkin lymphomas (HL) account for the remaining 10% [607]. HL is distinguishable from NHL due to the unique presence of a small number of neoplastic cells known as Reed-Sternberg cells (RS) [778-780].

NHL is the 10th most common cancer in the world [781] and is more common in developed countries, with the highest incidences reported in USA, Australia, and Northern Europe, and lowest incidences in eastern and South central Asia [607, 782]. NHL incidence has been increasing worldwide since the 1970s but has slowed in recent years (Figure 5.1) [783]. In 2014 the incidence in the UK was estimated to be 22.9 (men: 27.5, women: 19.1) events per 100,000 people [784]. HL, on the other hand, is less common than NHL in the general population, with an incidence rate of 3.3 (men: 3.9, women: 2.8) events per 100,000 people in the UK, 2014 [785]. HL incidence has increased in the UK general population since the early 1990s (Figure 5.2), driven by large increases in those aged over 70 years [785]. Slightly lower rates have been observed for HL and NHL in Europe [786]. Similar to NHL, HL incidence is higher in more developed countries [787].

Figure 5.1 Non-Hodgkin lymphoma (C82-C86), European age-standardised incidence rates, UK, 1993-2014 [784].

Figure 5.2 Hodgkin lymphoma (C81), European age-standardised incidence, UK, 1993-2014 [785].

Risk factors for NHL in the general population have been extensively investigated and include male gender, older age, high socioeconomic status, immune suppression (such as that caused by HIV infection, B-cell activating autoimmune disorders, or following an organ transplant), atopic disorders, family history of NHL, higher body mass index (BMI) when younger, recreational sun exposure, and skin cancer history [607, 788-794]. Infection with Epstein-Barr virus (EBV), Hepatitis C (HCV, although results are inconsistent), and T cell lymphoma virus 1 (HTLV1) are also associated with NHL risk. Additional risk factors in women have included oral contraception use before 1970, hormone therapy use initiated at age ≥50 years, low usual adult BMI, previous blood transfusion, and greater lifetime alcohol consumption [607, 788, 789]. Furthermore, certain occupations (including farmers, pesticide applicators, grain millers, wood and forestry workers, workers in the petroleum, rubber, plastic and synthetic industries and hair dressers) have been associated with increased risk of NHL attributed to exposure to harmful substances [607, 789].

Risk factors for HL in the general population include male gender, age, family history of lymphomas, HIV, and EBV infection. HL has a distinctive bi-modal age distribution, with one peak at around 30 years (known as the young adult peak) and a second at 50 years [794-798]. Some studies have indicated that smoking may play an etiological role in HL, particularly EBV positive HL [799, 800]. In addition, people with NHL also have excess risk of HL (and vice versa), likely due to somewhat shared aetiology [788, 801].

The strongest and most well established risk factors for both NHL and HL is suppression of the immune system, as reflected by the elevated incidence of both in HIV-positive (HIV+) people [607]. Recent estimates indicate that the incidence of NHL and HL remain elevated in HIV+

people compared to the general population, with NHL incidence estimated to be around 10-fold higher [436, 445, 802, 803] and HL incidence estimated to be around 11-times higher [15, 438, 470, 797]. This is despite a significant reduction of NHL (with the exception of Burkitt lymphomas) incidence following the introduction of combination antiretroviral therapy (cART) in 1996 [15, 16, 20, 21, 438, 439, 441-443, 450, 451, 604]. The basic epidemiology of both NHL and HL are described in Chapter 1 sections 1.2.2.1 and 1.2.2.4 and are also explored in chapter 3 as a component of infection related cancers (IRCs).

5.1.2 Non-Hodgkin and Hodgkin lymphoma in HIV-positive people

Lymphomas have different clinical characteristics in HIV+ compared with HIV-negative people. NHL in HIV+ people tend to be more aggressive, have a high proportion of the diffuse large B-cell subtype, extra-nodal location (meaning outside the lymph nodes and often in the gastro intestinal tract and central nervous system), and a stronger association with EBV than in HIV-negative people [607]). NHL can be further grouped into subtypes, including primary brain and central nervous system (PBCNS) lymphomas, and the systemic lymphomas which are Burkitt, diffuse large B-cell (DLBC, includes immunoblastic) and other less common subtypes [442, 450, 451]. HL in HIV+ people compared to HIV-negative people tend to also be more aggressive, presenting with B-cell symptoms, extra-nodal location, with poorer prognosis [471, 797], and have a higher proportion of RS cells [797]. In addition, nearly all are associated with EBV [471, 797, 804] compared with 30 – 50% of HL in HIV-negative people from the USA and western Europe [805]. The categories of HIV associated lymphomas are shown in Table 5.1.

Table 5.1 Immunological and Epstein-Barr virus (EBV) status in AIDS related lymphomas [804].

Lymphoma histology	HIV Specific	Association with Immunodeficiency	% in HIV	% EBV associated	EBV latency
Systemic AIDS related NHL					
Burkitt lymphomas (BL)		Mild	55	55%	Type I
Classic BL		Mild	30	30%	Type I
BL with plasmocitoid		Mild	20	50–70%	Type I
Atypical BL		Mild	<5	30–50%	Type I
Diffuse Large B-cell lymphomas					
Centroblastic type		Mild	20	30–40%	Type I
Immunoblastic type		Marked	10	90–100%	Type III
AIDS Primary central nervous system Lymphomas		Marked	<5	100%	Type III
Primary effusion Lymphomas	Yes	Marked	<5	90-100%	Type I
Plasmablastic lymphomas of the oral cavity	Yes	Not clear	<5	50%	Not clear
Hodgkin Disease Classical		Marked		80-100%	Type II

EBV: Epstein-Barr virus, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma.

In HIV+ people, the pathogenesis of NHL and HL is thought to be driven by a combination of latent EBV infection and HIV associated immune suppression and dysfunction [606-608]. The interaction between HIV infection, immune suppression, and dysfunction and EBV coinfection is not well characterised, however, it is thought to differ between NHL and HL, as well as by NHL subtype (Table 5.1) [452, 804].

For example: almost all HL is EBV related in HIV+ people and incidence is higher at lower CD4 cell counts [16, 427, 433, 804]. However, the association between HL and CD4 cell count is not as strong as that observed for PBCNS or DLBC lymphomas. Both PBCNS and immunoblastic DLBC lymphomas often occur at very low CD4 cell counts and are almost entirely EBV associated [804]. Furthermore, a lack of EBV-specific CD4 T-cell function has been shown in HIV+ people prior to PBCNS lymphoma development irrespective of absolute CD4 cell counts [806]. Finally, Burkitt lymphomas tend to occur at modest CD4 cell counts and approximately half are EBV related [450, 804, 807].

In the context of HL, some studies have indicated an association with other markers of immune deficiency in addition to low CD4 cell counts. For example, it has been hypothesised that an imbalance in the pool of EBV-specific CD4 and/or CD8 cells in peripheral blood may increase the risk of HL in HIV+ people [586]. It is thought that that EBV surveillance is largely controlled by CD8 cells, although CD4 cells are likely required for the expansion and long term

function of EBV-specific CD8 cells [808]. This has also been suggested for NHL [809], specifically PBCNS lymphomas [806] which is also predominantly EBV related.

A detailed description of the pathogenesis if NHL and HL is given in chapter 6.

5.1.2.1 Risk factors in HIV-positive people

Several prior studies have indicated that the risk factors for NHL and HL differ [474, 499, 500, 810-814]. A summary of risk factors explored and identified to date are given below.

- **Age:** Older age has been linked to NHL in HIV+ people [433, 435, 450, 815, 816]. The association between age and HL is less straight forward, with many studies reporting no association with age in HIV+ people [433, 586].

- **Gender:** Many studies have found no difference according to gender for NHL [817] and HL [586] in HIV+ people. However, differences have been observed according to HIV mode of HIV transmission (see below).

- **Ethnicity**: Incidence of NHL has been shown to be lower in non-white people in some studies [433, 606]. No association with HL has been shown [433].

- **Mode of HIV transmission:** higher NHL incidence has been reported in those who acquired HIV through sex between men [433, 435, 606, 815, 817], however, one study found this in treated people only [435, 817], and another in untreated patients only [815]. Similarly, higher HL incidence has also been found in men who acquired HIV through sex between men [586].

- **CD4:** Many studies have shown the dose response type of association between lower CD4 cell count and higher NHL, and to a lesser extent, HL incidence [16, 18, 20, 429, 433, 435, 441, 450, 451, 468, 470, 471, 606, 815-818]. For NHL, similar associations have been found for nadir CD4 cell count [435, 819] and time weighted CD4 cell count, however not after adjustment for current CD4 cell count [817]. Furthermore, the association between CD4 cell count and NHL varies by NHL subtype (as mentioned in section 5.1.2 and Table 5.1) [441]. For HL, many studies have shown that incidence peaks at low to moderate CD4 cell counts, rather than at very low CD4 cell counts [433, 468, 470, 820]. Some studies have found a dramatic decline in CD4 cell counts within 1-2 years prior to HL diagnosis in HIV+ [468, 586]. There was no evidence of a gradual decline in CD4 cell

count leading up to diagnosis and similar declines were observed in the CD8 and total lymphocyte cell count [821]. Therefore, it was suggested that this drop in CD4 cell count was a consequence of the tumour rather than a step in the tumour development [821].

- **Previous Kaposi's sarcoma (KS) diagnosis and NHL.** This association exists for several NHL subtypes and persists after adjusting for current CD4. The association is likely due to a combination of immune deficiency (which is a common risk factor for both NHL and KS), as well as additional immune suppression and chronic B-cell stimulation and transformation caused by uncontrolled replication of EBV and human herpes virus 8 (HHV8) [435, 500, 822-826]. Although uncommon, HHV8 is also aetiologically linked to some NHL subtypes [500].

- **HIV-VL**: Studies have shown an association between current, lagged, and cumulative HIV-VL and NHL incidence, independent of CD4 cell count and treatment status [16, 18, 433, 435, 451, 606, 816, 819]. Most studies have not shown an association between HL incidence and HIV-VL [16, 429, 433, 468].

- **HIV treatment**: When compared directly, the incidence of NHL in HIV+ people on cART has been estimated to be as low as half that of those not on cART [18, 20, 433, 435, 818]. However some studies showed only a borderline association [450, 815, 827]. A clear impact of HIV treatment on HL incidence has not been shown, with some studies showing lower HL incidence [586] particularly with longer duration of time on cART [828, 829] and others showing a higher incidence in treated people [20]. There is very little evidence of a difference in NHL or HL incidence according to cART regimen [435, 468, 827].

- **HCV**: An analysis within the COHERE database found an association between HCV (and hepatitis B [HBV]) and NHL incidence in treated patients, but not untreated patients [830]. This association has also been identified in two meta analyses [831, 832]. However, many other studies have failed to show an association between HCV and NHL incidence [433, 818, 833, 834]. One study found an association in men who acquired HIV through sex between men but no other subgroups [834]. A borderline association between HCV and HL incidence was identified in one meta-analysis [832].

5.1.2.2 Survival following non-Hodgkin lymphoma and Hodgkin lymphoma diagnosis in HIV-positive people

HIV+ people in the post cART era have a higher mortality rate following NHL diagnosis than HIV-negative people [835]. In treated HIV+ people in Europe, the 5 year survival rate for those diagnosed with systemic lymphomas is estimated to 55% (95%CI: 51–60%), with the highest mortality within the first year of diagnosis [807]. For PBCNS NHL, the 1 year survival was 54% (95%CI: 43–65%) and to too few people survived for 5 years or more to estimate the rate. Although the incidence of HL has not declined since the introduction of cART [19, 21, 430, 467], Survival of HIV+ people following HL diagnosis has dramatically improved in the cART era due to greater chemotherapy options and improved responses to cancer treatment [836]. Studies have shown a similar mortality rate in HIV+ and HIV-negative people after adjustment for age, cancer stage, and treatment, with a 5 year survival of 83% vs. 89%, respectively [535].

5.1.3 Implications of the START study

Although not specific to lymphomas, the START study (see chapter 1 section 1.1.8.7.1) demonstrated that immediate cART initiation significantly reduced the risk of IRC, independently of CD4 cell count and HIV-VL, indicating that IRC risk is mediated through other mechanisms such as immune activation and reduced immune surveillance, above and beyond the depletion of CD4 cell counts [141].

5.1.4 Aims

There are many studies which have investigated incidence of NHL and HL since the introduction of cART and associated risk factors. However, to my knowledge, few previous European studies have identified NHL and HL risk factors within the same population (with the exception of the French Hospital Database on HIV [433]). Furthermore, no studies have directly compared the risk factors for NHL and HL in order to identify and develop the understanding of different mechanistic pathways which may suggest different preventive approaches for reducing NHL and HL risk. Therefore this chapter had two aims:

- To identify independent risk factors for NHL and HL within the same population, focussing on immunological and virological related factors

- To directly compare and formally test factors that differently affect NHL and HL risk in order to develop the understanding of different mechanistic pathways, which may then suggest different preventive approaches for reducing NHL and HL risk.

5.2 Methods

This study was conducted within the Data Collection on Adverse events of Anti-HIV Drugs Study (D:A:D) collaboration using the 16th merger the D:A:D database (included follow-up on 49,709 people). NHL is classified as an AIDS defining cancer (ADC [133]) and such events have been collected in D:A:D since 1999. Non-AIDS defining cancers (NADC), such as HL, have been routinely collected and validated since 1 January 2004. The D:A:D study is described in detail in chapter 2 section 2.1.2.

5.2.1 Outcomes

This study investigated the association between multiple possible risk factors (with a focus on immunological and virological factors) and two main outcomes: the first diagnosis of NHL during follow-up and the first diagnosis of HL during follow-up. As a sensitivity analysis, immunological and virological factors associated with NHL subtypes (Burkitt, PBCNS, and immunoblastic NHL) were also investigated in the subset of people with NHL subtype reported (33.6%).

5.2.2 Inclusion criteria

People included in D:A:D were followed from their baseline date, defined as the latest of date of study entry, first reported CD4 cell count, or 1st January 2004, until the earliest of first NHL or HL diagnosis, last visit plus 6 months, death, or 1st February 2015.

There were 42,102 people in D:A:D with follow-up after 1 January 2004. I excluded a total of 682 people, of whom 509 had a prior NHL (N=408), HL (N=97) diagnosis or both (N=4), 10 had no recorded gender, and 163 had no CD4 measurement prior to end of follow-up. Those excluded were slightly older, and a higher proportion were male compared to those included. A flow chart for the inclusion criteria is shown in Figure 5.3.

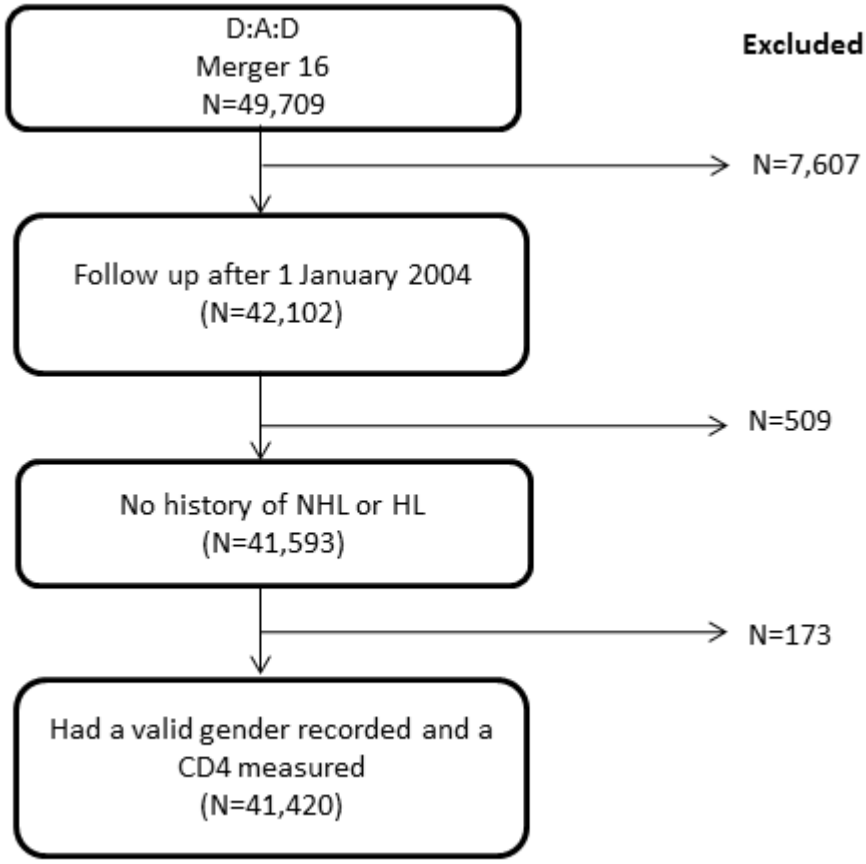


Figure 5.3 Flow chart for inclusion of D:A:D participants in analysis.

5.2.3 Measures of CD4 cell count and HIV viral load

Time updated current and historical measures of CD4 cell count and HIV-VL were considered and are outlined in Table 5.2. Historical measures of CD4 cell count included: nadir CD4 cell count, as a measure of the maximum level of immune suppression reached, and duration of time with CD4 cell count < 200 cells/mm^3, as a time varying measure of cumulative exposure to suppressed CD4 cell counts. Measures looking at time since last CD4<200 cells/mm^3 were also considered.

The area under the curve (AUC) of HIV-VL was considered as a time varying measure of accumulated exposure to replicating HIV. The measure refers to the area under each individuals' HIV-VL curve over time [837]. Formally, it can be interpreted as the total number of copies/mL of HIV-VL accumulated over time. For example, an AUC of HIV-VL of 10000 is equal to having a HIV-VL of 10000 copies/mL for 1 year or a HIV-VL of 1000 copies/mL for 10 years. The measure is calculated using the trapezoidal rule, a mathematical tool used to estimate the area under a curve (Equation 5.1):

$$AUC = \sum_{I=1}^{N} \frac{1}{2} \times \frac{D_i - D_{i-1}}{365.25} \times (V_i + V_{i-1})$$

Equation 5.1 Formula for area under the curve measures.

Where D_i is the date of the current HIV-VL measurement, V_i is the current HIV-VL level, D_{i-1} is the date of the previous HIV-VL measurement, V_{i-1} is the date of the previous HIV-VL measurement. For this chapter, I calculated the AUC for uncontrolled viremia (HIV-VL >50 copies/mm^3), meaning that 50 copies/mm^3 was subtracted from the HIV-VL measurements when using the equations. The AUC of HIV-VL was categorised into quintiles for analysis as detailed in Table 5.2: lowest 20%: lowest quintile (Q1), 21% - 40%: Q2, 41 – 60%: Q3, 61 – 80%: Q4, highest 20%: highest quintile– (Q5), where those in the lowest quintiles had the lowest accumulated exposure to HIV viral replication, and highest quintiles had the highest accumulated exposure to HIV viral replication since the latest of HIV-diagnosis (or entry into D:A:D).

For some analyses, current HIV-VL and AUC of HIV-VL were $\log_{10}$ transformed and current and nadir CD4 cell count were $\log_2$ transformed, due to the right skewed nature of the data, and also for interpretive reasons i.e. a one unit increase in $\log_{10}$ current HIV-VL is equivalent to a 10-fold increase, and a one unit increase in $\log_2$ CD4 cell count is equivalent to a 2-fold increase.

5.2.4 Variables included in analyses

Both baseline and time updated variables from the D:A:D database were included in this analysis, as detailed in Table 5.2. Use of cART was considered as both a current variable (i.e. on cART and not on cART) and according to duration of time on cART. Duration of time on cART was used for the main analysis as it was deemed a clinically important factor. Person years of follow-up (PYFU) were calculated per month of additional follow-up.

Table 5.2 Summary of baseline and time updated variables considered in analysis.

Variable	Time updated	Levels	Definitions and comments
Immunological markers			
Current CD4 cell count (cells/mm^3)	Yes	Continuous (per 2-fold higher) and <200, 200 – 349, 350 – 499, ≥500 cells/mm^3, unknown	
Nadir CD4 cell count (cells/ mm^3)	Yes	Continuous (per 2-fold higher) and <200, 200 – 349, 350 – 499, ≥500 cells/mm^3, unknown	Lowest recorded CD4 cell count measurement prior to date
Cumulative time spent with CD4 cell count < 200 cells/mm^3	Yes	Continuous (per year longer) and Categorised as 0, 0.1 – 2, 2 – 4, ≥ 4 years	
CD4 cell counts < 200 cells/mm^3 in the last 12 months	Yes	Yes, no, unknown	
Virological markers			
Current HIV-VL (copies/mL)	Yes	Continuous (per 10-fold higher) and <400, ≥ 400 copies/mL, unknown	
Area under the curve (AUC) of HIV-VL calculated from first visit into D:A:D	Yes	Continuous (per 10-fold higher) and categorised into quintiles: lowest 20%: lowest quintile (Q1), 21% - 40%: Q2, 41 – 60%: Q3, 61 – 80%: Q4, highest 20%: highest quintile– (Q5).	
Age (years)	Yes	Continuous (per 10 years older) and categorised into 16 – 29, 30 – 39, 40 – 49, 50 – 59, ≥ 60 years	
Calendar year of baseline		Continuous (per year) and categorised by year (2004/05, 2006/07, 2008/09, 2010/11, 2012/13/14/15)	Years were combined into categories due to the small amount of follow-up and events accrued within some years category.
Calendar year of follow-up	Yes	Continuous (per year) and Categorised by year (2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014/15	The years 2014 and 2015 were combined into one category due to the small amount of follow-up and events accrued within this category
Ethnicity		white, black, other or unknown	
Gender		Male, female	
HIV mode of transmission		Sex between men, heterosexual, IDU, other or unknown	
BMI (kg/m^2)	Yes	Under weight (<18), normal weight (18 – 25), Over weight (25 – 30), obese(30+)	Classified according to the WHO standard [597]

Smoking status	Yes	Non-smokers, current smokers, previous smokers, unknown	
Prior ADC diagnosis (excluding NHL)	Yes	Yes, no	Classified according to the 1993 CDC clinical definition [133]
Prior AIDS defining event (excluding ADC)	Yes	Yes , no	Classified according to the 1993 CDC clinical definition [133]
Prior cardiovascular disease	Yes	Yes , no	Defined as whether the person ever had a diagnosis since entry into D:A:D.
Hypertension	Yes	Yes, no	Defined as ever having systolic BP >140, diastolic BP >90 mm Hg or receiving any antihypertensive drugs or ACE inhibitors since entry into D:A:D
Diabetes	Yes	Yes, no	Defined as a reported diabetes diagnosis on a case-report form or use of anti-diabetic medication
HBV status	Yes	Positive, prior, negative, or unknown	Positive HBV status was defined by a prior positive HBsAG surface antigen test or presence of detectable HBV DNA, Prior HBV status was defined by a prior positive HBsAG surface antigen test or presence of detectable HBV DNA, with a negative latest HBsAG surface antigen test and/or undetectable HBV DNA.
HCV status	Yes	Positive, negative, or unknown	Positive hepatitis-C (HCV) status was defined as having a prior positive HCV surface antibody test.
cART use	Yes	On cART, not on cART	defined as being on at least 1 protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI)
cART regimen	Yes	PI or NNRTI	
Duration of time on cART (years)	Yes	Both continuous (per year longer) and categorised as 0, 1-2, 2-4, >4 years	
Duration of time on protease inhibitors (PI) (years)	Yes	Both continuous (per year longer) and categorised as 0, 1-2, 2-4, >4 years	
Duration of time on Non-nucleoside reverse transcriptase inhibitor (NNRTI) (years)	Yes	Both continuous (per year longer) and categorised as 0, 1-2, 2-4, >4 years	

HIV-VL: HIV viral load, AUC: area under the curve, IDU: injecting drug use, BMI: Body mass index, ADC: AIDS defining cancer, HBV: Hepatitis B, HCV; Hepatitis C, cART combination antiretroviral therapy.

5.2.5 Statistical methods

5.2.5.1 Characteristics at baseline.

Characteristics of people in D:A:D who had a NHL or a HL diagnosed during follow up were described using numbers and percentages for categorical variables and median with interquartile range (IQR) for numerical variables at baseline. All bivariate associations were tested using chi squared tests for categorical variables and Kruskal-Wallis tests for numerical variables.

5.2.5.2 Incidence rates of non-Hodgkin and Hodgkin lymphoma

Incidence rates of NHL and HL per 1000 PYFU were calculated according to categories of key variables of interest. These included calendar year of follow-up, current and nadir CD4 cell count, duration of time with CD4<200 cells/mm^3, current and AUC of HIV-VL, and cumulative time on treatment.

5.2.5.3 Identification of independent risk factors for non-Hodgkin and Hodgkin lymphoma

Separate Cox regression models were used to calculate both unadjusted hazard ratios (HR) and adjusted HR (aHR) to identify factors associated with NHL and HL, focussing on current and nadir CD4 cell count duration of time with CD4<200 cells/mm^3, and current and AUC of HIV-VL. Models were adjusted for baseline and time varying factors listed in Table 5.2.

Collinearity and over fitting of the models was a consideration due to the limited number of NHL and HL events that accrued during follow-up and the high correlation between the various immunological markers. To take this into account, I selected the single best fitting immunological marker to include in the models. Spearman's ρ was used to investigate the correlation between immunological factors and virological factors. Due to the high correlation between current and nadir CD4 cell count, and duration of time spent with CD4 cell count < 200 cells/mm^3 (Table 5.3), only one measure was selected to be included in the analysis according to the smallest Akaike information criterion (AIC) for both NHL and HL. Current and AUC of HIV-VL were not strongly correlated (Table 5.3) and therefore both were included in the same model. All other variables included in the model were identified a priori based on expert clinical input, availability of data, and the published literature.

Table 5.3 Correlations between immunological and virological variables at baseline[1].

	Current CD4 (cells/mm^3)		Nadir CD4 (cells/mm^3)		Time since last CD4 cell count < 200 cells/mm^3 (years)		Duration of time with CD4 <200 cells/mm^3 (years)		Current HIV-VL (copies/mL)	
	Spearman's r	**P**	**Spearman's ρ**	**P**	**Spearman's ρ**	**P**	**Spearman's ρ**	**P**	**Spearman's ρ**	**P**
Nadir CD4 (cells/mm^3)	0.60	<0.01	1	_						
Time since last CD4 < 200 cells/mm^3 (years)	0.82	<0.01	0.18	<0.01	1	_				
Duration of time with CD4 cell count <200 cells/mm^3 (years)	-0.38	<0.01	-0.80	<0.01	-0.05	<0.01	1	_		
Current HIV-VL (copies/mL)	-0.25	<0.01	0.20	<0.01	-0.32	<0.01	-0.26	<0.01	1	_
AUC of HIV-VL (copies/mL – year)	-0.06	<0.01	-0.28	<0.01	-0.07	<0.01	0.31	<0.01	-0.04	<0.01

HIV-VL: HIV viral load, AUC: area under the curve.

[1] Baseline was defined as the latest of 1 January 2004, entry into D:A:D, or first CD4 cell count.

5.2.5.4 Comparison of factors which distinguished between people at high risk of non-Hodgkin or Hodgkin lymphoma

The Wei, Lin, and Weissfeld method, based on the marginal Cox model [565], was used to identify factors that were differently associated with NHL and HL risk. The models were used to jointly calculate and compare the aHRs for NHL and HL for current and historical measures of HIV-VL and CD4, adjusting for variables listed above. In order to fit a more parsimonious model (i.e. reduce the number of parameters) variables not associated with either NHL or HL incidence were assumed to have a similar association for both NHL and HL. The remaining variables were allowed to vary according to outcome. It should be noted that the marginal Cox models are therefore different models to those used to identify independent risk factors (Cox models) with minor differences in adjustments (fixed and outcome specific variables) which give slightly different estimates.

The ratio of the aHRs (RHR) was used to identify factors with different risk profiles for NHL and HL. I refer to these factors as "differential factors". Variables for which the lower limit of the 95% confidence interval (95%CI) is > 1 indicates a relatively stronger association with NHL than HL for that factor. Conversely, when the upper limit of the 95%CI < 1 indicates a relatively stronger association with HL than NHL. Variables for which the 95%CI of the RHR crosses 1 indicates no evidence of a different association between the risk factor and HL and NHL risk.

5.2.5.5 Subtypes

Risk factors for NHL subtypes were investigated using separate adjusted Cox models only, however too few events were accrued to perform a direct comparison using marginal Cox models.

All statistical tests were two sided with a type I error rate of 5%. All statistical analyses were performed using SAS 9.4 (Statistical Analysis Software, Cary NC, USA).

5.3 Results

There were 41,420 people included in the analysis contributing 337,020 PYFU with a median follow-up of 9.2 (Interquartile range [IQR]: 6.3, 11.1) years per person. A total of 392 people developed NHL and 149 developed HL during follow-up (Table 5.4).

5.3.1 Baseline characteristics

Baseline characteristics of the study population, as well as those who developed NHL or HL during follow-up are shown in Table 5.4. Overall, 17.4% were aged ≥ 50 years at baseline and the median age was 40 (IQR: 34, 47) years, 72.9% were of male gender and 43.8% acquired HIV through sex between men. Almost half of those included (49.9%) were of white ethnicity and 40.9% were of unknown ethnicity. Furthermore, 40.7% were current smokers whereas 27.4% had never smoked. Approximately two thirds of people had a BMI within the healthy range of 18 – 26 kg/m^2, 1 in 5 people had a previous AIDS defining event (excluding ADCs), and 3.4% had a previous ADC (excluding NHL). HCV and HBV confection occurred in 19.2% and 18.9% of people respectively. A history of cardiovascular disease, diabetes or hypertension was reported in 2.1%, 2.5% and 14.8% of the study population, respectively. Just over half were on cART (51.8%) at baseline, with a median cART duration of 1.1 (IQR: 0.0, 4.9) years. The median CD4-cell count was 431 (IQR: 280, 620) cells/mm^3, nadir CD4 cell count was 248 (116, 403) cells/mm^3 and duration of time with CD4 < 200 cells/mm^3 was 0.0 (IQR: 0.0, 0.4) years. Just over half had a HIV-VL ≤ 500 copies/mL and the median AUC of log_{10} HIV-VL was 4.2 (3.0, 4.9) log_{10} copies/mL-years.

Table 5.4 Characteristics at baseline[1] of those included in the analysis and of those who developed either a non-Hodgkin (NHL) or Hodgkin (HL) lymphoma during follow-up.

Factors	All people (N=41,420)	people who developed NHL (n=392)	people who developed HL (N=149)
	N (%)	N (%)	N (%)
Age (years)			
<30	5,922 (14.3)	22 (5.6)	16 (10.7)
30 - 39	14,833 (35.8)	118 (30.1)	44 (29.5)
40 - 49	13,449 (32.5)	142 (36.2)	56 (37.6)
50 - 59	5,170 (12.5)	71 (18.1)	23 (15.4)
≥60	2,046 (4.9)	39 (9.9)	10 (6.7)
Gender			
Male	30,214 (72.9)	337 (86.0)	126 (84.6)
Female	11,206 (27.1)	55 (14.0)	23 (15.4)
HIV transmission mode			
Sex between men	18,124 (43.8)	200 (51.0)	87 (58.4)
IDU	5,926 (14.3)	53 (13.5)	15 (10.1)
Heterosexual	14,800 (35.7)	106 (27.0)	39 (26.2)
Other/Unknown	2,570 (6.2)	33 (8.4)	8 (5.4)
Race			
White	20,658 (49.9)	181 (46.2)	70 (47.0)
Black	2,963 (7.2)	10 (2.6)	6 (4.0)
Other	840 (2.0)	7 (1.8)	3 (2.0)
Unknown	16,959 (40.9)	194 (49.5)	70 (47.0)
smoking status			
Current	16,859 (40.7)	155 (39.5)	70 (47.0)
Ex	7,332 (17.7)	65 (16.6)	31 (20.8)
Never	11,364 (27.4)	98 (25.0)	28 (18.8)
Unknown	5,865 (14.2)	74 (18.9)	20 (13.4)
BMI (kg/m^2)			
<18	1,377 (3.3)	13 (3.3)	4 (2.7)
18 - 26	27,709 (66.9)	255 (65.1)	102 (68.5)
27 - 30	5,360 (12.9)	59 (15.1)	15 (10.1)
>30	1,833 (4.4)	16 (4.1)	10 (6.7)

Factors		All people (N=41,420)		people who developed NHL (n=392)		people who developed HL (N=149)
		N (%)		N (%)		N (%)
Unknown		5,141 (12.4)		49 (12.5)		18 (12.1)
Previous AIDS defining event (excluding ADC)		8,801 (21.2)		85 (21.7)		31 (20.8)
Previous ADC (excluding NHL)		1,419 (3.4)		26 (6.6)		8 (5.4)
On cART		21,436 (51.8)		161 (41.1)		70 (47.0)
HBV						
Negative		29,355 (70.9)		271 (69.1)		119 (79.9)
Positive/prior		7831 (18.9)		86 (21.9)		19 (12.8)
Unknown		4,234 (10.2)		35 (8.9)		11 (7.4)
HCV						
Negative		28,168 (68.0)		261 (66.6)		117 (78.5)
Positive		7,947 (19.2)		76 (19.4)		19 (12.8)
Unknown		5,305 (12.8)		55 (14.0)		13 (8.7)
Prior cardiovascular disease		857 (2.1)		5 (1.3)		6 (4.0)
Diabetes		1,055 (2.5)		12 (3.1)		2 (1.3)
Prior hypertension		6,121 (14.8)		68 (17.3)		30 (20.1)
Baseline year						
2004 - 2005		29,715 (71.7)		290 (74.0)		99 (66.4)
2006 - 2007		6,334 (15.3)		65 (16.6)		29 (19.5)
2008 - 2009		5,327 (12.9)		35 (8.9)		21 (14.1)
2010 - 2011		29 (0.1)		1 (0.3)		0 (0.0)
2012 - 2015		15 (0.0)		1 (0.3)		0 (0.0)
HIV-VL (copies/mL)						
≤500		21,546 (52.0)		113 (28.8)		65 (43.6)
>500		18,337 (44.3)		265 (67.6)		81 (54.4)
Unknown		1,537 (3.7)		14 (3.6)		3 (2.0)
	N	**Median (IQR)**	**N**	**Median (IQR)**	**N**	**Median (IQR)**
Age (years)	41420	40 (34,47)	392	43 (38,51)	149	42 (35,49)
CD4 count (cells/mm^3)	41420	431 (280,620)	392	342 (189,534)	149	409 (276,575)
Nadir CD4 count (cells/mm^3)	41420	248 (116,403)	392	197 (77,368)	149	259 (130,409)
Duration of time CD4 <200 cells/mm^3 (years)	37962	0.0 (0.0,0.4)	353	0.0 (0.0,0.6)	137	0.0 (0.0,0.2)

Factors	All people (N=41,420)		people who developed NHL (n=392)		people who developed HL (N=149)	
	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)
HIV viral load (copies/mL)	39883	260 (<50,20400)	378	13419 (80,90951)	146	2615 (<50,34000)
Log_{10} AUC viral load (copies/mL- years)	36840	4.2 (3.0,4.9)	345	4.5 (3.3,5.2)	135	4.3 (2.4,5.0)

NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, BMI: body mass index, ADC: AIDS defining cancer, cART: combination antiretroviral therapy, HBV: hepatitis B, HCV: hepatitis C, IQR: interquartile range, HIV-VL: HIV viral load, AUC: area under the curve.

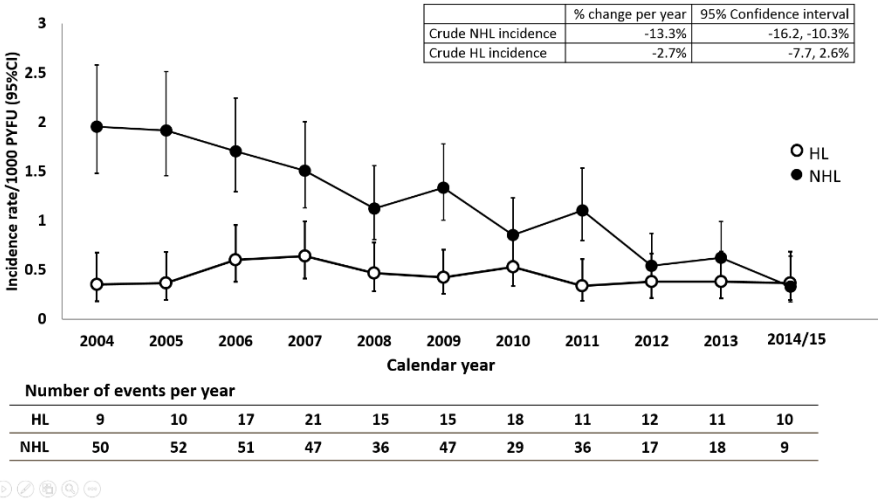
[1] Baseline was defined as the latest of 1 January 2004, entry into D:A:D, or first CD4 cell count.

5.3.2 Crude incidence rates

A total of 392 people developed NHL (crude incidence rate [IR] 1.17/1000 PYFU, 95%CI: 1.06, 1.30) and 149 developed HL (IR 0.44/1000 PYFU 95%CI: 0.38, 0.52) during follow-up.

5.3.2.1 Calendar time

The crude incidence of NHL and HL by calendar year is shown in Figure 5.4. The crude incidence of NHL declined over time by 13.3% per year (95%CI: 10.3, 16.2%) from an incidence rate of 1.96 (95%CI: 1.48, 2.58) events/1000 PYFU in 2004 to 0.32 (95%CI: 0.17, 0.2) in 2014/15. Conversely, crude HL incidence was stable over time (change per year: -2.7%, 95%CI: -7.7, 2.6%), with an incidence of 0.36 (95%CI: 0.19, 0.66) in 2014/15. An interesting observation is the convergence of the incidence of NHL and HL over the duration of this study up to 2014/15 where the incidence of NHL and HL are similar (and in fact, slightly higher for HL).



Number of events per year											
HL	9	10	17	21	15	15	18	11	12	11	10
NHL	50	52	51	47	36	47	29	36	17	18	9

Figure 5.4 Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by calendar time.
NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, PYFU: person years of follow-up.
The unadjusted percentage change per year (% change) incidence of NHL and HL per more recent calendar year is shown in the top right hand corner of the graph.

5.3.2.2 Immunological markers

The crude incidence of both NHL and HL according to immunological measures is shown in Figure 5.5-Figure **5.7**. A rapid decline in crude NHL incidence with increasing CD4 cell count category is demonstrated in Figure 5.5, which equates to a decline of 16 (95%CI: 14, 19)% per 2-fold increase in current CD4 cell count. Although less pronounced, a significant decline is also seen for crude HL incidence of 11 (95%CI: 5, 17)% per 2-fold increase in current CD4 cell count (Figure 5.5). NHL and HL incidence according to nadir CD4 cell count is shown in Figure 5.6. There is a significant

decline in crude NHL incidence of 7 (95%CI: 3, 11)% per 2-fold higher nadir CD4 cell count, however no significant association with crude HL incidence (% change per 2-fold higher: -1, 95%CI: -9, 7%). Figure **5.7** demonstrates an increase in crude NHL incidence with increasing duration of time with CD4 cell count < 200 cells/mm^3, which equates to an increase of 5 (95%CI: 3, 9)% per year longer. No association with crude HL incidence was found (% change per year longer: 0% 95%CI: -8, 8%).

5.3.2.3 Virological markers

The crude incidence of both NHL and HL according to virological measures is shown in Figure 5.8 and Figure **5.9**. A clear increase in crude NHL incidence is shown for both higher current HIV-VL category (Figure 5.8: % change per 10-fold higher: 74%, 95%CI: 62, 86%) and AUC of HIV-VL category (Figure **5.9**: % change per 10-fold higher: 81%, 95%CI: 59,106%). Conversely, incidence of HL was stable across current (% change per 2-fold higher: 5, 95%CI: -10, 22%, Figure 5.8) and AUC of HIV-VL (% change per 2-fold higher: 4, 95%CI: -12, 23%, Figure 5.9) category.

5.3.2.4 HIV treatment

Figure 5.10 shows the crude incidence rates of NHL and HL by treatment. Crude NHL and HL incidence was highest within the first 2 years of starting cART, and both declined with increasing time on cART, by 11 (95%CI: 9, 13)% and 7 (95%CI: 3, 11)% per year longer on cART respectively (Figure 5.10). Crude incidence of NHL was highest in the first 2 years on protease inhibitors (PIs) and declined by 6 (95%CI: 3, 8)% per year longer on PI, however no association between increasing time on PIs and crude HL incidence was found (% change per year longer -1% 95%CI:-5, 3%) (Figure 5.11). Crude incidence of both NHL and HL declined by 13% per year (95%CI: 10, 17% and 95%CI: 8, 19% respectively) with increasing time on non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Figure 5.12).

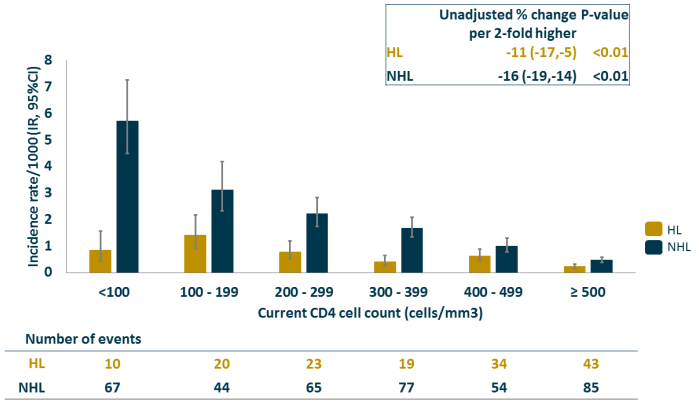


Figure 5.5 Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by current CD4 cell count.

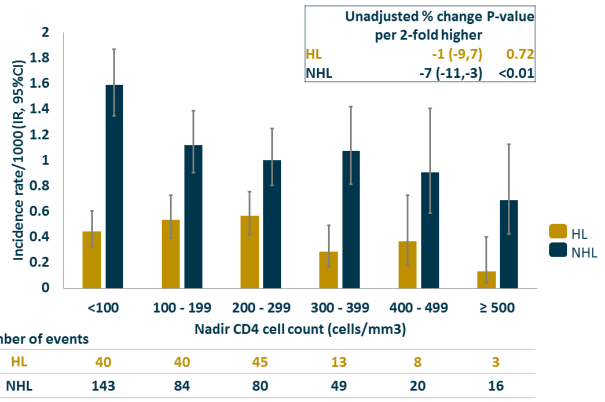
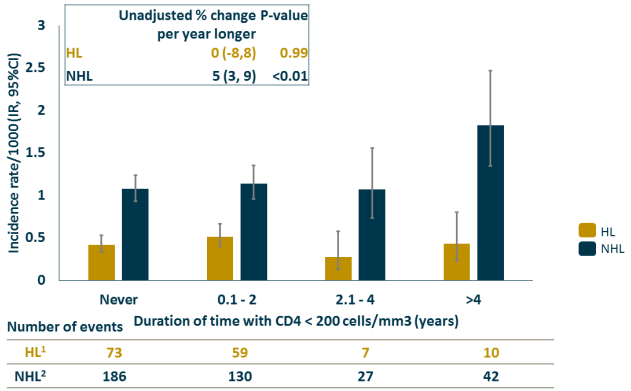
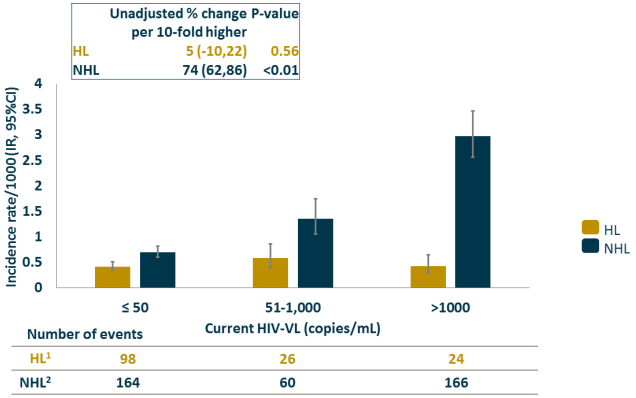


Figure 5.6 Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by nadir CD4 cell count.



[1] 0 HL events unknown status, [2] 7 NHL events with unknown status.

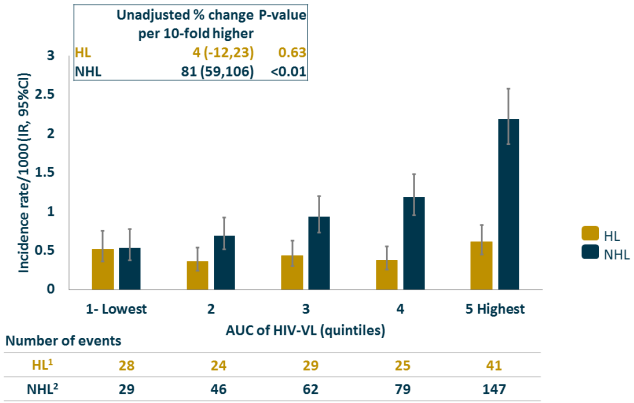
Figure 5.7 Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by duration of time with CD4<200 cells/mm³.



[1] 1 HL events unknown status, [2] 2 NHL events with unknown status.

Figure 5.8 Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by current HIV viral load (HIV-VL).

HL: Hodgkin lymphoma, NHL: non-Hodgkin lymphoma, AUC: area under the curve, HIV-VL: HIV viral load, cART: combination antiretroviral therapy, PI: protease inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor.



[1] 2 HL events unknown status, [2] 29 NHL events with unknown status.

Figure 5.9 **Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by current AUC of HIV-VL.**

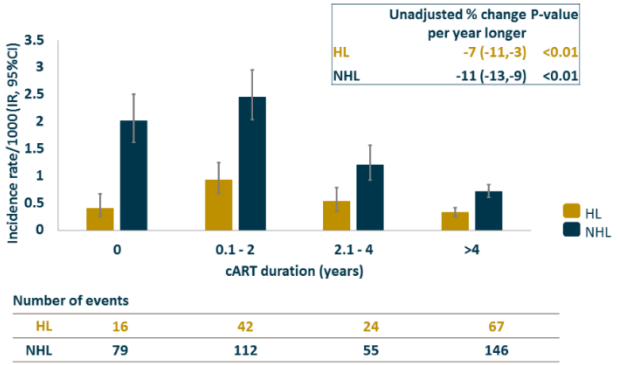


Figure 5.10 Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by duration of combination antiretroviral therapy (cART).

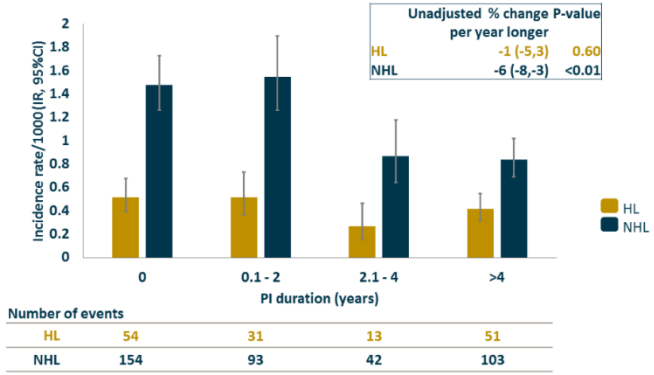


Figure 5.11 Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by duration of protease inhibitor (PI) use.

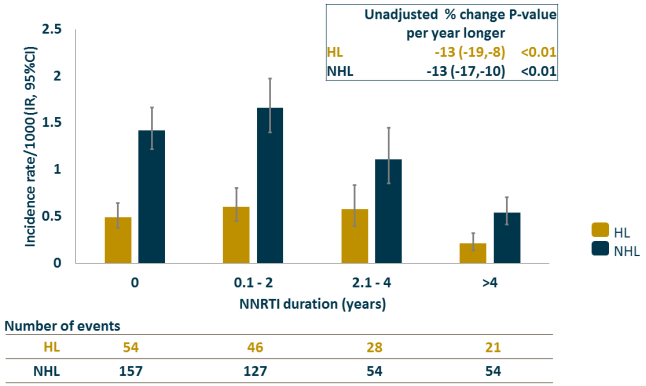


Figure 5.12 Crude incidence of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma by duration of non-nucleoside reverse transcriptase inhibitor (NNRTI) use.

HL: Hodgkin lymphoma, NHL: non-Hodgkin lymphoma, AUC: area under the curve, HIV-VL: HIV viral load, cART: combination antiretroviral therapy, PI: protease inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor.

5.3.3 Comparison and selection of immunological measures for multivariate model

The purpose of this section is to demonstrate the process of selecting one immunological marker to include in the analysis going forward. The aHRs for models containing each immunological measure modelled individually (not adjusting for other immunological factors) are shown in Table 5.5 and corresponding AICs in Table 5.6. All measures of immune deficiency were strongly associated with NHL. The rate of NHL declined with increasing current and nadir CD4, and increased with longer duration of time with CD4< 200 cells/mm^3 (Table 5.5). A lower rate of NHL was also associated with increasing time since last CD4 cell count < 200 cells/mm^3, however this was almost completely driven by the current CD4 level (Table 5.5). The best fit as given by the lowest AIC was for current CD4 cell count (AIC: 7779, Table 5.6). Rate of HL declined with increasing current and nadir CD4 cell count, but was not associated with duration of time with CD4< 200 cells/mm^3 (Table 5.5). Similarly to NHL, a lower rate of HL was also associated with increasing time since last CD4 cell count < 200 cells/mm^3, however this was also almost completely driven by the current CD4 cell count (Table 5.5). The best fit as given by the lowest AIC was for current CD4 cell count (AIC: 3064, Table 5.6). Based on these exploratory analyses and the AIC, the current CD4 cell count was included in analyses of both HL and NHL going forward.

Table 5.5 Adjusted Hazard ratios (aHR) and 95% confidence intervals for immunological factors (modelled separately) associated with non-Hodgkin (NHL) and Hodgkin lymphoma (HL).

	NHL		HL	
Immunological factors (fit separately)	**aHR[1] (95%CI)**	**P**	**aHR[1] (95%CI)**	**P**
Current CD4 cell count (cells/mm^3)				
<100	8.08 (5.63,11.61)	<.001	4.58 (2.22,9.45)	<.001
100 - 199	3.67 (2.49,5.39)	<.001	6.36 (3.62,11.20)	<.001
200 - 299	2.96 (2.12,4.13)	<.001	3.37 (1.99,5.69)	<.001
300 - 399	2.43 (1.78,3.33)	<.001	1.72 (0.99,2.97)	0.053
400 - 499	1.62 (1.15,2.29)	0.006	2.59 (1.64,4.08)	<.001
>=500	reference		reference	
Nadir CD4 cell count (cells/mm^3)				
<100	4.17 (2.36,7.35)	<.0001	5.03 (1.46,17.30)	0.0105
100 - 199	3.07 (1.74,5.41)	0.0001	5.83 (1.73,19.70)	0.0045
200 - 299	2.34 (1.34,4.09)	0.0028	5.41 (1.62,18.01)	0.0059
300 - 399	1.94 (1.09,3.43)	0.0235	2.47 (0.69,8.80)	0.1631
400 - 499	1.42 (0.74,2.75)	0.2938	2.99 (0.79,11.32)	0.1063
>=500	reference		reference	
Duration of time with CD4 cell count < 200 cells/mm^3				
0	reference		reference	
0.1 - 2	1.37 (1.06,1.76)	0.0146	1.32 (0.91,1.92)	0.1461
2.1 - 4	1.28 (0.82,1.98)	0.2743	0.84 (0.37,1.89)	0.672
>4	1.82 (1.22,2.72)	0.0034	1.37 (0.66,2.86)	0.3961
Unknown	4.06 (1.53,10.75)	0.0048	Not estimated	-
Time since last CD4 cell count < 200 cells/mm^3				
0	reference		reference	
0.1 - 2	0.48 (0.33,0.70)	0.0001	0.30 (0.15,0.57)	0.0003
2.1 - 4	0.36 (0.21,0.61)	0.0002	0.40 (0.19,0.81)	0.0111
>4	0.38 (0.26,0.57)	<.0001	0.20 (0.10,0.39)	<.0001
Unknown	0.39 (0.29,0.51)	<.0001	0.32 (0.20,0.51)	<.0001

aHR: adjusted hazard ratio, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma. [1]All models were adjusted for age, gender, ethnicity, mode of HIV acquisition, smoking status, body mass index, baseline year, cumulative time on combination antiretroviral therapy, Hepatitis B and C status, prior AIDS defining cancers (ADC), prior AIDS events (excluding ADC), hypertension, diabetes, cardiovascular disease, duration of time on treatment, current HIV Viral load and area under the curve of HIV viral load.

Table 5.6 AIC from adjusted models containing each immunological factor separately for non-Hodgkin (NHL) and Hodgkin lymphoma (HL).

Immunological factor	**NHL**		**HL**	
	AIC[1]	**Rank**	**AIC[1]**	**Rank**
Current CD4 cell count	7779	**1**	3064	**1**
Nadir CD4 cell count	7869	**2**	3096	**3**
Duration of time with CD4 cell count < 200 cells/mm^3	7895	**3**	3109	**4**
Time since last CD4 cell count < 200 cells/mm^3 (years)	7901	**4**	3084	**2**

AIC: Akaike information criterion, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma.
[1]All models were adjusted for age, gender, ethnicity, mode of HIV acquisition, smoking status, body mass index, baseline year, cumulative time on combination antiretroviral therapy, Hepatitis B and C status, prior AIDS defining cancers (ADC), prior AIDS events (excluding ADC), hypertension, diabetes, cardiovascular disease, duration of time on treatment, current HIV Viral load and area under the curve of HIV viral load.

5.3.4 Independent risk factors of non-Hodgkin and Hodgkin lymphoma

Both unadjusted and adjusted results of the Cox models to identify risk factors for NHL and HL are shown in Table 5.7 and Table 5.8 respectively.

Focussing on the adjusted associations with NHL (Table 5.7) a higher rate of NHL was strongly associated with lower current CD4 cell count category (CD4< 100 vs ≥ 500 cells/mm^3 aHR: 8.08 95%CI: 5.63, 11.61), higher current HIV-VL (HIV-VL> 1000 vs <50 copies/mL aHR: 1.97 95%CI: 1.50, 2.59) and higher AUC of HIV-VL (highest vs lowest quintile aHR: 2.91 95%CI: 1.92, 4.41). Higher rates of NHL were also observed in older people, where those aged over 60 years had a 3.15-fold higher rate (95%CI: 2.17, 4.58) compared to those aged 30-39 years, in men compared to women (aHR: 0.55 95%CI: 0.40, 0.76), and in those with a prior ADC (excluding NHL) (aHR: 1.49 95%CI: 1.02, 2.19).

The results for HL are shown in Table 5.8. Higher rates of HL were also associated with lower current CD4 cell count category (CD4< 100 vs ≥ 500 cells/mm^3 aHR: 4.58 95%CI: 2.22, 9.45), however, the highest rate was in those with a low-moderate CD4 cell count (CD4 cell count 100 – 199 vs ≥ 500 cells/mm^3 aHR: 6.36 95%CI: 3.62, 11.20), however no association was found for current or AUC of HIV-VL. Other factors associated with the rate of HL included current smokers, who had almost a 2-fold higher rate compared to non-smokers (aHR: 1.97 95%CI: 1.23, 3.16).

No association was found for HCV or HBV status with either NHL or HL. The HIV-VL associations were consistent when considering other markers of CD4 cell count as measures of immune deficiency.

Table 5.7 Unadjusted (HR) and adjusted Hazard ratios (aHR) and 95% confidence intervals for factors associated with non-Hodgkin lymphoma (NHL).

Factors	HR (95%CI)	P	aHR[2] (95%CI)	P
Age (years)[1]				
<30	0.53 (0.29,0.95)	0.032	0.49 (0.27,0.89)	0.019
30 - 39	reference		reference	
40 - 49	1.41 (1.08,1.85)	0.013	1.53 (1.16,2.01)	0.003
50 - 59	1.77 (1.30,2.40)	<.001	2.02 (1.47,2.78)	<.001
≥60	2.56 (1.82,3.62)	<.001	3.15 (2.17,4.58)	<.001
Female gender	0.44 (0.33,0.59)	<.001	0.55 (0.40,0.76)	<.001
Race				
White	reference		reference	
Other	0.51 (0.31,0.84)	0.009	0.65 (0.39,1.10)	0.109
Unknown	1.22 (1.00,1.50)	0.053	0.99 (0.80,1.24)	0.949
HIV transmission mode				
Sex between men	reference		reference	
IDU	0.89 (0.66,1.20)	0.451	0.91 (0.59,1.40)	0.677
Heterosexual	0.67 (0.53,0.84)	<.001	0.91 (0.70,1.20)	0.523
Unknown	1.23 (0.85,1.77)	0.279	1.35 (0.92,1.98)	0.125
Smoking[1]				
Current	1.08 (0.83,1.39)	0.58	0.91 (0.69,1.19)	0.48
Ex	1.05 (0.78,1.41)	0.747	0.89 (0.66,1.21)	0.461
Never	reference		reference	
Unknown	1.76 (1.28,2.42)	<.001	1.35 (0.96,1.89)	0.08
BMI group[1] (kg/m^2)				
<18	1.70 (1.08,2.68)	0.022	1.42 (0.89,2.26)	0.142
18 - 26	reference		reference	
37 - 30	1.10 (0.84,1.44)	0.495	1.13 (0.86,1.49)	0.384
>30	0.54 (0.31,0.95)	0.031	0.63 (0.35,1.10)	0.104
Unknown	1.34 (0.93,1.91)	0.113	1.16 (0.80,1.67)	0.437
Current CD4 cell count (cells/mm^3) [1]				
<100	13.25 (9.57,18.35)	<.001	8.08 (5.63,11.61)	<.001
100 – 199	5.17 (3.58,7.47)	<.001	3.67 (2.49,5.39)	<.001
200 – 299	3.83 (2.77,5.31)	<.001	2.96 (2.12,4.13)	<.001
300 – 399	3.00 (2.20,4.10)	<.001	2.43 (1.78,3.33)	<.001
400 – 499	1.91 (1.36,2.69)	<.001	1.62 (1.15,2.29)	0.006
≥500	reference		reference	
Current HIV-VL[1]				
≤ 50	reference		reference	
51 - 1,000	1.76 (1.31,2.37)	<.001	1.35 (1.00,1.83)	0.051
>1,000	3.19 (2.54,4.01)	<.001	1.97 (1.50,2.59)	<.001
Unknown	0.70 (0.17,2.83)	0.617	0.65 (0.15,2.81)	0.563
AUC of HIV-VL (Quintiles) [1]				
1 - Lowest	reference		reference	
2	1.32 (0.83,2.11)	0.237	1.10 (0.69,1.76)	0.685
3	1.84 (1.18,2.86)	0.007	1.47 (0.94,2.30)	0.088
4	2.48 (1.62,3.80)	<.001	1.81 (1.17,2.80)	0.007
5 - Highest	4.84 (3.24,7.21)	<.001	2.91 (1.92,4.41)	<.001
unknown	2.30 (1.37,3.88)	0.002	1.63 (0.93,2.85)	0.088
Previous ADC diagnosis (excl. NHL)[1]	1.83 (1.26,2.65)	0.002	1.49 (1.02,2.19)	0.041
Factors	**HR (95%CI)**	**P**	**aHR[2] (95%CI)**	**P**
Previous AIDS diagnosis (excl. ADC) [1]	1.27 (1.02,1.59)	0.03	1.01 (0.79,1.28)	0.967

HBV status[1]				
Positive/prior	1.19 (0.93,1.52)	0.178	1.10 (0.85,1.41)	0.479
Negative	reference		reference	
Unknown	1.11 (0.75,1.64)	0.598	0.97 (0.62,1.53)	0.906
HCV status[1]				
Positive/prior	1.13 (0.88,1.44)	0.342	1.13 (0.81,1.59)	0.462
Negative	reference		reference	
Unknown	1.24 (0.86,1.80)	0.25	1.20 (0.78,1.84)	0.416
Prior cardiovascular disease[1]	1.15 (0.67,1.96)	0.616	0.97 (0.55,1.70)	0.917
Diabetes[1]	1.22 (0.81,1.85)	0.338	1.16 (0.75,1.78)	0.499
Prior hypertension[1]	0.96 (0.75,1.22)	0.72	0.83 (0.63,1.08)	0.158

NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, HR: hazard ratio, aHR: adjusted hazard ratio, IDU: injecting drug use, BMI: body mass index, HIV-VL: HIV viral load, AUC: area under the curve, ADC: AIDS defining cancer, HBV: hepatitis B, HCV: hepatitis C.

[1]Time updated variables.

[2] Models adjusted for all factors in the table as well as for time on cART (results in Table 5.9).

Table 5.8 Unadjusted (HR) and adjusted Hazard ratios (aHR) and 95% confidence intervals for factors associated with Hodgkin lymphoma (HL).

Factors		HR (95%CI)	P	aHR[2] (95%CI)	P
Age (years)[1]					
	<30	0.88 (0.41,1.90)	0.742	0.90 (0.41,1.95)	0.781
	30 - 39	reference		reference	
	40 - 49	1.20 (0.78,1.85)	0.395	1.25 (0.81,1.93)	0.322
	50 - 59	1.42 (0.87,2.31)	0.159	1.37 (0.82,2.28)	0.224
	≥60	1.15 (0.60,2.20)	0.68	1.06 (0.53,2.15)	0.865
Female gender		0.50 (0.32,0.77)	0.002	0.73 (0.43,1.25)	0.253
Race					
	White	reference		reference	
	Other	0.70 (0.35,1.41)	0.318	0.98 (0.47,2.04)	0.948
	Unknown	1.12 (0.80,1.56)	0.502	0.95 (0.66,1.35)	0.758
HIV transmission mode					
	Sex between men	reference		reference	
	IDU	0.59 (0.34,1.02)	0.058	0.72 (0.32,1.59)	0.413
	Heterosexual	0.57 (0.39,0.83)	0.003	0.67 (0.42,1.06)	0.084
	Unknown	0.69 (0.33,1.42)	0.314	0.78 (0.37,1.66)	0.525
Smoking[1]					
	Current	1.91 (1.22,3.00)	0.005	1.97 (1.23,3.16)	0.005
	Ex	1.55 (0.94,2.57)	0.086	1.54 (0.92,2.58)	0.098
	Never	reference		reference	
	Unknown	1.45 (0.75,2.77)	0.266	1.25 (0.63,2.47)	0.523
BMI group[1] (kg/m^2)					
	<18	0.61 (0.19,1.92)	0.398	0.59 (0.18,1.86)	0.366
	18 - 26	reference		reference	
	37 - 30	0.83 (0.53,1.32)	0.43	0.89 (0.56,1.42)	0.631
	>30	1.03 (0.55,1.92)	0.921	1.28 (0.67,2.43)	0.45
	Unknown	0.52 (0.21,1.29)	0.159	0.54 (0.22,1.33)	0.181
Current CD4 cell count (cells/mm^3) [1]					
	<100	3.81 (1.90,7.64)	<.001	4.58 (2.22,9.45)	<.001
	100 – 199	5.54 (3.24,9.48)	<.001	6.36 (3.62,11.20)	<.001
	200 – 299	3.07 (1.84,5.11)	<.001	3.37 (1.99,5.69)	<.001
	300 – 399	1.62 (0.94,2.79)	0.081	1.72 (0.99,2.97)	0.053
	400 – 499	2.53 (1.61,3.97)	<.001	2.59 (1.64,4.08)	<.001
	≥500	reference		reference	
Current HIV-VL (copies/mL)[1]					
	≤50	reference		reference	
	51 - 1,000	1.35 (0.87,2.08)	0.177	1.08 (0.69,1.69)	0.724
	>1,000	0.87 (0.55,1.38)	0.559	0.67 (0.39,1.17)	0.158
	Unknown	0.70 (0.10,5.07)	0.727	3.73 (0.22,62.35)	0.36
AUC of HIV-VL (Quintiles) [1]					
	1 - Lowest	reference		reference	
	2	0.70 (0.41,1.21)	0.205	0.65 (0.37,1.12)	0.12
	3	0.86 (0.51,1.44)	0.556	0.79 (0.47,1.34)	0.379
	4	0.76 (0.44,1.30)	0.308	0.65 (0.38,1.14)	0.133
	5 - Highest	1.26 (0.78,2.04)	0.349	1.01 (0.61,1.68)	0.965
	unknown	0.22 (0.05,0.95)	0.042	0.14 (0.02,1.04)	0.055

Previous ADC diagnosis (excl. NHL)[1]		1.36 (0.69,2.66)	0.377	1.10 (0.55,2.18)	0.792
Previous AIDS diagnosis (excl. ADC) [1]		1.08 (0.75,1.55)	0.691	0.99 (0.67,1.46)	0.956
HBV status[1]					
	Positive/prior	0.75 (0.47,1.19)	0.22	0.75 (0.47,1.20)	0.232
	Negative	reference		reference	
	Unknown	0.63 (0.28,1.43)	0.265	0.94 (0.40,2.25)	0.897
HCV status[1]					
	Positive/prior	0.68 (0.44,1.06)	0.091	0.66 (0.35,1.24)	0.193
	Negative	reference		reference	
	Unknown	0.30 (0.10,0.95)	0.041	0.33 (0.10,1.10)	0.07
Prior cardiovascular disease[1]		1.60 (0.78,3.27)	0.196	1.56 (0.73,3.34)	0.251
Diabetes[1]		0.73 (0.32,1.66)	0.452	0.72 (0.31,1.68)	0.451
Prior hypertension[1]		1.10 (0.76,1.60)	0.617	1.01 (0.67,1.51)	0.972

NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, HR: hazard ratio, aHR: adjusted hazard ratio, IDU: injecting drug use, BMI: body mass index, HIV-VL: HIV viral load, AUC: area under the curve, ADC: AIDS defining cancer, HBV: hepatitis B, HCV: hepatitis C.

[1]Time updated variables.

[2] Models adjusted for all factors in the table as well as for time on cART (results in Table 5.9).

5.3.5 HIV treatment

The aHR for NHL and HL associated with being on treatment and increasing time on treatment are shown in Table 5.9. There was no difference in rate of NHL or HL in those on cART vs those not on cART, however, risk of both NHL (aHR per year longer on cART: 0.92 95%CI: 0.89, 0.95) and HL (aHR per year longer on cART: 0.92 95%CI: 0.87, 0.97) were reduced with each additional year on cART. Rate of NHL was significantly lower in those with 2 or more years of PI exposure compared to those who had never used PIs. The rate of HL did not decline with increasing time on PIs (aHR per year longer: 0.98 95%CI: 0.93, 1.03). The rate of both NHL and HL were significantly lower in those with more than 4 years of NNRTI exposure compared to those who had never used NNRTIs.

Table 5.9 Adjusted hazard ratios for non-Hodgkin (NHL) and Hodgkin (HL) lymphoma for current use of cART and duration of time on cART overall and according to regimen.

Cumulative time on treatment	aHR[1] (95%CI)	
	NHL	HL
On cART[2]	1.06 (0.81, 1.37)	1.11 (0.69, 1.81)
cART duration (Years)[3]		
Never on cART	Reference	Reference
0.1 - 2	1.22(0.87,1.7)	1.31(0.67,2.55)
2.1 - 4	0.72(0.49,1.08)	0.8(0.39,1.68)
>4	0.49(0.34,0.7)	0.61(0.3,1.21)
Per year longer	0.92 (0.89,0.95)	0.92 (0.87,0.97)
On PI vs Not on PI[4]	1.09 (0.85, 1.39)	1.38 (0.90, 2.13)
PI duration (Years)[5]		
Never on PI	Reference	Reference
0.1 - 2	1.01(0.77,1.32)	0.87(0.55,1.38)
2.1 - 4	0.62(0.43,0.88)	0.48(0.26,0.91)
>4	0.55(0.41,0.74)	0.75(0.47,1.19)
Per year longer	0.95 (0.92,0.98)	0.98 (0.93,1.03)
On NNRTI vs Not on NNRTI[4]	1.19 (0.92, 1.53)	0.92 (0.59, 1.43)
NNRTI duration (Years)[5]		
Never on NNRTI	Reference	Reference
0.1 - 2	1.12(0.87,1.44)	0.96(0.63,1.45)
2.1 - 4	0.87(0.63,1.21)	0.93(0.57,1.51)
>4	0.65(0.46,0.92)	0.42(0.24,0.74)
Per year longer	0.93 (0.89,0.97)	0.86 (0.8,0.92)

NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, aHR: adjusted hazard ratio, cART: combination antiretroviral therapy, PI: protease inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor.

[1]All models were adjusted for age, gender, ethnicity, mode of HIV acquisition, smoking status, body mass index, baseline year, cumulative time on cART, HCV and HBV status, prior AIDS defining cancers (ADC), prior AIDS events (excluding ADC), hypertension, diabetes, cardiovascular disease, current CD4 cell count, current HIV-viral load and AUC of HIV-viral load.

5.3.6 Differences in risk factors for non-Hodgkin and Hodgkin lymphoma

Factors that were associated with either NHL or HL in Table 5.7 and Table 5.8 were considered as potential differential factors, that is, they were jointly modelled to determine if they were differently associated with NHL and HL (Figure 5.13). For each factor, the ratio of the aHR for NHL relative to the aHR for HL was calculated. Current HIV-VL was a differential factor, as demonstrated by the increase in ratio of the aHRs with higher HIV-VL category. For example, the ratio of the aHRs (of NHL relative to HL) was 2.52 (95%CI: 1.32, 4.81) and 4.57 (95%CI: 2.20, 9.50) in those with current HIV-VL 50 – 1000 copies/mL and >1000 copies/mL relative to <50 copies/mL respectively. This indicates a progressively stronger relative association between current HIV-VL and NHL than for HL, where current HIV-VL >1000 copies/ml was a more than 4.5-fold stronger predictor for NHL compared to HL. A similar result was found for the AUC of HIV-VL, which was also a differential factor. Current CD4 cell count was not a differential factor

as the 95%CI of the ratio of the aHRs for each CD4 category relative to CD4 cell count 200 - 299 cells/mm^3 contained 1, indicating no evidence that lower CD4 cell count was differently associated with NHL and HL. Age was a differential risk factor as the ratio of the aHRs of people aged > 60 relative to those aged 30 – 39 was more than 3 (95%CI: 1.43, 6.55), indicating a stronger relative association between current HIV-VL and NHL than for HL for people in this older age group. Finally, smoking status was a differential factor, as the ratio of the aHRs for current smokers relative to never smokers was 0.49 (95%CI: 0.28, 0.83), indicating a stronger relative association with HL than NHL in this group. None of the other factors investigated were differential factors, including gender, HIV transmission mode, or time on cART either overall or stratified by use of a PI or NNRTI.

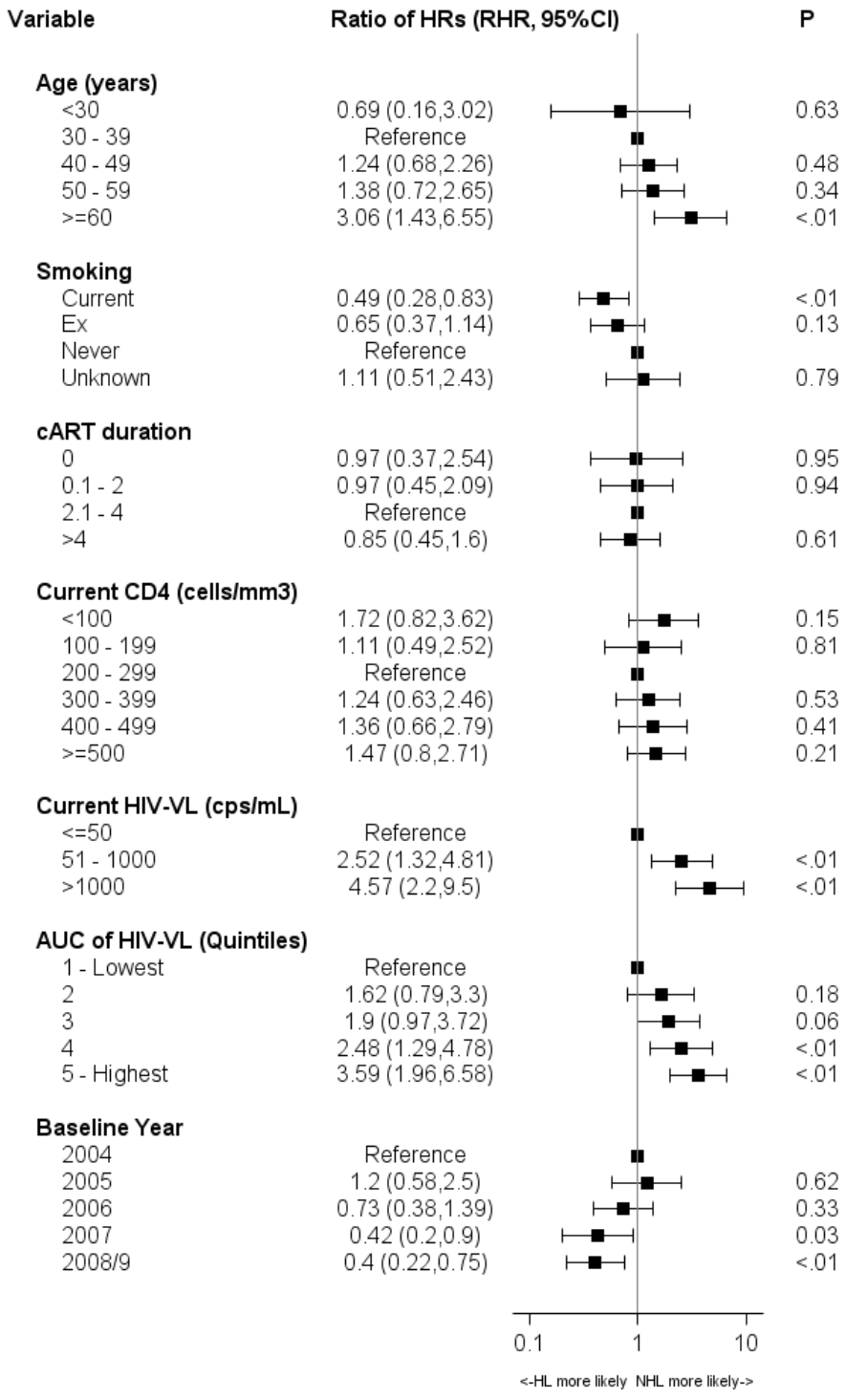


Figure 5.13 Adjusted ratio of the hazard ratios of NHL (NHL HR) and HL (HL HR) for each considered risk factor, and the ratio of the HR (RHR).

NHL: non Hodgkin lymphoma, HL: Hodgkin lymphoma, HR: adjusted hazard ratios, RHR: ratio of the HRs, cART: combination antiretroviral therapy, HIV-VL: HIV viral load, AUC: area under the curve. Model was also adjusted for gender, ethnicity, mode of HIV acquisition, body mass index, baseline year, hepatitis C and B status, prior AIDS defining cancers (ADC), prior AIDS events (excluding ADC), cardiovascular disease, diabetes, hypertension. Variables for which the 95%CI for the ratio of the hazard ratios of NHL to HL cross 1 indicate no evidence of a difference in HRs for NHL relative to HL. A P value < 0.05 corresponds to a ratio of the HR significantly different to 1, i.e. a significantly different HR for NHL relative to HL (or vice versa).

5.3.7 Non-Hodgkin lymphoma subtypes

Of the 392 NHL, 42 were Burkitt (10.7%), 25 were PBCNS (6.4%), 65 were immunoblastic (16.6%), and 260 were unknown (66.3%). Those with known and unknown subtype were similar in age, gender, mode of transmission, smoking status, comorbidities, immunological and virological markers and use of cART at diagnosis (all P>0.05). Slightly more people with known subtype reported being on PIs (known subtype: 46.2% vs unknown subtype: 38.6%, P=0.05), and slightly fewer had a previous cancer diagnosis (3.1% vs 10.6%, P=0.02). In the 33.7% of people who had a subtype recorded, associations between immunological and virological risk factors modelled separately for each subtype are shown in Table 5.10. For example, lower risk of PBCNS NHL was associated with higher current CD4 cell count (aHR for 2-fold higher: 0.59 95%CI: 0.51, 0.70) and nadir CD4 cells count (aHR for 2-fold higher: 0.68 95%CI: 0.58, 0.80) and lower AUC of HIV-VL (aHR for 10-fold higher: 2.21 95%CI: 1.22, 4.00) but not current HIV-VL. Lower risk of immunoblastic NHL was associated with higher current and nadir CD4 and lower current and AUC of HIV-VL. Lower Burkitt NHL risk was associated with higher current and AUC of HIV-VL but not immunological markers. Nadir CD4 cell count was not associated with PBCNS, immunoblastic or Burkitt lymphoma after additional adjustment for current CD4 cell count.

Table 5.10 Adjusted Hazard ratios (aHR) and 95% confidence intervals for factors associated with non-Hodgkin lymphoma (NHL) subtypes.

	PBCNS (N=25)		Immunoblastic (N=65)		Burkitt (N=42)	
	aHR[1] (95%CI)	**P**	**aHR[1] (95%CI)**	**P**	**aHR[1] (95%CI)**	**P**
log_2 current CD4[2]	0.59 (0.51,0.70)	<0.01	0.75 (0.67,0.85)	<0.01	0.88 (0.74,1.05)	0.15
log_2 nadir CD4[2]	0.68 (0.58,0.80)	<0.01	0.87 (0.77,0.98)	0.02	0.97 (0.82,1.14)	0.68
log_{10} current HIV-VL[3]	1.11 (0.80,1.54)	0.54	1.32 (1.08,1.61)	0.01	1.38 (1.08,1.76)	0.01
Log_{10} of AUC of HIV-VL[3]	2.21 (1.22,4.00)	<0.01	1.60 (1.16,2.21)	<0.01	2.14 (1.35,3.38)	<0.01

NHL: non-Hodgkin lymphoma, PBCNS: Primary brain and central nervous system, HIV-VL: HIV viral load, AUC: area under the curve.
[1]Models were adjusted age, gender, mode of HIV acquisition, race, current smoking status, cumulative time on combination antiretroviral therapy.
[2]Models were additionally adjusted for log_{10} current HIV-VL and Log_{10} of AUC of HIV-VL in addition to variables listed in (1).
[3]Models were additionally adjusted for log_2 current CD4 in addition to variables listed in (1).

5.4 Discussion

This study is the first to directly compare the risk factors for NHL and HL in order to identify differences in the underlying pathology of both in HIV+ people. Higher current and accumulated HIV-VL were stronger risk factors for NHL than HL. Although current CD4 cell count and gender were strong predictors of both, they were equally associated with risk of NHL and HL.

Furthermore, these results identified independent risk factors for NHL and HL, which were consistent with those described in the existing literature. In this study, the crude incidence of NHL declined steadily over time while HL incidence remained stable, and as a result, incidence of NHL and HL has been similar in HIV+ people in recent years.

Before discussing the main results of this chapter, it is important to note that most analyses presented treat NHL as a single entity, however, there is considerable heterogeneity in aetiology between NHL subtypes [804, 838]. This is somewhat demonstrated in that the risk of NHL subtypes differed according to immunological and virological factors [804], however, the low number of NHL with known subtype prevented me from exploring this in depth. This should be kept in mind when interpreting the results for NHL.

5.4.1 Changes in incidence of non-Hodgkin and Hodgkin lymphoma over time

The incidence of NHL declined over time by 13% per year while HL incidence remained stable at around 0.36/1000 PYFU. The NHL decline is consistent with previous studies which have shown a clear decline for non-Burkitt subtypes (i.e. DLBC and PBCNS NHL) [15, 16, 19-21, 430, 438, 439, 441-443, 451, 467]. The trends over time are not surprising and have been reported before, however, my study is one of the first to show a convergence of incidence of NHL and HL in HIV+ people in recent years. A convergence in NHL and HL has also been suggested in a German study in the context of sustained viral suppression and limited immune suppression [839]. This result demonstrates a shift away from the cancers with a strong link with immune deficiency, and highlights the need for a better understanding of how generalised immune dysfunction above and beyond low CD4 cell counts can facilitate lymphoma development.

5.4.2 Immunological and virological factors

This chapter shows that the risk of NHL and HL differs according to current and accumulated HIV-VL, but not current CD4. It is believed that HIV infection facilitates lymphoma development by inducing immune suppression [608, 840, 841]. This has a number of consequences including reduced control of oncogenic viruses (such as EBV) [608, 840, 841]. My results indicate a similar role of immunodeficiency in both NHL and HL development, however, an important distinction is the involvement of HIV-VL in NHL but not HL development.

My findings that NHL is independently associated with both current CD4 and current and accumulated HIV-VL are consistent with previous research [429, 433, 435, 441, 468, 818] and indicates that NHL behaves like a typical opportunistic disease. The risk of opportunistic disease is higher at very low CD4 cell counts, but is also higher in people with uncontrolled HIV replication regardless of CD4 cell count [842]. This was experimentally shown by the results of the START trial, which demonstrated that risk of AIDS defining events was not zero among those on immediate cART indicating that immune damage may occur early in HIV infection and lead to ongoing immune dysfunction and AIDS defining events [141]. One clinician I spoke to speculated that he would not be surprised if I observed a biphasic association between CD4 and NHL incidence, due to the vastly different associations between DLBC and Burkitt lymphomas and CD4 cell count, however, this was not observed. Subtype information is missing on two thirds of the NHL events. However, close to two thirds of those with known subtype were PBCNS or DLBCL NHL, both of which are associated with very low CD4. Therefore, if we assume the distribution of NHL in the unknown subtypes is similar to that in the known, it is likely these subtypes are driving the strong association with low CD4 cell count.

The association between NHL and current and cumulative HIV-VL indicate that HIV infection may contribute to lymphomagenesis of NHL through additional immune dysfunction, not captured by a supressed CD4 cell count [141, 840]. These may include reduced immune surveillance for proteins expressed by cells infected with latent EBV, immune activation, cytokine over production, and CD8-dysfunction leading to depleted response from EBV specific CD8 cytotoxic cells [606-608, 806, 840, 843-845]. Some studies have found that levels of circulating free light chains are predictive of NHL in HIV+ people, which supports the involvement of immune activation [846, 847]. This is further explored in chapter 6, which investigates several markers of immune activation preceding NHL and HL diagnosis. Recent data have suggested that HIV may play a more direct role in lymphomagenesis [840, 848], with HIV-derived p17 secreted within lymphoid tissues, possibly promoting lymphoma development by inducing changes to the microenvironment [848].

The finding that HL incidence increases with lower current CD4 cell count with highest incidence in CD4 cell counts ranging from 100 – 199 cells/mm^3, but no association with virological measures is also consistent with previous research [433, 471, 500]. Almost all cases of HL are associated with EBV in the context of HIV [474, 499, 500, 810-814], and thus, the impact of HIV-infection on HL development may be primarily driven by a balance between impaired immune function and loss of control of EBV as well as remaining immune activity to allow for the influx

of immune cells to the tumour microenvironment [849]. Low availability of CD4 cells in the severely immune suppressed may explain the lower HL incidence observed in those with CD4 cell counts < 100 cells/mm^3 [470]. The RS cells secrete chemokines and chemokines which attract activated CD4 cells (and other cells) to their microenvironment which are induced to proliferate near the tumour cells in order to produce molecules required for RH cell proliferation and to prevent apoptosis [470, 850, 851]. The cytokine and chemokines secreted contribute to both the growth of RS cells as well as the maintenance of an environment in which a sufficient immune response to RH cells cannot be achieved [470, 850, 851]. This clinically manifests as a decline in CD4 (as well as total lymphocyte and CD8) cell counts, independent of HIV-VL level, due to influx of CD4 cell counts to the tissue of the HL often within 1-2 years prior to diagnosis [468, 586]. In addition, it has been suggested that an unbalanced pool of EBV-specific CD4 and or CD8 cells may increase the risk of HL in HIV+ people [586].

5.4.3 HIV treatment

The link between increasing treatment duration and reduced incidence of NHL and HL is consistent with the previous literature [18, 20, 433, 435, 818, 828, 829]. Longer duration of PI and NNRTI based regimens both resulted in a decline in NHL risk. Longer duration of NNRTI use was associated with lower HL risk, however, the decline in HL risk for longer duration of PI use was not significant. A previous study from the D:A:D collaboration showed that that use of NNRTIs was associated with a slight reduction in HL incidence but no association with PIs was shown, which is consistent with my results. However in my study, there was no evidence that PI use or NNRTI use were differential factors for NHL or HL risk. The use of measures of cART duration in the D:A:D study are based on clinical notes of starting, continuing, swapping, and stopping cART regimens. This does not take into account the actual adherence of the individual and it is possible that people are misclassified as being on cART when they do not actively take their treatment.

5.4.4 Smoking status

Current smoking was a differential factor, where those who were current smokers had a higher risk of HL but not NHL. Previous research in the general population has indicated that smoking may play an etiological role in HL, specifically, EBV positive HL [799, 800]. This is particularly relevant as almost all HL in HIV+ people are EBV related, and therefore smoking presents as one of the few modifiable risk factors for this cancer. A possible biological explanation for this association is the known negative impacts of smoking on the immune system [852] which could

amplify the effects of HIV-induced immune deficiency and thus loss of control of latent EBV. These results provide additional evidence for the importance of smoking cessation strategies in HIV+ people. The benefits of smoking cessation on cancer incidence in HIV+ people are explored in chapter 4.

5.4.5 Hepatitis B and C status

This study did not find a significant association between HCV or HBV coinfection and increased risk of lymphomas. This is in contrast to results from the COHERE study, which found a significant association between both HCV and HBV and NHL risk in treated patients [830], and two meta analyses [831, 832]. However, my results are consistent with the bulk of the literature that found a negative association with HCV [433, 818, 833, 834]. Interestingly, the direction of effect for the association between HCV and HL was protective, likely due to competing risks of death or higher rates of loss to follow-up in HCV coinfected people. Furthermore, low number of people on treatment in the early years of follow-up may further mask a possible association.

5.4.6 Other factors

Risk of NHL and HL differed according to age, where the people aged over 50 years had a stronger relative association with NHL than HL, which is consistent with previous studies [433, 450, 586, 606, 815, 853]. This may reflect age associated immune activation and inflammation and further supports the hypothesis that immune dysfunction beyond a low CD4 cell count is involved in the pathology of NHL [721]. Alternatively, this may also reflect longer exposure to EBV infection and associated impacts on the immune system (for more detail on HIV and aging see chapter 1 section 1.2.4) [3, 617]. NHL incidence is strongly associated with age in the general population [784], and therefore, the association in HIV+ people may just mirror that in the background population. The higher NHL and HL (although not significant) incidence in men is interesting. A similar but less marked observation has been made in the general population [784]. Furthermore, a consistent association between men who acquired HIV through sex between men and elevated risk for both NHL and HL have been shown in HIV+ people [433, 435, 606, 815, 817]. Some studies have suggested that this difference may reflect differences in exposures to known environmental risk factors for NHL or higher prevalence of EBV in this population [606]. After adjustment for gender, no association with mode of HIV transmission was found in this study. I also attempted to replace gender and mode of transmission with a gender specific HIV transmission mode variable grouped as follows: sex between men, female

IDU, and male IDU, female heterosexual, and male heterosexual, other/unknown, which had no impact on the results.

5.4.7 Strengths of these analyses

The major strengths of this study include the extensive follow-up on over 40,000 people available within the D:A:D data study. This allowed for the analysis of a relatively large number of prospectively collected and validated NHL and HL events. The end point review process in D:A:D (detailed in chapter 2 section 2.1.2.5) minimises the risk of misclassification of lymphomas as other cancers, as lymphomas can develop in almost any part of the body. Furthermore, the D:A:D study contains highly detailed individual level data on demographic, HIV, and health related factors collected over a long follow-up period. In addition, this is one of the only studies to directly compare NHL and HL risk factors within the same cohort, which further strengthens my results.

5.4.8 Limitations of these analyses

However, the study has a number of limitations which need to be kept in mind when interpreting these data. First, there were several known risk factors for NHL and HL that are not collected in D:A:D and could not be taken into account. For example, environmental exposures, autoimmune disorders, family history, and EBV coinfection [607, 788, 789]. Second, NHL subtype is only reported by a subset of cohorts in D:A:D, and as a result it was missing on 66% of people, however I did compare those with and without subtype information, and they were similar in terms of demographics, HIV-related and treatment information. In addition, D:A:D does not collect information on cancer treatment or care following diagnosis and analyses of patient outcomes following diagnosis are not possible. Furthermore, other markers of immune suppression (such as CD8 cell counts), markers of immune dysfunction, or EBV viral load are not collected. Although D:A:D has extensive follow-up, data on CD4 and HIV-VL are only available since date of entry into D:A:D or HIV-diagnosis. This means that estimated cumulative and historical measures of HIV-VL and CD4 will underestimate true exposure to replicating HIV-VL and immune suppression due to unknown levels during their time living with undiagnosed and untreated HIV as well as time prior to entry into D:A:D.

The D:A:D dataset is a collaboration of 11 cohorts, each of which have differences in terms of the data collected and data quality. For example, demographic and clinic data is reported differently to each cohort and this may impact on the quality and consistency of the data.

However, all contributing cohorts perform random monitoring of at least 10% of study participants' clinical records to ensure events are not missed. Finally, this is an observational study and I cannot rule out the effects of residual, unknown, and unmeasured confounding on the results and causality of the associations presented cannot be determined.

5.5 Conclusions

In conclusion, this chapter investigated and compared the variations in factors associated with NHL and HL development in HIV+ people. It has demonstrated that although CD4 cell depletion increased risk of both types of lymphomas, current and accumulated HIV-VL had a stronger relative association with NHL than HL. This suggests that NHL development is related to both CD4 cell depletion and added immune dysfunction derived from ongoing HIV replication, which is consistent with opportunistic disease. This latter factor is not affecting HL risk. In addition, these results support that HL in HIV+ people is the result of a balance between impaired immune function and loss of control of EBV as well as remaining immune activity to allow for the influx of immune cells to the tumour microenvironment. Furthermore, the incidence of NHL and HL are now similar within HIV+ people. These findings stress the importance of early HIV diagnosis and treatment, and of ensuring sustained viral suppression.

5.6 Publications

A manuscript of this chapter was submitted to the Journal of the National Cancer Institute (JNCI) in 2017. The results were presented at the HIV drug therapy Glasgow conference in 2016. Preliminary results from the EuroSIDA study were also presented at CROI 2015. The manuscript and slides are included in Appendix VI, Appendix VII, and Appendix VIII respectively.

6 The extent of B-cell activation preceding lymphoma development in HIV-positive people

6.1 Introduction

In the previous chapter (chapter 5), I investigated and compared the involvement of HIV associated immune suppression and viral replication in the development of non-Hodgkin (NHL) and Hodgkin (HL) lymphoma in HIV-positive (HIV+) people. In this chapter, I will expand on the results of chapter 5 by exploring markers of immune activation leading up to the diagnosis of lymphomas in HIV+ people.

As explored in chapter 3, HIV+ people have particularly elevated risk of infection related cancers (IRC) [8, 15, 18, 616, 854]. In the setting of HIV infection, the oncogenic virus Epstein-Barr Virus (EBV) has been associated with almost all cases of HL [474, 499, 500, 810-814] and between 30-100% of cases of NHL, depending on subtype [474, 499, 500, 855, 856]. As shown in chapter 3, NHL is the most commonly occurring cancer since 2001, and HL the 4th most common, and together they accounted for approximately 40% of all diagnosed cancers in EuroSIDA. Similar results were found in the Swiss cohort study [430], the French Hospital Database on HIV cohort [433] and studies from the USA [436].

6.1.1 Pathogenesis of lymphomas in HIV-positive people

Although several processes are thought to contribute to lymphoma development in HIV+ people, the mechanisms driving pathogenesis are poorly understood. One of the main drivers of B-cell lymphomas is thought to be disruption to the immune system due to untreated HIV infection [507, 608, 857], characterised by polyclonal B-cell activation, hypergammaglobulinaemia, immune deficiency, immune dysfunction, impaired immune surveillance, senescence, and inflammation [475, 505-507, 608, 841, 858, 859]. Another is the loss of immunoregulatory control of EBV due to HIV mediated immune suppression and dysfunction which can result in the uncontrolled growth and transformation of EBV infected B-cells [608, 841]. It should be noted that lymphomas in HIV+ people are heterogeneous across NHL subtypes and HL in terms of pathology, pathogenic pathways, and cellular derivation [860]. Furthermore, infectious origin of a tumour can only be definitively determined through in situ hybridisation studies in tumour samples.

6.1.1.1 HIV infection and the development of lymphomas

Chronic B-cell activation due to HIV infection is thought to be one of the main mechanisms for B-cell lymphoma development in HIV+ people [608, 841]. Until recently, HIV was thought to largely play an indirect role in the development of lymphomas, as HIV infection causes immune suppression and facilitates the loss of control EBV-positive B-cells [608]. However, a more direct role for HIV beyond immune suppression is emerging [840, 848]. HIV derived p17 is secreted within lymphoid tissues, possibly promoting lymphoma development by inducing changes to the microenvironment of lymphoid tissue [840, 848, 861]. Furthermore, HIV virions themselves might induce B-cell activation, thus increasing the likelihood of chromosomal translocations and of oncogene mutations, leading to DNA damage that can result in lymphomas [608, 862]. It has been shown that accumulation of exposure to HIV replication is predictive of NHL development in HIV+ people, which further supports this theory [608, 816]. Finally, recent findings indicate viral cooperation between EBV and HIV in lymphoma development [841, 863]. This is the mechanism by which coinfection with 2 or more viruses simultaneously have synergistic or regulatory effects on the tumour microenvironment and promote carcinogenesis [863]. For example, highly oncogenic latency patterns (see section 6.1.1.2 and Figure 6.1) are observed during untreated HIV infection due to loss of immune surveillance against EBV [864], however, studies have indicated a shift towards lymphomas associated with lower latency patterns during the post combination antiretroviral therapy (cART) era [844]. Loss of T-cell response to EBV may also play a role, as HIV+ people with EBV-positive lymphomas have been shown to have compromised CD4 and CD8 T-cell responses to EBV protein EBNA1 [809, 845].

6.1.1.2 Epstein-Barr virus and the development of lymphomas

EBV is a gamma herpes virus that is estimated to infect >90% of the world's adult population. It has been implicated in the development of a wide range of B and T-cell NHL and HL as well as various cancers including nasopharynx and stomach carcinomas and smooth muscle tumours [471, 499, 865]. Despite widespread infection, EBV associated cancers are uncommon in people with preserved immunity [499].

EBV is transmitted through bodily fluids, in particular saliva, and infects the B-cells of the oral mucosa [499, 866]. Early after primary infection, EBV drives proliferation of various types of infected B-cells and the pool of infected cells expands [867]. The immune system quickly targets the proliferating cells for destruction by virus specific cytotoxic T-cells (or killer T-cells), however, EBV infection is maintained by a reservoir of resting latently infected B-cells [499, 867, 868].

These latent cells are not cycling, not targeted for immune destruction, and persist indefinitely [608, 869].

The EBV life cycle includes two states: lytic and latent [499]. New virions are produced during the lytic cycle [499]. Production of virions cease during latent infection, however, proliferation continues and the virus spreads through the B-cell compartment following primary infection [499]. There are three stages of latency (latency I, II and III) which are characterised by the different patterns of genes and viral proteins expressed (Figure 6.1). These different gene patterns are also found in tumours and indicate the stage of EBV infection in which they occurred [499]. EBV viral proteins produced during higher latency patterns are highly immunogenic (meaning they induce a strong immune response), and as a result, cancers associated with these latency patterns tend to occur in immune compromised people [499, 870].

Figure 6.1 Major patterns of Epstein-Barr virus latent gene expression in lymphoproliferative disorders [870].

The exact mechanisms of how EBV infection leads to lymphoma genesis is not understood. However, it is likely that EBV plays both a direct and indirect role in lymphoma genesis [608]. For example, EBV infection can induce B-cell activation either directly through its viral genes (such as Epstein–Barr nuclear antigen 1 [EBNA-1], which is a viral protein produced during all stages of latency and present in all B-cell lymphomas) or indirectly by regulating host gene expression in order to immortalise B-cells [608, 871, 872]. As mentioned in section 6.1.1, HIV+ people with poorly controlled HIV infection and reduced immune function are at higher risk of lymphoma due to loss of control of EBV-positive B-cells. This is the case for primary brain and central nervous system (PBCNS) lymphomas which are almost all EBV+ and strongly linked to immune deficiency [608, 804]. On the other hand, the role that EBV plays in the genesis of other lymphoma subtypes in the context of HIV, such as Burkitt or diffuse large B-cell (DLBC) lymphoma, is less clear [608]. For example, Burkitt lymphomas develop in HIV+ people with relatively preserved CD4 cell counts and around half are EBV-positive. Burkitt lymphoma tends

to occur as a consequence of errors in Ig gene rearrangement during B-cell hyperproliferation with hypergamaglobulinaemia, which is a feature of early HIV infection [873-875]. In addition, Immunoblastic DLBC lymphomas tend to occur at very low CD4 cell counts due to failure to eradicate EBV latency type III infected lymphocytes [500, 804, 876], whereas centroblastic DLBC lymphomas are weakly associated with low CD4 cell counts and less than 40% are EBV related [804]. Finally, EBV latent membrane protein 1 (LMP1) is expressed in almost all HL in HIV+ people, suggesting an etiological role of EBV in these cancer in the context of HIV [477].

Primary brain and central nervous system (PBCNS) lymphomas are almost all EBV+ and strongly linked to immune deficiency [16, 427, 433, 608, 804]. On the other hand, the role that EBV plays in the genesis of other lymphoma subtypes in the context of HIV, such as Burkitt or diffuse large B-cell (DLBC) lymphoma, is less clear [608]. For example, Burkitt lymphomas develop in HIV+ people with relatively preserved CD4 cell counts and around half are EBV-positive. Burkitt lymphoma tends to occur as a consequence of errors in Ig gene rearrangement during B-cell hyperproliferation with hypergamaglobulinaemia, which is a feature of early HIV infection [873-875]. In addition, Immunoblastic DLBC lymphomas tend to occur at very low CD4 cell counts due to failure to eradicate EBV latency type III infected lymphocytes [500, 804, 876], whereas centroblastic DLBC lymphomas are weakly associated with low CD4 cell counts and less than 40% are EBV related [804]. Finally, EBV latent membrane protein 1 (LMP1) is expressed in almost all HL in HIV+ people, suggesting an etiological role of EBV in these cancer in the context of HIV [477].

6.1.2 The lymphatic system

Lymphomas are cancers of the lymphatic system. The lymphatic system provides protection against pathogens and also produce immunoregulatory cytokines to coordinate other parts of the immune system [607, 877, 878]. Components of the lymphatic system run throughout the whole body, and for this reason, lymphomas can arise in almost any part of the body.

The immune system has two categories of responses: the innate and adaptive immune system [879]. The Innate response is the first line of defence to infection and occurs rapidly [879]. If the innate immune system fails to eliminate a pathogen, the adaptive immune system is initiated [879]. The adaptive immune response involves pathogen specific lymphocytes, which increases protection against reinfection with the same pathogen [879]. Lymphocytes are created in the central lymphatic organs (the thymus and bone marrow) and can differentiate into 3 lines: T-lymphocytes which operate in antibody and cellular immunity, B-lymphocytes which can

differentiate into plasma cells and secrete antibodies (or immunoglobulins), and natural killer (NK) cells [816, 878, 879].

6.1.3 B lymphocytes and the production of immunoglobulins

Mature B-lymphocytes primarily remain in peripheral lymphoid organs (the lymph nodes, tonsils, spleen, thymus, and mucosal and submucosal tissues of the alimentary and respiratory systems), where they can be activated by specific antigens and induce an immune response [877, 878]. The lymphocyte receptor repertoire is made up of millions of lymphocytes, each carrying a different receptor (a surface immunoglobulin molecule) which is specific to a single antigen (a protein on the surface of pathogens) [879, 880]. If the B-cell surface receptor binds to the specific antigen then the B-cell will become activated [816, 878-880]. At this point the cellular structure changes and the B-cell becomes a lymphoblast. The lymphoblast starts to proliferate (creating around 1000 identical clones) [878, 879]. This process is called “clonal expansion” and takes around 4-5 days to complete [878, 879]. The clones then differentiate into plasma cells which are able to secrete immunoglobulins [816, 878, 879].

6.1.4 The production of Immunoglobulins

Immunoglobulins are an essential part of the immune system [816, 878, 879]. Immunoglobulin molecules are made up 2 identical heavy chains and two identical light chains (Figure 6.2) [881]. There are 5 classes of antibodies: IgA, IgD, IgE, IgG, and IgM and two classes of light chains: κ or λ [816, 881]. Either type of light chain may be associated with any of the heavy chains [881]. Elevated total serum globulins are thought to reflect hypergammaglobulinaemia and generalised B-cell activation [882]. Immunoglobulins as markers of B-cell activation are present years before lymphoma diagnosis [846, 883], making them potentially useful markers of future lymphoma development which may also provide insight into the aetiology of these cancers [608]. Furthermore, polyclonal elevation of FLCs (a marker of polyclonal B-cell activation and indicates the presence of many different immunoglobulin molecules made by many different B-cells) is a marker of general immune activation and polyclonal hypergammaglobulinamia and has been linked to malignant disease [884]. Monoclonal elevation of FLCs indicates the presence of many identical immunoglobulins produced by many clones of a single parent B-cell and is indicative of several plasma cell proliferative disorders, including monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma [885, 886].

Figure 6.2 A typical immunoglobulin molecule [881].

6.1.4.1 Immunoglobulin M

IgM is the first class of immunoglobulin to be made by a developing B-cell (although many subsequently switch to IgG) and is the principal antibody produced during the early stages of a primary antibody response to infection [878, 881]. IgM accounts for 5-10% of serum immunoglobulins in adults and has a half-life of 5 days[885]. IgM levels can be detected in the blood 3–14 days after the first exposure to an antigen and peaks after around 10-14 days. IgM returns pre immune response levels after several weeks [878, 881]. Subsequent repeated exposure to an antigen induce a response involving IgG molecules (see Figure 6.3), and the antibody response is more rapid and for a longer duration. IgM is mainly found in the blood [878, 885].

Figure 6.3 Isotypes of serum antibodies in primary and secondary immunization [878].

6.1.4.2 Immunoglobulin D

IgD exists alongside IgM primarily as a surface receptor on early mature B-cell membranes to help initiate antibody responses by activating B-cell growth [878, 881, 885]. IgD is only secreted in small amounts (< 1% of serum immunoglobulins) [878].

6.1.4.3 Immunoglobulin G

IgG is the main immunoglobulin class in the blood and extracellular fluid. It accounts for around 85% of serum immunoglobulins in adults and has the longest half-life of 23 days [879, 885]. IgG primarily operates in tissues where it acts as an efficient opsonin, meaning it efficiently binds with surface antigens on pathogens to mark them for elimination [879]. In order for a plasma cell to produce IgG it must undergo a process called “class switching” which is initiated through an interaction between activated B-cells and T helper cells [885]. During this process, a region of the immunoglobulin molecule on the B-cell surface undergoes gene-rearrangement (or recombination) in order to switch from producing IgM (and IgD) to IgG [885]. There are four subclasses of IgG (IgG1 – IgG4) [878].

6.1.4.4 Immunoglobulin A

IgA at mucosal sites is one of the first lines of defence against infection [878]. IgA is the principal class of immunoglobulin in secretions, including saliva, tears, milk, and respiratory and intestinal secretions, and is the second most common immunoglobulin in serum (accounting for 5 – 15% of serum immunoglobulins) [878, 879, 881, 885]. IgA has a half-life of 6 days [885]. IgA primarily operates on epithelial surfaces and acts as a neutralising antibody [878], however, IgA also acts as an opsonin (but much less potent than IgG) [879]. The production of IgA by plasma cells involves the input of T-helper cells [885].

6.1.4.5 Free light chain kappa and lambda

The heavy and light chains are produced separately within the plasma cells and are then assembled to form a whole (“intact”) immunoglobulin. During immunoglobulin production, the B-cells produce more light chains relative to heavy chains, and excess unbound light chains (known as free light chains, FLCs) enter circulation. Both immunoglobulins and FLCs can be detected in serum [887, 888]. The relative concentrations of κ to λ (i.e. the FLC-κ/λ ratio) can be used as a numerical measure of clonality [886]. During polyclonal B-cell activation and immunoglobulin production, both FLC-κ and FLC-λ concentrations can be 30 to 40-fold higher

[886], however, the FLC-κ/λ ratio remains relatively unchanged [886]. In contrast, monoclonal B-cell activation is characterised by the excess production of one FLC type and an abnormal FLC-κ/λ ratio occurs [886].

6.1.4.6 Immunoglobulins and free light chains in HIV-positive people

Hypergammaglobulinaemia occurs early in HIV infection [889]. Ongoing B-cell dysfunction in HIV+ people is characterised by abnormally low levels of antibodies to specific pathogens and poor immune responses to vaccines. Paradoxically, total serum levels of IgG are actually elevated, reflecting a non-specific polyclonal activation of B-cells [858, 859, 882]. Immunoglobulin levels have been implicated as markers of B-cell hypergammaglobulinaemia and generalised B-cell activation [882]. However, studies linking immunoglobulin levels and lymphoma development in HIV+ people have had mixed results, with some finding elevated risk with higher level of IgG [890], and others finding no association (serum globulin) [606, 846, 891]. Studies in the general population have found lower levels of IgM, IgA and IgG prior to lymphoma [892], and levels declined with more advanced disease [893], speculated to be a consequence of lymphoma development.

The level of individual FLCs (FLC-κ and FLC-λ), the ratio (FLC-κ/λ), and the sum of FLC-κ and FLC-λ (FLC-κ+λ) are markers of B-cell activation and hypergammaglobulinaemia [846, 847, 894]. Non-specific polyclonal B-cell activation has been shown to be strong and sensitive predictor of the risk of lymphomas in HIV+ people [846, 847, 894]. Polyclonal FLC elevation levels have also been associated with AIDS [895], HIV severity [896], and are reduced in those on cART [896].

6.1.5 Objectives

HIV associated immune deficiency, B-cell dysfunction, B-cell activation, as well as reactivation of latent EBV infection all play a role in lymphoma development. This chapter aims to investigate the relationship between B-cell activation, as demonstrated by increased levels of immunoglobulins and FLCs, and the subsequent risk of developing lymphomas in HIV+ people.

6.2 Methods

6.2.1 The EuroSIDA study

Analyses for this chapter were conducted within the EuroSIDA cohort and utilised the EuroSIDA plasma biobank of prospectively stored plasma samples. Both the EuroSIDA study and the plasma biobank are described in detail in in chapter 2 sections 2.1.1 and 2.1.1.9.

6.2.2 Study design

A 1:2 nested case control study utilising stored plasma samples to investigate the kinetics and predictive value of several markers of immune activation: FLC-κ, FLC-λ, IgG, IgA, IgM and IgD.

6.2.3 Inclusion criteria

Those with follow-up after the 1 of January 2001 with no history of a lymphoma diagnosis prior to this date were eligible for inclusion. Both cases and controls were required to have at least one plasma sample available for analysis in the plasma biobank (the plasma biobank is explained in detail in chapter 2 section 2.1.1.9) prior to lymphoma diagnosis (or equivalent in controls). Date of earliest plasma sample was considered as baseline.

6.2.4 Cases and controls

All eligible people with a primary diagnosis of lymphoma after 1 January 2001 were considered as cases. For each case, 2 matched controls (where available) were selected using incidence density sampling (see Figure 6.4). If two controls were not available, 1 control was selected. Controls were selected from eligible people with prospective follow-up after 1 January 2001 with no history of NHL or HL at the time of diagnosis for each case. Both cases and controls were HIV+.

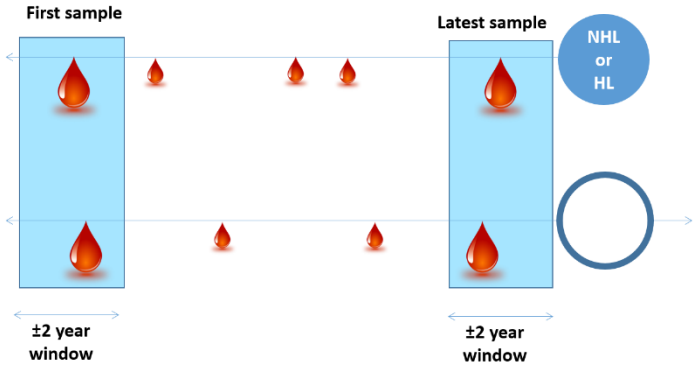


Figure 6.4 Selection of cases and controls using incidence density sampling.
NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma

6.2.5 Matching variables

Cases and controls were matched on region of Europe, gender, date of earliest plasma sample date (±2 years), date of latest plasma sample date (±2 years), age at earliest plasma sample date (± 5 years), and CD4 cell count at earliest plasma sample (taken from closest visit) (± 200 cells/mm^3). The windows used for matching were selected to allow suitable identification of controls while ensuring as few cases as possible were excluded from analyses.

An example of the selection of a case control pair is shown in Figure 6.4. For each case of lymphoma (top person), a similar person who was lymphoma free at the date of diagnosis of the case (represented by the open circle) was selected. Incidence density sampling means the cases and controls are matched on follow-up time. In the example, the date of each plasma sample is represented by a red drop. The date of earliest plasma sample for the case and control had to be within a 2 year window (represented by the blue region), and the date of the plasma latest plasma sample for the case and control had to be within a 2 year window (within the blue region).

6.2.6 Plasma samples and measurement of markers

All available serial plasma samples for cases and controls prior to the date of lymphoma diagnosis (or matched date in controls) were considered for inclusion. Where more than one plasma sample was available during the same calendar year, one plasma sample was randomly selected for inclusion in order to preserve plasma for future analyses.

Serial plasma samples for cases and controls (up to a maximum of 1 per calendar year) were analysed for FLC-κ, FLC-λ, IgG, IgA, IgM and IgD. All biomarkers were centrally measured by a

technician blinded to case control status on frozen stored plasma at the Department of Clinical Biochemistry at Rigshospitalet. FLC (the κ and λ Freelite® turbidimeteric/nephelometric immunoassay, product code: LK016.S and KL018.S), IgG (NK004.S), IgA (NK010.S), IgM (NK012.S), and IgD (LK013.S) concentrations were measured on plasma in all patients using Immunoassay from the Binding Site Group Ltd, Birmingham, UK on the SPAPLUS ®. There were initially 73 cases and 143 controls (3 cases had only 1 suitable control available) selected for analysis with 600 plasma samples for inclusion. However 6 plasma samples were not in the freezer, therefore 594 plasma samples were analysed. This study was performed in collaboration with the Binding Site, who provided the kits free of charge. Information and contact details for the Binding Site are available at http://www.bindingsite.com/en.

In this chapter, I considered the relationship between FLC-κ, FLC-λ, the ratio of FLC-κ to FLC-λ (FLC-κ/λ), the sum of FLC-κ and λ (FLC-κ+λ), IgG, IgA, IgM and IgD and lymphoma development. I also assessed whether monoclonal or polyclonal elevations were associated with lymphoma development. Many other studies have used the upper limit of the normal to identify high marker levels (Table 6.1), however, it was decided not to use this approach as these limits have been validated in the HIV-negative population only. Instead, marker levels were investigated on the log2 scale, giving an odds ratio which corresponded to a 2-fold higher marker level. However, I did use the definitions for combined FLC elevations in order to classify monoclonal or polyclonal elevations (as defined in Table 6.1).

Table 6.1 Marker reference ranges in the general population.

Marker	Normal range
FLC-κ	3.3 – 19.4 mg/L [897]
FLC-λ	5.71 – 26.3 mg/L [898]
FLC-κ/λ ratio	0.26–1.65 [897, 898]
FLC-κ+λ	9.01- 45.7 mg/L [897, 898]
Combined FLC elevations	**Monoclonal elevations:** FLC-κ>19.4 mg/L or FLC-λ > 26.3 mg/L and FLC-K/L not between 0.26 – 1.65. [897, 898] **Polyclonal elevations:** FLC-κ >19.4 mg/L or FLC-λ > 26.3 mg/L and FLC-K/L between 0.26 mg/L – 1.65 mg/L. [897, 898] **Normal levels:** FLC-κ <19.4 mg/L and FLC-λ <26.3 mg/L. [897, 898]
IgG	6.103 - 16.16 g/L [899]
IgA	0.845-4.990g/L [900]
IgM	0.35-2.42 g/L [901]
IgD	7.7-132.1 g/mL [902]

FLC: free light chain, Ig: Immunoglobulin

6.2.7 Variables included in analyses

Both baseline and time updated variables from the EuroSIDA database were included in this analysis, as detailed in Table 6.2. Both current and area under the curve (AUC) of HIV viral load

(HIV-VL) were used. In Brief, AUC of HIV-VL is a measure of accumulated exposure to replicating HIV [837]. The calculation and interpretation of AUC HIV-VL is described in detail in chapter 5 section 5.2.3. When referring to time updated variables, the term "current" was used, for example "current CD4 cell count" refers to time updated CD4 cell count.

Table 6.2 Summary of baseline and time updated variables.

Variable	Time updated	Levels	Definitions and comments
Age (years)	Yes	Continuous (per 10 years older)	
Region of Europe		East, east central, south, west central, north Europe	See chapter 2 section 2.1.1.2
Ethnicity		White, non-white	
Mode of HIV transmission		Sex between men, other transmission modes	Other transmission modes included through IDU, heterosexual sex, and other or unknown
Gender		Male, female	
Current CD4 cell count (cells/mm^3)	Yes	Continuous (per 2–fold higher)	Within 6 months prior to date of interest
Nadir of CD4 cell count	Yes	Continuous (per 2–fold higher)	Lowest recorded CD4 cell count measurement prior to date of interest. Calculated since entry into EuroSIDA
Current HIV-VL (copies/mL)	Yes	Continuous (per 10–fold higher) and categorised as ≤ 500, > 500 copies/mL, and unknown	Within 6 months prior to date of interest.
Area under the curve of HIV-VL (copies/mL – year)	Yes	Continuous (per 10–fold higher) and categorised as ≤ 50%, > 50%, unknown. 50% represents the median value	Calculated since entry into EuroSIDA. Explained in detail in chapter 5 section 5.2.3.
Prior AIDS defining event (excluding NHL)	Yes	Yes , no	Classified according to the 1993 CDC clinical definition [133]
Prior non-AIDS defining event (excluding HL)	Yes	Yes , no	Pancreatitis, grade 3 or 4 hepatic encephalopathy or liver-related death, non-AIDS defining cancers, myocardial infarction, stroke, coronary artery bypass graft, coronary angioplasty, carotid endarterectomy, and end-stage renal disease [221]

Variable	Time updated	Levels	Definitions and comments
HBV coinfection	Yes	Positive, negative, or unknown	Most recent positive HBsAG surface antigen test or presence of detectable HBV DNA. Those without a HBV test were categorised as unknown
HCV coinfection	Yes	Positive, negative, or unknown	A prior positive HCV surface antibody test. Those without a HCV test were categorised as unknown
cART use	Yes	Yes, no	Defined as use of ≥ 3 antiretroviral drugs from any class.
Duration of time on cART	Yes	Continuous (per year longer)	
Date of first plasma sample		Continuous date	Date of first plasma sample available for analysis in the plasma biobank
estimated glomerular filtration rate (eGFR) (ml/min)		Continuous	Cockcroft–Gault formula[903] standardised for body surface area [904]

IDU: injecting drug use, HIV-VL: HIV viral load, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, cART: combination antiretroviral therapy, HBV: Hepatitis B, HCV: hepatitis C,

6.2.8 Statistical methods

6.2.8.1 Characteristics of cases and controls at earliest plasma sample and matching variables

The characteristics of cases and controls were compared using numbers and percentages for categorical variables and median with interquartile range (IQR) for numerical variables at baseline. All bivariate associations were tested using univariate conditional logistic regression due to the matched nature of the data.

6.2.8.2 Association of each marker with likelihood of lymphoma diagnosis

Unadjusted conditional logistic regression models were used to investigate the association between the odds of developing lymphoma and each marker using plasma samples that were collected ≤ 2 and > 2 years prior to lymphoma diagnosis (in cases) or matched date in controls. In the case where ≥ 1 plasma sample was available within the same time period, one was randomly selected.

6.2.8.3 Predictive value of each marker for lymphoma diagnosis

The area under the receiver operator curve statistic (c-statistic) was calculated to determine the predictive value of each marker, where a c-statistic = 0.5 indicates no predictive ability (i.e. the

marker is no better at determining lymphoma than flipping a coin) and a c-statistic = 1 indicates perfect predictability [905]. Predictive ability was classified as follows (0.51 – 0.6: Poor, 0.61 – 0.7: Poor-moderate, 0.71 – 0.8: Moderate, 0.81 – 0.9: Good, 0.9 – 1: Excellent). This was performed for each marker using plasma samples collected ≤ 2 and > 2 years prior to lymphoma diagnosis in cases or matched date in controls, as described in section 6.2.8.2.

6.2.8.4 Kinetics of each marker in the time before lymphoma diagnosis

The percentage change for each marker over calendar time (% change per year) in cases or matched date in controls in the period prior to diagnosis or matched date in controls was investigated using mixed models with random slopes and intercepts (accounting for multiple measurements within each person). Differences in the trajectory of cases and controls were investigated by testing for an interaction between time until lymphoma diagnosis or matched date in controls and case and control status. The magnitude of change in marker levels over time were compared using three different approaches:

1. Unadjusted models
2. Models adjusted for region of Europe, gender, age at first plasma sample and log2 CD4 cell count at first plasma sample (i.e. the matching variables)
3. Models adjusted for region of Europe, gender, current age, current log2 CD4 cell count, current use of cART, and duration of time on cART

6.2.8.5 Factors associated with elevated marker levels

Patient factors associated with higher B-cell activation marker levels were assessed using mixed models (accounting for multiple measurements within each person). In order to minimise bias due to the nested-case control study design (leading to a non-representative patient population where 1 in 3 develop lymphomas) this analysis of factors was restricted to controls only. Factors investigated included current age, gender, region of Europe, current use of cART (defined as ≥ 3 antiretroviral drugs), current and nadir CD4 cell count, and current and AUC of HIV viral load (HIV-VL).

6.3 Results

6.3.1 Baseline characteristics

Characteristics of cases (N=73, 52 NHL and 21 HL) and controls (N=143) are shown in Table 6.3. Overall, there was a median of 2.0 years (IQR: 0.4, 3.1) between first and last plasma sample (cases: 2.1 years IQR: 0.6, 4.4; controls: 1.8 years IQR: 0.0, 4.3, P=0.72) and in cases there was 1.3 years (IQR: 0.3, 2.9) between last plasma sample and date of lymphoma diagnosis. Cases differed from controls according to HIV and treatment related factors. Median HIV-VL level was higher in cases (672 1QR: 80, 10,994 copies/mL) than controls (140 IQR: <50, 848 copies/mL), and a lower proportion of cases (63.0%) were on cART at baseline compared to controls (78.3%). The median duration of cART was also shorter in cases (0.9 IQR: 0.0, 2.5 years) than controls (1.7 IQR: 0.4, 3.3 years). In addition, a lower proportion of cases were HCV positive compared to controls (9.6 vs 27.3%). Cases and controls were well balanced on other non-matched demographic characteristics. At earliest plasma sample, median levels of FLC-κ, FLC-λ and FLC-κ+λ were elevated in cases relative to controls, however the median FLC-κ/λ ratio was similar. Levels of IgG and IgM were also elevated in cases, however levels of IgA and IgD were similar in cases and controls.

6.3.2 Characteristics at latest plasma sample

Immunological, HIV, and B-cell related markers at latest plasma sample prior to lymphoma diagnosis in cases or matched date in controls is shown in Table 6.4. Cases had a higher median HIV-VL (cases: <200 IQR: <50, 6,560; controls: <50.0 IQR: <50, 600 copies/mL) and a shorter duration of cART (cases: 2.7 IQR: 0.8, 4.4; controls: 4.2 IQR: 1.7, 6.6 years) relative to controls at latest plasma sample. There was no difference in levels of any B-cell markers at latest plasma sample (all P>0.05).

Table 6.3 Baseline[1] characteristics of cases and controls.

Factors		Overall (N=216)		Cases (N=73)		Controls (N=143)	p-value
Categorical variables							
Male gender[2]		193 (89.4)		65 (89.0)		128 (89.5)	Na
HIV transmission group							
Sex between men		114 (52.8)		41 (56.2)		73 (51.0)	0.39
Other		102 (47.2)		32 (43.8)		70 (49.0)	
White ethnicity		180 (83.3)		56 (76.7)		124 (86.7)	0.06
Region of Europe[2]							
South		46 (21.3)		16 (21.9)		30 (21.0)	Na
West-central		75 (34.7)		25 (34.2)		50 (35.0)	
North		72 (33.3)		24 (32.9)		48 (33.6)	
East central		21 (9.7)		7 (9.6)		14 (9.8)	
East		2 (0.9)		1 (1.4)		1 (0.7)	
Prior AIDS event (excluding NHL)		60 (27.8)		23 (31.5)		37 (25.9)	0.33
Prior non-AIDS event (excluding HL)		5 (2.3)		2 (2.7)		3 (1.4)	0.88
HCV							
Positive		46 (21.3)		7 (9.6)		39 (27.3)	0.02
Negative		128 (59.3)		50 (68.5)		78 (54.5)	
Unknown		42 (19.4)		16 (21.9)		26 (18.2)	
HBV							
Positive		17 (7.9)		8 (11)		9 (6.3)	0.22
Negative		171 (79.2)		53 (72.6)		118 (82.5)	
Unknown		28 (13.0)		12 (16.4)		16 (11.2)	
On cART		158 (73.1)		46 (63.0)		112 (78.3)	0.02
Numerical variables	**N**	**Median (IQR)**	**N**	**Median (IQR)**	**N**	**Median (IQR)**	**p-value**
Age (years) [2]	216	42.2 (36.9,49.7)	73	42.6 (37.0,50.5)	143	41.8 (36.6,49.3)	0.47
CD4 cell count (cells/mm^3) [2]	216	317.5 (208.0,477.0)	73	316.0 (180.0,500.0)	143	319.0 (213.0,461.0)	0.06[3]

First plasma sample date[2]	216	11DEC1999 (20JUL1998,09OCT2004)	73	15NOV1999 (24JAN1998,16JUL2004)	143	25JAN2000 (28JUL1998,14OCT2004)	0.67
Nadir CD4 cell count (cells/mm^3)	216	120 (44,228)	73	179 (55,280)	143	101 (40,200)	0.37[3]
HIV-VL (copies/mL)	206	215 (<50,2100)	68	672 (80,10993.5)	138	140 (<50,848)	<.01[4]
Log 10 of AUC of HIV-VL (copies/mL - years)	206	4.3 (3.6,5.0)	68	4.5 (3.6,5.1)	138	4.2 (3.6,5.0)	0.51
Duration of time on cART (years)	216	1.4 (0.2,3.1)	73	0.9 (0.0,2.5)	143	1.7 (0.4,3.3)	<.01
eGFR	53	97.1 (77.2, 104.4)	19	98.6 (61.9, 107.4)	34	95.4 (79.9, 104.1)	0.82
Markers							
FLC—κ (mg/L)	213	32.0 (23.4,50.5)	71	39.9 (28.1,62.1)	142	29.4 (20.8,41.0)	<.01[3]
FLC—λ (mg/L)	214	21.8 (14.5,31.4)	72	27.7 (19.2,45.0)	142	18.6 (13.7,27.2)	<.01[3]
FLC—κ/λ	213	1.6 (1.2,2.0)	71	1.6 (1.2,2.0)	142	1.6 (1.2,2.1)	0.12[3]
FLC—κ+λ	213	53.4 (39.3,80.8)	71	69.6 (49.6,99.5)	142	48.2 (36.7,67.6)	<.01[3]
IgG (g/L)	214	14.3 (11.1,17.8)	72	15.8 (12.5,19.6)	142	13.2 (10.3,16.9)	<.01[3]
IgA (g/L)	214	2.2 (1.5,3.6)	72	2.7 (1.5,3.8)	142	2.2 (1.6,3.3)	0.34[3]
IgM (g/L)	214	0.8 (0.5,1.3)	72	1.1 (0.7,1.7)	142	0.8 (0.5,1.1)	0.02[3]
IgD (g/L)	214	28.5 (6.7,58.9)	72	39.2 (9.0,60.3)	142	26.0 (6.7,58.2)	0.12[3]

NHL: non-Hodgkin lymphoma. HL: Hodgkin lymphoma, HCV: hepatitis C, HBV: hepatitis B, cART: combination antiretroviral therapy, HIV-VL: HIV viral load, AUC: area under the curve, eGFR estimated Glomerular Filtration Rate, IQR: interquartile range, FLC: free light chain, Ig: immunoglobulin.

[1] Baseline defined as the date of earliest plasma sample.

[2] Matching variable.

[3] p-value calculated on the log 2 scale.

[4] p-value calculated on the log 10 scale.

Table 6.4 Marker levels at latest plasma sample.

	Overall		Cases		Controls		p-value
	N	Median(IQR)	N	Median(IQR)	N	Median(IQR)	
CD4 (cells/mm^3) [1]	216	378 (238,541)	73	380 (223,530)	143	373 (255,547)	0.07[2]
Nadir CD4 (cells/mm^3)	216	104 (34,211)	73	140 (30,254)	143	95 (36,196)	0.86[2]
HIV-VL (copies/mL)	215	60 (<50,1700)	73	<200 (<50,6560)	142	<50.0 (<50,600)	0.02[3]
Log 10 AUC of HIV-VL (copies/mL - years)	215	4.6 (3.9,5.3)	73	4.9 (4.0,5.5)	142	4.4 (3.8,5.2)	0.10
Duration of time on cART (years)	216	3.5 (1.3,6.3)	73	2.7 (0.8,4.4)	143	4.2 (1.7,6.6)	<.01
B-cell Markers							
FLC—κ (mg/L)	213	34.9 (21.3,55.1)	71	36.7 (24.5,57.0)	142	33.1 (20.1,54.8)	0.68[2]
FLC—λ (mg/L)	214	21.8 (13.7,34.3)	72	24.5 (17.2,40.1)	142	21.3 (12.9,31.7)	0.87[2]
FLC—κ/λ	214	1.6 (1.3,2.1)	72	1.6 (1.2,2.0)	142	1.7 (1.3,2.2)	0.81[2]
FLC—κ+λ	214	56.9 (38.3,90.3)	72	63.5 (41.1,92.0)	142	53.9 (35.5,84.9)	0.75[2]
IgG (g/L)	214	13.0 (10.5,17.1)	72	14.4 (11.5,18.2)	142	12.8 (10.1,16.4)	0.18[2]
IgA (g/L)	214	2.3 (1.4,3.4)	72	2.5 (1.3,3.5)	142	2.2 (1.5,3.4)	0.28[2]
IgM (g/L)	214	0.8 (0.5,1.3)	72	0.8 (0.5,1.5)	142	0.8 (0.5,1.2)	0.86[2]
IgD (g/L)	214	21.9 (7.2,59.8)	72	24.5 (10.7,87.1)	142	21.0 (6.7,53.9)	0.287

IQR: interquartile range, HIV-VL: HIV viral load, AUC: area under the curve, FLC: free light chain, Ig: immunoglobulin.

[1] Matched at first plasma sample.

[2] p-value calculated on the log 2 scale.

[3] p-value calculated on the log 10 scale.

6.3.3 Odds of developing a lymphoma during prospective follow-up

The odds of developing lymphoma for a 2-fold higher marker level both ≤ 2 and > 2 years before lymphoma diagnosis or matched date in controls are shown in Figure 6.5. A 2-fold higher level of FLC-κ (OR: 1.84 95%CI: 1.19, 2.84), FLC-λ (OR: 2.15 95%CI: 1.34, 3.46), IgG (OR: 3.05 95%CI: 1.41, 6.59), and IgM (OR: 1.46 95%CI: 1.01, 2.11) were predictive of lymphoma development >2 years prior to diagnosis. However, associations were not evident ≤ 2 years prior to diagnosis. No association was found for 2-fold higher IgA or IgD (although, the p-value was close to 0.05 for the association > 2 years prior for IgD) at either time point. FLC-κ+λ (OR: 2.08 95%CI: 1.30, 3.35) was predictive > 2 years but not ≤ 2 years prior to diagnosis prior to lymphoma diagnosis. The ratio of FLC-κ/λ was not predictive at either > 2 years or ≤ 2 years prior to diagnosis.

Proportionately high levels of both FLC-κ and FLC-λ (a marker of polyclonal expansion) was associated with lymphoma >2 years prior to diagnosis (OR: 4.74 95%CI: 1.71 – 27.56), but not ≤ 2 years prior (1.62 94%CI: 0.54, 5.05). Having a disproportionately high level of one FLC (a marker of monoclonal expansion) was not associated at either time point.

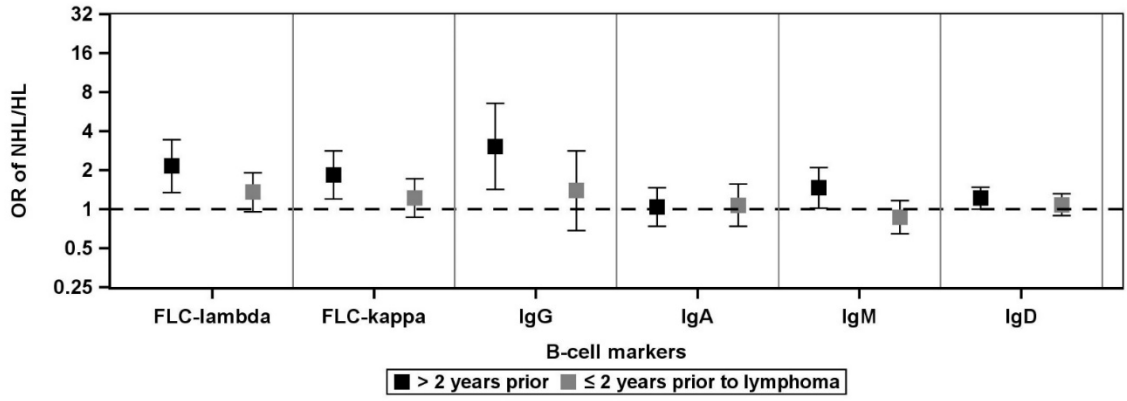


Figure 6.5 Odds ratio (OR) of lymphoma associated with a 2-fold increase in B-cell markers, ≤2 and >2 years prior to diagnosis.
OR: Odds ratio, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, FLC: free light chain, Ig: immunoglobulin.

The odds of developing lymphoma for both a 10-fold higher current and AUC of HIV-VL level ≤2 and > 2 years before lymphomas are shown in Figure 6.6. In those who had HIV-VL measured (N = 586 samples in 214 people), a 10-fold higher AUC of HIV-VL was associated with a higher risk of lymphoma in plasma samples ≤ 2 years prior to diagnosis (OR: 1.68, 95%CI: 1.08, 2.62), however HIV-VL was predictive >2 years prior (OR: 1.51 95%CI: 1.1, 2.08).

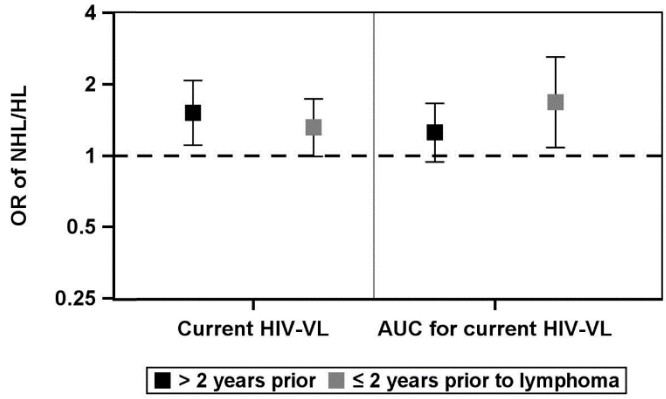


Figure 6.6 Odds ratio of lymphoma associated with a 2-fold increase in current HIV viral load (HIV-VL) and area under the curve (AUC) of HIV-VL, ≤ 2 and > 2 years.
OR: Odds ratio, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, HIV-VL: HIV viral load, AUC: area under the curve.

6.3.4 Predictive value of B-cell markers

The marker with the best predictability ≥ 2 years prior to lymphoma diagnosis was FLC-λ (Table 6.5). This marker predicted lymphoma diagnosis with better accuracy than chance alone (P<0.01), however the c-statistic of 0.67 suggests only poor to moderate classification power. The following markers also had some predictive power (all P<0.05), however prediction was poor to moderate at best: FLC-κ+λ (c-statistic = 0.67, poor-moderate prediction), and IgG (c-statistic: 0.64, poor-moderate). Only FLC-λ (c-statistic: 0.61) predicted lymphoma within 2 years of diagnosis, however accuracy was poor. No other markers were predictive (all P>0.05).

Table 6.5 c-statistics for prediction of lymphoma diagnosis for each marker, stratified by < 2 years prior to diagnosis and ≤ 2 years prior to diagnosis.

B-cell markers	≥ 2 years		<2 years	
	c-statistic	P[1]	c-statistic	P[1]
FLC–κ	0.65 (0.56,0.74)	0.29	0.56 (0.45,0.66)	0.29
FLC–λ	0.67 (0.58,0.76)	<.01	0.61 (0.51,0.71)	0.04
Ratio FLC–κ/λ	0.53 (0.43,0.62)	0.60	0.54 (0.43,0.64)	0.48
Sum FLC–κ+λ	0.67 (0.58,0.76)	<.01	0.58 (0.47,0.68)	0.15
IgG	0.64 (0.55,0.73)	<.01	0.55 (0.45,0.65)	0.32
IgA	0.52 (0.42,0.62)	0.65	0.50 (0.40,0.61)	0.94
IgM	0.59 (0.49,0.69)	0.09	0.52 (0.41,0.63)	0.70
IgD	0.58 (0.49,0.68)	0.10	0.54 (0.44,0.64)	0.43

FLC: free light chain, Ig: immunoglobulin.
[1] P compares the c-statistic for each marker to 0.5 (i.e. prediction is no better than chance).

6.3.5 Trajectories of each B-cell marker prior to diagnosis

The trajectories of each marker in the cases and controls and unadjusted percentage change per year for each marker in the time leading up to diagnosis or matched date in controls are shown in Figure 6.7 to Figure 6.16. In unadjusted analysis, the largest difference was observed for IgM, which was declining in cases by 6.42% (95%CI: 3.12, 9.61) per year, but levels were stable in controls (%Change per year: 0.40 95%CI:-2.09, 2.95%) (Figure 6.13). The difference in the rates of change per year between cases and controls was statistically significant (P for interaction < 0.01). Levels of IgG were also declining in cases, but stable in controls (Figure 6.11), which was a borderline significant difference in the rates of change per year when comparing cases and controls (P for interaction=0.10). Although levels of FLC-κ were stable in cases but increasing in controls (Figure 6.7), the difference in the rates of change per year between cases and controls were non-significant (P for interaction = 0.20). The ratio of FLC-κ/λ was increasing in cases, but stable in controls (Figure 6.9), and conversely, FLC-κ+λ level was increasing in controls but stable in cases (Figure 6.10), however the differences in the rates of change per year between cases and controls were not-significant for either (P for interaction = 0.44 and 0.16, respectively). Levels of FLC-λ (Figure 6.8), IgA (Figure 6.12), and IgD (Figure 6.14) did not change over time in either cases or controls. The trajectories were similar after adjustment for matching variables (Table 6.6, adjusted model 1). Further adjustment for current CD4 cell count, current age and HIV treatment variables also produced consistent results (Table 6.6, adjusted model 2).

6.3.6 Trajectories of HIV-VL prior to diagnosis

In those who had a HIV-VL measured (N = 586 samples in 214 people), in unadjusted analysis, HIV-VL levels were high many years prior to diagnosis (Figure 6.15), and significantly declined in cases in the time leading up to diagnosis, whereas levels were stable in controls, however this difference was not significant (P=0.11). The level of current CD4 cell count was stable in both cases and controls (Figure 6.16). The trajectories for HIV-VL were similar after adjustment for matching variables (Table 6.6, adjusted model 1) and after further adjustment for current CD4 cell count, current age and HIV treatment variables also produced consistent results (Table 6.6, adjusted model 2). Trajectories for CD4 attenuated after adjustment for HIV treatment variables, however the conclusions remained unchanged (Table 6.6, adjusted model 2).

% change[1] cases 0.28 (-2.91,3.55)

% change[1] controls 2.90 (0.53,5.31)*

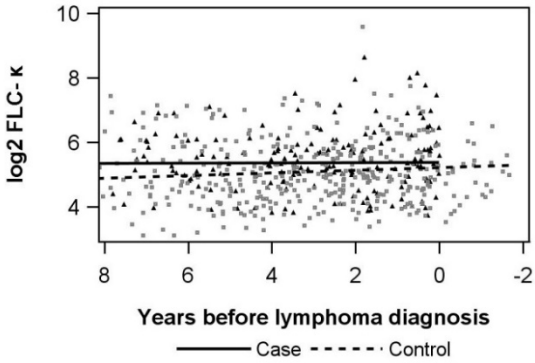


Figure 6.7 Trajectory of FLC-κ prior to lymphoma diagnosis in cases and controls.

% change[1] cases -1.54 (-4.63,1.66)

% change[1] controls 1.74 (-0.58,4.11)

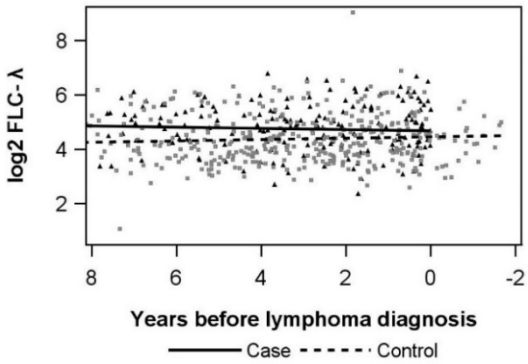


Figure 6.8 Trajectory of FLC-λ prior to lymphoma diagnosis in cases and controls.

% change[1] cases 2.22 (0.23,4.25)*

% change[1] controls 1.26 (-0.17,2.7)

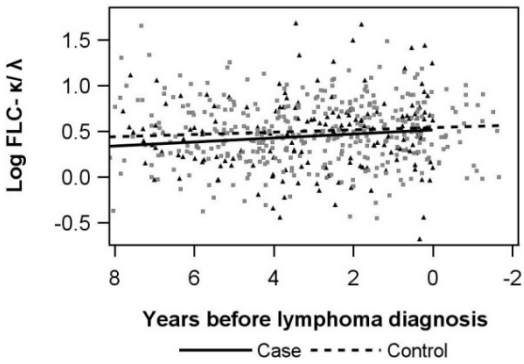


Figure 6.9 Trajectory of FLC-κ/λ prior to lymphoma diagnosis in cases and controls.

% change[1] cases -0.4 (-3.43,2.73)

% change[1] controls 2.38 (0.11,4.68)*

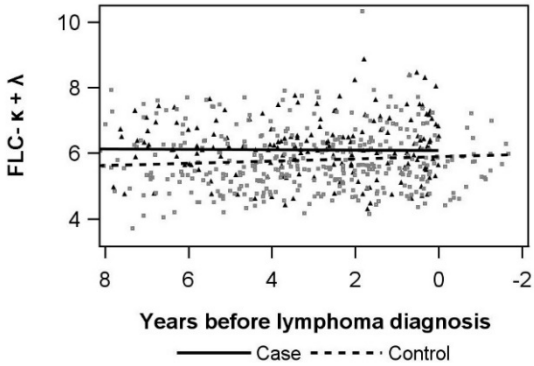


Figure 6.10 Trajectory of FLC-κ+λ prior to lymphoma diagnosis in cases and controls.

FLC: free light chain

[1] The unadjusted percentage change per year (% change) in each marker per year (i.e. the slope) in the time leading up to diagnosis is shown at the top of each panel.

* P for change<0.05: This is testing whether there is a significant increase or decline in the markers over time.

P for interaction: This tests whether the % change per year is different in cases and controls. FLC-κ: 0.2, FLC-λ: 0.1, Ratio FLC- κ/λ: 0.44, FLC- κ+λ: 0.16.

% change[1] cases -2.54 (-4.18,-0.88)*

% change[1] controls -0.84 (-2.04,0.38)

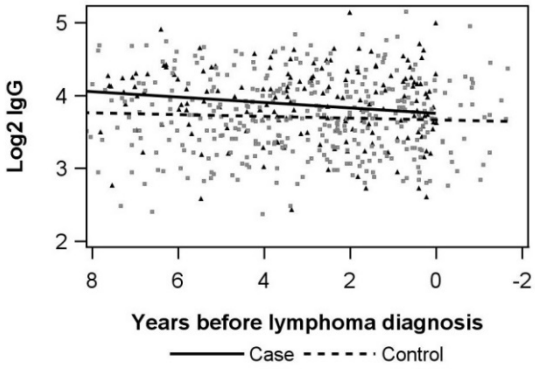


Figure 6.11 Trajectory of IgG prior in cases and controls.

% change[1] cases -1.46 (-4.00,1.14)

% change[1] controls -1.26 (-3.11,0.61)

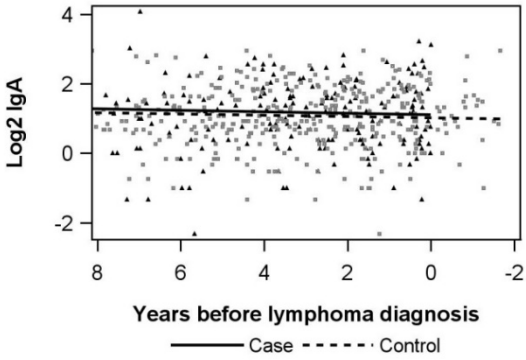


Figure 6.12 Trajectory of IgA prior in cases and controls.

% change[1] cases -6.42 (-9.61,-3.12)*

% change[1] controls 0.40 (-2.09,2.95)

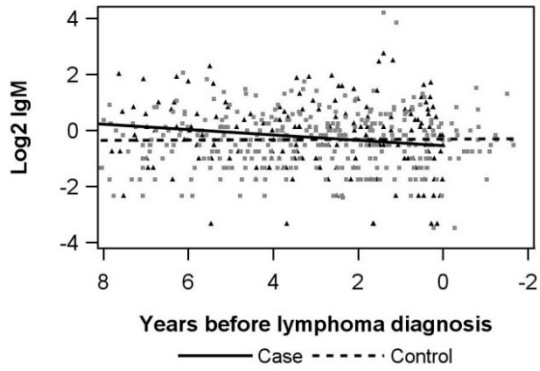


Figure 6.13 Trajectory of IgM in cases and controls.

% change[1] cases -1.46 (-5.99,3.29)

% change[1] controls -0.76 (-4.09,2.68)

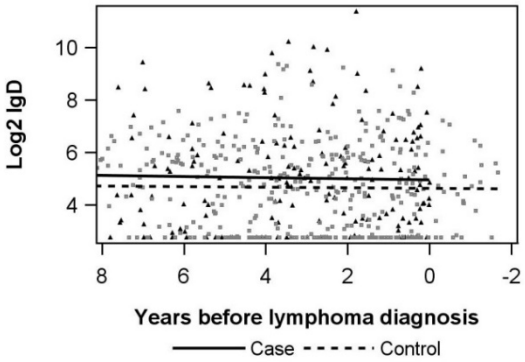


Figure 6.14 Trajectory of IgD in cases and controls.

% change[1] cases -20.88 (-33.04,-6.52)*

% change[1] controls -6.50 (-16.85,5.13)

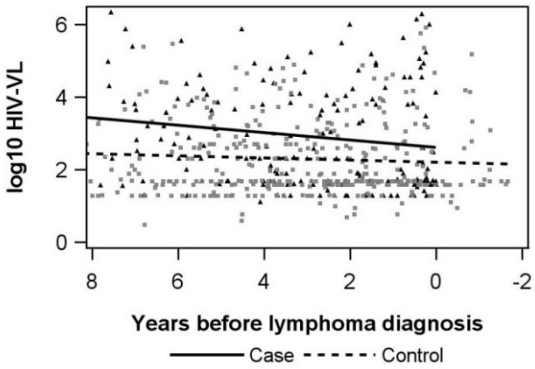


Figure 6.15 Trajectory of HIV viral load (HIV-VL) in cases and controls.

% change[1] cases 3.78 (-0.88,8.66)

% change[1] controls 3.30 (-0.07,6.77)

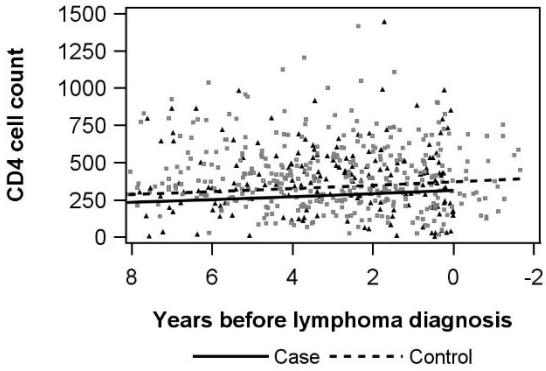


Figure 6.16 Trajectory of CD4 cell count in cases and controls.

Ig: immunoglobulin, HIV-VL: HIV viral load,

[1] The unadjusted percentage change per year in each marker per year (i.e. the slope) in the time leading up to diagnosis is shown at the top of each panel.

* P for change<0.05: This is testing whether there is a significant increase or decline in the markers over time.

P for interaction: This tests whether the % change per year is different in cases and controls. IgG: 0.10, IgA: 0.90, IgM<0.01, IgD: 0.81, current HIV-VL: 0.11, current CD4 cell count: 0.87.

Table 6.6 Percent (%) change per year in FLC-κ and FLC-λ, FLC-κ/λ, FLC-κ+λ, IgG, IgA, IgM, IgD, current HIV viral load and CD4 cell count (unadjusted and adjusted).

Marker		Unadjusted model		Adjusted model 1[2]		Adjusted model 2[3]	
		% change per year (95%CI)	P for interaction[1]	% change per year (95%CI)	P for interaction[1]	% change per year (95%CI)	P for interaction[1]
FLC–κ							
	Case	0.28 (-2.91,3.55)	0.20	0.4 (-2.78,3.69)	0.21	1.9 (-1.33,5.22)	0.32
	Control	2.9 (0.53,5.31)		2.96 (0.58,5.41)		3.82 (1.07,6.63)	
FLC–λ							
	Case	-1.54 (-4.63,1.66)	0.10	-1.3 (-4.42,1.94)	0.10	0.78 (-2.54,4.22)	0.12
	Control	1.74 (-0.58,4.11)		2.02 (-0.35,4.43)		3.92 (1.07,6.84)	
Ratio FLC–κ/λ							
	Case	2.22 (0.23,4.25)	0.44	2.1 (0.1,4.13)	0.44	1.46 (-0.59,3.53)	0.30
	Control	1.26 (-0.17,2.7)		1.14 (-0.31,2.59)		0.18 (-1.47,1.87)	
Sum FLC–κ+λ							
	Case	-0.4 (-3.43,2.73)	0.16	-0.18 (-3.23,2.96)	0.16	1.56 (-1.56,4.79)	0.24
	Control	2.38 (0.11,4.68)		2.52 (0.25,4.86)		3.78 (1.11,6.5)	
IgG							
	Case	-2.54 (-4.18,-0.88)	0.10	-2.5 (-4.13,-0.83)	0.10	-1.06 (-2.71,0.61)	0.07
	Control	-0.84 (-2.04,0.38)		-0.82 (-2.03,0.41)		0.7 (-0.71,2.14)	
IgA							
	Case	-1.46 (-4,1.14)	0.90	-1.72 (-4.25,0.89)	0.89	-0.5 (-3.27,2.34)	0.73
	Control	-1.26 (-3.11,0.61)		-1.5 (-3.34,0.39)		0.04 (-2.38,2.51)	
IgM							
	Case	-6.42 (-9.61,-3.12)	<0.01	-6.72 (-9.91,-3.43)	<0.01	-4.26 (-7.5,-0.92)	<0.01
	Control	0.4 (-2.09,2.95)		0 (-2.5,2.57)		2.34 (-0.62,5.38)	
IgD							
	Case	-1.46 (-5.99,3.29)	0.81	-0.78 (-5.35,4.02)	0.79	0.36 (-4.77,5.76)	0.89
	Control	-0.76 (-4.09,2.68)		0 (-3.39,3.5)	.	0.76 (-3.75,5.5)	
Current CD4 cell count							
	Case	3.78 (-0.88,8.66)	0.87	4.18 (-0.51,9.07)	0.85	1.44 (-3.2,6.31)[4]	0.49
	Control	3.3 (-0.07,6.77)		3.62 (0.22,7.13)		-0.5 (-4.23,3.37)[4]	
Current HIV-VL							
	Case	-20.88 (-33.04,-6.52)	0.11	-21.88 (-33.81,-7.79)	0.09	-17.06 (-28.96,-3.15)	0.13
	Control	-6.5 (-16.85,5.13)		-6.94 (-17.27,4.67)		-4.68 (-15.57,7.61)	

FLC: free light chain, Ig: immunoglobulin, HIV-VL: HIV viral load. [1] P for interaction: This tests whether the % change per year is different in cases and controls. [2]Adjusted Model 1: Age and CD4 at earliest plasma sample, region of Europe and gender, [3]Adjusted Model 2: Time updated Age, time updated CD4 (with the exception of current CD4 cell count trajectory), region of Europe, gender, currently on cart, duration of time on cART, [4] Model adjusted for CD4 at earliest plasma sample instead of time updated CD4.

6.3.7 Patient factors associated with B-cell marker levels in controls

Adjusted associations between demographic, HIV, and immunological related factors and marker levels in the control population are shown in Table 6.7 and Table 6.8. For each factor, the adjusted fold change in marker level is presented. For example, those from southern Europe had on average a 1.46-fold higher marker level compared to those from west-central Europe (Table 6.7). The results for FLC-κ, FLC-λ, and IgG are shown in Table 6.7, and for IgA, IgM, and IgD in Table 6.8. Higher FLC-κ, FLC-λ, and IgG were all associated with HIV transmission modes other than sex between men, lower current CD4 cell count, higher current HIV-VL and not being on cART (borderline for FLC-λ) (Table 6.7). FLC-κ and FLC-λ levels also increased with older age. Higher IgA was associated with lower CD4 cell count and higher AUC of HIV-VL (Table 6.8). Higher IgM was as associated with higher HIV-VL, not being on cART, and HIV transmission mode other than sex between men. Higher IgD level was associated with higher AUC of HIV-VL and higher nadir CD4 cell count (borderline).

6.3.8 Collaboration with the Binding Site

This study was performed in collaboration with the Binding Site. The Binding Site provided the kits free of charge. As part of an academic collaboration and have not been involved in the presentation of these data or the decision to publish these data. Contact details for the Binding Site are below.

The Binding Site Group Ltd
8 Calthorpe Road
Edgbaston
Birmingham
B15 1QT

Tel: 44 (121) 456 9500
Fax: 44 (121) 456 9749

Email: info@bindingsite.co.uk

Table 6.7 Multivariate analysis of the fold change in FLC-κ, FLC-λ, and IgG associated with patient factors.

Effect	FLC-κ		FLC- λ		IgG	
	Fold change (95%CI)	P	Fold change (95%CI)	P	Fold change (95%CI)	P
Age (1 year older)	1.25 (1.13 , 1.38)	<.01	1.14 (1.03 , 1.26)	0.01	1.00 (0.94 , 1.05)	0.81
Region						
South	1.46 (1.10 , 1.95)	0.01	1.45 (1.08 , 1.95)	0.01	1.31 (1.12 , 1.52)	<.01
North	1.20 (0.95 , 1.52)	0.12	1.25 (0.98 , 1.59)	0.07	1.20 (1.06 , 1.37)	<.01
East and East central	1.11 (0.77 , 1.59)	0.58	0.99 (0.69 , 1.44)	0.98	1.15 (0.95 , 1.40)	0.13
West-central	Reference		Reference		Reference	.
Female Gender	1.03 (0.73 , 1.46)	0.87	0.88 (0.61 , 1.25)	0.47	0.97 (0.81 , 1.17)	0.77
Non-white ethnicity	0.90 (0.65 , 1.23)	0.5	0.92 (0.66 , 1.27)	0.6	1.06 (0.89 , 1.26)	0.46
Non-sex between men HIV transmission mode	1.33 (1.07 , 1.66)	0.01	1.36 (1.09 , 1.70)	<.01	1.19 (1.06 , 1.34)	<.01
Current CD4 cell count (2-fold higher)	0.91 (0.86 , 0.97)	<.01	0.91 (0.86 , 0.96)	<.01	0.97 (0.94 , 0.99)	0.04
Nadir of CD4 (2-fold higher)	1.00 (0.95 , 1.06)	0.92	1.01 (0.96 , 1.08)	0.63	0.99 (0.96 , 1.02)	0.14
Current HIV-VL						
Missing	1.39 (0.76 , 2.53)	0.29	1.54 (0.88 , 2.68)	0.13	1.15 (0.85 , 1.55)	0.54
low: < 500	0.86 (0.78 , 0.94)	<.01	0.86 (0.79 , 0.94)	<.01	0.92 (0.88 , 0.96)	<.01
high: ≥ 500	Reference		Reference		Reference	
AUC of HIV-VL						
Missing	0.81 (0.48 , 1.36)	0.42	0.73 (0.45 , 1.19)	0.21	0.82 (0.63 , 1.07)	0.22
low < 50%	0.91 (0.78 , 1.05)	0.2	0.96 (0.83 , 1.10)	0.53	0.95 (0.88 , 1.02)	0.1
high ≥ 50%	Reference		Reference		Reference	
Not on cART	1.25 (1.07 , 1.47)	<.01	1.16 (0.99 , 1.35)	0.06	1.15 (1.06 , 1.25)	<.01

FLC: free light chain, Ig: immunoglobulin, HIV-VL: HIV viral load, AUC: area under the curve, cART: antiretroviral therapy.
Models were adjusted for all variables listed in the table.

Table 6.8 Multivariate analysis of the fold change in IgA, IgM, and IgD associated with patient factors.

Effect	IgA		IgM		IgD	
	Fold change (95%CI)	P	Fold change (95%CI)	P	Fold change (95%CI)	P
Age (1 year older)	1.00 (0.94 , 1.05)	0.89	0.96 (0.87 , 1.06)	0.47	1.06 (0.95 , 1.17)	0.29
Region						
South	1.31 (1.12 , 1.52)	<.01	0.98 (0.74 , 1.30)	0.91	1.24 (0.92 , 1.67)	0.15
North	1.20 (1.06 , 1.37)	<.01	1.16 (0.92 , 1.46)	0.22	1.12 (0.88 , 1.44)	0.35
East and East central	1.15 (0.95 , 1.40)	0.14	1.11 (0.78 , 1.59)	0.55	1.66 (1.15 , 2.41)	<.01
West-central	Reference		Reference		Reference	
Female Gender	0.97 (0.81 , 1.17)	0.76	0.90 (0.64 , 1.27)	0.55	1.17 (0.81 , 1.67)	0.41
Non-white ethnicity	1.06 (0.89 , 1.26)	0.51	0.74 (0.54 , 1.02)	0.07	0.84 (0.60 , 1.17)	0.3
Non-sex between men HIV transmission mode	1.19 (1.06 , 1.34)	<.01	0.87 (0.70 , 1.07)	0.19	1.34 (1.07 , 1.68)	0.01
Current CD4 cell count (2-fold higher)	0.97 (0.94 , 0.99)	0.02	0.92 (0.88 , 0.97)	<.01	0.96 (0.90 , 1.02)	0.15
Nadir of CD4 (2-fold higher)	0.99 (0.96 , 1.02)	0.68	0.98 (0.93 , 1.04)	0.53	1.06 (1.00 , 1.13)	0.05
Current HIV-VL						
Missing	1.15 (0.85 , 1.55)	0.37	0.99 (0.59 , 1.66)	0.97	1.36 (0.75 , 2.49)	0.31
low: < 500	0.92 (0.88 , 0.96)	<.01	1.02 (0.94 , 1.11)	0.63	0.72 (0.66 , 0.80)	<.01
high: ≥ 500	Reference		Reference		Reference	
AUC of HIV-VL						
Missing	0.82 (0.63 , 1.07)	0.15	0.82 (0.52 , 1.28)	0.38	0.66 (0.39 , 1.11)	0.12
low < 50%	0.95 (0.88 , 1.02)	0.16	0.85 (0.74 , 0.97)	0.02	0.88 (0.76 , 1.02)	0.09
high ≥ 50%	Reference		Reference		Reference	
Not on cART	1.15 (1.06 , 1.25)	<.01	0.98 (0.85 , 1.14)	0.83	1.29 (1.09 , 1.52)	<.01

Ig: immunoglobulin, HIV-VL: HIV viral load, AUC: area under the curve, cART: antiretroviral therapy.
Models were adjusted for all variables listed in the table.

6.4 Discussion

This study investigated the trajectories of FLC-κ, FLC-λ, IgG, IgA, IgM, and IgD over time prior to lymphoma diagnosis. I show that the strength of association diminishes consistently with time leading up to diagnosis. Levels of FLC-κ, FLC-λ, and IgG were associated with lymphoma development in HIV+ people more than 2 years prior to lymphoma development, however, predictive power for lymphoma development was poor. The markers investigated in this chapter, therefore, are unlikely to be strong candidates for risk assessment for targeted interventions for monitoring of high risk patients and early diagnosis.

6.4.1 Levels of markers of B-cell activation in cases and controls

In my study, proportionately higher levels of both FLC-κ and FLC-λ (a marker of polyclonal expansion) were associated with lymphoma >2 years prior to diagnosis. Two main studies have also demonstrated that elevated levels of FLC-κ and FLC-λ are associated with higher likelihood of lymphoma development in HIV+ people [846, 847]. The study by Landgren *et al* (2010) [846] found that elevated FLC-κ and FLC-λ were associated with NHL 2–5 years prior to diagnosis, conversely only FLC-λ was associated 0–2 years prior [846]. However, a later study by the same group subsequently found FLC-κ and FLC-λ levels to be similarly predictive of all AIDS defining events and not specifically NHL [895]. Results were consistent in the more recent study by Bibas *et al* (2012) [847], who found FLC-κ and FLC-λ to be predictive of both NHL and HL independently of CD4 cell count and HIV-VL [847]. My finding that FLC-κ and λ are predictive of lymphoma in the long term are somewhat concurrent with those of Landgren *et al* and Bibas *et al* [846, 847], however, I did not find an association with FLC-λ closer to diagnosis. Furthermore, my finding the polyclonal FLC elevations preceded lymphoma development is also concurrent with both studies [846, 847].

The results presented in this chapter demonstrate an association between IgG and IgM >2 years prior to date of lymphoma diagnosis (although the association with IgM was borderline), which attenuated closer to this date. This was driven by a faster decline in levels in cases while controls remained stable. This was not identified in study by Landgren *et al* (2010), which reported no association between IgG, IgA or IgM and NHL diagnosis (possibly due to lower prevalence of cART use in that study which may have masked this association) [846]. Studies have found mixed associations between immunoglobulins and lymphomas in HIV. For example, an Australian study

found high levels of serum globulin, mainly IgG, were predictive of NHL [890]. However, other studies found no association between serum globulin, immunoglobulins and NHL [606, 891].

In this study, levels of FLC-κ and λ were higher in cases and controls long before diagnosis, however the difference between cases and controls attenuated closer to the matched date. For FLC-κ and λ, this was driven by an increase in levels in controls while levels in cases remained constant. The attenuation in the difference between cases and controls over time is intriguing and is consistent with previous findings [846, 847, 890]. This result could simply reflect a 2 year lag period for the increase in B-cell activity to manifest as a clinically detectable lymphoma [847]. However, I hypothesise that the observed trends are probably reflecting the concurrent, but very different, immune consequences induced by HIV infection and lymphoma development and disentangling this relationship is not straight forward. HIV associated immunosuppression causes hypergammaglobulinaemia with elevated serum levels of immunoglobulins, mainly IgG but also IgA and IgD [858]. Conversely, studies in the general population have found lower levels of IgM, IgA and IgG prior to lymphoma diagnosis [892], and levels declined with more advanced disease [892, 893, 906-908], speculated to be driven by the developing lymphoma. Additionally, transformed B-cells may have compromised immunoglobulin production and levels may not reflect the immune environment in which the lymphoma initiated [893]. Therefore, it is possible the decline in marker levels ≤2 years may be a consequence of early undiagnosed lymphoma. A similar phenomenon has been reported for undiagnosed HL and declining CD4 cell counts within 1–2 years prior to diagnosis [468, 586].

Current HIV-VL was found to be a strong predictor of lymphoma risk > 2 years prior to diagnosis however, AUC of HIV-VL was a predictor ≤2 years prior to diagnosis. This indicates that exposure to uncontrolled HIV replication in the past and the associated effects on the immune system, may play a role in lymphomagenesis. This result is independent of CD4 cell count which was a matching factor. Furthermore, these results are in keeping with the study by Bibas *et al* (2012), which found duration of undetectable HIV-VL, but not current CD4 cell count or current VL, to be associated with lymphoma risk [847]. Additionally, NHL has been repeatedly linked with HIV-VL in the literature [16, 18, 433, 435, 451, 606, 816, 819], and the results in this chapter add to the growing body of literature supporting the involvement of HIV-induced immune dysfunction, above and beyond immune deficiency, in the development of NHL in HIV+ people [816, 840].

In chapter 5, I demonstrated that NHL was associated with both current and AUC of HIV-VL, but HL was not associated with HIV-VL. In this chapter, I have again demonstrated a link between

previous HIV-VL level and lymphoma, probably driven by the high proportion of NHL in this analysis. Furthermore, in this chapter, I have shown that markers of polyclonal rather than monoclonal B-cell expansion precedes lymphoma diagnosis. This implies that HIV induced B-cell activation precedes lymphoma development in HIV+ people, rather than or in addition to EBV induced activation [857]. Furthermore, elevated levels of IgG and IgM many years prior to diagnosis indicate HIV associated hyperglobulinaemia, which further supports the involvement of HIV induced B-cell dysfunction. Although not evident in this chapter, HL is almost completely EBV related in HIV+ people and lymphomagenesis is likely driven by low CD4 cell counts (as shown in chapter 5) allowing for loss of EBV control and HIV itself does not seem to play a direct role.

6.4.2 Predictive value of B-cell markers

Although FLC-λ, Sum FLC-κ+λ, and IgG were predictive of lymphoma development ≥ 2 years prior to lymphoma development, the predictive power was poor. This is likely reflecting the complexity of the processes surrounding lymphomagenesis.

6.4.3 Factors associated of markers of B-cell activation

6.4.3.1 Age

The Higher levels of FLC-κ and FLC-λ were associated with older age, which may reflect the increased immune dysfunction and inflammation associated with aging (see chapter 1 section 1.2.4) [909]. This finding adds further evidence to the conclusions in chapter 5, that immune dysfunction, such as chronic activation and inflammation, which occurs with older age is involved in the pathology of NHL [721]. This is further supported by recent results that link polyclonal FLCs to impaired dendritic cell maturation, which could indicate that elevated polyclonal FLCs may be a marker of innate immune dysfunction and reduced immune surveillance and immune senescence [909].

6.4.3.2 Immunological and Virological factors

Elevated levels of FLC-λ, FLC-κ, and IgG were associated with higher current HIV-VL and lower current CD4 cell count in controls, which is consistent with other studies [847, 896]. Higher levels of IgM were correlated with higher current HIV-VL only. The markers were not associated with historical measures of HIV-VL or CD4, indicating that levels reflect the immediate immune environment, rather than long term immune dysfunction. When considered in light of the

discussion in section 6.4.1, these findings may indicate that exposure to uncontrolled HIV-VL is playing an integral role in lymphoma development [816], and elevated FLCs may be reflecting HIV specific B-cell dysfunction [857] occurring long before diagnosis. In addition, IgA was also associated with both higher current CD4 and lower AUC of HIV-VL. IgD was associated with lower AUC of HIV-VL and borderline associated with higher nadir CD4. However these two markers were not associated with lymphoma development.

6.4.3.3 HIV treatment

Levels of FLC-λ, FLC-κ, IgG, and IgM were higher in those not on cART, after adjustment for CD4 cell count and HIV-VL. This is in line with another study which found that FLCs in HIV+ people were markers of disease severity and antiretroviral use [896]. Results from the START study have demonstrated that cART use is associated with lower HIV-VL, higher CD4 and reduced inflammation [910]. My results may reflect the benefit of cART by reducing the activation and dysfunction of B-cells HIV+ people. This further supports the findings of the START study indicating that HIV-treatment improves immune function beyond that of low CD4 cell counts [141].

6.4.3.4 Other factors

Several markers varied according to region of Europe, for example, of FLC-λ and FLC-κ were higher in southern relative to west central Europe, where as IgM was higher in east or east central Europe. To my knowledge, there is no reason for this biologically and likely reflects confounding in the data due to unmeasured or unknown factors. There was no variation by gender or ethnicity. Levels of FLC-λ, FLC-κ, IgG, and IgM were higher in those who acquired HIV through exposure other than sex between men, possibly indicating that immune activation and dysfunction is higher in these groups. This could reflect increased activation due to higher prevalence of HCV and HBV in those who inject drugs [911]. HCV infection is associated with several B-cell disorders, including cryoglobulinaemia and B-cell NHL, and elevated levels of FLC-κ and an abnormal FLC-ratio have been associated with the severity of B-cell dysfunction in HCV-positive people [912, 913].

6.4.4 Strengths of these analyses

The major strength of this study is the availability of serial plasma samples collected prior to and independently of lymphoma diagnosis, as well as the inclusion of a comparatively large number of lymphomas from contemporary HIV+ individuals. Furthermore, although this is a case control

study, cases and controls were nested within the EuroSIDA cohort. This design minimised the risk of some biases common to case-control studies, such as selection and recall bias. Selection bias is reduced as the cases and controls were drawn from the same population. In addition, although the number of lymphomas were low, the matching between cases and controls improved the efficiency of this analysis.

6.4.5 Limitations of these analyses

However, this chapter has a number of limitations which need to be kept in mind when interpreting from the results. This is an observational study and a comparatively small nested case-control study. Therefore, there is a risk the associations presented are affected by confounding and conclusions regarding causality cannot be determined. There are many known risk factors for lymphomas that are not collected in EuroSIDA and therefore could not be taken into account. For example, environmental exposures, autoimmune disorders, family history and EBV coinfection [607, 788, 789]. The original design of this study also involved measuring EBV DNA in stored plasma samples. A laboratory was identified to perform the analysis, however, no agreement on the time frame and cost of plasma sample analyses were attained within a timely manner (further discussed in chapter 8). Therefore, I made the executive decision to progress the study without the EBV component. Further adjustment for potential confounders was not possible due to low numbers, although only minor significant imbalances were evident at baseline. These included a lower proportion of cases on treatment and a lower proportion HCV positive. The higher HCV prevalence in the control group may result in an underestimation of the effects. Finally, the use of the matched design reduces risk of confounding of important risk factors by design, but also increases the complexity of the data, requiring specialist methods for analysis. Baseline CD4 was included as a matching factor in order to investigate the independent associations between B-cell activation and lymphoma development, however, it should be kept in mind that this may result in an underestimate of the association between markers of B-cell activation and lymphoma development.

In this chapter NHL and HL were combined as the numbers were not sufficient to investigate them separately. HL and NHL subtypes are a very heterogeneous group of cancers and considering them together ignores important pathological differences [804, 838]. Bibas *et al* (2012) [847] found that the elevated FLCs seems to be more associated with the risk of NHL than HL, possibly suggesting a different pathological mechanism (although only 16 HL cases were included). Another previous study investigated whether FLCs and immunoglobulins differed according to NHL subtypes and found HL had slightly higher levels of IgA and lower levels of IgM,

but no different in FLCs than NHL subtypes [894]. I repeated my analysis excluding HL cases and their respective controls and the results were unchanged. For this chapter, associations could not be investigated by NHL subtype due to insufficient numbers.

Another possible limitation is that FLCs levels may be affected by renal function as FLCs are excreted and catabolised by the kidneys [888, 914]. Measurements of eGFR were only available on one third of measurements (175/592, prospective collection of serum creatinine to calculate eGFR started in 2004). However, in people with eGFR measurements available, there was no evidence of a difference in eGFR between cases and controls at baseline. It is also possible that changes in FLCs and immunoglobulins reflect undiagnosed or late diagnosed cancer rather than preceding cancer development, however, the extended period over which plasma samples were taken make this unlikely. And finally, the assays used to measure B-cell markers were not validated in Plasma (validated in serum and whole blood) at the time of this study. Therefore it is possible that these results are due to lack of sensitivity to the markers levels in the plasma samples rather than true reflections of circulating levels at the time the plasma samples were drawn.

6.5 Conclusions

In conclusion, FLC-λ, FLC-λ and IgG were higher more than 2 years before lymphoma diagnosis, but the difference diminished nearer diagnosis. B-cell dysfunction, as demonstrated by polyclonal hyperglobulinemia, occurs many years prior to lymphoma development. The trajectories of FLC-κ and λ IgG, IgA, IgM, and IgD over time prior to lymphoma diagnosis show that the strength of association diminishes consistently with time leading up to diagnosis. The magnitude of the associations was moderate at best, and poorly predicted lymphoma development. The markers investigated in this chapter are unlikely to be strong candidates for risk assessment for targeted interventions

6.6 Publications

A manuscript for this chapter was accepted for publication in HIV Medicine in 2017. The results were presented at the HIV drug therapy Glasgow conference in 2016. The manuscript and presentation are included in Appendix IX and Appendix X respectively.

7 Testing patterns and predictive value of prostate specific antigen in a European HIV–positive cohort: does one size fit all?"

7.1 Introduction

7.1.1 Prostate cancer in the general population

Prostate cancer (PCa) is the most common solid neoplasm in older European men, with an incidence of >200 cases per 100,000 men in the general population from north and west-central Europe [915]. Over 41,700 men are diagnosed with PCa each year in the UK, accounting for almost 1 in 4 of all cancers in men [916], with an estimated 1 in 8 men developing PCa in their lifetime [916, 917].

7.1.2 Risk factors for prostate cancer

The causes of PCa in the general population are widely unknown, however, several factors associated with a higher risk of PCa have been identified.

- **Age** is thought to be one of the strongest risk factors. Clinical PCa is relatively rare in men aged <50 years, with approximately 1 in 3 of all diagnosis in men aged over 75 years [916].
- **Family history:** Men with a first degree relative diagnosed with PCa also have higher risk of PCa, and the risk increases with the number of first degree relatives diagnosed [915, 918, 919]. Risk is also increased in men whose mother was diagnosed with breast cancer [916]. It is estimated that 9% of PCa is truly hereditary [915].
- **BRCA1 and BRCA2 mutations:** Men with a mutation on the BRCA2 gene have increased risk of early onset PCa and more aggressive disease [920-922]. Mutations in the BRCA1 may also increase risk of PCa [916, 922].
- **Ethnicity:** Risk of PCa is 2-fold higher in men of black ethnicity relative to white ethnicity [916, 917].
- **Hormone levels:** Previous research suggested that high total testosterone levels may be associated with higher risk of PCa and more rapid progression [923, 924], however, recent studies have found no impact [431, 925-928]. Furthermore, no association has been found in hypergonadal men (a condition with a higher prevalence in HIV-positive [HIV+] men)[929].

- **Prostatitis:** Two meta analyses indicated that prostatitis is associated with PCa development, however they included studies that were heterogeneous and as well as case-control studies [916, 930, 931]. Inflammation of the prostate has been found to be predictive of PCa risk in prospective studies, however this may be driven by an association with higher PSA levels [932].

- **Diabetes:** Diabetes has been associated with lower PCa risk. Recent studies have attributed this to generally lower PSA levels in diabetic men and a lower rate of PCa diagnosed through PSA testing (i.e. PSA > 4 ng/mL) compared to non-diabetic men [916, 933, 934].

7.1.3 Early detection and diagnosis of prostate cancer

European guidelines on the early detection and management of PCa in the general population is provided by the European Association of Urology [915]. Men at high risk of PCa are generally identified though elevated prostate specific antigen (PSA) tests and digital rectal exams (DRE). A PSA ≥4 ng/mL and/or an abnormal DRE are commonly used as clinical indicators of PCa risk [935-937]. A DRE can be used to detect PCas in the peripheral area of the prostate and around 18% of PCas are detected using this method alone (regardless of PSA level) [915, 938]. The introduction of PSA testing revolutionised PCa diagnosis. PSA level is thought to be a better predictor of cancer than DRE alone [935, 939] and is the most common reason why PCa is detected in the United States [935]. Men with an elevated PSA and/or abnormal DRE are then required to have a biopsy to confirm the presence of PCa [915, 937, 939]. The biopsy involves taking several small cores of prostate tissue which are then examined by a pathologist [939]. The biopsy results are summarised using the Gleason score which is a measure of PCa grade (ranging from 2 – 10) based on the mix of cancer cells present [940]. Less aggressive cancers tend to have lower Gleason scores (2 – 4), while more aggressive PCa tend to have higher Gleason scores (7 – 10) [940].

7.1.4 Screening for prostate cancer

Screening is often initiated by health authorities with the intention of identifying PCa early to maximise treatment options, reduce the PCa associated mortality, and to maintain or improve future quality of life [935, 941]. There are two main approaches to screening: population based (or mass) screening which is defined as the systematic examination of asymptomatic men at risk for a disease, and opportunistic (or early detection) screening which involves individual case finding initiated through communication between patient and physician [935]. Population based

screening for PCa is currently one of the most controversial topics in the urological literature. Population based screening is not currently recommended by most urological societies and associated medical entities, however, recommendations on the appropriate use of screening in PCa detection and management is conflicting [915, 937, 939, 942-944].

PCa is often asymptomatic while it remains localised to the prostate gland, however, if diagnosed after the cancer has spread beyond the prostate, treatments are less effective [941]. Therefore, early detection and treatment could be highly beneficial [941]. It has been estimated that screening for PCa can result in a diagnosis of PCa between five to 13 years earlier (referred to as the lead time) [945]. There is currently no high level evidence that population based screening using PSA testing reduces PCA mortality [915, 939, 941, 946, 947]. A Cochrane review concluded that population based PCa screening leads to a 30% higher rate of diagnosis of PCa as well as a higher rate of localised and less advanced PCa, however, no overall or PCa specific survival benefit was found [488, 941, 948]. Furthermore, screening was associated with a high degree of overdiagnosis, invasive and expensive follow-up tests, and unnecessary treatment in some men (which can cause more adverse events than the PCa itself) [941]. One large RCT did report a 20% significant reduction of PCa specific mortality in a pre-specified subgroup of men aged 55 to 69 years (results were inconclusive in other age groups), but an overdiagnosis rate of 50% in those screened [945, 946, 949, 950]. Overdiagnosis refers to the situation where a man is diagnosed with a clinically non-significant PCa though PSA testing that would not have been apparent in the absence of PSA testing. Autopsy studies have shown many men living with undiagnosed PCa often go on to die from another cause, therefore, overdiagnosis puts men at risk of unnecessary treatment [945, 951, 952]. The European Randomised study of Screening for Prostate Cancer Study (ERSPC) have estimated that the number needed to invite for PSA screening to avoid one prostate cancer death was 781 and the number needed to detect and treat was 27 [946], which is comparable to some breast cancer trials [953]. The benefits and harms of screening are summarised in Figure 7.1

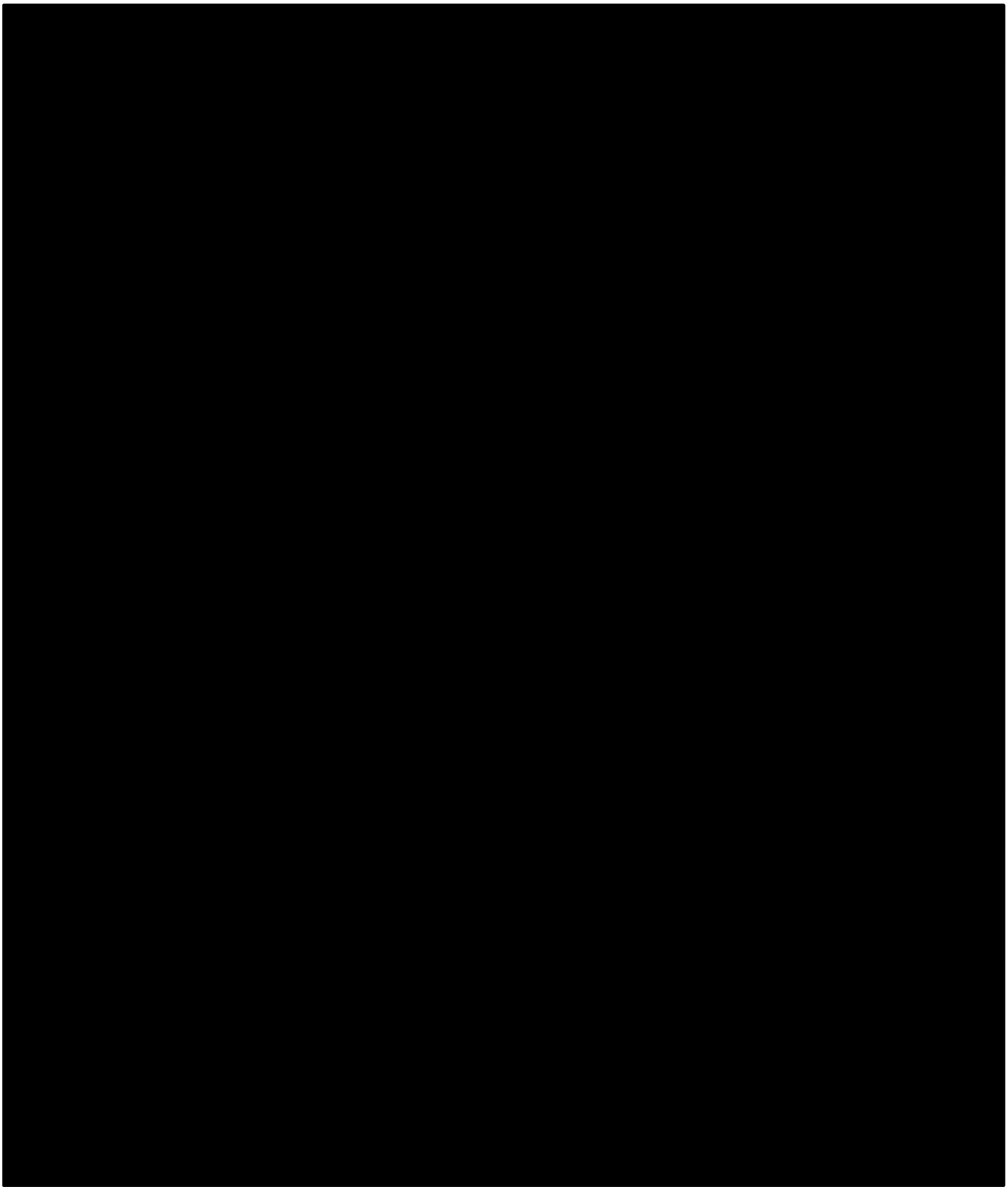


Figure 7.1 Benefits and harms of prostate specific antigen (PSA) screening for prostate cancer (PCa) diagnosis in men aged 55 or older from the US preventative task force 2017 draft recommendation statement on screening for prostate cancer [954].

* This includes men who choose surgery or radiation at diagnosis, as well as men who choose to monitor their cancer initially and later have surgery or radiation when it progresses.

7.1.5 Survival after prostate cancer diagnosis

The 5 year survival for prostate cancer has been estimated to be 83.4 (83.1 – 83.6)% in Europe and has increased over time from 76% to 87% between 1999 – 2007 [955]. The high 5 year survival for PCa in Europe is closely linked to increasing diagnoses of early stage cancers and longer lead time (the time by which the diagnosis is brought forward due to screening [956]) due to PSA testing [957]. Men with Low grade (Gleason < 6) and low volume PCa have less than a <6% risk of disease progression within a decade of diagnosis and rate of progression is slow [957].

7.2 Prostate cancer in HIV-positive men

Recent studies have consistently found a protective effect of HIV infection on PCa incidence in the combination antiretroviral therapy (cART) era [15, 16, 21, 431, 438, 469, 486-488], independently of PSA screening patterns and risk factors for PCa. Nonetheless, the frequency of PCa diagnoses in HIV+ men has increased and is expected to continue as the proportion of HIV+ men aged >50 years grow [7, 436, 438, 958]. Furthermore, HIV+ people are estimated to have a higher 2-fold higher mortality rate following PCa diagnosis than HIV-negative people after adjustment for various patient factors, including age, cancer stage, and cancer treatment [535]. As PCa becomes more prevalent, urologist, and oncologists will increasingly contribute to HIV care, with early PCa detection, diagnosis, and treatment likely to become an integral component of management of HIV+ men [959, 960].

7.2.1 Guidelines on prostate specific antigen testing in HIV-positive men

There are no clear guidelines on the use of PSA tests and screening for PCa in HIV+ men. The British HIV association (BHIVA) recommend managing PCa in HIV+ men in a similar fashion to those HIV-negative, however, detection and diagnosis of PCa is not addressed [961]. The European AIDS Clinical society (EACS) guidelines are based on those for the general population. They suggest that screening should be limited to men aged >50 years (unless considered high risk) and to men with a life expectancy of more than 10 years. Recommended methods of diagnosis include using a DRE, with or without a PSA test [140, 915]. Furthermore, it is recommended that a decision to use PSA testing is performed on a per patient basis, where the decision is between the patient and physician [140, 915]. The lack of clear or consistent guidelines facilitates variations in screening patterns across clinics and regions.

7.3 Markers associated with prostate cancer

7.3.1 Prostate specific antigen

PSA is a glycoprotein enzyme produced by epithelial cells of the prostate gland. It is elevated in the presence of PCa, however PSA is organ specific rather than cancer specific, thus it is also elevated in the presence of other prostate disorders [915]. Level of PSA is a continuous parameter of PCa risk, meaning that the higher the PSA level, the higher the likelihood of PCa. Unfortunately this is not useful in the context of clinical practice, and a PSA cut-off of PSA >4 ng/mL is often applied to identify "high risk" men [935, 936]. The sensitivity (true positive rate) and specificity (true negative rate) of using a cut-off of PSA >4 ng/mL in the general population has been estimated to be 21% and 90%, respectively, with a positive predictive value of 30% [935, 942]. Indeed 79% of PCa will exist with PSA <4 ng/mL and can even exist even at very low levels of PSA (Table 7.1) which may be missed in the screening process [915, 962].

Table 7.1 Prevalence of PCa in relation to low PSA values in the general population in the control arm of the Prostate Cancer Prevention Trial [962].

PSA level (ng/mL)	Prevalence of PCa (%)	Prevalence of high grade PCa (Gleason > 7) (%)
0.0 – 0.5	6.6	0.8
0.6 – 1.0	10.1	1.0
1.1 – 2.0	17.0	2.0
2.1 – 3.0	23.9	4.6
3.1 – 4.0	26.9	6.7

PSA: Prostate specific antigen, PCa: prostate cancer.

Serum Total PSA (tPSA) is made up of complexed PSA (cPSA) and free PSA (fPSA) [963]. How the presence of PCa impacts on the prostate and PSA related processes is shown in Figure 7.2 and Figure 7.3. In a healthy prostate (Figure 7.2), PROpsa is converted into active PSA in the lumen region of the prostate. Some of the active PSA diffuses into circulation where it forms cPSA with protease inhibitors (cPSA consists of three different forms: PSA-ACT, PSA-A2M and PSA-API), however the majority undergoes proteolysis in the lumen to produce inactive PSA. Inactive PSA enters circulation as fPSA (inactive and uncomplexed) and is made up of intact PSA and BPSA. The presence of PCa can cause break down in basal cells, basement membrane and normal lumen architecture (Figure 7.3). This results in a reduction of inactive PSA production in the lumen and an increased amounts of active PSA leak into circulation and is converted into cPSA [964-966]. This effectively increases the concentration of tPSA overall, but also increases ratio

of cPSA to fPSA present in circulation. Therefore both fPSA and tPSA concentrations increase, however fPSA increases at a slower rate and the proportion of free PSA declines.

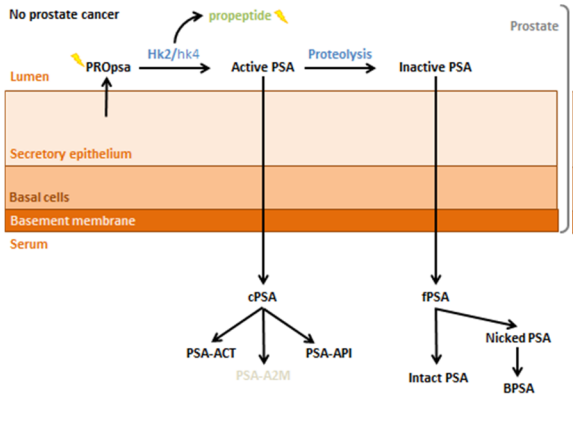


Figure 7.2 Production of various molecular forms of PSA within the prostate gland for men without PCa (adapted from [964-966]). PSA-A2M is not easily measured and is coloured in grey to demonstrate this.

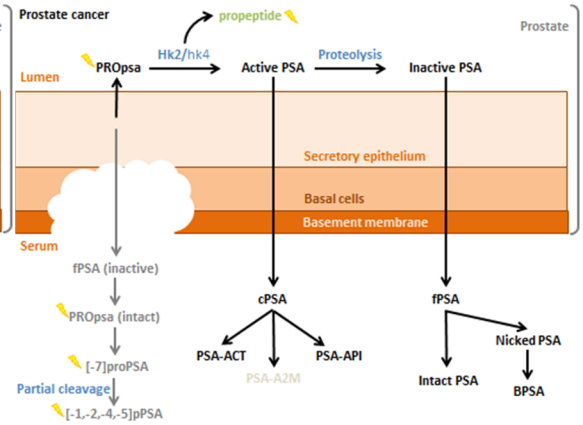


Figure 7.3 Production of various molecular forms of PSA within the prostate gland for men with PCa (adapted from [964-966]). PSA-A2M is not easily measured and is coloured in grey to demonstrate this.

PSA: Prostate specific antigen, PCa: prostate cancer.

Various derivatives of PSA have been suggested in order to improve the predictive power of PSA alone for PCa detection and these are outlined in Table 7.2.

Table 7.2 PSA derivatives and alternate measurements.

PSA derivatives	Definition	Association
fPSA/tPSA ratio (f/tPSA) [915]	fPSA Divided by tPSA	In the presence of PCa, the amount of both tPSA and fPSA in the blood increase, however fPSA levels increases at a slower rate than tPSA, resulting in a lower ratio of fPSA to tPSA [915, 936, 967, 968] f/tPSA is often used to differentiate benign prostate hyperplasia from PCa in men with moderately elevated tPSA 4 – 10 ng/mL and a negative DRE. F/tPSA is not predictive in men with PSA >10 ng/mL [915, 969, 970]
PSA density [915]	tPSA divided by prostate volume (determined by trans-rectal ultrasound) (TRUS)	The higher the PSA density, the more likely that PCa is clinically significant [971, 972]
PSA velocity (PSAV) [973, 974].	absolute annual increase in tPSA (ng/mL/year)	High levels of background noise limit use as a diagnostic marker. Potentially useful as prognostic markers [975]
PSA doubling time (PSA-DT) [974, 976].	measures the exponential increase in tPSA over time	High levels of background noise limit use as a diagnostic marker. Potentially useful as prognostic markers [975]
Prostate health index (PHI)	Combines three isotypes of PSA: pro[-2]PSA, fPSA and tPSA. PHI = (pro[-2]PSA /fPSA)*sqrt(tPSA)	Some studies have shown PHI to be a better diagnostic marker than tPSA or fPSA as well as having improved detectability of clinically significant PCa. [977, 978]

PSA: Prostate specific antigen, PCa: Prostate cancer, tPSA: total PSA, fPSA: free PSA, DRE: digital rectal exam.

7.3.2 Testosterone

Testosterone is the most important testicular androgen in men [979]. Deficient sex hormone production, or hypogonadism, is common in HIV+ men (prevalence 12 - 20% in the cART era) and may be treated with testosterone replacement therapy (TRT) to relieve symptoms [980, 981]. Some studies in the general population have linked elevated testosterone levels to increased risk of PCa [923, 924], however, recent studies have found no impact [431, 925-927]. Very low testosterone levels have been linked with lower PSA values and therefore may mask PCa detection through PSA testing [982], however, recent studies have shown that testosterone replacement therapy does not raise PSA concentration in the long term [983]. In the adult general population, testosterone levels tend to decline with older age [984].

7.3.3 Sex hormone binding globulin

Sex hormone binding globulin (SHBG) regulates the plasma levels and biological actions of testosterone within the body [985]. In the adult general population, most circulating testosterone is bound to SHBG (50-60%) [979]. When SHBG levels are low, this indicates that a higher proportion of total testosterone will be bioavailable (not bound to SHBG) [985]. SHBG has been modestly inversely associated with PCa risk as well as the velocity and doubling time [925]. SHBG levels also tend to increase with older age as testosterone levels decline [984].

7.4 Aims of this chapter

There is limited data available on variations in PSA testing practices in HIV+ men. Most studies are US-based, where PSA testing has been widely used since 1980, and may not be generalizable to European settings [431, 546]. No previous studies have investigated how PSA behaves prior to PCa diagnosis in HIV+ men and appropriate use of a cut-off in HIV+ men remain largely unverified. Therefore, these analyses had two aims.

- **Aim 1:** To perform a cohort analysis to assess variations in PSA testing patterns in European HIV+ men.
- **Aim 2:** To perform a nested-case-control study to assess the diagnostic ability of tPSA and fPSA associated with PCa risk, including changes in marker levels prior to diagnosis. As PCa is a hormone dependent cancer, plasma levels of testosterone and SHBG were also measured.

7.5 Methods

7.5.1 Patients and methods

This study was conducted within the EuroSIDA cohort and utilised the EuroSIDA plasma biobank of prospectively stored plasma samples. Both the EuroSIDA study and the biobank are described in detail in chapter 2 sections 2.1.1 and 2.1.1.9. The EuroSIDA study has collected the results of PSA tests performed locally at clinics (i.e. as part of patient care) since 1 January 2008. These PSA tests will be referred to as reported PSA to distinguish them from centrally measured PSA on plasma samples as part of the nested-case-control study. This study was done on the D38 version of the EuroSIDA database.

7.5.2 Aim 1: Reported testing for prostate specific antigen in Europe

The first component of this study investigated reported PSA testing rates in a cohort of European men. Baseline was defined as the later of either first visit, 1 January 2008 or 1 January of the first year the clinic reported PSA testing in ≥5% of men. Men were followed until the earliest of PCa diagnosis, last visit or death. The primary outcome was the incidence rate of reported PSA testing during follow-up. All PSA tests reported to EuroSIDA during the follow-up period were included and multiple PSA tests were allowed per person.

7.5.2.1 Inclusion criteria

Men enrolled in EuroSIDA with prospective follow-up after 1 January 2008 were included. Men who were aged <16 years, without a CD4 cell count, or HIV viral load (HIV-VL) measured during follow-up were excluded. Not all clinics use PSA testing as part of routine care or have routinely reported PSA to EuroSIDA. Therefore, the main analysis was restricted to clinics that reported PSA testing in ≥5% of attending men per year (N=4,482). Sensitivity analyses investigated how the results changed when including all clinics, regardless of PSA testing practices, and centers that reported PSA testing in ≥10% and ≥25% of men per year. Prospective data collection for all cancers diagnosed in EuroSIDA has been in place since 2001. All cancers were classified using the International Classification of Diseases and Related Health Problems, 10th edition [986]. Men with PCa prior to baseline, metastases of any cancer and recurrences of PCa during follow-up were excluded. A flow chart for the inclusion criteria is shown in Figure 7.4.

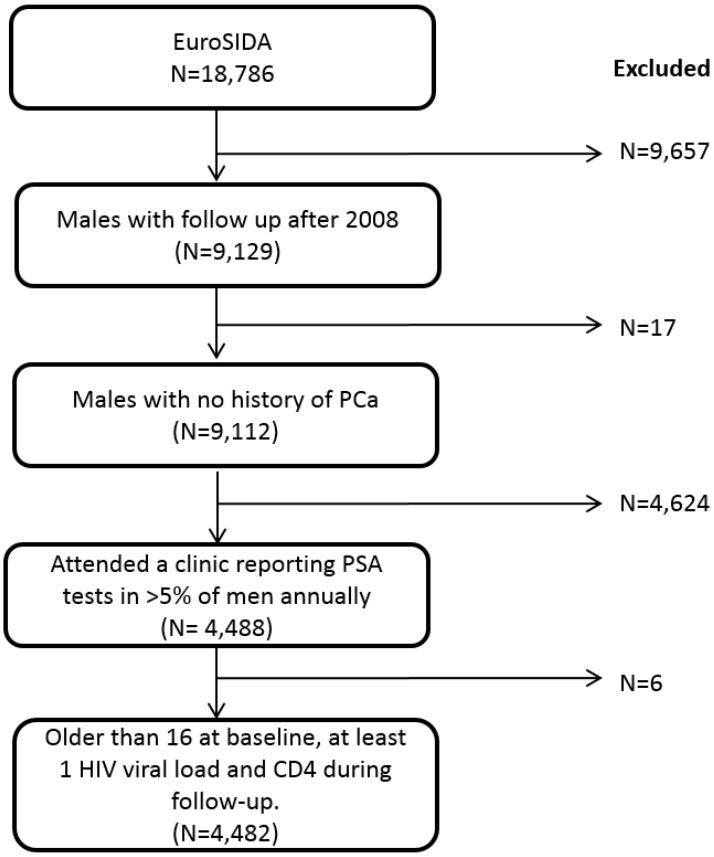


Figure 7.4 Flow chart for inclusion of EuroSIDA participants in cohort analysis.
PCa: Prostate cancer, PSA: prostate specific antigen.

7.5.2.2 Variables included in analyses

Both baseline and time updated variables from the EuroSIDA database were included in this analysis, as detailed in Table 7.3.

Table 7.3 Summary of baseline and time updated variables.

Variable	Time updated	Levels	Definitions and comments
Age (years)	Yes	Categorised into ≤35, 36 – 40, 41 – 50, ≥ 51 years	
Calendar year	Yes	Categorised by year	
Region of Europe		East, east central, south, west central, north Europe, and Argentina	See chapter 2 section 2.1.1.2
Ethnicity		White, Non-white and other (includes Asian, black and unknown race)	

Variable	Time updated	Levels	Definitions and comments
HIV mode of transmission		Sex between men, heterosexual, IDU other or unknown	
BMI (kg/m^2)		Under weight (<18), normal weight (18 – 25), Over weight (25 – 30), obese(30+)	Classified according to the WHO standard [597]
Smoking status	Yes	Non-smokers, current smokers, previous smokers, unknown	
Current CD4 cell count (cells/mm^3)	Yes	Categorised into <200, 200 – 349, 350 – 499, ≥500 cells/mm^3, unknown	Within 6 months prior to date of interest
Current HIV-VL (copies/mL)	Yes	Categorised into <400, ≥ 400 copies/mL, unknown	Within 6 months prior to date of interest
Prior AIDS defining cancer (ADC) diagnosis	Yes	Yes , no	Classified according to the 1993 CDC clinical definition [133]
Prior AIDS defining event (excluding ADC)	Yes	Yes , no	Classified according to the 1993 CDC clinical definition [133]
Prior non-AIDS defining cancer (NADC) diagnosis	Yes	Yes , no	
Prior non-AIDS defining event (excluding NADC)	Yes	Yes , no	Pancreatitis, grade 3 or 4 hepatic encephalopathy or liver-related death, myocardial infarction, stroke, coronary artery bypass graft, coronary angioplasty, carotid endarterectomy, and end-stage renal disease [221]
HBV coinfection	Yes	Positive, negative, or unknown	Most recent positive HBsAG surface antigen test or presence of detectable HBV DNA. Those without a HBV test were categorised as unknown
HCV coinfection	Yes	Positive, negative, or unknown	A prior positive HCV surface antibody test. Those without a HCV test were categorised as unknown
Hypertension	Yes	Yes, no	systolic blood pressure >140 mmHG, diastolic blood pressure >90 mmHg or receiving any hypertensive drugs
Antiretroviral use	Yes	Yes, no	Antiretroviral regimen containing ≥1 drugs from any class

IDU: through injecting drug use, BMI: Body mass index, HIV-VL: HIV viral load, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, HBV: Hepatitis B, HCV; Hepatitis C.

7.5.2.3 Statistical methods

7.5.2.3.1 Characteristics at baseline

Characteristics of men who had ≥1 reported PSA test recorded were compared to men who had no reported PSA test using numbers and percentages for categorical variables and median with interquartile range (IQR) for numerical variables at baseline. All bivariate associations were tested using chi squared tests for categorical variables and Kruskal-Wallis tests for numerical variables.

7.5.2.3.2 Patient factors associated with prostate specific antigen testing

Poisson regression with generalised estimating equations assuming auto-regressive (AR1) correlation allowing for repeated tests per person were used to investigate the association between various demographic, HIV, and lifestyle related characteristics and PSA testing. The AR(1) correlation structure was selected in order to assume that correlation diminished with increasing time between observations. Variables included in the model were identified a priori based on expert clinical input, availability of data, and the published literature.

7.5.3 Aim 2: The kinetics of and predictive value of prostate specific antigen and other biomarkers for prostate cancer

The second component of this project involved designing and analysing a 1:2 nested-case-control study within the EuroSIDA cohort to assess the kinetics and predictive value of PCa biomarkers. The methodology used for the design of this study was similar to that described in chapter 6.

7.5.3.1 Inclusion criteria

Men with follow-up after the 1 of January 2001 with no history of PCa were eligible for inclusion. Both cases and controls were required to have at least one sample in the prospective sample repository (the plasma repository is explained in detail in chapter 2 section 2.1.1.9) available prior to PCa diagnosis (or equivalent in controls). Date of earliest plasma sample was considered as baseline.

7.5.3.2 Cases and controls

All eligible men with a primary diagnosis of PCa after 1 January 2001 were considered as cases. For each case, 2 matched controls (where available) were selected using incidence density sampling (see Figure 7.5). Controls were selected from eligible men with prospective follow-up after 1 January 2001 with no history of PCa at the date of diagnosis for each case. If two controls were not available, 1 control was selected. The date of diagnosis of each case and equivalent date of latest sample in controls will be referred to as the "matched date".

7.5.3.3 Matching variables

Cases and controls were matched on region of Europe, date of earliest plasma sample (±2 years), date of latest plasma sample (±2 years), age at earliest plasma sample (± 5 years), CD4 cell count at earliest plasma sample (± 200 cells/mm^3). The windows used for matching were selected to allow suitable identification of controls while ensuring as few cases as possible were excluded from analyses.

An example of the selection of a case-control pair is shown in Figure 7.5. For each case of PCa (top person), a similar person who was PCa free at the date of diagnosis of the case (represented by the open circle) was selected. Incidence density sampling means the cases and controls are matched on follow-up time. In the example, the date of each plasma sample is represented by a red drop. The date of earliest sample for the case and control had to be within a 2 year window (represented by the blue region), and the date of the latest sample for the case and control had to be within a 2 year window (within the blue region).

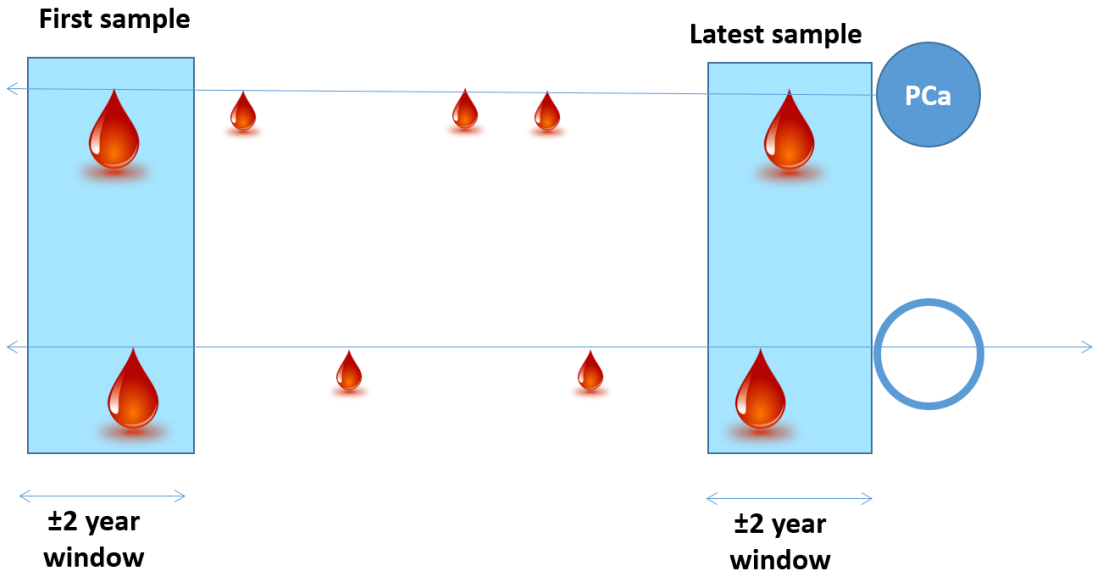


Figure 7.5 Selection of cases and controls using incidence density sampling.
PCa: prostate cancer.

7.5.3.4 Plasma samples and measurement of markers

All available serial plasma samples for cases and controls prior to the matched date were considered for inclusion. Where more than one plasma sample was available during the same calendar year, one sample was randomly selected for inclusion in order to preserve plasma samples for future analysis in other biomarker studies. There were 284 samples selected for analysis, however 2 were missing, 1 sample ID did not match to the correct patient and 4 were not in the freezer. This resulted in 277 samples that were analyzed.

Serial plasma samples were analysed for tPSA, fPSA, SHBG, and testosterone. All biomarkers were centrally measured by a technician blinded to case control status and other information on frozen stored plasma at the Department of Clinical Biochemistry at Rigshospitalet. tPSA, fPSA, and testosterone were measured by competitive electrochemiluminescense immunoassays using Cobas 8000 (Roche Diagnostics, Indianapolis, IN). SHBG was measured by a sandwich chemiluminescence immunometric assay using Immunulite 2000 (Siemens Healthcare Diagnostics, Flanders, NJ). Lower limits of detection were 0.03 µg/L for tPSA, 0.02 µg/L for fPSA, 0.42 nmol/L for testosterone, and 2 nmol/L for SHBG.

I considered the relationship between tPSA, fPSA, testosterone, SHBG and the ratio of fPSA to tPSA (f/tPSA) and PCa. In men with ≥2 plasma samples available, the doubling time and the average percentage change in marker level were calculated for each marker. Doubling time measures the exponential increase in serum PSA over time, in other words, the time in years it takes for the PSA level to double (years).

7.5.3.5 Statistical methods

7.5.3.5.1 Characteristics of cases and controls at earliest sample and matching variables

The characteristics of cases and controls were compared using numbers and percentages for categorical variables and median with IQR for numerical variables at baseline. All bivariate associations were tested using univariate conditional logistic regression due to the matched nature of the data.

7.5.3.5.2 Kinetics of each marker in the time before matched date

Mixed models with random slopes and intercepts were used to describe kinetics of each marker in the period prior to the matched date (accounting for multiple measurements within each person). Differences in the trajectory of each marker in cases and controls were investigated by testing for an interaction between time since first sample and case control status. Models were adjusted for age at first sample, and current log2 CD4 cell count and log10 HIV-VL. Models for fPSA and tPSA were further adjusted for current testosterone and SHBG level.

7.5.3.5.3 Association of each marker with likelihood of PCa

Unadjusted conditional logistic regression models were used to investigate the association between the odds of PCa and levels of each marker at baseline and latest sample. A log2 transformation was applied to each marker. The average percentage change per year and doubling time of each marker were also considered.

7.5.3.5.4 Sensitivity and specificity of total PSA for predicting PCa diagnosis

Receiver operator curve (ROC) analyses were used to assessed the predictive performance of each marker [905]. The area under the ROC curve (c-statistic) can be used to assess which marker (or combination of markers) best predicts cases and controls [905]. The c-statistic ranges from

0.5 to 1, where a c-statistic = 0.5 indicates no predictive ability (i.e. the marker is no better at determining PCa than flipping a coin) and a c-statistic = 1 indicates perfect predictability [905]. This was performed for each marker individually, all four markers combined, f/t PSA ratio, and the average percentage change per year and doubling time of each marker. The sensitivity and specificity of the widely used cut-off of tPSA>4 ng/mL was assessed and alternate cut-offs which jointly optimised sensitivity and specificity were investigated. For the purposes of this study, I considered sensitivity and specificity to be equally as important. For men with ≥2 samples available for analysis, the furthest sample from diagnosis was selected. I also considered age specific cut-offs for men aged <50 and ≥50 years.

All statistical tests were two sided with a type I error rate of 5%. All statistical analyses were performed using SAS 9.3 (Statistical Analysis Software, Cary NC, USA).

7.6 Results

7.6.1 Aim 1: Reported testing for prostate specific antigen in Europe

Out of the 110 clinics who provide data to EuroSIDA, 59 clinics reported PSA test results in >5% of men who attended their clinic annually, resulting in 4,482 men who were included in the study. Of the 4,482 men who were included, 1,318 (29.4%) of them had ≥1 reported PSA test recorded during follow-up. A total of 3,368 PSA reported tests were reported across all men with a median of 2 (IQR: 1, 3) tests per man.

I compared the 4,482 men included in this analysis who attended a clinic with a PSA testing rate of 5% or higher annually to those who were excluded but under follow-up after 2008 (N=6,957). Comparing men who were included to those who were not, a higher proportion were treatment experienced (87.4 vs 78.7%, respectively), whereas a lower proportion had a CD4 cell count <200 cell/mm^3 (6.7 vs 14.8%), HIV-VL >400 copies/mL (15.5 vs 34.9%), were HCV coinfected (13.8 vs 20.6%), or acquired their HIV through IDU (12.5% vs 20.1). A lower proportion of men from Eastern Europe were included (1.9 vs 11.9%). Age was similar in both groups.

7.6.1.1 Prostate cancer incidence

There were 14 reported diagnoses of PCa during 14,169 person-years of follow-up (incidence rate 1.0/1000 PYFU, 95%CI: 0.6, 1.7), and 10/14 PCa events which occurred in men who were PSA tested, with a median most recent PSA level of 7.9 (IQR: 3.8, 10.4) ng/mL, measured a median of 7 (IQR: 2, 13) months prior to PCa diagnosis.

7.6.1.2 Baseline characteristics

The baseline characteristics of the cohort are shown in Table 7.4. Of the men included in the analysis, 54% were aged 41 or older, 60% acquired HIV through sex between men, over 90% were of white ethnicity and over three quarters were from south, west central or northern Europe. Close to 80% of men has a baseline year of 2008. Over half had a CD4 cell count of 500 cells/mm^3 or higher and almost 85% were virally suppressed (HIV-VL≤ 400 copies/mL). Approximately 5% had ever had an AIDS defining cancer (ADC) diagnosis and just over one quarter had a prior AIDS event (other than ADC). Approximately 2% had been diagnosed with a non-AIDS defining cancer (NADC), and just under 5% had had a non-AIDS defining event (other than NADC). Just over one quarter had a history of hypertension while 5% were coinfected with HBV and 14% of HCV. Close to 90% of the men included were antiretroviral experienced. Almost 70% of PSA testing in HIV+ men occurred in those aged under 50, and approximately one third occurred in men aged under 40 years.

Table 7.4 Baseline[1] characteristics of men attending a clinic where >5% of men have at least one rouptinely reported prostate specific antigen (PSA) test result per year.

Characteristics	Baseline[1] N(%)	
	All patients	≥1 PSA test[2]
Overall	4,482 (100)	1,318 (100)
Age (years)		
≤ 35	1,100 (24.5)	218 (16 .5)
36 – 40	943 (21.0)	216 (16.4)
41 – 50	1,520 (33.9)	478 (36.3)
51 or older	919 (20.5)	406 (30.8)
Region		
East central	610 (13.6)	289 (21.9)
East	84 (1.9)	13 (1.0)
Argentina	262 (5.8)	27 (2.0)
South	1,389 (31.0)	393 (29.8)
West central	814 (18.2)	284 (21.5)
North	1,323 (29.5)	312 (23.7)
Calendar year of baseline[1]		
2008	3,625 (80.9)	1,187 (90.1)
2009	17 (0.4)	8 (0.6)
2010	2 (0.0)	0 (0.0)
2011	82 (1.8)	20 (1.5)
2012	692 (15.4)	103 (7.8)
2013/14	64 (1.4)	0 (0.0)
Mode of HIV transmission		
Sex between men	2,701 (60.3)	860 (65.3)
IDU	561 (12.5)	141 (10.7)
Heterosexual	925 (20.6)	242 (18.4)
Other/missing	295 (6.6)	75 (5.7)
Non-white ethnicity	348 (7.8)	78 (5.9)
Smoking status		
Never	1,777 (39.6)	520 (39.5)
Current	1,508 (33.6)	422 (32.0)
Former	24 (0.5)	9 (0.7)
Unknown	1,173 (26.2)	367 (27.8)
prior ADC[3]	240 (5.4)	77 (5.8)
prior AIDS event (excluding ADC)[3]	1,202 (26.8)	413 (31.3)
prior NADC	96 (2.1)	36 (2.7)
prior Non-AIDS event (excluding NADC) [4]	198 (4.4)	66 (5.0)
Prior Hypertension[5]	1,207 (26.9)	380 (28.8)
HBV[6]		
Yes	247 (5.5)	69 (5.2)
No	3,575 (79.8)	1,059 (80.3)
Unknown	660 (14.7)	190 (14.4)
HCV [7]		
Yes	619 (13.8)	150 (11.4)
No	3,045 (67.9)	889 (67.5)
Unknown	818 (18.3)	279 (21.2)
CD4 cell count cells/mm^3		
≤ 200	300 (6.7)	81 (6.2)
200 – 349	760 (17.0)	218 (16.5)
350 – 499	1,077 (24.0)	307 (23.3)
≥ 500	2,345 (52.3)	712 (54.0)
HIV-VL > 400 copies/mL	695 (15.5)	168 (12.7)
Antiretroviral experienced[8]	3,917 (87.4)	1,205 (91.4)

PSA: prostate specific antigen, IDU: injecting drug use, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, HBV: hepatitis B, HCV: hepatitis C, HIV-VL: HIV viral load.

[1] Baseline was defined as the latest of first visit, 01 January 2008, or the 1 of January of the first year in which the attending clinic reported PSA testing rates >5% per year.
[2] Reported PSA testing done in the clinic.

7.6.1.3 Factors associated with prostate specific antigen testing across Europe

Unadjusted (IRR) and adjusted incidence rate ratios (aIRR) for reported PSA testing are shown in Table 7.5. After adjustment, reported PSA testing in Europe was stable over time (aIRR for 1 year later: 1.00; 95%CI: 0.98, 1.02), although lower rates were observed in 2008 relative to 2010 and stabilized thereafter. The rate of reported PSA testing significantly increased with older age, with the highest rate in those aged 51 or older. Testing was also elevated in men aged 41 – 50 compared to those aged 36 – 40 years. East central Europe had very high rates of reported PSA testing, more than 7-fold higher than north Europe, and rates were also elevated in south and west-central Europe. Testing rates were higher in men who acquired their HIV through sex between men compared to other modes of HIV transmission, and were lower in those with lower current CD4 cell counts, current smokers relative to never smokers, a history of hypertension, or HCV coinfection. PSA testing was not associated with current HIV-VL, ethnicity, prior ADC, NADC, AIDS or non-AIDS events or HBV coinfection.

Table 7.5 Unadjusted (IRR) and adjusted incidence (aIRR) rate ratios of reported prostate specific antigen (PSA) testing in EuroSIDA clinics where >5% of men have at least one PSA test result per year.

Characteristics	Univariate analysis		Multivariate analysis[1]	
	IRR (95%CI)	P	aIRR (95%CI)	P
Overall				
Age (years)				
≤35	1.14 (0.82,1.59)	0.438	0.96 (0.73,1.26)	0.76
36 – 40	reference		reference	
41 – 50	0.89 (0.68,1.17)	0.402	1.37 (1.10,1.70)	<0.01
51 or older	1.39 (1.06,1.82)	0.017	2.37 (1.92,2.94)	<0.01
Region				
East central	6.25 (5.34,7.32)	<.001	7.49 (6.42,8.73)	<0.01
East	0.52 (0.29,0.92)	0.025	0.96 (0.56,1.66)	0.89
Argentina	0.48 (0.27,0.83)	0.009	0.74 (0.42,1.30)	0.3
South	1.31 (1.11,1.54)	0.001	1.72 (1.46,2.02)	<0.01
West central	1.51 (1.28,1.79)	<.001	1.54 (1.31,1.81)	<0.01
North	reference		reference	
Calendar year				
2008	0.54 (0.46,0.63)	<.001	0.67 (0.57,0.79)	<0.01
2009	1.12 (1.02,1.23)	0.016	1.11 (1.01,1.22)	0.03
2010	reference		reference	
2011	0.95 (0.87,1.04)	0.251	0.95 (0.87,1.04)	0.26
2012	0.98 (0.90,1.08)	0.725	0.96 (0.88,1.05)	0.41
2013/14	1.19 (1.07,1.32)	0.001	0.96 (0.86,1.06)	0.42
Mode of HIV transmission				
Sex between men	reference		reference	
IDU	0.41 (0.34,0.51)	<.001	0.64 (0.49,0.83)	<0.01
Heterosexual	0.68 (0.57,0.81)	<.001	0.74 (0.64,0.87)	<0.01
Other/missing	0.74 (0.55,0.98)	0.038	0.73 (0.55,0.96)	0.03
Non-white ethnicity	0.65 (0.51,0.85)	0.001	1.28 (0.93,1.76)	0.13
Smoking status				
Never	reference		reference	
Current	0.71 (0.62,0.82)	<.001	0.80 (0.71,0.90)	<0.01
Former	0.75 (0.62,0.90)	0.002	0.96 (0.82,1.13)	0.61
Unknown	0.82 (0.58,1.16)	0.262	0.80 (0.60,1.06)	0.12
prior ADC	0.99 (0.78,1.26)	0.939	1.05 (0.84,1.32)	0.66
prior AIDS event (excluding ADC)	0.91 (0.80,1.05)	0.2	1.02 (0.89,1.16)	0.82
prior NADC	1.13 (0.90,1.41)	0.303	1.26 (0.94,1.70)	0.12
prior Non-AIDS event (excluding NADC)	0.95 (0.80,1.14)	0.602	0.84 (0.69,1.03)	0.1
Prior Hypertension	0.92 (0.81,1.04)	0.191	0.89 (0.79,0.99)	0.03
HBV				
Yes	1.01 (0.80,1.27)	0.954	1.06 (0.89,1.27)	0.5
No	reference		reference	
Unknown	0.67 (0.49,0.92)	0.013	0.69 (0.52,0.92)	0.01
HCV				
No	reference		reference	
Yes	0.55 (0.46,0.65)	<.001	0.75 (0.61,0.93)	<0.01
Unknown	1.53 (1.24,1.89)	<.001	1.12 (0.94,1.33)	0.19
CD4 cell count cells/mm^3				
≤ 200	0.71 (0.55,0.91)	0.006	0.76 (0.61,0.95)	0.015
200 – 349	0.83 (0.71,0.98)	0.026	0.86 (0.75,0.99)	0.03
350 – 499	0.84 (0.75,0.94)	0.003	0.87 (0.79,0.97)	0.011
≥ 500	reference		reference	
HIV-VL > 400 copies/mL	1.17 (0.95,1.44)	0.134	1.11 (0.92,1.33)	0.28
Antiretroviral experienced	0.78 (0.56,1.09)	0.15	0.79 (0.58,1.09)	0.15

PSA: prostate specific antigen, IDU: injecting drug use, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, HBV: hepatitis B, HCV: hepatitis C, HIV-VL: HIV viral load.

[1] Models adjusted for all variables listed

7.6.1.4 Sensitivity analysis: varying inclusion criterion of the proportion of men tested for prostate specific antigen in each center per year

Sensitivity analyses varying the cut-off for the proportion of men tested in each center per year to ≥ 0 (i.e. all centers), 5, 10 and 25% are shown in Table 7.6. Only key variables are shown. Overall, the main difference in results was an attenuation in the aIRR with an increasingly restrictive definition. Starting with age, the association between older age and a higher rate of reported PSA testing attenuates as the inclusion criteria moves from ≥0% to ≥25%, however the result was still significant. For calendar year, the lower rate of reported PSA testing observed in 2008 was similar for all sensitivity analyses, however a general increase in reported PSA testing over time was observed when all centers were included, and when the analysis was restricted to centers that tested ≥25% of men. The higher rate of reported PSA testing in east central Europe remained, regardless of changes in the entry criteria.

Table 7.6 Sensitivity analyses varying the cut-off inclusion: proportion of men tested for prostate specific antigen (PSA) in each center per year to ≥ 0 (i.e. all centers), 5, 10 and 25%.

Center screening rate	≥0% (All centers) Men: 11439 Men with ≥1 PSA test: 1381		Main results: ≥5% Men: 4482 Men with ≥1 PSA test: 1318		≥10% Men: 2688 Men with ≥1 PSA test: 1026		≥25% Men: 973 Men with ≥1 PSA test: 575	
Variable	*aIRR*	*P-value*	*aIRR*	*P-value*	*aIRR*	*P-value*	*aIRR*	*P-value*
Age at baseline								
≤ 35 years	1.01 (0.75,1.36)	0.942	0.96 (0.73,1.26)	0.76	1.13 (0.92,1.40)	0.247	1.03 (0.85,1.23)	0.789
36 – 40 years	reference		reference		reference		reference	
41 – 50 years	1.52 (1.20,1.92)	<.001	1.37 (1.10,1.70)	<0.01	1.28 (1.08,1.52)	0.004	1.17 (1.01,1.35)	0.037
51 + years	2.57 (2.03,3.25)	<.001	2.37 (1.92,2.94)	<0.01	1.97 (1.66,2.34)	<.001	1.77 (1.53,2.05)	<.001
Calendar year								
2008	0.07 (0.06,0.08)	<.001	0.67 (0.57,0.79)	<0.01	0.64 (0.53,0.78)	<.001	0.06 (0.05,0.07)	<.001
2009	0.84 (0.76,0.93)	<.001	1.11 (1.01,1.22)	0.03	1.07 (0.97,1.18)	0.191	0.91 (0.83,1.01)	0.072
2010	reference		reference		reference		reference	
2011	1.11 (1.01,1.21)	0.024	0.95 (0.87,1.04)	0.26	0.91 (0.82,1.01)	0.079	1.07 (0.97,1.17)	0.198
2012	1.20 (1.10,1.32)	<.001	0.96 (0.88,1.05)	0.41	0.83 (0.75,0.92)	<.001	1.24 (1.13,1.36)	<.001
2013/14	1.47 (1.32,1.63)	<.001	0.96 (0.86,1.06)	0.42	0.91 (0.81,1.02)	0.104	1.58 (1.43,1.75)	<.001
Region								
East central	5.95 (5.03,7.03)	<.001	7.49 (6.42,8.73)	<0.01	4.77 (4.11,5.53)	<.001	5.66 (4.89,6.56)	<.001
East	0.32 (0.19,0.53)	<.001	0.96 (0.56,1.66)	0.89				
Argentina	0.78 (0.51,1.20)	0.262	0.74 (0.42,1.30)	0.3				
South	2.00 (1.69,2.37)	<.001	1.72 (1.46,2.02)	<0.01	1.09 (0.91,1.31)	0.33	0.77 (0.63,0.93)	0.008
West-central	1.29 (1.08,1.55)	0.005	1.54 (1.31,1.81)	<0.01	1.06 (0.89,1.26)	0.531	0.83 (0.70,0.99)	0.034
North	reference		reference		reference		reference	

aIRR: adjusted incidence rate ratio, PSA: prostate specific antigen,

Models were further adjusted for mode of HIV transmission, ethnicity, smoking status, AIDS defining cancers (ADC), AIDS defining events (excluding ADC), non-AIDS defining cancers (NADC), non-AIDS defining events (excluding NADC), hypertension, Hepatitis B, Hepatitis C, CD4 cell count, HIV viral load.

7.6.2 Aim 2: The kinetics of PSA biomarkers and predictive value of PSA and other biomarkers

7.6.2.1 Baseline characteristics and matching variables

Characteristics of cases (N=21) and controls (N=40) are shown in Table 7.7. Cases and controls were well balanced on both matched and non-matched variables, with a median of 6 years (IQR: 1, 9) between first and last plasma sample. Cases only differed from controls according to nadir CD4 cell count, which was higher in cases than controls.

7.6.2.2 Marker levels at earliest and latest sample in cases and controls

Of men with tPSA available at first sample, 53/59 (89.8%) of cases and controls had tPSA ≤4 ng/mL. Levels of tPSA were elevated in cases relative to controls at earliest sample and even more so at latest sample (Table 7.8). The average change per year of tPSA was increasing in cases, but was stable in controls. Doubling time for tPSA was similar for cases and controls. A similar trend was observed for fPSA. Cases had a lower f/tPSA than controls at baseline and even more so at latest sample, however no differences in average change per year or doubling time were detected (both P>0.05). Baseline and latest testosterone and SHBG levels, as well as doubling time and average change per year were similar for cases and controls.

Table 7.7 Baseline[1] characteristics for cases and controls.

Factors	*Cases*	*Controls*	*p-value*
	N (%)	**N (%)**	
All	21 (100)	40 (100)	-
Region[2]			
East central	5 (23.8)	10 (25.0)	Na
Argentina	1 (4.8)	1 (2.5)	
South	2 (9.5)	3 (7.5)	
North	6 (28.6)	12 (30.0)	
west-central	7 (33.3)	14 (35.0)	
Mode of HIV transmission			
Sex between men	17 (81.0)	30 (75.0)	0.98
IDU	0 (0.0)	2 (5.0)	
Heterosexual	2 (9.5)	5 (12.5)	
Other/Missing	2 (9.5)	3 (7.5)	
Non-white ethnicity	0 (0.0)	4 (10.0)	0.99
Smoking status			
Never	6 (28.6)	14 (35.0)	0.48
Current	2 (9.5)	1 (2.5)	
Former	0 (0)	0 (0)	
Missing	13 (61.9)	25 (62.5)	
prior ADC	21 (100)	34 (85.0)	0.99
prior AIDS (excluding ADC)	4 (19.0)	13 (32.5)	0.17
prior NADC	19 (90.5)	40 (100)	0.99
prior Non-AIDS event (excluding NADC)	17 (81.0)	39 (97.5)	0.1
Prior Hypertensive	8 (38.1)	15 (37.5)	0.56
HBV	3 (14.3)	1 (2.5)	0.12
HCV	0 (0.0)	1 (2.5)	1
Antiretroviral experienced	20 (95.2)	38 (95.0)	1
HIV-VL > 400 copies/mL	4 (19.0)	10 (25.0)	0.58
Median (IQR)	***Median (IQR)***	***Median (IQR)***	
Age at sample[2]	51.9 (48.6,56.7)	51.1 (47.3,55.5)	0.18
Earliest sample date[2]	OCT 1999 (MAR 1999, APR 2006)	AUG 2000 (JAN 1998, JUN 2006)	0.909
Latest sample date[2]	JUL 2008 (DEC 2004, MAR 2011)	SEP 2007 (SEP 2004, OCT 2010)	0.524
Time till PCa	6.6 (2.8,10.1)	-	-
CD4 cell count/mm^3[2]	460.0 (260.0,610.0)	426.0 (229.5,595.0)	0.07
Nadir CD4 count/mm^3	202.0 (144.0,320.0)	143.0(57.0,276.0)	0.05

IQR: interquartile range, ADC: AIDS defining cancer, NADC: non-AIDS defining cancer, HBV: hepatitis B, HCV: hepatitis C, HIV-VL: HIV viral load, PCa: Prostate cancer.

[1] Baseline defined as the date of earliest plasma sample.

[2] Matching variables.

Table 7.8 Marker levels at baseline, latest sample, and changes over time for cases and controls.

	Cases	*Controls*[1]	*p-value*
Marker level	***Median (IQR)***	***Median (IQR)***	
tPSA			
Baseline (ng/mL)	2.8 (1.6,4.6)	0.8 (0.5,1.2)	<0.01
latest (ng/mL)	6.1 (4.7,9.5)	0.8 (0.5,1.4)	0.04
Doubling time (years)	4.5 (2.8, 5.2)	4.1 (-12.6, 15.7)	0.83
Average change per year (ng/mL/year)	0.76 (0.37,0.86)	0.00 (-0.02,0.39)	Not estimated
fPSA			
Baseline (ng/mL)	0.4 (0.2,0.8)	0.3 (0.2,0.4)	<0.01
Latest (ng/mL)	0.9 (0.6,1.3)	0.2 (0.2,0.4)	<0.01
Doubling time (years)	5.59 (3.84,7.2)	13.43 (-7.31,31.05)	0.79
Average change per year (ng/mL/year)	0.07 (0.05,0.1)	0 (0,0.01)	0.01
f/t PSA ratio			
Baseline	0.2 (0.1,0.3)	0.3 (0.3,0.4)	<0.01
Latest	0.1 (0.1,0.2)	0.3 (0.2,0.5)	0.008
Doubling time (years)	-7.07 (-16.49,-3.07)	-2.2 (-16.13,18.55)	0.58
Average change per year (/year)	1.72 (0.33,29.48)	0.18 (-0.15,1.09)	0.54
Testosterone (nmol/L)			
Baseline (nmol/L)	19.3 (14.5,22.2)	19.7 (15.8,25.9)	0.73
Latest (nmol/L)	17.7 (16.6,21.7)	18.0 (14.0,23.7)	0.917
Doubling time (year)	0.53 (-15.25,29.73)	-6.8 (-17.1,23.78)	0.52
Average change per year	0.01 (-0.87,0.49)	-0.32 (-1.01,0.3)	1
SHBG (nmol/L)			
Baseline (nmol/L)	48.0 (34.0,69.0)	53.5 (34.0,64.0)	0.83
Latest (nmol/L)	54.0 (34.0,63.0)	49.0 (36.0,65.0)	0.83
Doubling time (year)	10.66 (-5.72,31.98)	-4.01 (-13.24,12.82)	0.49
Average change per year (nmol/L/year)	0.9 (-2.17,2.48)	-0.58 (-3.08,1.59)	0.64

IQR: interquartile range, PSA: prostate specific antigen, tPSA: total PSA, fPSA: free PSA, SHBG: Sex hormone binding globulin.

[1] Marker levels were available on all cases at baseline and latest sample, and 38/41 and 39/41 controls at baseline and latest sample.

7.6.2.3 Kinetics of each marker in time prior to prostate cancer diagnosis

The trajectories of each marker for cases and controls in the time leading up to the date of diagnosis or matched date in controls are shown in Figure 7.6 - Figure 7.10. Figure 7.6 shows a clear increase tPSA level leading up to PCa diagnosis for cases, however levels were stable in controls and a similar pattern was observed for fPSA (Figure 7.7). Conversely, a clear decline in f/tPSA in the years prior to diagnosis in cases was demonstrated in cases, but not controls (Figure 7.8). Levels of testosterone and SHBG were similar in cases and controls prior to the matched date (Figure 7.9 and Figure 7.10).

The percentage change over time of each marker is summarised in Table 7.9. TPSA and fPSA was increasing over time in the cases, but not in controls, and this difference was significant (both p<0.01). For example, level of tPSA and fPSA increased over time in univariate analyses in cases by 13.7 and 7.9 % per year, respectively, however levels of both were stable in controls. The difference in change in tPSA and fPSA between cases and controls remained significant after adjustment (both p<0.01). In unadjusted analyses, the rate of decline in f/tPSA differed in cases and controls (p=0.04), where f/tPSA declined by 3.5% per year in cases but was stable in controls, however the difference between the cases and controls was not significant after adjustment (p=0.11). There was little change over time in testosterone and SHBG, which was similar when comparing cases and controls both before and after adjustment.

Table 7.9 Average percentage (%) change in markers over time between persons developing prostate cancer (cases) and those remaining prostate cancer free (controls).

	Univariate			*Multivariate*[1]		
	% Change per year	*p-value (slope)*	*p-value* [2]	*% Change per year*	*p-value (slope)*	*p-value* [2]
tPSA						
controls	-0.44 (-2.49, 1.65)	0.67	<.01	1.19 (-1.38, 3.82)	0.37	<.01
cases	13.74 (10.29,17.29)	<.01		15.47 (10.69,20.46)	<.01	
fPSA						
controls	-0.95 (-2.97, 1.10)	0.36	<.01	0.97 (-1.42, 3.42)	0.43	<.01
cases	7.93 (4.73,11.21)	<.01		11.84 (7.62,16.24)	<.01	
t/fPSA ratio						
controls	-0.52 (-2.18, 1.16)	0.54	0.04	-0.38 (-2.21, 1.49)	0.69	0.11
cases	-3.49 (-5.70,-1.23)	<.01		-3.15 (-5.98,-0.23)	0.03	
testosterone						
controls	-3.01 (-4.37,-1.63)	<.01	0.09	0.37 (-1.24, 2.00)	0.65	0.96
cases	-0.89 (-2.94, 1.21)	0.40		0.29 (-2.18, 2.82)	0.82	
SHBG						
controls	-0.87 (-2.55, 0.83)	0.31	0.11	-2.42 (-3.56,-1.26)	<.01	0.58
cases	1.51 (-0.83, 3.91)	0.21		-1.79 (-3.78, 0.25)	0.09	

PCa: prostate cancer, PSA: prostate specific antigen, tPSA: total PSA, fPSA: free PSA, SHBG: Sex hormone binding globulin.

[1]Adjusted for current age, log2 CD4 cell count, log10 HIV viral load. Models for PSA related variables were also adjusted for current log2 SHBG and testosterone. Models for testosterone and SHBG were additionally adjusted for current SHBG and testosterone only.

[2] P-value for difference in % change per year in each marker between cases and controls.

PCa: prostate cancer, PSA: prostate specific antigen, tPSA: total PSA, fPSA: free PSA, SHBG: Sex hormone binding globulin.

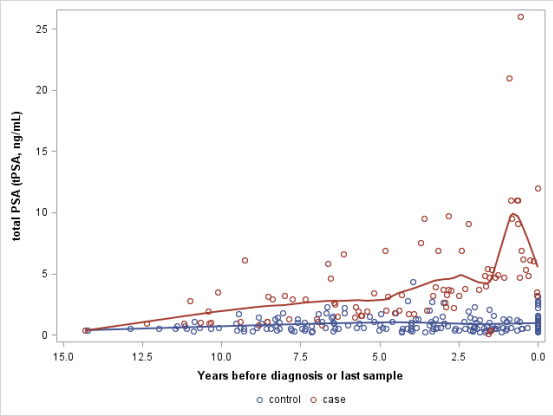


Figure 7.6 Scatter plot of tPSA by time till prostate cancer (or last sample) with superimposed loess curves for cases and controls.

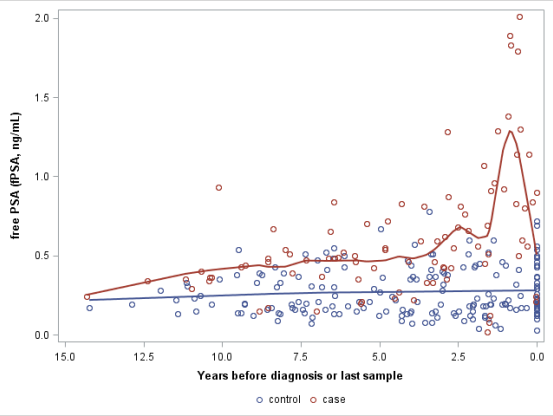


Figure 7.7 Scatter plot of fPSA by time till prostate cancer (or last sample) with superimposed loess curves for cases and controls.

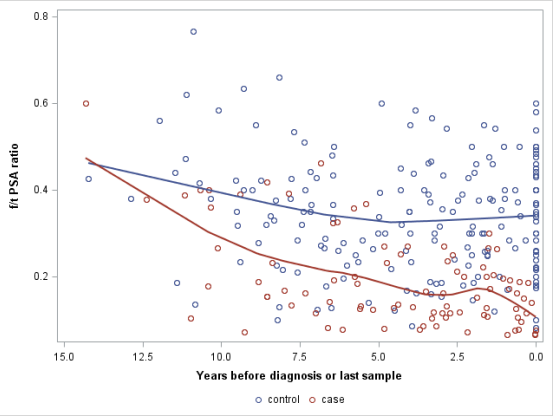


Figure 7.8 Scatter plot of f/tPSA by time till prostate cancer (or last sample) with superimposed loess curves for cases and controls.

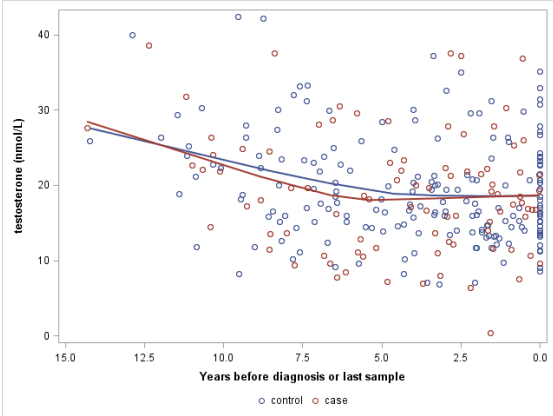


Figure 7.9 Scatter plot of testosterone by time till prostate cancer (or last sample) with superimposed loess curves for cases and controls.

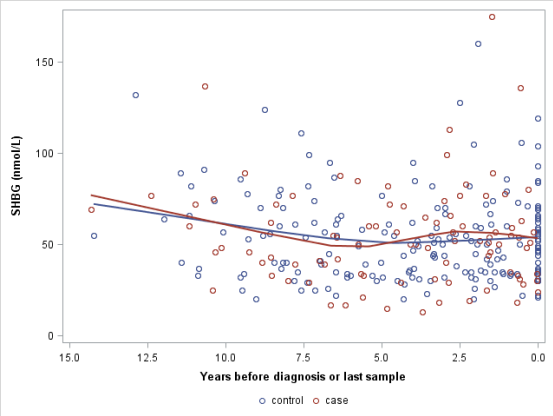


Figure 7.10 Scatter plot of SHBG by time till prostate cancer (or last sample) with superimposed loess curves for cases and controls.

7.6.2.4 Association between prostate cancer and marker levels

Higher levels of tPSA and fPSA at earliest and latest sample were associated with higher odds of PCa (Table 7.10), where a 2-fold higher baseline tPSA was associated with almost 5-fold higher odds of PCa and latest tPSA with an 8-fold higher in odds of PCa. Conversely, a higher f/tPSA ratio was associated with lower conditional odds of PCa at both first and latest sample. A 1 unit faster percentage change in tPSA and fPSA over time were associated with higher odds of PCa, however, no association with the percentage change in the f/tPSA ratio over time was identified. The time for tPSA level to double (doubling time) was not associated with PCa, nor doubling time of fPSA or f/tPSA ratio. There was no significant association between odds of PCa and testosterone and SHBG level at earliest or latest sample, or according to average percentage change per year or doubling time.

Given the pattern of changes seen in Figure 7.6 - Figure 7.10 and that the levels of tPSA and fPSA appeared to be significantly elevated in cases relative to controls many years prior to PCa diagnosis, I investigated how long before PCa the differences in tPSA and fPSA appeared. Both fPSA and tPSA at 5 years prior to PCa were associated with significantly higher odds of PCa (OR for 2-fold higher tPSA: 2.92; 95%CI: 1.38, 8.07; P<0.01 and fPSA: 5.46; 95%CI: 1.54, 29.05; P<0.01, respectively). A similar pattern was seen at 6, 7 and up to 10 years prior to PCa, but the number of cases and controls with results at these time points were too small to allow formal statistical analyses.

7.6.2.5 Predictive ability of markers for prostate diagnosis

C-statistics reflecting the predictive value of each marker are shown in Table 7.11. Looking at the absolute marker values, the most informative predictor of PCa was tPSA (c-statistic= 0.90), followed by fPSA (c-statistic= 0.82) and the ratio of fPSA to tPSA (c-statistic= 0.82). Testosterone (0.51) and SHBG (0.51) did not predict PCa. Combining the four main markers investigated in this study of tPSA, fPSA, SHBG, and testosterone resulted in a c-statistic of 0.91, which was only a marginal improvement on tPSA alone. In those who had ≥2 samples available, I also looked at the c-statistic associated with average percentage average change per year of tPSA and doubling time, however this was only based on 16 cases and 32 controls, and the model did not converge, and therefore these results are not shown. However, for completeness, average percentage change in tPSA and fPSA per year were highly predictive of PCa (c-statistic = 0.97 and 0.92).

Percentage change in testosterone and SHBG per year and doubling time of all markers were poor predictors of PCa.

Table 7.10 Unadjusted conditional odds ratio of prostate cancer according baseline, latest, average percentage change per year and doubling time.

Variable		tPSA		fPSA		f/tPSA ratio	
		OR (95%CI)	***P-value***	***OR (95%CI)***	***P-value***	***OR (95%CI)***	***P-value***
Baseline	2-fold higher	4.67 (1.69,12.89)	<.01	5.41 (1.68,17.44)	<.01	0.31 (0.14,0.67)	<.01
Latest	2-fold higher	8.09 (1.11,58.86)	0.04	10.38 (1.94,55.55)	<.01	0.08 (0.01,0.4)	<.01
Average percentage change per year	1 unit faster	1.18 (1.01,1.37)	0.04	1.09 (1.01,1.17)	0.03	0.94 (0.89,1)	0.06
Doubling time	1 year longer	1.05 (0.96,1.15)	0.27	0.97 (0.93,1.01)	0.09	1 (1,1.01)	0.58
		Testosterone		**SHBG**			
		OR (95%CI)	***P-value***	***OR (95%CI)***	***P-value***		
Baseline	2-fold higher	0.83 (0.29,2.35)	0.73	0.91 (0.4,2.08)	0.83		
Latest	2-fold higher	1.06 (0.34,3.31)	0.92	1 (0.37,2.68)	0.99		
Average percentage change per year	1 unit faster	1.02 (0.98,1.06)	0.31	0.99 (0.95,1.02)	0.45		
Doubling time	1 year longer	1 (0.98,1.01)	0.53	1 (0.98,1.02)	0.96		

OR: odds ratio, PSA: prostate specific antigen, tPSA: total PSA, fPSA: free PSA, SHBG: Sex hormone binding globulin.

Table 7.11 C-statistics for the prediction of cases and controls.

Marker level	**c-statistic (95% CI)**
Total PSA	0.90 (0.85,0.94)
Free PSA	0.82 (0.77,0.88)
f/t PSA ratio	0.82 (0.76,0.87)
Testosterone	0.51 (0.44,0.59)
SHBG	0.51 (0.430.58)
tPSA + fPSA + testosterone + SHBG	0.91 (0.870.95)

PSA: prostate specific antigen, tPSA: total PSA, fPSA: free PSA, SHBG: Sex hormone binding globulin.

7.6.2.6 Sensitivity and specificity of tPSA

The usual cut-off of tPSA >4 ng/mL to identify men who were at risk of a PCa diagnosis had 99% specificity and 38% sensitivity (Figure 7.11), meaning 99% of men who were PCa free were correctly identified as "low risk" using a PSA test (I.e. had a PSA ≤4 ng/mL), however only 38% with PCa were correctly identified as "high risk" (i.e. had a PSA >4 ng/mL). The specificity and sensitivity were highest in the year prior to diagnosis (specificity = 100%, sensitivity = 88%, 42 men, 45 samples). The optimal cut-off which maximised both sensitivity and specificity was 1.5 ng/mL overall (specificity=84%, sensitivity=81%) although cut-offs between 1.4 – 1.6 ng/mL provided >80% sensitivity and specificity. This was similar in those aged <50 years, with an optimal cut-off 1.4 ng/mL (specificity=94%,sensitivity=86%, in 22 men and 41 samples, with cut-offs 1.2 – 2.8 ng/mL providing >80% sensitivity and specificity), and ≥50 years with an optimal cut-off 1.5 ng/mL (specificity=82%, sensitivity=81%, N=54 men and 236 samples, no other cut-offs had >80% sensitivity and specificity) at most recent sample.

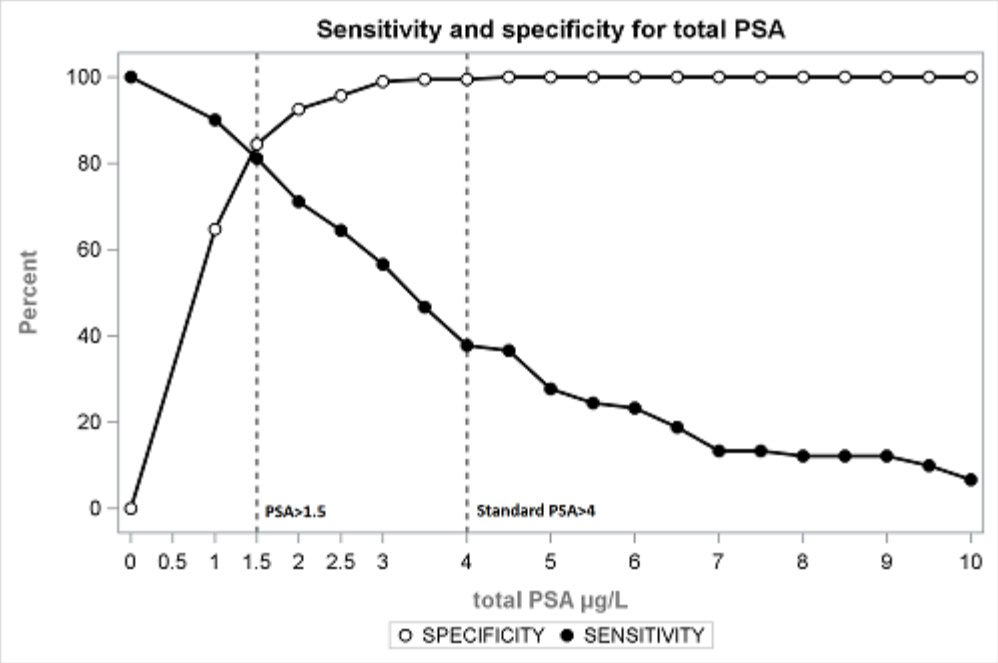


Figure 7.11 Sensitivity and specificity for tPSA considering varying thresholds from 0.1 – 10.
PSA: prostate specific antigen, tPSA: total PSA.

7.7 Discussion

I have divided the discussion according to the two separate aims of the study.

7.7.1 Aim 1: Reported testing for PSA in Europe

These results show considerable heterogeneity in PSA testing reported by the clinics across Europe. Testing was concentrated in men aged 41 or older, varied according to region of Europe, and rates were stable from 2010 onwards but lower previously. Interestingly, although PSA testing was highest in those aged over 50, almost one in three tests were in men aged ≤40 years, a population in which PCa is considered a relatively rare disease [916]. This is in conflict with the recommendations in the EACS guidelines for use of PSA screening in HIV+ men, which do not recommend use in men aged 50 or younger [140].

Reported PSA testing varied by region of Europe, partly driven by variable use at the clinic level. Geographic variations in PSA testing rates also have been reported for the general population [987]. Reported testing rates were highly variable between clinics, ranging from testing no HIV+ men (0%) to testing all men as part of routine care (100% of men). I contacted clinics with reported very high or low screening rates to validate the reported testing rates. Furthermore, I asked the clinics whether there was a reason for the observed rates. Although many clinics confirmed their reported PSA testing rates to be accurate, many did not offer an explanation. Very low testing rates were recorded for east Europe, with many clinics testing <5% of men. This could be driven by a general lack of testing in this region or a lack of reporting. On the other hand, very high rates were observed for central east Europe, due to a handful of clinics testing more than 70% of men. Two clinics responded to my email. One physician said he tested all men as standard care (i.e. 100% of men), however, this was his own practice and did not reflect the practice of other physicians at his clinic. Another was running a study that involved PSA testing. Other possible explanations for the regional variations may include poor reporting to EuroSIDA, varying testing practices between physicians, varying testing practices between clinics, and targeted testing of men who were older, had PCa risk factors (i.e. a family history), or symptoms suggestive of PCa. Large variations in the use of PSA testing in HIV+ men in Europe is driven by lack of clear guidelines on the role of PSA in PCa management [140, 915]. This is reflected in the large variations seen in this study.

Reported PSA testing rates were low in 2008, but stable in the later years of this study. PSA testing was introduced into clinical practice in the late 1980s/early 1990s [988], with rapid uptake in the general population in both the USA and Europe [987-990]. Major changes to US guidelines in 2008 (and also 2012) and publication of results from two major trials in 2009 led to a modest but sustained decline in testing rates in the general population [991, 992], however some studies still report an increase[990]. It is plausible that the increase in reported PSA testing in EuroSIDA since 2008 reflects a delay in the uptake of PSA testing, relative to the general population, due to the relatively recent aging of the HIV+ male population. Alternatively, the results may reflect a lag in the reporting of PSA test results in EuroSIDA after 2008.

Men who were smokers, acquired HIV through IDU, were HCV coinfected, had a history of hypertension, and had lower CD4 cell count had a lower rate of reported PSA testing. This could indicate that PSA testing tends to be less of a priority in sicker patients or those with more complex needs and where other health issues or patient management is of higher priority. In addition, reported PSA tests had higher recorded PSA levels than those measured from plasma samples and most PCa diagnoses in during follow-up were preceded by PSA testing. Taken together, this suggests that on the whole, PSA testing may be symptom driven rather than a component of routine care. The rate of PCa was much higher in those who had a previous PSA test, which may further support that PSA testing is symptom, or alternatively this could reflect over-diagnosis (and thus over treatment) of clinically insignificant PCa when PSA testing is used (estimated to be up to 67% in the general population) [993].

The major strengths of this component of this chapter include the use of EuroSIDA, a large prospective study which collects rich demographic, clinical, and HIV related information on a heterogeneous group of HIV+ men. Furthermore, clinics have reported PSA tests since 2008, and to date there is very little published data on the use of PSA testing in clinics in HIV+ men (or HIV-negative men for that matter). However the limitations need to be taken into account. Firstly, this is an observational study, which means the results may be influenced by residual and unmeasured confounding. Reported PSA tests in EuroSIDA are not routinely collected and there is no way to distinguish between a man where no test was done and a man where a test was performed but not reported. PSA tests were inconsistently reported across centers and no information on the prevalence of unreported tests or tests performed outside the cohort were available. I attempted to limit the impact of this by restricting the analysis to centers with reported testing rates of >5% per year, however under reporting in some centers is likely, particularly in early study years. Furthermore, reasons on why reported PSA tests were

performed by clinicians are unknown and results presented here reflect clinical practice rather than whether true PSA screening is implemented by clinics. Therefore, the results presented here may not be generalizable to PSA testing practices in HIV+ men in Europe. Finally, no information is collected on the diagnostic methods used for PCa identification, stage, or subsequent treatment or prognosis, therefore the survival benefit of PSA testing could not be assessed.

7.7.2 Aim 2: The kinetics of PSA biomarkers and predictive value of PSA and other biomarkers

The strongest predictor of PCa was tPSA in this study with elevations detectable more than 5 years before diagnosis, however, various forms of PSA were predictive. These results show that the commonly used PSA threshold >4 ng/mL as a marker of high PCa risk in HIV+ men was substantially less sensitive (38%) than a lower threshold of 1.5 ng/mL or more (81%).

The strongest marker of PCa was tPSA when considering absolute marker levels and t/fPSA only, which is consistent with the literature [974]. Elevated tPSA levels was found to be detectable more than 5 years prior to PCa diagnosis, indicating that tPSA has the potential to be a helpful longer term marker of PCa in HIV+ men. Similar estimates have been shown in the general population [945, 994]. However, it should be noted that earlier diagnosis is only beneficial if it results in either improved survival or quality of life. Increased time living with a diagnosis can be harmful due to unnecessary treatments and increased mental stress including major depression and anxiety for both the person affected and their families [995, 996]. Testosterone and SHBG levels were not associated with PCa and did not affect PSA predictability. This finding suggests that circulating androgens do not influence the risk of PCa and is also consistent with other studies [431, 925]. I also considered velocities and density of each marker, however this was only available in 16 cases and 32 controls, and estimates were unreliable.

An interesting observation was that the median tPSA levels measured from plasma samples were relatively low for the men included in my study. Two American studies reported similar PSA ranges in HIV+ men to that reported here [997, 998] and previous studies have shown similar or slightly lower PSA levels in otherwise healthy HIV+ and HIV-negative men in the absence of PCa[999-1001], but lower latest PSA levels prior to PCa diagnosis in HIV+ men [431]. There are several possible explanations for the lower tPSA levels in my study. It could reflect the higher prevalence of hypogonadism in HIV+ men (12 - 20%), characterised by reduced androgen levels [980, 981, 1002]. It is also possible that the lower levels are driven by the comparatively

younger age at PCa diagnosis since PSA level is known to increase with age [1003]. If this is the case, the PSA concentration in HIV+ men would expect to approach that of the general population as they continue to age, and age specific PSA risk ranges may be useful. Finally, there could be an alternative biological relationship between PCa and PSA in the presence of HIV which causes PSA levels to be systematically lower in HIV+ men. If this is the case, lower PSA levels could mask the presence of PCa and delay diagnosis, therefore, HIV specific cut-offs should be considered.

The commonly used cut-off of tPSA >4 ng/mL to identify men at high risk for PCa was notably less sensitive than the lower cut-off of tPSA >1.5 ng/mL in HIV + men. However, the sample size is small and hence this data needs to be validated in larger studies before being applied in clinical practice. Furthermore, this cut-off was similar for men aged <50 and >50 years, however, I suspect this is mainly due to the men in the aged over 50 group having a relatively low age (most aged < 60) and a small numbers. I expect that differences in age specific cut offs will become apparent as the proportion of HIV+ men aged 50 years or older increase.

Serum tPSA level is measured by a minimally invasive blood test making it an ideal candidate for a cancer screening tool. However, this study was hypothesis generating, with the aim of assessing whether PSA concentration has merit as a diagnostic marker in HIV+ men and to determine whether the commonly used cut-off of >4 ng/mL is appropriate for this population. For this analysis, I assumed that sensitivity and specificity were equally important. Ideally, a screening tool with 100% sensitivity and specificity is desired, however in the real world this is almost never a reality. Prioritising sensitivity leads to tests that correctly identify more PCa cases while allowing for lower specificity which could lead to overdiagnosis (as is often the case with PSA testing). On the other hand, prioritising specificity would minimise overdiagnosis but possibly lead to missed clinical disease due to lowered sensitivity. How to balance these in the context of PSA testing in HIV+ men is an ongoing controversial issue with no straight forward answer. In order to properly determine how to properly prioritise sensitivity and specificity, a cost effectiveness analysis could be a possible route for future research. It is important to remember that screening tools are to be used as indicators of disease but are not used for diagnosis alone.

When presenting this work at conferences there has been much interest from clinicians in use of PSA testing as a screening tool in HIV+ men. This study was not designed or intended to advise clinical practice on how to use PSA testing as a screening tool or to advise whether population

based screening is appropriate. The limitations of PSA testing as a screening tool still hold in HIV+ men and need to be kept in mind. These limitations include that PSA is a continuous marker of PCa risk where higher PSA level is associated with higher risk and therefore no "ideal" cut-off exists [915]. Raised PSA levels can be caused by a number of prostate related conditions, such as chronic prostatitis, and PSA testing alone cannot distinguish between them [1004]. In addition, PSA levels are influenced by many other facts such as older age, prostate volume, race [1005], and recent ejaculation[1006]. It is also worthwhile noting that two large RCTs in the general population found no effect of PSA screening on overall mortality [946, 947]. Taken together, these limitations have collectively prevented the recommendation of a population wide PSA based screening program in the general population, and therefore HIV+ men.

The major strengths of this component of this chapter include the large number of prospectively diagnosed PCa in EuroSIDA, relative to other prospective studies, and access to prospectively stored plasma samples collected independent of PCa. The frequency of PCa was low from a statistical point of view, however matching between cases and controls improved the efficiency of our analysis. Cases and controls were nested within the EuroSIDA cohort. This design minimised the risk of some biases common to case-control studies, such as selection and recall bias. Selection bias is reduced as the cases and controls were drawn from the same population and both information about PCa diagnoses and plasma samples were independently and prospectively collected. It is unknown if the cases became symptomatic at some point prior to the date of PCa diagnosis, however, the use of prospectively collected plasma samples to measure PSA rather than clinically measured PSA prior to PCa diagnosis avoids bias due to symptom driven PSA testing. However, the study has a number of limitations which need to be kept in mind when interpreting from the results. This is an observational study and a small nested case-control study. Therefore, there is a risk the associations presented are affected by confounding and causality cannot be determined. There are many known risk factors for PCa are not collected in EuroSIDA and therefore could not be taken into account, for example: family history [2]. Further adjustment for potential confounders was not possible due to low numbers, although only minor significant imbalances were evident at baseline. Finally, the use of the matched design reduces risk of confounding of important risk factors by design, but also increases the complexity of the data, requiring specialist methods for analysis.

7.8 Conclusions

This chapter has demonstrated that there was considerable heterogeneity in PSA testing in clinics across Europe, and PSA testing was particularly high in older men, as expected.

Standardisation of PSA testing patterns in European HIV+ men is inhibited by lack of clear guidelines on the role of PSA in PCa screening and management. Although overall PSA levels were low, a clinically relevant increase in tPSA and fPSA in the years preceding PCa diagnosis was observed, and was evident at least 5 years prior to diagnosis. Attention to symptoms of localised and disseminated PCa even at lower PSA levels is needed, and DRE and rectal ultrasounds should be performed. The commonly used PSA>4ng/mL to indicate high PCa risk was not sensitive in our study and use of the lower cut-off of PSA>1.5ng/mL warrants consideration. Given the limited sample of the study, these potential implications should be investigated in larger studies.

7.9 Publications

A manuscript was published in Antiviral Therapy in 2016 [1007]. Results from this chapter were presented at the HIV Glasgow drug therapy conference in 2014, the European AIDS clinical society (EACS) conference in 2015. The manuscript and slides are included in Appendix XI, Appendix XII, and Appendix XIII, respectively.

8 Discussion

8.1 Summary of main findings

Effective and durable cART has led to the increased longevity of HIV-positive (HIV+) people [1-6]. The burden of cancers in the aging HIV+ population raises multiple issues in regard to future HIV healthcare, including the detection, diagnosis, comanagement, and treatment of cancers and increased complexity of patient management [504]. The use of cohort studies to understand the emerging trends of cancers in HIV+ people, to identify and characterise associated factors, and to describe the underlying mechanisms of cancer genesis are important in order to advise clinical guidelines, allocate resources, and target interventions to optimally meet the needs of this population. Therefore, the aims of this thesis were to describe the changing epidemiology of commonly occurring cancers in HIV+ people and to explore and characterise plasma biomarkers of common cancers in HIV+ people in order to better understand the mechanisms leading to cancer development. This thesis focusses on specific cancers or groups of cancers that are expected to become a major sources of morbidity and mortality as the population ages.

8.2 My role in this thesis

I was the lead researcher for each of the pieces of work presented in this thesis, from the study conception through to publication. I designed and implemented each of the studies, which involved the conception and development of the research objective, composing and developing the research proposal for approval by the EuroSIDA steering committee, designing each study, planning and performing the statistical analysis, interpretation of results, and writing and publication of the manuscript. I also composed multiple abstracts and delivered presentations of the work in these chapters at international conferences. A group of clinicians from The Center of Excellence for Health, Immunity and Infections (CHIP) in Copenhagen provided guidance in terms of the clinical relevance of research questions, the clinical interpretation of results, and translation for use in clinical practice. In addition, my supervisor Amanda Mocroft answered questions and provided guidance in terms of statistical analyses and study design, and also provided feedback on abstracts, presentations and manuscripts. For the nested case control studies (chapters 6 and 7), I designed each of the studies, identified the cases and controls, and selected samples for the nested case controls studies. Furthermore, I also set up an academic collaboration in order to ensure funding for the measurement of immunoglobulins and free light chains on plasma samples for the nested case control study in chapter 6. As my thesis focuses primarily on cancers in HIV+ people, I put together a special interest group composed of

clinicians and epidemiologists with specialist knowledge of cancers in HIV+ people. They provided additional guidance on some studies presented here and were involved in discussions surrounding the selection of markers of B-cell activation prior to non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) diagnosis. Throughout my PhD I have been centrally involved in the EuroSIDA research agenda, an active member of the EuroSIDA steering committee, participated in regular meetings with colleagues from multidisciplinary back grounds, and also provided statistical support for other projects within my research area.

8.2.1 Infection related and unrelated cancers, HIV, and the aging population

HIV+ people are at higher risk of infection related cancers (IRCs), however, increased risk of some non-infection related cancers (IURCs) have been reported in recent years. This is due to a combination of aging of the HIV+ population and high prevalence of traditional cancer risk factors (such as smoking) [8, 14-18, 22, 23, 420, 421, 427, 570]. In chapter 3 I demonstrate the changing incidence of IRCs and IURCs in HIV+ people in Europe between 2001 and 2012 and also identify and describe factors that are driving higher risk. This chapter demonstrates that the demographic and HIV related risk factors that are driving elevated IURC (aging and smoking) and IRC (immunodeficiency and ongoing viral replication) risk differ markedly. Furthermore, I was able to demonstrate an expected decline in IRC incidence, likely due to control of HIV related factors through effective cART, and a rise in IURC incidence, due to aging of the HIV population.

These findings suggest that the burden of cancer is expected to shift towards IURC due to the non-declining incidence and the increasing proportion of HIV+ people aged over 50 years, as well as higher prevalence of known cancer risk factors, such as smoking, and low prevalence of untreated HIV-infection [405, 503]. This highlights the need to consider how to optimise HIV care for older HIV+ people when planning for the future. In addition, preparing for an increase in IURC burden allows time to investigate possible strategies for integration of services, screening and detection strategies, and optimal treatment regimens for the concurrent use of both cART and chemotherapy. Efforts to minimise the impact of avoidable cancers will dramatically reduce the strain on the health care system, not just at the HIV clinic but also on oncological services and potentially free up resources for use elsewhere.

This was the largest study in Europe to investigate the changing incidence of IRCs and IURCs over time and to investigate associated risk factors. Several other studies had performed similar analyses at the time of this study, however most were performed within HIV+ people in the USA,

where the patient demographics and health care structure is considerably different to those in Europe. Furthermore, many studies had compared incidence in HIV+ people to the background population or investigated changes in incidence since the introduction of cART, however, few had considered the impact of individual level risk factors. At the time of this study, reclassification of cancers into IRC and IURC rather than AIDS defining cancers (ADCs) and non-AIDS defining cancers (NADCs) was a novel approach.

This chapter was the earliest analyses looking at cancers in HIV+ people that I undertook. I now have a more in depth understanding of cancers in HIV, including the aetiology, risk factors, and mechanisms and have a greater appreciation of their heterogeneous nature. If I were to do this project again, I would try to reduce the scope and focus on a few cancers rather than all IRC and IURC combined. However, even using a large database such as EuroSIDA (or D:A:D for that matter), such analyses are limited as cancers are a rare event which take many years to develop and therefore power is often an issue. I have tried to be more focused in later chapters by looking at smaller groups of cancers where numbers allow.

This chapter was published in HIV Medicine in 2016 [636] and the manuscript is included in Appendix III. This chapter was presented at the European AIDS clinical society (EACS) conference in 2013 and the slides are included in Appendix IV.

8.2.2 Cessation of cigarette smoking and the impact on cancer incidence in the D:A:D Study

As mentioned in chapter 3, HIV+ people often have elevated rates of smoking, and as a result, are at risk of various smoking related conditions, including cancers [420, 421]. In addition, incidence of lung cancer has been shown to be elevated in HIV+ people independently of higher rates of smoking [725]. Despite the well characterised harms of smoking in HIV+ people, the clinical benefits of smoking cessation on cancer risk have not, to my knowledge, been reported. In chapter 4, I estimated the change in incidence of lung, other smoking related cancers, and smoking unrelated cancers after smoking cessation in HIV+ people from the D:A:D study. This study demonstrates that the incidence of lung cancers is 10-fold higher in current smokers relative to never smokers. In those who quit smoking, lung cancer incidence remains at a similar level to current smokers >5 years after cessation. The incidence of smoking related cancers (excluding lung) returned to a similar level to never smokers after 1 year of cessation (although the magnitude of association remained elevated possible reflecting ongoing risk), and smoking status did not impact on the incidence of smoking unrelated cancers.

The results of this study have various implications for HIV+ people. Firstly, not only is lung cancer incidence highly elevated following cessation, there is little evidence of a decline with increasing time since cessation after the first year. This is in contrast to the general population where a decline is evident after 5 – 9 years and increases with cessation duration. This implies that the harms of smoking maybe more severe and take longer to reduce in HIV+ people than the general population. These results highlight the urgent need for effective smoking cessation efforts in HIV+ populations as well as efforts into smoking prevention. Furthermore, smoking in the context of HIV is complex and is intertwined with mental health issues, alcohol, and drug use. Therefore, programs that focus on smoking alone may not be effective in this population and methods that address multiple substance use issues and mental health may be beneficial.

The D:A:D collaboration was one of the few HIV+ studies which could address this question due to the long history of clinically obtained smoking information collected, the large size, and long follow-up time which allowed for the sufficient acquisition of cancer events (particularly for lung cancers). No decline in lung cancer incidence was detected following cessation after the first year, however, the follow-up was relatively short compared to studies in the general population. These results should therefore not be misinterpreted as a lack of benefit for smoking cessation on lung cancer risk, rather that the decline in HIV+ people does not manifest within the first 5 years of follow-up. The reason for the lack of reduction in risk could be due to multiple reasons, including longer and more intense smoking histories in HIV+ people, higher susceptibility to inflammation in the lung and lung infections, or a more prominent residual effect of long term tobacco use relative to the HIV-negative population. This should be the focus of future research efforts.

If I were to repeat this study, the main thing that I would change would be to utilise an observational study with a much longer follow-up time (i.e. at least 20 years). Unfortunately, this is not currently possible with the HIV+ cohorts that currently exist. In addition, either adjustment for or stratification by smoking history (such as smoking duration and intensity) would further improve this study, however this information was not available in D:A:D.

This chapter was awarded a young investigator scholarship and presented at CROI 2017 as an oral presentation slides are in Appendix V. A manuscript has been drafted with the intention to submit to JAMA.

8.2.3 Differences in virological and immunological risk factors for non-Hodgkin and Hodgkin lymphoma in the D:A:D study

Despite a clear decline in NHL incidence since the introduction of cART, NHL remains the most common ADC in HIV+ people (as shown in chapter 3) [430, 437, 441, 443, 451]. On the other hand HL, incidence has not declined and remains elevated in HIV+ people since the introduction of cART [8, 14-16, 19, 438, 469]. There are many studies which have investigated incidence of NHL and HL since the introduction of cART and associated risk factors. However, few previous studies had identified NHL and HL risk factors within the same population and no studies had directly compared the risk factors for NHL and HL in order to identify and develop the understanding of different mechanistic pathways which may suggest different preventive approaches for reducing NHL and HL risk. In chapter 5 I demonstrate that higher current and accumulated HIV viral load (HIV-VL) are relatively stronger risk factors for NHL than HL. Although current CD4 cell count and gender are strong predictors of both, they are similarly associated with risk of NHL and HL. In addition, I show that current smoking is associated with HL but not NHL risk. Finally, this is one of the first studies to demonstrate a convergence of the incidence of NHL and HL in recent years, due to the ongoing decline in the crude incidence of NHL and unchanging HL incidence.

The results of this chapter suggest different mechanistic pathways for the involvement of HIV in the development of NHL and HL. My finding that NHL is independently associated with both CD4 and HIV-VL is similar to that observed in opportunistic disease, where risk is higher in people with uncontrolled HIV replication regardless of CD4 cell count, which has been confirmed in both observational studies [842] and the START trial [141, 616]. This indicates that ongoing immune dysfunction (beyond the low CD4 cell count) may play a role. Conversely, the association between CD4, but not HIV-VL, and HL indicates that the impact of HIV-infection on HL development may be primarily driven by a balance between impaired immune function and loss of control of Epstein-Barr virus (EBV) as well as remaining immune activity to allow for the influx of immune cells to the tumour microenvironment [849]. Furthermore, this study implicates a link between smoking and HL incidence which further supports the conclusions of chapters 3 and 4 regarding the need for smoking cessation efforts. These findings provide new insights into the pathogenesis of these lymphomas and call for the characterisation of biological markers of immune dysfunction before and during the evolution of these cancers to further understand these processes. This would have implications for other immune impaired populations, other than HIV+ people, such as transplant recipients. Furthermore, these results stress the importance of early HIV diagnosis and treatment, and of ensuring sustained viral suppression.

Initially, this project was performed within EuroSIDA, however due to small numbers of NHL and HL, it was decided to instead use data from the D:A:D collaboration to improve the number of outcomes ascertained with the hope of looking at NHL subgroups. Unfortunately, subgroup information was missing on 66% of NHL in D:A:D which only allowed for very limited analyses by NHL subtype. The primary results treat NHL as a single entity, however, I am aware there is considerable heterogeneity in aetiology between NHL subtypes which is not captured by these results.

A manuscript of this chapter was submitted to the Journal of the National Cancer Institute (JNCI) in 2017. The results were presented at the HIV drug therapy Glasgow conference in 2016. Preliminary results from the EuroSIDA study were also presented at CROI 2015. The manuscript and slides are included in Appendix VI, Appendix VII, and Appendix VIII respectively.

8.2.4 The extent of B-cell activation preceding lymphoma development in HIV-positive people

The genesis of lymphomas in HIV+ people are thought to be driven by HIV mediate B-cell dysfunction and infection with EBV, however, how these factors interact to facilitate lymphoma development is unclear. In chapter 6 I investigate the trajectory of various markers of B-cell activation in the time leading up to lymphoma diagnosis and explore the associations between levels of markers of B-cell activation and lymphoma development in HIV+ people. I demonstrate that various markers of B-cell activation are elevated many years prior to lymphoma diagnosis in HIV+ people, however, the levels of these markers attenuate with closer time to lymphoma diagnosis. Levels of HIV-VL were also higher more than 2 years prior to lymphoma diagnosis and correlated with elevated levels of B-cell activation markers, implying that previous exposure to HIV-VL may be inducing ongoing B-cell activation and dysfunction which could facilitated lymphoma development. The predictive ability of these markers to identify people at high risk of lymphomas is modest at best, and they are unlikely to be candidates for clinic based risk assessment for lymphomas.

The analyses presented in this chapter took much longer than planned to develop and execute. There were several reasons for this. Firstly, the kits used to measure the markers of B-cell activation in the plasma samples were provided by a third party (the Binding Site). This meant that all proposals, agreed analyses, data ownership, conferences, and manuscripts had to go through an additional round of discussions, negotiations, and review on top of the usual EuroSIDA procedures. The original design of this study involved measuring EBV DNA in addition

to markers of B-cell activation in stored plasma samples. However, this was not feasible due to laboratory time and cost restraints and I made the executive decision to proceed with the analysis without the EBV DNA component. Originally, it was not planned to investigate CD4 cell count and HIV-VL as part of this study. Therefore, it was decided to match on CD4 cell count in order to control for differences in severity and stage in HIV infection when looking at markers of B-cell activation. However, this soon became of interest. In hindsight, I would reconsider whether or not to match on CD4 cell count. Although, it is important to note that replicating HIV can stimulate B-cell proliferation and antibody production and further controlling for the contribution from this effect (which is beyond that due to the malignant B-cell replication) would be of interest.

A manuscript for this chapter was accepted for publication in HIV Medicine in 2017. The results were presented at the HIV drug therapy Glasgow conference in 2016. The manuscript and presentation are included in Appendix IX and Appendix X respectively.

8.2.5 Testing patterns and predictive value of prostate specific antigen in a European HIV–positive cohort: does one size fit all?"

Prostate cancer (PCa) is the most common cancer to occur in older European men and is emerging as a major contributor to cancer burden in the HIV+ people as they age [7, 436, 438, 958]. It is common practice in the general population to use prostate specific antigen (PSA) ≥4.0 µg/L as a clinical indicator for men at risk of PCa [935-937], however this has been unverified in HIV + men and investigation into factors associated with PSA testing in HIV+ men is unknown. In Chapter 7 I identify factors associated with higher rates of PSA testing in HIV+ men in European clinics. In addition, I investigate the trajectory of PSA levels prior to PCa diagnosis in HIV+ men and assess the predictive value of PSA for identifying men at high risk for PCa. Finally, I explore whether the commonly used cut off of PSA≥4.0 µg/L is appropriate for use in HIV+ men and whether lower PSA cut-offs should be considered.

This chapter demonstrates that the use of PSA testing is highly variable in clinics across Europe. This highlights the need for clear guidelines on the role of PSA in PCa screening and management which may help to optimise use. In addition, PSA levels were highly predictive of PCa presence in HIV+ men more than 5 Years prior to PCa diagnosis, however, levels of PSA in HIV+ men were surprisingly low. This indicates that, while PSA presents as a useful clinical indicator of PCa risk, using PSA cut-offs intended for the general population may result in underdiagnosis of clinically significant PCa. When comparing PSA cut-offs to identify men with elevated risk of PCa to

undergo further testing, it was found that the lower cut-off of 1.5 µg/L dramatically improved the sensitivity (true positive rate) for PCa with a small reduction in the specificity (true negative rate). It was unclear in this study whether the lower PSA levels in HIV+ men observed were possibly driven by the younger age in HIV+ men at diagnosis (due to the younger age of the population overall), or if PSA operates differently in the presence of HIV-infection. Therefore, a future avenue of research would be to investigate age-appropriate cut offs as a larger proportion of HIV+ people live into the older ages. The results presented in chapter 7 were exploratory and do not sufficiently support use of a lower cut off in clinical practice. However, they do raise the question of whether a lower PSA cut-off would be beneficial for HIV+ people and this needs to be independently validated in larger studies before these finding have clinical meaning. It should be kept in mind that use of PSA testing as a population based screening tool for PCa remains highly controversial and is not recommended by most urological societies and associated medical entities [915, 937, 939, 942-944].

The component of this chapter looking at rates of PSA testing across Europe used yearly rates of PSA testing to identify and reduce the impact of clinics that do not report PSA testing in EuroSIDA. I suspect that most clinics only tested for PSA after PCa was already symptomatic and not as part of routine screening and it is likely that handling of those with elevated levels was highly heterogeneous. If repeating this study with a larger budget, I would have liked to study PSA as a marker for emerging prostate cancer in a HIV+ cohort with routine screening for PSA levels and with pre-specified criteria on how to react to an elevated level. In addition, I would also have liked to measure other forms of PSA that have been shown to be associated with PCa risk, mainly the isotope Pro[-2]PSA [1008, 1009]. This form is a specific marker of PCa in the general population, and can be combined with total and free PSA to calculate the prostate health index (PHI), which some studies have demonstrated to be more predictive of clinically significant PCa than each measure in isolation [1008, 1009]. In addition, at the time of this study, tests for urine PSA levels (PCA3) were emerging as markers for PCa which could better differentiate between PCa and benign prostate disorders than circulating PSA [1010]. Unfortunately, urine samples were not available in EuroSIDA and this could not be explored.

A manuscript was published in Antiviral Therapy in 2016 [1007]. Results from this chapter were presented at the HIV Glasgow drug therapy conference in 2014, the European AIDS clinical society (EACS) conference in 2015. The manuscript and slides are included in Appendix XI, Appendix XII, and Appendix XIII, respectively.

.

8.3 Limitations

The specific limitations of each individual analysis has been discussed within each chapter, however, here I will discuss a selection of broad limitations that are common to several chapters.

8.3.1 Observational studies

The chapters presented in this thesis use data from observational studies. Chapters 3, 6, and 7 utilise data from the EuroSIDA study, which is a large prospective observational cohort study. Chapters 4 and 5 utilise data from the D:A:D study, which is a collaboration between 11 large prospective observational cohort studies from Europe, USA, and Australia.

Observational data is very useful in the context of clinical research. In particular, both EuroSIDA and D:A:D are examples of observational studies which follow a large number of heterogeneous people (due to liberal inclusion criteria) and accumulate information across a wide range of demographic, lifestyle, and heath related factors. Furthermore, EuroSIDA periodically recruits new people to replace those who may have died or are lost to follow-up. A major strength of both these data sources is the large number of people enrolled and the long period of follow-up. This increases the power to study the occurrence of rare events (in this case cancers) and the long term implications of many factors on clinical outcomes.

Use of data from observational studies to answer research questions is extremely useful in situations where experimental data is not available or not appropriate. In addition, data from large prospective observational cohorts reflect real world clinical situations. However, observational studies often have issues with confounding and various types of bias, including selection and information bias (which are discussed in sections 8.3.1.2 and 8.3.1.3). Lead time bias refers specifically to screening and is where a patient's survival time is artificially increased due to being diagnosed earlier than they otherwise would have. However, this is not relevant to the results presented here.

8.3.1.1 Confounding

In many situations, randomised control trials (RCTs) are the gold standard to establish causal relationships between an exposure and an outcome. RCTs require that participants are allocated to a regimen at random, and therefore, they control for confounding by design. In observational

studies, the researcher does not intervene in the clinical care of participants. This means that confounding is an issue as allocation to exposure groups is not random, and as a result, demographic, lifestyle, and health related factors are likely to differ between the groups. The use of RCTs would not be suitable and not feasible for any of the chapters in this thesis. For example, studies which investigate the association between smoking and cancer (such as chapter 4) are one of the few situations where a RCT is not possible. This is because of the ethical issues of assigning people to a "current smokers" arm, the large sample size, and long follow-up required to accrue sufficient numbers of cancers (a rare event which take a long time to develop), and the high cost of running such a study. In this situation observational cohort data is required.

Methods to account for confounding, such as use of multivariate models and matching of case control studies, were implemented in the chapters of this thesis. However, I could only attempt to account for confounders which were known and for which data was available. Both EuroSIDA and D:A:D collect a wide range of demographic, life style, HIV, laboratory, and health related variables, however there are many known cancer risk factors not collected in either database (such as family history). In addition, it is possible that residual confounding remains after statistical adjustment due to inaccuracies or lack of appropriate detail in the measurement and reporting of some variables. I cannot rule out that the results presented in this thesis are affected by confounding due to unknown, unmeasured, or residual confounding variables [1011].

8.3.1.2 Selection bias

Selection bias is a broad term used to describe the situation where the people included in a study are not representative of the population from which they are selected [1012]. This can arise due to the way people were selected into the study (i.e. non-random selection), refusal to take part in the study, or high or differential dropout rates. Selection bias becomes an issue when the selection of people is related to the outcome of interest, and therefore, the associations explored in the study are different to that in the wider population. For example, it is known that those enrolled in EuroSIDA are a generally healthy group of HIV+ people compared to the wider HIV+ population. This is partly because clinics which contribute to EuroSIDA tend to be larger, high performing clinics which offer above average standards of care. In addition, recruitment into EuroSIDA requires the individual to be in contact with a participating clinic to be included. Therefore, the associations presented in this thesis may not be generalizable to the entire population of HIV+ people across Europe.

Selection bias is also an issue in case-control studies if the cases and controls are either not representative of the background population or not drawn from the same population. The risk of this was minimised in the nested case control studies in chapters 6 and 7 as both the cases and controls were drawn from the EuroSIDA cohort and the controls were randomly selected.

8.3.1.3 Information bias

Information bias refers to the situation where information is inaccurately or wrongly recorded on some patients in measured variables with continuous values, such as differences in PSA, CD4 cell count, or blood pressure. Misclassification refers to a similar error where an individual is incorrectly allocated to the wrong group. For example, in D:A:D smoking data is only recorded at the clinic visit and therefore may not accurately reflect the day to day smoking behaviour of the person. This could lead to misclassification of people who quit smoking but shortly relapse as previous smokers and bias results.

8.3.1.4 Recall bias

This is a common bias in case control studies and refers to systematic differences in the ability of people to accurately recall historical exposures. Nested case control studies were used in chapters 6 and 7, however the variables of interest were measured in plasma samples and therefore this was not an issue. Recall bias can also occur in cohort studies. Both EuroSIDA and D:A:D are based on data that is captured as part of routine clinical care and relies of recall of history of behaviours at the clinic visit. For example, the ability of the patient to recall recent smoking and alcohol use as well as to accurately report changes in behaviour (a person may feel embarrassed if they quit smoking and relapse). Furthermore, in EuroSIDA, each center completes a follow-up form on each patient every 12 (formerly 6) months and the accuracy of this data depends on the quality and detail of the clinical notes for each patient. Therefore, it is possible that clinicians are more likely to record or report certain information in some instances.

8.3.2 Rare events as clinical outcomes

Cancers were the main outcome in each of the chapters presented here. Many cancers are rare events in HIV+ people, particularly those which occur in the older ages. As a result, the number of clinical events observed during follow-up were sometimes too low to consider individual cancer types as separate endpoints and composite endpoints were used. For example, NHL subtypes were grouped into a single NHL outcome in the analyses in chapter 5, and NHL and HL were grouped into a single lymphoma outcome in chapter 6. This approach assumes that risk

factors associated with the composite outcome are the same across the contributing end points, which is not always the case. In addition, the associations with composite outcomes are more likely to reflect those of the more common endpoints included and factors associated with rarer outcomes may be over looked. For example, the associations between B-cell markers and lymphoma presented in chapter 6 were more in line with what is known of the pathogenesis of NHL than HL and may not accurately reflect the associations with HL. Similarly, analyses in chapter 3 used two composite outcomes: IRC and IURC. The risk factors identified are likely reflecting the high frequency of NHL and Kaposi's sarcoma (KS) in the IRC group, and lung, prostate and breast cancers in the IURC group and may not represent the associations present for less common cancers. Although cancers are rare events, they are fairly easy to ascertain as a cancer diagnosis leads to further contact with the health system and often admission to hospital, conversely, benign events are less straight forward to ascertain and typically require standardised reporting.

8.3.3 Missing data

The EuroSIDA and D:A:D studies were both set up in order to assess HIV alone. As a result, historical cancer information is often not available. Missing data is a common side effect of using routine clinical data and is an issue in both the EuroSIDA and D:A:D databases. If the data are missing at random then missing values will have a negligible impact on the results. However, some of the missing values may be due to differences in clinical practice, monitoring strategies, or availability of tests or diagnostic facilities across the centres. For example, missing CD4 cell counts and HIV-VL values in EuroSIDA are largely due to less frequent monitoring of people in certain regions, such as eastern Europe. Another example is missing smoking information in D:A:D. In many cases, smoking information is only recorded in the follow-up form if it is recorded in the clinic notes, therefore, smoking status will not be updated in patients who are not asked about smoking during their clinic visit. In addition, differences in when the collection of smoking status commenced in each individual cohort may contribute. A major limitation of both the EuroSIDA and D:A:D studies is the high proportion of cancer diagnoses that are missing cancer subtype information. In D:A:D this is partially due to some cohorts not collecting this information, however, a large proportion were simply not reported or reported as free text and not incorporated into the main database. Furthermore, information on cancer treatment and prognosis was not collected and this limits the ability to investigate the clinical outcomes of HIV+ people following their cancer diagnosis. This will become an increasing area of interest as the population ages.

The analysis in chapter 7 looked at PSA testing rates in EuroSIDA. PSA testing is not routinely reported in EuroSIDA and therefore not all centers consistently provide this data. To account for this, I only included centers that screened more than 5% of men per year. However, there is no way to distinguish between a center that does not report PSA and another that does not use PSA testing as part of routine clinical practice. Therefore, this method likely excludes some centers that truly have low testing rates, and includes some centers with higher rates but which provide incomplete data (but still fulfils the inclusion criteria). Another limitation is that there is no information on why tests were or were not reported, for example, some centers may not have the facilities to perform PSA testing. This could potentially lead to biased results if the reported PSA testing rates do not reflect what is actually performed in the clinic.

There are several methods available to deal with missing data, however, no post hoc methods are equivalent to having a complete dataset. In most analyses presented here, missing data was dealt with by either including a missing category, using last value carried forward, or using a complete case approach. For example, in chapter 4, people missing smoking information were dealt with using last value carried forward. People who never had smoking information available were excluded. Both these approaches are simple and widely used methods to deal with missing data. Missing data in predictor variables do not cause bias in analyses of complete cases if the reasons for the missing data are unrelated to the outcome (i.e. if the data is missing at random) [1013], however, if this assumption is not true then these methods can lead to biased results [1013].

EuroSIDA implements several processes to minimise missing data. The number of people lost to follow-up is reported to each center after each data collection. Centers with high rates of lost to follow-up are contacted and often a member of staff from the coordinating center will visit the site to assist with any issues with completing follow-up forms that the site may have. However loss to follow-up in EuroSIDA is low and is reported as 5% per year [557].

8.3.4 Changes in data collection

The EuroSIDA database has changed over time due to a shift in focus of the research areas surrounding HIV infection as well as the characteristics of people living with HIV. For example, NADC have been collected in EuroSIDA since 2001 and in D:A:D since 2004 in response to the emergence of cancers as a major source of morbidity and mortality in the context of cART. Therefore, it is possible that any increasing incidence of cancer events over time may actually reflect changes in data collection and improved ascertainment of events rather than a true

increase. For example, there has been a steep increase in the number of liver cancers reported in EuroSIDA over time, which has been in part attributed to improvements in case finding mechanisms, however, an increase has also been observed in other studies [436, 464]. The inclusion criteria in EuroSIDA has changed over time, however, this only impacts on people enrolled in cohort I and cohort X and is unlikely to impact on the results presented here. The largest change has been the incorporation of cohort X which enrolled hepatitis C (HCV) coinfected patients. This includes the collection of many HCV specific variables not previously included. This also led to a restructuring of the follow-up forms to allow a more integrated collection of HIV and HCV related information. The focus of recruitment has changed over time, with an increased focus on eastern Europe and women as the study progressed, however, both gender and region of Europe has been adjusted for in all multivariate analysis. There have been multiple changes to the follow-up forms over time as various risk factors for not only HIV, but other important conditions, became apparent.

8.3.5 Availability of resources

Several of the chapters presented here were restricted by the availability of resources. Chapters 6 and 7 involved measuring biological markers in stored plasma samples. This is an expensive exercise in terms of cost as well as use of a finite biological resource. As a result, the markers I could feasibly measure were restricted to those performed routinely in laboratories (such as total PSA) or those subsidised through academic collaboration (such as the measurement of markers of B-cell activation). In addition, samples were selected in such a way to conserve biological material.

As already mentioned, the NHL subtype information was missing in a high proportion of people. At the time of this study, it was not feasible to return to the pathology records and obtain this information as D:A:D was no longer funded and the resources were not available to perform this task. However, I do think this exercise is important for future research.

During my PhD, the funding for EuroSIDA also came to an end and, in an effort to conserve resources, the items collected as part of the core EuroSIDA database were reduced to those deemed essential and data collection moved from biannual to annual. However, this does not impact on the analyses presented here as this transition only affected later versions of the data.

8.4 Further research

HIV infection is entering a new era where a substantial proportion of HIV+ people are older and at risk of various age related comorbidities. Although it is clear that this poses a complex challenge in terms of integration of healthcare services, healthcare planning, and resource allocation, this is uncharted territory. It is not yet clear how the HIV epidemic will change in the context of an aging population and how to best cater to their needs. This will potentially manifest as a substantial burden on the healthcare system, which may not yet be set up to treat people with HIV as a chronic with associated health complications. For this reason, it is essential that research into risk factors for high burden conditions and mechanisms for disease are continued.

8.4.1 Future steps in HIV and cancer

One possible avenue for future would be to develop the analyses in chapter 5 and investigate the risk factors for each NHL subtype individually. As mentioned in section 8.3.5, at the time of this study, it was not feasible to return to the pathology records and obtain subtype information on those missing this information. Furthermore these results warrant a systematic biological characterisation of patterns in personal "-omics" data (such as genes, mRNAs, proteins, and metabolites) in people with and without lymphomas. This would deepen the understanding of biological differences between NHL subtypes and HL and shed light on the how these cancers develop. Smoking status was established as a risk factor for HL, which has been suggested in EBV associated HL in the general population. It would be very interesting to investigate this further and possibly look at the impacts of cessation on cancer incidence. Another future project that would be of great interest would be to develop a risk score for NHL and HL, as has been previously done for cardiovascular and chronic kidney disease in HIV+ people.

Another interesting avenue for future research would be to investigate EBV DNA levels preceding lymphoma diagnosis in HIV+ people and to correlate this with markers of activation. Several studies have shown that EBV infection occurs prior to lymphoma diagnosis, [1014-1016], however none have shown an elevation of EBV replication prior to the development of lymphomas. This would indicate whether EBV drives lymphoma genesis or acts as a passenger virus. An extension of this would also be to compare how levels of EBV DNA and markers of B-cell activation differ across various immune suppressed populations. For example, transplant recipients have high risk of lymphoma and post transplant lymphoproliferative disorder (PTLD) and high rates of EBV infection. It has been found that EBV can be reactivated without the

development of malignant lymphomas (1 in 3 people develop PTLD following EBV reactivation) in transplant recipients [1017]. If a similar result was found within HIV+ people, this would warrant the exploration of other factors in addition to EBV that drives the malignant process.

Additionally, investigating the incidence of lung cancer with a longer time since cessation in studies that follow HIV+ people for long periods of follow up would be a significant contribution to the literature. Ideally, studies following HIV+ people throughout their lifetimes are needed to determine when the benefit of cessation will be seen. I speculate that a decline in incidence may be evident with longer follow-up time. Unfortunately, no additional follow-up in D:A:D will be accrued as D:A:D funding has ended and the database closed. Furthermore, the epidemiology and pathology of lung cancers differ according to histology and several studies have suggested that adenocarcinoma is more prevalent in the HIV+ population. It would be interesting to see if cessation benefit differs according to subtype and whether cessation benefit differs by previous AIDS defining events. In addition, a previous study within D:A:D demonstrated an association between protease inhibitors (PIs) and cancer risk (mainly driven by anal cancers) [577]. PI use both inhibits and induces various parts of the cytochrome P450 (CYP450) system, which has been linked with cancer risk. It would be of great interest to investigate this in relation to lung cancer.

It would also be interesting to investigate age-specific PSA cut offs as a larger proportion of HIV+ people live into the older ages. Furthermore, larger studies to validate a lower cut off for PSA are needed to inform clinical practice, and a cost effectiveness analysis on the use of PSA testing for PCa diagnosis could be a possible route for future research.

8.4.2 The RESPOND consortium

A new research platform has been established to answer question about HIV+ people going forward. RESPOND is a newly formed international cohort consortium of infectious disease to allow for continued research collaboration between HIV cohorts. RESPOND will consist of a number of research modules each with a focus on a separate scientific agenda. It is a possibility that cancers in HIV+ people will be included as a module going forwards and may present as an appropriate setting to monitor the long term epidemiology of cancers in HIV+ people.

8.5 Concluding remarks.

This thesis had two aims. First, to describe the changing epidemiology of commonly occurring cancers in HIV+ people, particularly focussing on specific cancers or groups of cancers that are expected to become a major source of morbidity and mortality as the population ages. Second, to explore and characterise plasma biomarkers of common cancers in HIV+ people in order to better understand the mechanisms leading to cancer development. Cancer is now a major area of research in HIV + people and is gathering a lot of interest. As the HIV+ population age, the burden of cancers not traditionally associated with HIV are expected to increase and open up a new set of obstacles and issues for patient treatment and longevity. This thesis provides three major contributions to the field of HIV. The first is a description of the current distribution of cancers in HIV+ people in Europe and an indication that IURCs are expected to increase in coming years, driven by the increasing age of HIV+ population and higher prevalence of traditional smoking risk cancers, such as smoking. The second is the characterisation of the benefits of smoking on cancer incidence in HIV+ people. Smoking is a key modifiable risk factor for excess lung cancer risk, however the benefits of smoking cessation are not evident within the first 5 years of cessation. This suggests that HIV+ people may be at increased risk of smoking harms and prevention of smoking uptake should be a public health focus. The third is the characterisation and identification of risk factors which differ between NHL and HL as well as the suggestion that ongoing HIV replication may be driving lymphoma genesis in some HIV related lymphomas. Data presented here have also shown that PSA is highly predictive of PCa in HIV+ men, however, lower cut offs may be useful and need to be validated in larger studies. The findings from my research have contributed to the understanding of the epidemiology of cancers in HIV+ people and it is hoped that the results presented in this thesis will provide evidence to advise patient management guidelines and health care planning as HIV+ people age.

Continued monitoring of HIV+ people will be possible through the newly established RESPOND consortium. RESPOND will allow for a flexible research agendas as well as data collection through to the introduction of research modules. This will facilitate more powerful and detailed analysis on specific clinical events especially as follow-up time accrues.

Appendix I. EuroSIDA study group and steering committee

The EuroSIDA Study Group

The multi-centre study group, EuroSIDA (national coordinators in parenthesis).

Argentina: (M Losso), M Kundro, Hospital JM Ramos Mejia, Buenos Aires. Austria: (B Schmied), Pulmologisches Zentrum der Stadt Wien, Vienna; R Zangerle, Medical University Innsbruck, Innsbruck. Belarus: (I Karpov), A Vassilenko, Belarus State Medical University, Minsk, VM Mitsura, Gomel State Medical University, Gomel; D Paduto, Regional AIDS Centre, Svetlogorsk. Belgium: (N Clumeck), S De Wit, M Delforge, Saint-Pierre Hospital, Brussels; E Florence, Institute of Tropical Medicine, Antwerp; L Vandekerckhove, University Ziekenhuis Gent, Gent. Bosnia-Herzegovina: (V Hadziosmanovic), Klinicki Centar Univerziteta Sarajevo, Sarajevo. Croatia: (J Begovac), University Hospital of Infectious Diseases, Zagreb. Czech Republic: (L Machala), D Jilich, Faculty Hospital Bulovka, Prague; D Sedlacek, Charles University Hospital, Plzen. Denmark: G Kronborg,T Benfield, Hvidovre Hospital, Copenhagen; J Gerstoft, T Katzenstein, Rigshospitalet, Copenhagen; NF Møller, C Pedersen, Odense University Hospital, Odense; L Ostergaard, Skejby Hospital, Aarhus, L Wiese, Roskilde Hospital, Roskilde; L N Nielsen, Hillerod Hospital, Hillerod. Estonia: (K Zilmer), West-Tallinn Central Hospital, Tallinn; Jelena Smidt, Nakkusosakond Siseklinik, Kohtla-Järve. Finland: (M Ristola), I Aho, Helsinki University Central Hospital, Helsinki. France: (J-P Viard), Hôtel-Dieu, Paris; P-M Girard, Hospital Saint-Antoine, Paris; C Pradier, E Fontas, Hôpital de l'Archet, Nice; C Duvivier, Hôpital Necker-Enfants Malades, Paris.Germany: (J Rockstroh), Universitäts Klinik Bonn; R Schmidt, Medizinische Hochschule Hannover; O Degen, University Medical Center Hamburg-Eppendorf, Infectious Diseases Unit, Hamburg; HJ Stellbrink, IPM Study Center, Hamburg; C Stefan, JW Goethe University Hospital, Frankfurt; J Bogner, Medizinische Poliklinik, Munich; G. Fätkenheuer, Universität Köln, Cologne. Georgia: (N Chkhartishvili) Infectious Diseases, AIDS & Clinical Immunology Research Center, Tbilisi. Greece: (P Gargalianos), G Xylomenos, K Armenis, Athens General Hospital "G Gennimatas"; H Sambatakou, Ippokration General Hospital, Athens. Hungary: (J Szlávik), Szent Lásló Hospital, Budapest.Iceland: (M Gottfredsson), Landspitali University Hospital, Reykjavik. Ireland: (F Mulcahy), St. James's Hospital, Dublin. Israel: (I Yust), D Turner, M Burke, Ichilov Hospital, Tel Aviv; E Shahar, G Hassoun, Rambam Medical Center, Haifa; H Elinav, M Haouzi, Hadassah University Hospital, Jerusalem; D Elbirt, ZM Sthoeger, AIDS Center (Neve Or), Jerusalem. Italy: (A D'Arminio Monforte), Istituto Di Clinica Malattie Infettive e Tropicale, Milan; R Esposito, I Mazeu, C Mussini, Università Modena, Modena; F Mazzotta, A Gabbuti, Ospedale S Maria Annunziata, Firenze; V Vullo, M Lichtner, University di Roma la Sapienza, Rome; M Zaccarelli, A

Antinori, R Acinapura, M Plazzi, Istituto Nazionale Malattie Infettive Lazzaro Spallanzani, Rome; A Lazzarin, A Castagna, N Gianotti, Ospedale San Raffaele, Milan; M Galli, A Ridolfo, Osp. L. Sacco, Milan.Latvia: (B Rozentale), Infectology Centre of Latvia, Riga. Lithuania: (V Uzdaviniene) Vilnius University Hospital Santariskiu Klinikos, Vilnius; R Matulionyte, Center of Infectious Diseases, Vilnius University Hospital Santariskiu Klinikos, Vilnius. Luxembourg: (T Staub), R Hemmer, Centre Hospitalier, Luxembourg. Netherlands: (P Reiss), Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam. Norway: (V Ormaasen), A Maeland, J Bruun, Ullevål Hospital, Oslo. Poland: (B Knysz), J Gasiorowski, M Inglot, Medical University, Wroclaw; A Horban, E Bakowska, Centrum Diagnostyki i Terapii AIDS, Warsaw; R Flisiak, A Grzeszczuk, Medical University, Bialystok; M Parczewski, K Maciejewska, B Aksak-Was, Medical Univesity, Szczecin; M Beniowski, E Mularska, Osrodek Diagnostyki i Terapii AIDS, Chorzow; T Smiatacz, M Gensing, Medical University, Gdansk; E Jablonowska, E Malolepsza, K Wojcik, Wojewodzki Szpital Specjalistyczny, Lodz; I Mozer-Lisewska, Poznan University of Medical Sciences, Poznan. Portugal: (L Caldeira), Hospital Santa Maria, Lisbon; K Mansinho, Hospital de Egas Moniz, Lisbon; F Maltez, Hospital Curry Cabral, Lisbon. Romania: (R Radoi), C Oprea, Spitalul de Boli Infectioase si Tropicale: Dr. Victor Babes, Bucarest. Russia: (A Panteleev), O Panteleev, St Petersburg AIDS Centre, St Peterburg; A Yakovlev, Medical Academy Botkin Hospital, St Petersburg; T Trofimora, Novgorod Centre for AIDS, Novgorod, I Khromova, Centre for HIV/AIDS & and Infectious Diseases, Kaliningrad; E Kuzovatova, Nizhny Novgorod Scientific and Research Institute of Epidemiology and Microbiology named after Academician I.N. Blokhina, Nizhny Novogrod; E Borodulina, E Vdoushkina, Samara State Medical University, Samara. Serbia: (D Jevtovic), The Institute for Infectious and Tropical Diseases, Belgrade. Slovenia: (J Tomazic), University Clinical Centre Ljubljana, Ljubljana. Spain: (JM Gatell), JM Miró, Hospital Clinic Universitari de Barcelona, Barcelona; S Moreno, J. M. Rodriguez, Hospital Ramon y Cajal, Madrid; B Clotet, A Jou, R Paredes, C Tural, J Puig, I Bravo, Hospital Germans Trias i Pujol, Badalona; P Domingo, M Gutierrez, G Mateo, MA Sambeat, Hospital Sant Pau, Barcelona; JM Laporte, Hospital Universitario de Alava, Vitoria-Gasteiz. Sweden: (K Falconer), A Thalme, A Sonnerborg, Karolinska University Hospital, Stockholm; A Blaxhult, Venhälsan-Sodersjukhuset, Stockholm; L Flamholc, Malmö University Hospital, Malmö.Switzerland: (A Scherrer), R Weber, University Hospital Zurich; M Cavassini, University Hospital Lausanne; A Calmy, University Hospital Geneva; H Furrer, University Hospital Bern; M Battegay, University Hospital Basel; P Schmid, Cantonal Hospital St. Gallen.Ukraine: A Kuznetsova, Kharkov State Medical University, Kharkov; G Kyselyova, Crimean Republican AIDS centre, Simferopol; M Sluzhynska, Lviv Regional HIV/AIDS Prevention and Control CTR, Lviv. United Kingdom: (B Gazzard), St. Stephen's Clinic, Chelsea and Westminster Hospital, London; AM Johnson, E Simons, S Edwards, Mortimer Market Centre, London; A Phillips, MA Johnson, A

Mocroft, Royal Free and University College Medical School, London (Royal Free Campus); C Orkin, Royal London Hospital, London; J Weber, G Scullard, Imperial College School of Medicine at St. Mary's, London; A Clarke, Royal Sussex County Hospital, Brighton; C Leen, Western General Hospital, Edinburgh.

The following centers have previously contributed data to EuroSIDA:

Infectious Diseases Hospital, Sofia, Bulgaria; Hôpital de la Croix Rousse, Lyon, France; Hôpital de la Pitié-Salpétière, Paris, France; Unité INSERM, Bordeaux, France; Hôpital Edouard Herriot, Lyon, France; Bernhard Nocht Institut für Tropenmedizin, Hamburg, Germany; 1st I.K.A Hospital of Athens, Athens, Greece; Ospedale Riuniti, Divisione Malattie Infettive, Bergamo, Italy; Ospedale di Bolzano, Divisione Malattie Infettive, Bolzano, Italy; Ospedale Cotugno, III Divisione Malattie Infettive, Napoli, Italy; Dérer Hospital, Bratislava, Slovakia; Hospital Carlos III, Departamento de Enfermedades Infecciosas, Madrid, Spain; Kiev Centre for AIDS, Kiev, Ukraine; Luhansk State Medical University, Luhansk, Ukraine;Odessa Region AIDS Center, Odessa, Ukraine.

EuroSIDA Steering Committee

Chair: J Rockstroh, **Study Co-leads**: A Mocroft, O Kirk**, Steering Committee**: J Gatell, B Gazzard, A Horban, I Karpov, M Losso, A d'Arminio Monforte, C Pedersen, M Ristola, A Phillips, P Reiss, J Lundgren, J Rockstroh

Appendix II. D:A:D Study group and steering committee.

D:A:D Steering Committee: Names marked with *, Chair with ¢

Cohort PIs: W El-Sadr* (CPCRA), G Calvo* (BASS), F Bonnet and F Dabis* (Aquitaine), O Kirk* and A Mocroft* (EuroSIDA), M Law* (AHOD), A d'Arminio Monforte* (ICONA), L Morfeldt* (HivBIVUS), C Pradier* (Nice), P Reiss* (ATHENA), R Weber* (SHCS), S De Wit* (Brussels)

Cohort coordinators and data managers: A Lind-Thomsen (coordinator), R Salbøl Brandt, M Hillebreght, S Zaheri, FWNM Wit (ATHENA), A Scherrer, F Schöni-Affolter, M Rickenbach (SHCS), A Tavelli, I Fanti (ICONA), O Leleux, J Mourali, F Le Marec, E Boerg (Aquitaine), E Thulin, A Sundström (HIVBIVUS), G Bartsch, G Thompsen (CPCRA), C Necsoi, M Delforge (Brussels), E Fontas, C Caissotti, K Dollet (Nice), S Mateu, F Torres (BASS), K Petoumenos, A Blance, R Huang, R Puhr (AHOD), K Grønborg Laut, D Kristensen (EuroSIDA)

Statisticians: CA Sabin*, AN Phillips*, DA Kamara, CJ Smith, A Mocroft*

D:A:D coordinating office: CI Hatleberg, L Ryom, A Lind-Thomsen, RS Brandt, D Raben, C Matthews, A Bojesen, AL Grevsen, JD Lundgren*¢

Member of the D:A:D Oversight Committee: B Powderly*, N Shortman*, C Moecklinghoff*, G Reilly*, X Franquet*

D:A:D working group experts:

Kidney: L Ryom, A Mocroft*, O Kirk *, P Reiss*, C Smit, M Ross, CA Fux, P Morlat, E Fontas, DA Kamara, CJ Smith, JD Lundgren *¢

Mortality: CJ Smith, L Ryom, CI Hatleberg, AN Phillips*, R Weber*, P Morlat, C Pradier*, P Reiss*, FWNM Wit, N Friis-Møller, J Kowalska, JD Lundgren*¢

Cancer: CA Sabin*, L Ryom, CI Hatleberg, M Law*, A d'Arminio Monforte*, F Dabis*, F Bonnet*, P Reiss*, FWNM Wit, CJ Smith, DA Kamara, J Bohlius, M Bower, G Fätkenheuer, A Grulich, JD Lundgren*¢

External endpoint reviewers: A Sjøl (CVD), P Meidahl (oncology), JS Iversen (nephrology)

The current members of the 11 Cohorts are as follows:

ATHENA (AIDS Therapy Evaluation Project Netherlands):
Central coordination: P. Reiss*, S. Zaheri, M Hillebregt, F.W.N.M. Wit;
CLINICAL CENTRES *(¤ denotes site coordinating physician)* **Academic Medical Centre of the University of Amsterdam:** J.M. Prins¤, T.W. Kuijpers, H.J. Scherpbier, J.T.M. van der Meer, F.W.N.M. Wit, M.H. Godfried, P. Reiss, T. van der Poll, F.J.B. Nellen, S.E. Geerlings, M. van Vugt, D. Pajkrt, J.C. Bos, W.J. Wiersinga, M. van der Valk, A. Goorhuis, J.W. Hovius, J. van Eden, A. Henderiks, A.M.H. van Hes, M. Mutschelknauss, H.E. Nobel, F.J.J. Pijnappel, S. Jurriaans, N.K.T. Back, H.L. Zaaijer, B. Berkhout, M.T.E. Cornelissen, C.J. Schinkel, X.V. Thomas. **Admiraal De Ruyter Ziekenhuis, Goes:** M. van den Berge, A. Stegeman, S. Baas, L. Hage de Looff, D. Versteeg. **Catharina Ziekenhuis, Eindhoven:** M.J.H. Pronk¤, H.S.M. Ammerlaan, E.S. de Munnik. A.R. Jansz, J. Tjhie, M.C.A. Wegdam, B. Deiman, V. Scharnhorst. **Emma Kinderziekenhuis:** A. van der Plas, A.M. Weijsenfeld. **Erasmus MC, Rotterdam:** M.E. van der Ende¤, T.E.M.S. de

Vries-Sluijs, E.C.M. van Gorp, C.A.M. Schurink, J.L. Nouwen, A. Verbon, B.J.A. Rijnders, H.I. Bax, M. van der Feltz. N. Bassant, J.E.A. van Beek, M. Vriesde, L.M. van Zonneveld. A. de Oude-Lubbers, H.J. van den Berg-Cameron, F.B. Bruinsma-Broekman, J. de Groot, M. de Zeeuw- de Man, C.A.B. Boucher, M.P.G Koopmans, J.J.A van Kampen, S.D. Pas. **Erasmus MC–Sophia, Rotterdam:** G.J.A. Driessen, A.M.C. van Rossum, L.C. van der Knaap, E. Visser. **Flevoziekenhuis, Almere:** J. Branger¤, A. Rijkeboer-Mes, C.J.H.M. Duijf-van de Ven. **HagaZiekenhuis, Den Haag:** E.F. Schippers¤, C. van Nieuwkoop. J.M. van IJperen, J. Geilings. G. van der Hut. P.F.H. Franck. **HIV Focus Centrum (DC Klinieken):** A. van Eeden¤. W. Brokking, M. Groot, L.J.M. Elsenburg, M. Damen, I.S. Kwa. **Isala, Zwolle:** P.H.P. Groeneveld¤, J.W. Bouwhuis, J.F. van den Berg, A.G.W. van Hulzen, G.L. van der Bliek, P.C.J. Bor, P. Bloembergen, M.J.H.M. Wolfhagen, G.J.H.M. Ruijs. **Leids Universitair Medisch Centrum, Leiden:** , F.P. Kroon¤, M.G.J. de Boer, M.P. Bauer, H. Jolink, A.M. Vollaard, W. Dorama, N. van Holten, E.C.J. Claas, E. Wessels. **Maasstad Ziekenhuis, Rotterdam:** J.G. den Hollander¤, K. Pogany, A. Roukens, M. Kastelijns, J.V. Smit, E. Smit, D. Struik-Kalkman, C. Tearno, M. Bezemer, T. van Niekerk, O. Pontesilli. **Maastricht UMC+, Maastricht:** S.H. Lowe¤, A.M.L. Oude Lashof, D. Posthouwer, R.P. Ackens, J. Schippers, R. Vergoossen, B. Weijenberg-Maes, I.H.M. van Loo, T.R.A. Havenith. **MCH-Bronovo, Den Haag:** E.M.S. Leyten¤, L.B.S. Gelinck, A. van Hartingsveld, C. Meerkerk, G.S. Wildenbeest, J.A.E.M. Mutsaers, C.L. Jansen. **MC Slotervaart, Amsterdam:** J.W. Mulder, S.M.E. Vrouenraets, F.N. Lauw, M.C. van Broekhuizen, H. Paap, D.J. Vlasblom, P.H.M. Smits. **MC Zuiderzee, Lelystad:** S. Weijer¤, R. El Moussaoui, A.S. Bosma. **Medisch Centrum Leeuwarden, Leeuwarden:** M.G.A.van Vonderen¤, D.P.F. van Houte, L.M. Kampschreur, K. Dijkstra, S. Faber, J Weel. **Medisch Spectrum Twente, Enschede:** G.J. Kootstra¤, C.E. Delsing, M. van der Burg-van de Plas, H. Heins, E. Lucas. **Noorwest Ziekenhuisgroep, Alkmaar:** W. Kortmann¤, G. van Twillert¤, J.W.T. Cohen Stuart, B.M.W. Diederen, D. Pronk, F.A. van Truijen-Oud, W. A. van der Reijden, R. Jansen. **OLVG, Amsterdam:** K. Brinkman¤, G.E.L. van den Berk, W.L. Blok, P.H.J. Frissen, K.D. Lettinga W.E.M. Schouten, J. Veenstra, C.J. Brouwer, G.F. Geerders, K. Hoeksema, M.J. Kleene, I.B. van der Meché, M. Spelbrink, H. Sulman, A.J.M. Toonen, S. Wijnands, M. Damen, D. Kwa, E. Witte. **Radboudumc, Nijmegen:** P.P. Koopmans, M. Keuter, A.J.A.M. van der Ven, H.J.M. ter Hofstede, A.S.M. Dofferhoff, R. van Crevel, M. Albers, M.E.W. Bosch, K.J.T. Grintjes-Huisman, B.J. Zomer, F.F. Stelma, J. Rahamat-Langendoen,D. Burger. **Rijnstate, Arnhem:** C. Richter¤, E.H. Gisolf, R.J. Hassing, G. ter Beest, P.H.M. van Bentum, N. Langebeek, R. Tiemessen, C.M.A. Swanink. **Spaarne Gasthuis, Haarlem:** S.F.L. van Lelyveld¤, R. Soetekouw, N. Hulshoff, L.M.M. van der Prijt, J. van der Swaluw, N. Bermon, W.A. van der Reijden, R. Jansen, B.L. Herpers, D.Veenendaal. **Medisch Centrum Jan van Goyen, Amsterdam:** D.W.M. Verhagen, M. van Wijk. **St Elisabeth Ziekenhuis, Tilburg:** M.E.E. van Kasteren¤, A.E. Brouwer, B.A.F.M. de Kruijf-van de Wiel, M. Kuipers, R.M.W.J. Santegoets, B. van der Ven, J.H. Marcelis, A.G.M. Buiting, P.J. Kabel. **Universitair Medisch Centrum Groningen, Groningen:** W.F.W. Bierman¤, H. Scholvinck, K.R. Wilting, Y. Stienstra, H. de Groot-de Jonge, P.A. van der Meulen, D.A. de Weerd, J. Ludwig-Roukema, H.G.M. Niesters, A. Riezebos-Brilman, C.C. van Leer-Buter, M. Knoester. **Universitair Medisch Centrum Utrecht, Utrecht:** A.I.M. Hoepelman¤, T. Mudrikova, P.M. Ellerbroek, J.J. Oosterheert, J.E. Arends, R.E. Barth, M.W.M. Wassenberg, E.M. Schadd, D.H.M. van Elst-Laurijssen, E.E.B. van Oers-Hazelzet, S. Vervoort, M. van Berkel, R. Schuurman, F. Verduyn-Lunel, A.M.J. Wensing. **VUmc, Amsterdam:** E.J.G. Peters¤, M.A. van Agtmael, M. Bomers, J. de Vocht, M. Heitmuller, L.M. Laan, A.M. Pettersson, C.M.J.E. Vandenbroucke-Grauls, C.W. Ang. **Wilhelmina Kinderziekenhuis, UMCU, Utrecht:** S.P.M. Geelen, T.F.W. Wolfs, L.J. Bont, N. Nauta. **COORDINATING CENTRE** P. Reiss, D.O. Bezemer, A.I. van Sighem, C. Smit, F.W.N.M. Wit., T.S. Boender, S. Zaheri, M. Hillebregt, A. de Jong, D. Bergsma, P. Hoekstra, A. de Lang, S. Grivell, A. Jansen, M.J. Rademaker, M. Raethke, R. Meijering, S. Schnörr, L. de Groot, M. van den Akker, Y. Bakker, E. Claessen, A. El Berkaoui, J. Koops, E. Kruijne, C. Lodewijk, L. Munjishvili, B. Peeck, C. Ree, R. Regtop, Y. Ruijs, T. Rutkens, L. van de Sande, M. Schoorl, A. Timmerman, E. Tuijn, L. Veenenberg, S. van der Vliet, A. Wisse, T. Woudstra, B. Tuk.

Aquitaine Cohort (France)
Composition du Conseil scientifique :

Coordination: F. Bonnet, F. Dabis

Scientific committee: M. Dupon, V. Gaborieau, D. Lacoste, D. Malvy, P. Mercié, P. Morlat, D. Neau, JL. Pellegrin, S. Tchamgoué, E. Lazaro, C. Cazanave, M. Vandenhende, M.O. Vareil, Y. Gérard, P. Blanco, S. Bouchet, D. Breilh, H. Fleury, I. Pellegrin, G. Chêne, R. Thiébaut, L. Wittkop, L. Wittkop, O. Leleux, S. Lawson-Ayayi, A. Gimbert, S. Desjardin, L. Lacaze-Buzy, V. Petrov-Sanchez

Epidemiology and Methodology: F. Bonnet, G. Chêne, F. Dabis, R. Thiébaut, L. Wittkop

Infectious Diseases and Internal Medicine: K. André, N. Bernard, F. Bonnet, O. Caubet, L. Caunegre, C. Cazanave, I. Chossat, C. Courtault, FA. Dauchy, S. De Witte, D. Dondia, M. Dupon, P. Duffau, H. Dutronc, S. Farbos, I. Faure, H. Ferrand, V. Gaborieau, Y. Gerard, C. Greib, M. Hessamfar, Y. Imbert, D. Lacoste , P. Lataste, E. Lazaro, D. Malvy, J. Marie, M. Mechain, P. Mercié, E.Monlun, P. Morlat, D. Neau, A. Ochoa, JL. Pellegrin, T. Pistone, I. Raymond,MC. Receveur, P. Rispal, L. Sorin, S. Tchamgoué, C. Valette, MA. Vandenhende, MO. Vareil, JF. Viallard, H. Wille, G. Wirth.

Immunology: I. Pellegrin, P. Blanco

Virology: H. Fleury, Me. Lafon, P. Trimoulet, P. Bellecave, C. Tumiotto

Pharmacology: S. Bouchet, D. Breilh, F. Haramburu, G. Miremeont-Salamé

Data collection, Project Management and Statistical Analyses: MJ. Blaizeau, M. Decoin, C. Hannapier, E. Lenaud et A. Pougetoux; S. Delveaux, C. D'Ivernois, F. Diarra B. Uwamaliya-Nziyumvira, O. Leleux; F. Le Marec, Eloïse Boerg, S. Lawson-Ayayi;

IT department and eCRF development: G. Palmer, V. Conte, V. Sapparrart

AHOD (Australian HIV Observational Database, Australia):
Central coordination: M. Law *, K. Petoumenos, R Puhr, R Huang (Sydney, New South Wales). **Participating physicians (city, state):** R. Moore, S. Edwards, J. Hoy, K. Watson, N. Roth, H Lau (Melbourne, Victoria); M Bloch, D. Baker, A. Carr, D. Cooper, (Sydney, New South Wales);M O'Sullivan (Gold Coast, Queensland), D. Nolan, G Guelfi (Perth, Western Australia).

BASS (Spain):
Central coordination: G. Calvo, F. Torres, S. Mateu (Barcelona);
Participating physicians (city): P. Domingo, M.A. Sambeat, J. Gatell, E. Del Cacho, J. Cadafalch, M. Fuster (Barcelona); C. Codina, G. Sirera, A. Vaqué (Badalona).

The Brussels St Pierre Cohort (Belgium):

Coordination: S. De Wit*, N. Clumeck, M. Delforge, C. Necsoi.

Participating physicians: N. Clumeck, S. De Wit*, AF Gennotte, M. Gerard, K. Kabeya, D. Konopnicki, A. Libois, C. Martin, M.C. Payen, P. Semaille, Y. Van Laethem.

CPCRA (USA):
Central coordination: J. Neaton, G. Bartsch, W.M. El-Sadr*, E. Krum, G. Thompson, D. Wentworth;
Participating physicians (city, state): R. Luskin-Hawk (Chicago, Illinois); E. Telzak (Bronx, New York); W.M. El-Sadr (Harlem, New York); D.I. Abrams (San Francisco, California); D. Cohn (Denver, Colorado); N. Markowitz (Detroit, Michigan); R. Arduino (Houston, Texas); D. Mushatt (New Orleans, Louisiana); G. Friedland (New Haven, Connecticut); G. Perez (Newark, New Jersey); E. Tedaldi (Philadelphia, Pennsylvania); E. Fisher (Richmond, Virginia); F. Gordin (Washington, DC); L.R. Crane (Detroit, Michigan); J. Sampson (Portland, Oregon); J. Baxter (Camden, New Jersey).

EuroSIDA (multinational**)**

Steering Committee: J Gatell, B Gazzard, A Horban, I Karpov, M Losso, A d'Arminio Monforte, C Pedersen, M Ristola, A Phillips, P Reiss, J Lundgren, J Rockstroh

Chair: J Rockstroh

Study Co-leads: A Mocroft, O Kirk

Coordinating Centre Staff: O Kirk, L Peters, C Matthews, AH Fischer, A Bojesen, D Raben, D Kristensen, K Grønborg Laut, JF Larsen, D Podlekareva

Statistical Staff: A Mocroft, A Phillips, A Cozzi-Lepri, L Shepherd, A Schultze, S Amele

The multi-centre study group, EuroSIDA (national coordinators in parenthesis).

Argentina: (M Losso), M Kundro, Hospital JM Ramos Mejia, Buenos Aires.

Austria: (B Schmied), Pulmologisches Zentrum der Stadt Wien, Vienna; R Zangerle, Medical University Innsbruck, Innsbruck.

Belarus: (I Karpov), A Vassilenko, Belarus State Medical University, Minsk, VM Mitsura, Gomel State Medical University, Gomel; D Paduto, Regional AIDS Centre, Svetlogorsk.

Belgium: (N Clumeck), S De Wit, M Delforge, Saint-Pierre Hospital, Brussels; E Florence, Institute of Tropical Medicine, Antwerp; L Vandekerckhove, University Ziekenhuis Gent, Gent.

Bosnia-Herzegovina: (V Hadziosmanovic), Klinicki Centar Univerziteta Sarajevo, Sarajevo.

Croatia: (J Begovac), University Hospital of Infectious Diseases, Zagreb.

Czech Republic: (L Machala), D Jilich, Faculty Hospital Bulovka, Prague; D Sedlacek, Charles University Hospital, Plzen.

Denmark: G Kronborg,T Benfield, Hvidovre Hospital, Copenhagen; J Gerstoft, T Katzenstein, Rigshospitalet, Copenhagen; NF Møller, C Pedersen, Odense University Hospital, Odense; L

Ostergaard, Skejby Hospital, Aarhus, L Wiese, Roskilde Hospital, Roskilde; L N Nielsen, Hillerod Hospital, Hillerod.

Estonia: (K Zilmer), West-Tallinn Central Hospital, Tallinn; Jelena Smidt, Nakkusosakond Siseklinik, Kohtla-Järve.

Finland: (M Ristola), I Aho, Helsinki University Central Hospital, Helsinki.

France: (J-P Viard), Hôtel-Dieu, Paris; P-M Girard, Hospital Saint-Antoine, Paris; C Pradier, E Fontas, Hôpital de l'Archet, Nice; C Duvivier, Hôpital Necker-Enfants Malades, Paris.

Germany: (J Rockstroh), Universitäts Klinik Bonn; R Schmidt, Medizinische Hochschule **Hannover;** O Degen, University Medical Center Hamburg-Eppendorf, Infectious Diseases Unit, **Hamburg;** HJ Stellbrink, IPM Study Center, Hamburg; C Stefan, JW Goethe University Hospital, **Frankfurt;** J Bogner, Medizinische Poliklinik, Munich; G. Fätkenheuer, Universität Köln, Cologne.

Georgia: (N Chkhartishvili) Infectious Diseases, AIDS & Clinical Immunology Research Center, Tbilisi

Greece: (P Gargalianos), G Xylomenos, K Armenis, Athens General Hospital "G Gennimatas"; H Sambatakou, Ippokration General Hospital, Athens.

Hungary: (J Szlávik), Szent Lásló Hospital, Budapest.

Iceland: (M Gottfredsson), Landspitali University Hospital, Reykjavik.

Ireland: (F Mulcahy), St. James's Hospital, Dublin.

Israel: (I Yust), D Turner, M Burke, Ichilov Hospital, Tel Aviv; E Shahar, G Hassoun, Rambam Medical Center, Haifa; H Elinav, M Haouzi, Hadassah University Hospital, Jerusalem; D Elbirt, ZM Sthoeger, AIDS Center (Neve Or), Jerusalem.

Italy: (A D'Arminio Monforte), Istituto Di Clinica Malattie Infettive e Tropicale, Milan; R Esposito, I Mazeu, C Mussini, Università Modena, Modena; F Mazzotta, A Gabbuti, Ospedale S Maria Annunziata, Firenze; V Vullo, M Lichtner, University di Roma la Sapienza, Rome; M Zaccarelli, A Antinori, R Acinapura, M Plazzi, Istituto Nazionale Malattie Infettive Lazzaro Spallanzani, Rome; A Lazzarin, A Castagna, N Gianotti, Ospedale San Raffaele, Milan; M Galli, A Ridolfo, Osp. L. Sacco, Milan.

Latvia: (B Rozentale), Infectology Centre of Latvia, Riga.

Lithuania: (V Uzdaviniene) Vilnius University Hospital Santariskiu Klinikos, Vilnius; R Matulionyte, Center of Infectious Diseases, Vilnius University Hospital Santariskiu Klinikos, Vilnius.

Luxembourg: (T Staub), R Hemmer, Centre Hospitalier, Luxembourg.

Netherlands: (P Reiss), Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam.

Norway: (V Ormaasen), A Maeland, J Bruun, Ullevål Hospital, Oslo.

Poland: (B Knysz), J Gasiorowski, M Inglot, Medical University, Wroclaw; A Horban, E Bakowska, Centrum Diagnostyki i Terapii AIDS, Warsaw; R Flisiak, A Grzeszczuk, Medical University, Bialystok; M Parczewski, K Maciejewska, B Aksak-Was, Medical Univesity, Szczecin; M Beniowski, E Mularska, Osrodek Diagnostyki i Terapii AIDS, Chorzow; T Smiatacz, M Gensing, Medical University, Gdansk; E Jablonowska, E Malolepsza, K Wojcik, Wojewodzki Szpital Specjalistyczny, Lodz; I Mozer-Lisewska, Poznan University of Medical Sciences, Poznan.

Portugal: (L Caldeira), Hospital Santa Maria, Lisbon; K Mansinho, Hospital de Egas Moniz, Lisbon; F Maltez, Hospital Curry Cabral, Lisbon.

Romania: (R Radoi), C Oprea, Spitalul de Boli Infectioase si Tropicale: Dr. Victor Babes, Bucarest.

Russia: (A Panteleev), O Panteleev, St Petersburg AIDS Centre, St Peterburg; A Yakovlev, Medical Academy Botkin Hospital, St Petersburg; T Trofimora, Novgorod Centre for AIDS, Novgorod, I Khromova, Centre for HIV/AIDS & and Infectious Diseases, Kaliningrad; E Kuzovatova, Nizhny Novgorod Scientific and Research Institute of Epidemiology and Microbiology named after Academician I.N. Blokhina, Nizhny Novogrod; E Borodulina, E Vdoushkina, Samara State Medical University, Samara.

Serbia: (D Jevtovic), The Institute for Infectious and Tropical Diseases, Belgrade.

Slovenia: (J Tomazic), University Clinical Centre Ljubljana, Ljubljana.

Spain: (JM Gatell), JM Miró, Hospital Clinic Universitari de Barcelona, Barcelona; S Moreno, J. M. Rodriguez, Hospital Ramon y Cajal, Madrid; B Clotet, A Jou, R Paredes, C Tural, J Puig, I Bravo, Hospital Germans Trias i Pujol, Badalona; P Domingo, M Gutierrez, G Mateo, MA Sambeat, Hospital Sant Pau, Barcelona; JM Laporte, Hospital Universitario de Alava, Vitoria-Gasteiz.

Sweden: (K Falconer), A Thalme, A Sonnerborg, Karolinska University Hospital, Stockholm; A Blaxhult, Venhälsan-Sodersjukhuset, Stockholm; L Flamholc, Malmö University Hospital, Malmö.

Switzerland: (A Scherrer), R Weber, University Hospital Zurich; M Cavassini, University Hospital Lausanne; A Calmy, University Hospital Geneva; H Furrer, University Hospital Bern; M Battegay, University Hospital Basel; P Schmid, Cantonal Hospital St. Gallen.

Ukraine: A Kuznetsova, Kharkov State Medical University, Kharkov; G Kyselyova, Crimean Republican AIDS centre, Simferopol; M Sluzhynska, Lviv Regional HIV/AIDS Prevention and Control CTR, Lviv.

United Kingdom: (B Gazzard), St. Stephen's Clinic, Chelsea and Westminster Hospital, London; AM Johnson, E Simons, S Edwards, Mortimer Market Centre, London; A Phillips, MA Johnson, A Mocroft, Royal Free and University College Medical School, London (Royal Free Campus); C Orkin, Royal London Hospital, London; J Weber, G Scullard, Imperial College School of Medicine at St. Mary's, London; A Clarke, Royal Sussex County Hospital, Brighton; C Leen, Western General Hospital, Edinburgh.

The following centers have previously contributed data to EuroSIDA:

Infectious Diseases Hospital, Sofia, Bulgaria

Hôpital de la Croix Rousse, Lyon, France

Hôpital de la Pitié-Salpétière, Paris, France

Unité INSERM, Bordeaux, France

Hôpital Edouard Herriot, Lyon, France

Bernhard Nocht Institut für Tropenmedizin, Hamburg, Germany

1st I.K.A Hospital of Athens, Athens, Greece

Ospedale Riuniti, Divisione Malattie Infettive, Bergamo, Italy

Ospedale di Bolzano, Divisione Malattie Infettive, Bolzano, Italy

Ospedale Cotugno, III Divisione Malattie Infettive, Napoli, Italy

Dérer Hospital, Bratislava, Slovakia

Hospital Carlos III, Departamento de Enfermedades Infecciosas, Madrid, Spain

Kiev Centre for AIDS, Kiev, Ukraine

Luhansk State Medical University, Luhansk, Ukraine

Odessa Region AIDS Center, Odessa, Ukraine

The ICONA Foundation (Italy):
BOARD OF DIRECTORS
A d'Arminio Monforte (President), A Antinori, A Castagna, F Castelli, R Cauda, G Di Perri, M Galli, R Iardino, G Ippolito, GC Marchetti, CF Perno, F von Schloesser, P Viale
SCIENTIFIC SECRETARY
A d'Arminio Monforte, A Antinori, A Castagna, F Ceccherini-Silberstein, A Cozzi-Lepri, E Girardi, S Lo Caputo, C Mussini, M Puoti
STEERING COMMITTEE
M Andreoni, A Ammassari, A Antinori, C Balotta, A Bandera, P Bonfanti, S Bonora, M Borderi, A Calcagno, L Calza, MR Capobianchi, A Castagna, F Ceccherini-Silberstein, A Cingolani, P Cinque, A Cozzi-Lepri, A d'Arminio Monforte, A De Luca, A Di Biagio, E Girardi, N Gianotti, A Gori, G Guaraldi, G Lapadula, M Lichtner, S Lo Caputo, G Madeddu, F Maggiolo, G Marchetti, S Marcotullio, L Monno, C Mussini, S Nozza, M Puoti, E Quiros Roldan, R Rossotti, S Rusconi, MM Santoro, A Saracino, M Zaccarelli.
STATISTICAL AND MONITORING TEAM
A Cozzi-Lepri, I Fanti, L Galli, P Lorenzini, A Rodano, M Shanyinde, A Tavelli
BIOLOGICAL BANK INMI
F Carletti, S Carrara, A Di Caro, S Graziano, F Petrone, G Prota, S Quartu, S Truffa
PARTICIPATING PHYSICIANS AND CENTERS

Italy A Giacometti, A Costantini, V Barocci (Ancona); G Angarano, L Monno, C Santoro (Bari); F Maggiolo, C Suardi (Bergamo); P Viale, V Donati, G Verucchi (Bologna); F Castelli, C Minardi, E Quiros Roldan (Brescia); T Quirino, C Abeli (Busto Arsizio); PE Manconi, P Piano (Cagliari); B Cacopardo, B Celesia (Catania); J Vecchiet, K Falasca (Chieti); A Pan, S Lorenzotti (Cremona); L Sighinolfi, D Segala (Ferrara); F Mazzotta, F Vichi (Firenze); G Cassola, C Viscoli, A Alessandrini, N Bobbio, G Mazzarello (Genova); C Mastroianni, V Belvisi (Latina); P Bonfanti, I Caramma (Lecco); A Chiodera, P Milini (Macerata); A d'Arminio Monforte, M Galli, A Lazzarin, G Rizzardini, M Puoti, A Castagna, G Marchetti, MC Moioli, R Piolini, AL Ridolfo, S Salpietro, C Tincati, (Milano); C Mussini, C Puzzolante (Modena); A Gori, G Lapadula (Monza); N Abrescia, A Chirianni, G Borgia, R Orlando, G Bonadies, F Di Martino, I Gentile, L Maddaloni (Napoli); AM Cattelan, S Marinello (Padova); A Cascio, C Colomba (Palermo); F Baldelli, E Schiaroli (Perugia); G Parruti, F Sozio (Pescara); G Magnani, MA Ursitti (Reggio Emilia); M Andreoni, A Antinori, R Cauda, A Cristaudo, V Vullo, R Acinapura, G Baldin, M Capozzi, S Cicalini, A Cingolani, L Fontanelli Sulekova, G Iaiani, A Latini, I Mastrorosa, MM Plazzi, S Savinelli, A Vergori (Roma); M Cecchetto, F Viviani (Rovigo); G Madeddu, P Bagella (Sassari); A De Luca, B Rossetti (Siena); A Franco, R Fontana Del Vecchio (Siracusa); D Francisci, C Di Giuli (Terni); P Caramello, G Di Perri, S Bonora, GC Orofino, M Sciandra (Torino); M Bassetti, A Londero (Udine); G Pellizzer, V Manfrin (Vicenza) G Starnini, A Ialungo(Viterbo).

Nice HIV Cohort (France):

Central coordination: C. Pradier*, E. Fontas, K. Dollet, C. Caissotti.

Participating physicians: P. Dellamonica, E. Bernard, J. Courjon, E. Cua, F. De Salvador-Guillouet, J.Durant, C. Etienne, S. Ferrando, V. Mondain-Miton, A. Naqvi, I. Perbost,S. Pillet , B. Prouvost-Keller, P. Pugliese, V. Rio, K. Risso, P.M. Roger.

SHCS (Swiss HIV Cohort Study, Switzerland):

The data are gathered by the Five Swiss University Hospitals, two Cantonal Hospitals,15 affiliated hospitals and 36 private physicians (listed in http://www.shcs.ch/180-health-care-providers).

Members of the Swiss HIV Cohort Study :

Aubert V, Battegay M, Bernasconi E, Böni J, Braun DL, Bucher HC, Calmy A, Cavassini M, Ciuffi A, Dollenmaier G, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Günthard HF (President of the SHCS), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hoffmann M, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kouyos RD, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Marzolini C, Metzner KJ, Müller N, Nicca D, Pantaleo G, Paioni P, Rauch A (Chairman of the Scientific Board), Rudin C (Chairman of the Mother & Child Substudy), Scherrer AU (Head of Data Centre), Schmid P, Speck R, Stöckle M, Tarr P, Trkola A, Vernazza P, Wandeler G, Weber R*, Yerly S.

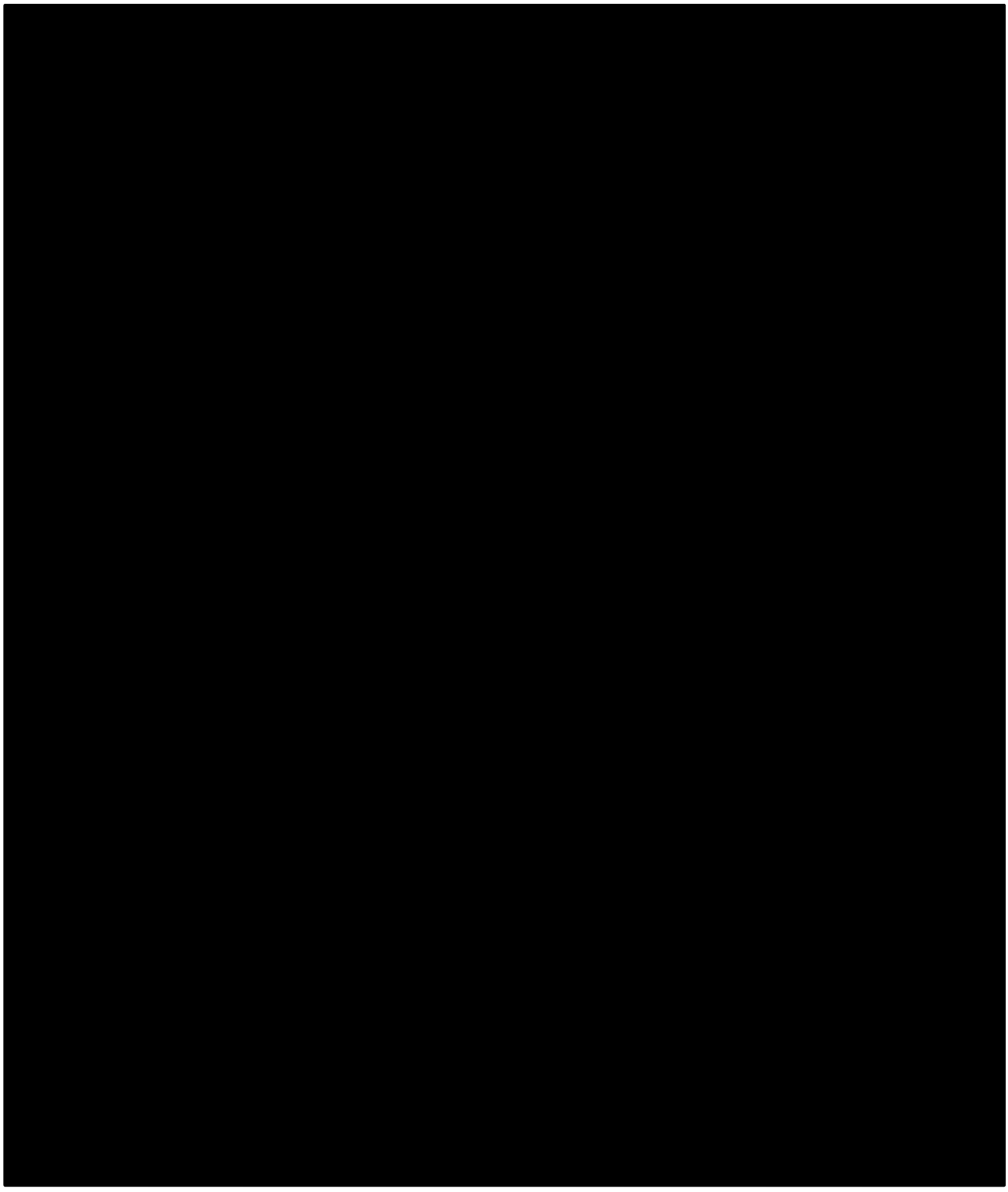
Financial acknowledgements:

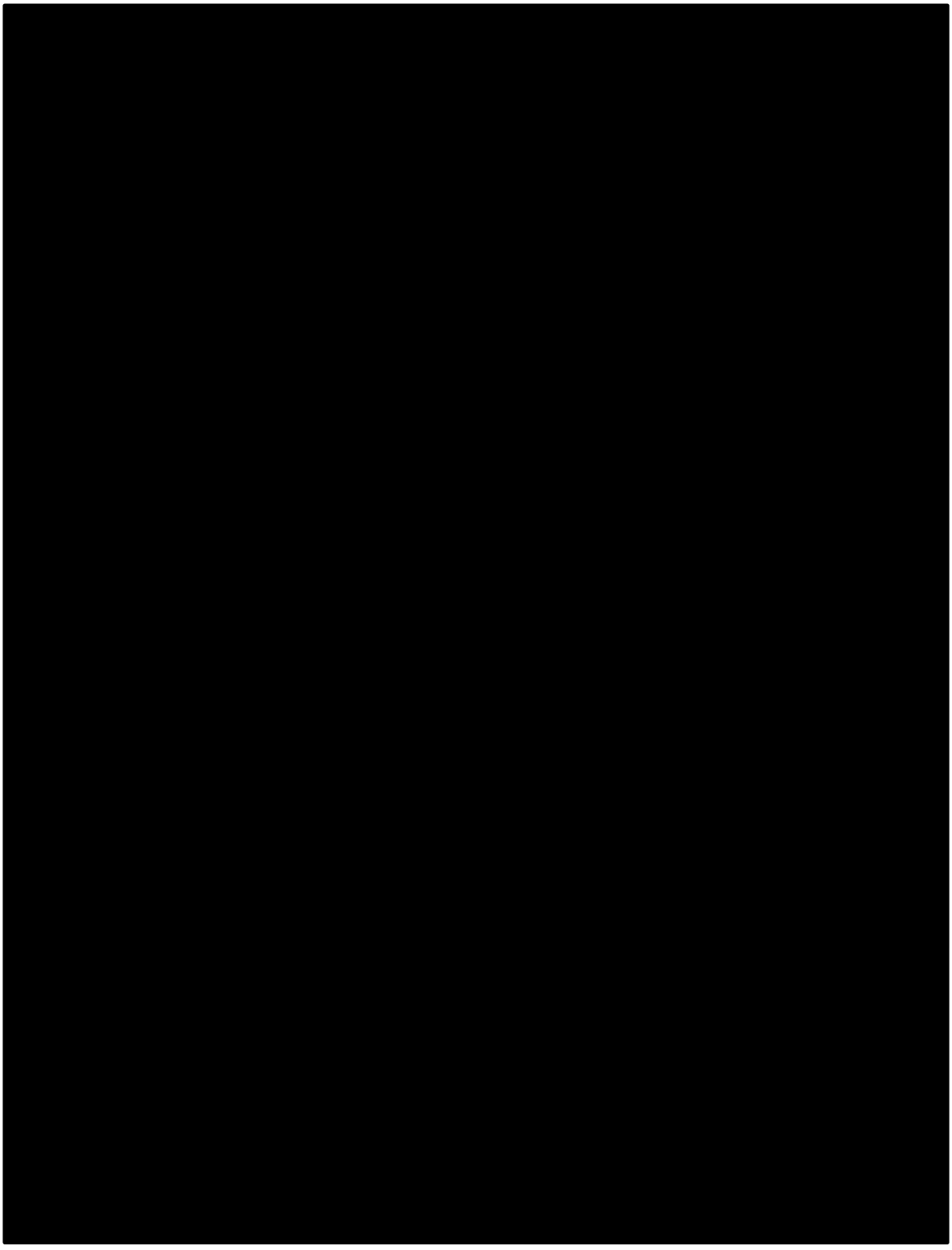
Data on Adverse Events (D:A:D) Study:The D:A:D study was supported by the Highly Active Antiretroviral Therapy Oversight Committee (HAARTOC), a collaborative committee with representation from academic institutions, the European Agency for the Evaluation of Medicinal Products, the United States Food and Drug Administration, the patient community, and pharmaceutical companies with licensed anti-HIV drugs in the European Union: AbbVie,

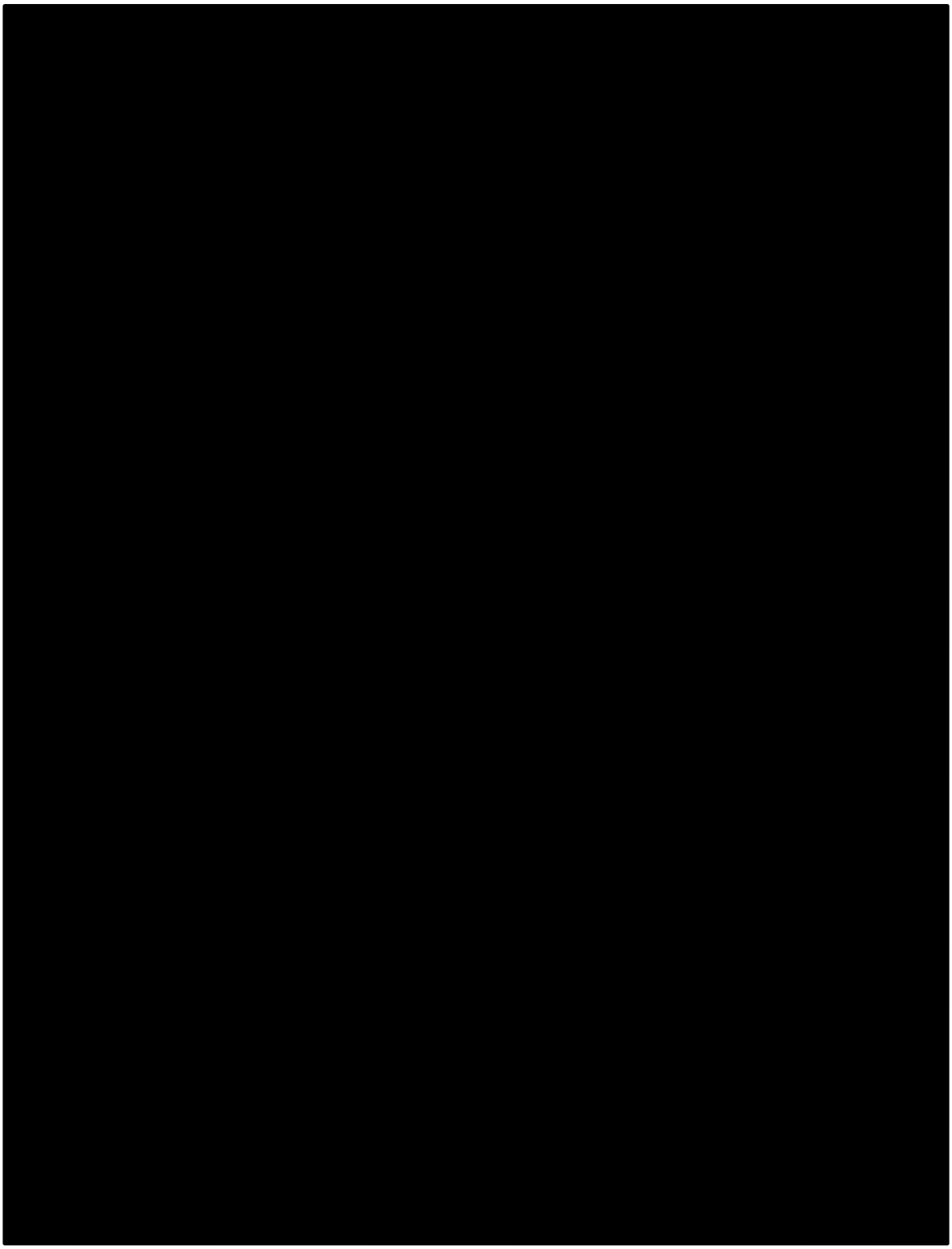
Bristol-Myers Squibb, Gilead Sciences Inc., ViiV Healthcare, Merck & Co Inc. and Janssen Pharmaceuticals. Supported also by a grant [grant number DNRF126] from the Danish National Research Foundation (CHIP & PERSIMUNE); by a grant from the Dutch Ministry of Health, Welfare and Sport through the Center for Infectious Disease Control of the National Institute for Public Health and the Environment to Stiching HIV Monitoring (ATHENA); by a grant from the Agence nationale de recherches sur le sida et les hépatites virales [ANRS, Action Coordonnée no.7, Cohortes] to the Aquitaine Cohort; The Australian HIV Observational Database (AHOD) is funded as part of the Asia Pacific HIV Observational Database, a program of The Foundation for AIDS Research, amfAR, and is supported in part by a grant from the U.S. National Institutes of Health's National Institute of Allergy and Infectious Diseases (NIAID) [grant number U01-AI069907] and by unconditional grants from Merck Sharp & Dohme; Gilead Sciences; Bristol-Myers Squibb; Boehringer Ingelheim; Janssen-Cilag; ViiV Healthcare. The Kirby Institute is funded by The Australian Government Department of Health and Ageing, and is affiliated with the Faculty of Medicine, The University of New South Wales; by grants from the Fondo de Investigación Sanitaria [grant number FIS 99/0887] and Fundación para la Investigación y la Prevención del SIDA en Espanã [grant number FIPSE 3171/00], to the Barcelona Antiretroviral Surveillance Study (BASS); by the National Institute of Allergy and Infectious Diseases, National Institutes of Health [grants number 5U01AI042170-10, 5U01AI046362-03], to the Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA); by primary funding provided by the European Union's Seventh Framework Programme for research, technological development and demonstration under EuroCoord grant agreement n˚ 260694 and unrestricted grants by Bristol-Myers Squibb, Janssen R&D, Merck and Co. Inc., Pfizer Inc., GlaxoSmithKline LLC, (the participation of centres from Switzerland is supported by The Swiss National Science Foundation (Grant 108787)) to the EuroSIDA study; by unrestricted educational grants of AbbVie, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Pfizer, Janssen Pharmaceuticals to the Italian Cohort Naive to Antiretrovirals (The ICONA Foundation); and financed within the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation (grant #148522) and by the SHCS research foundation.

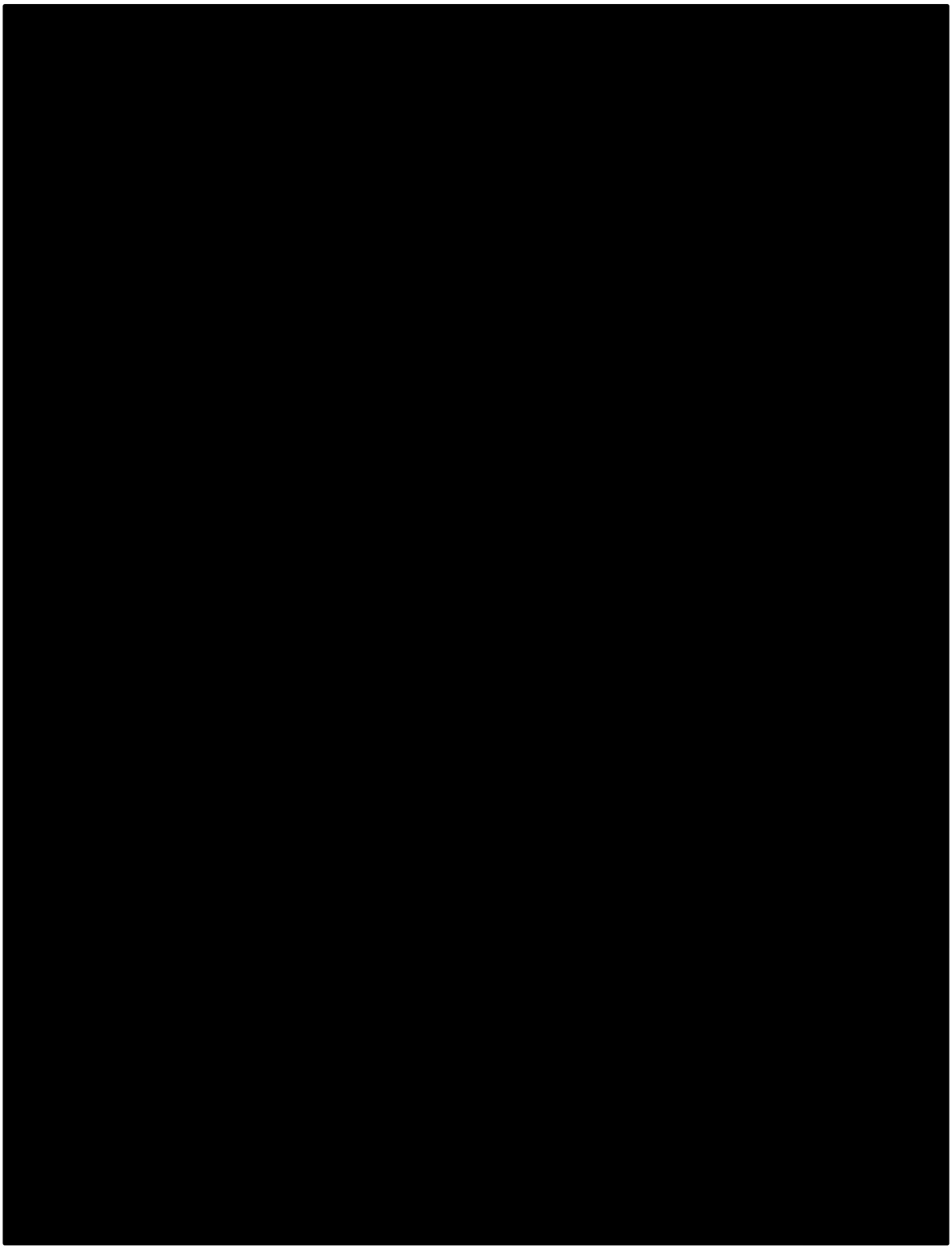
The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

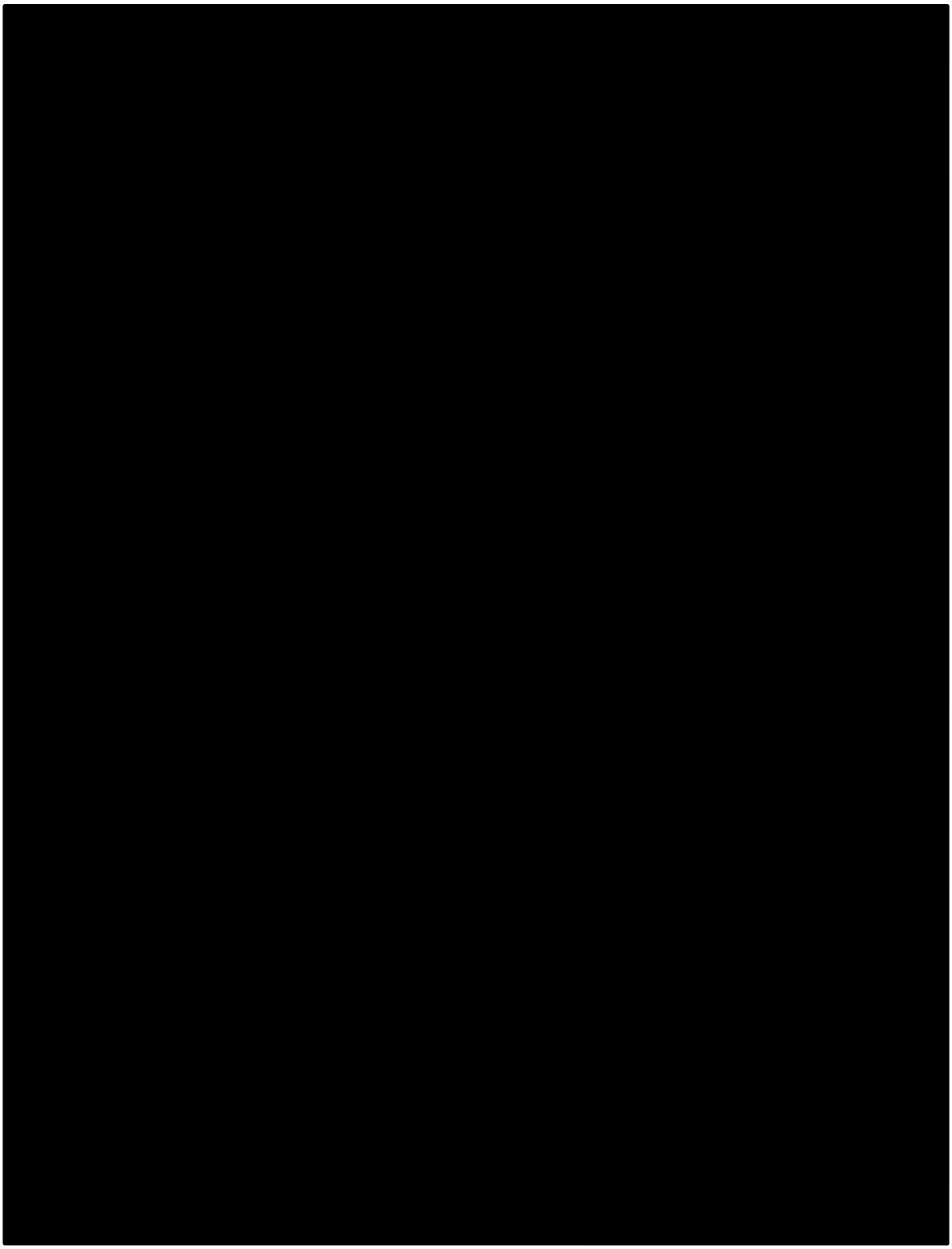
Appendix III. Published manuscript entitled “Infection-related and –unrelated malignancies, HIV and the aging population”

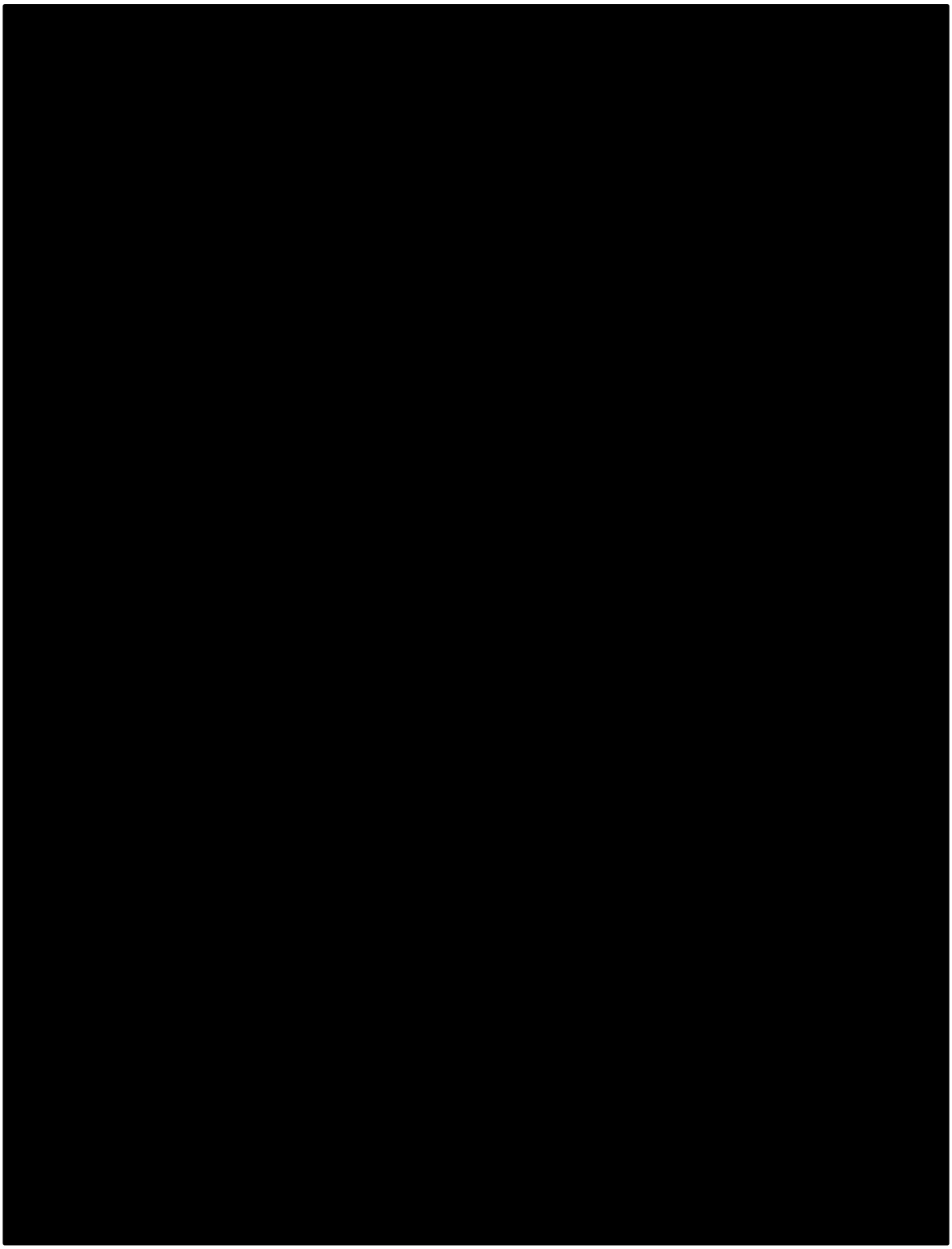


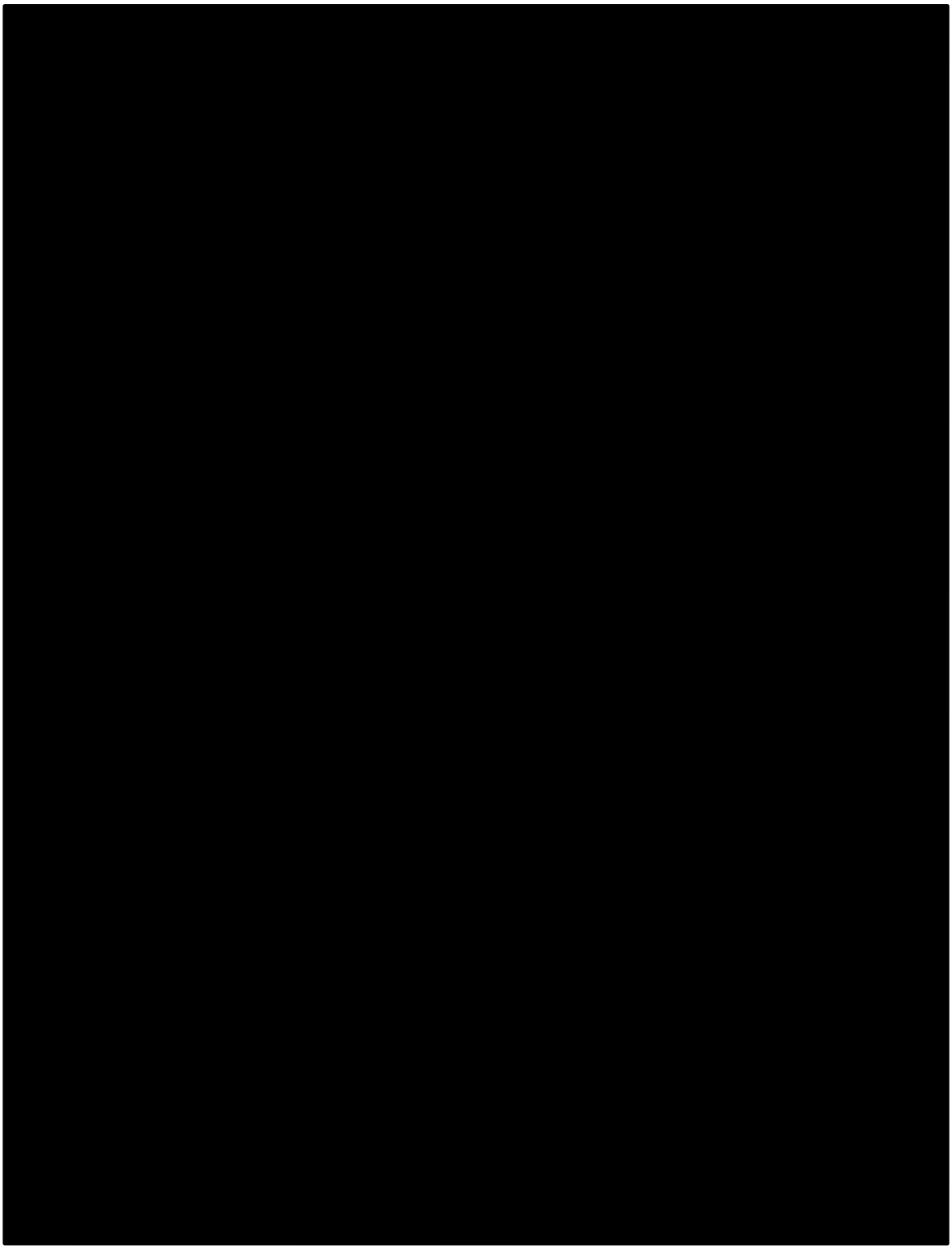


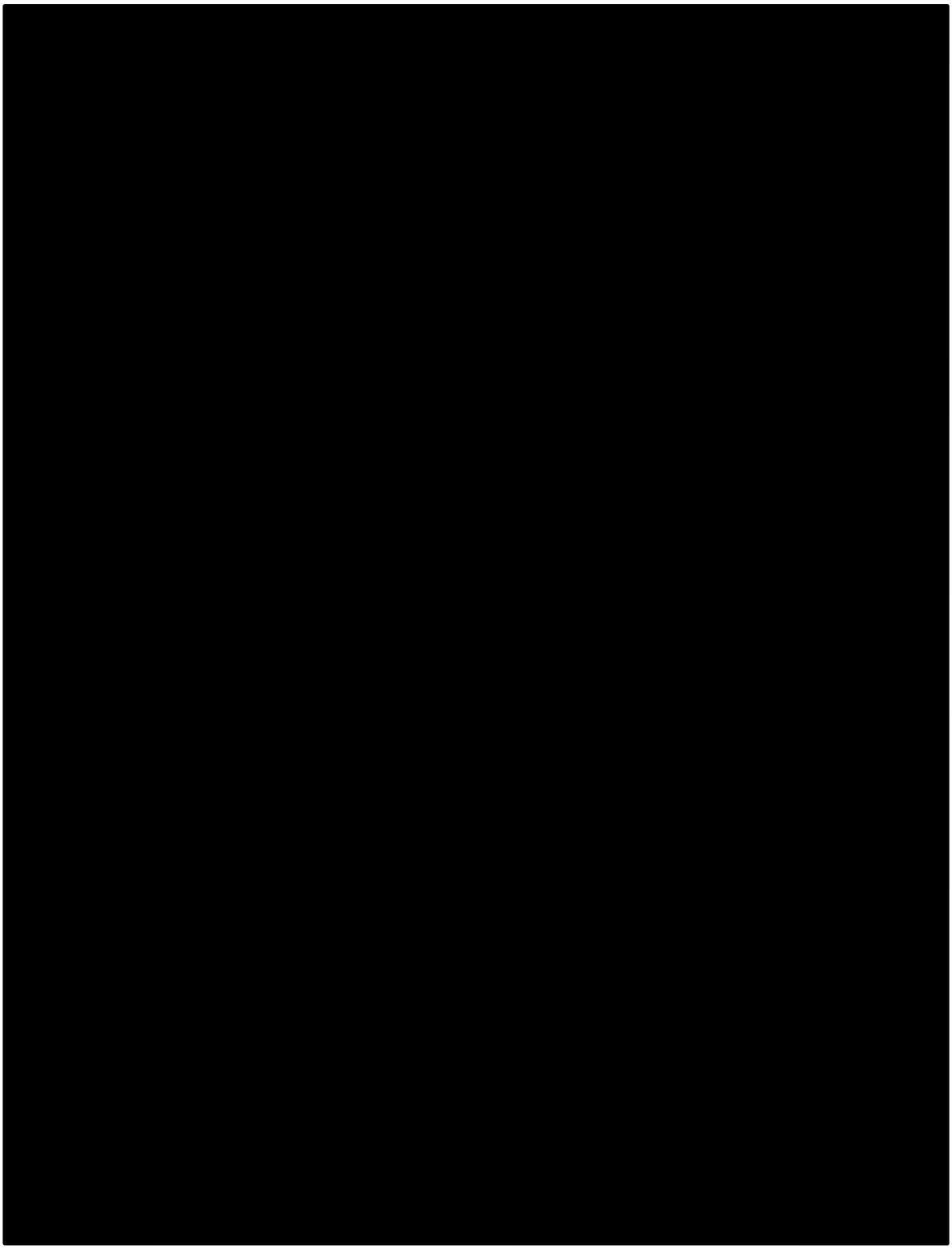


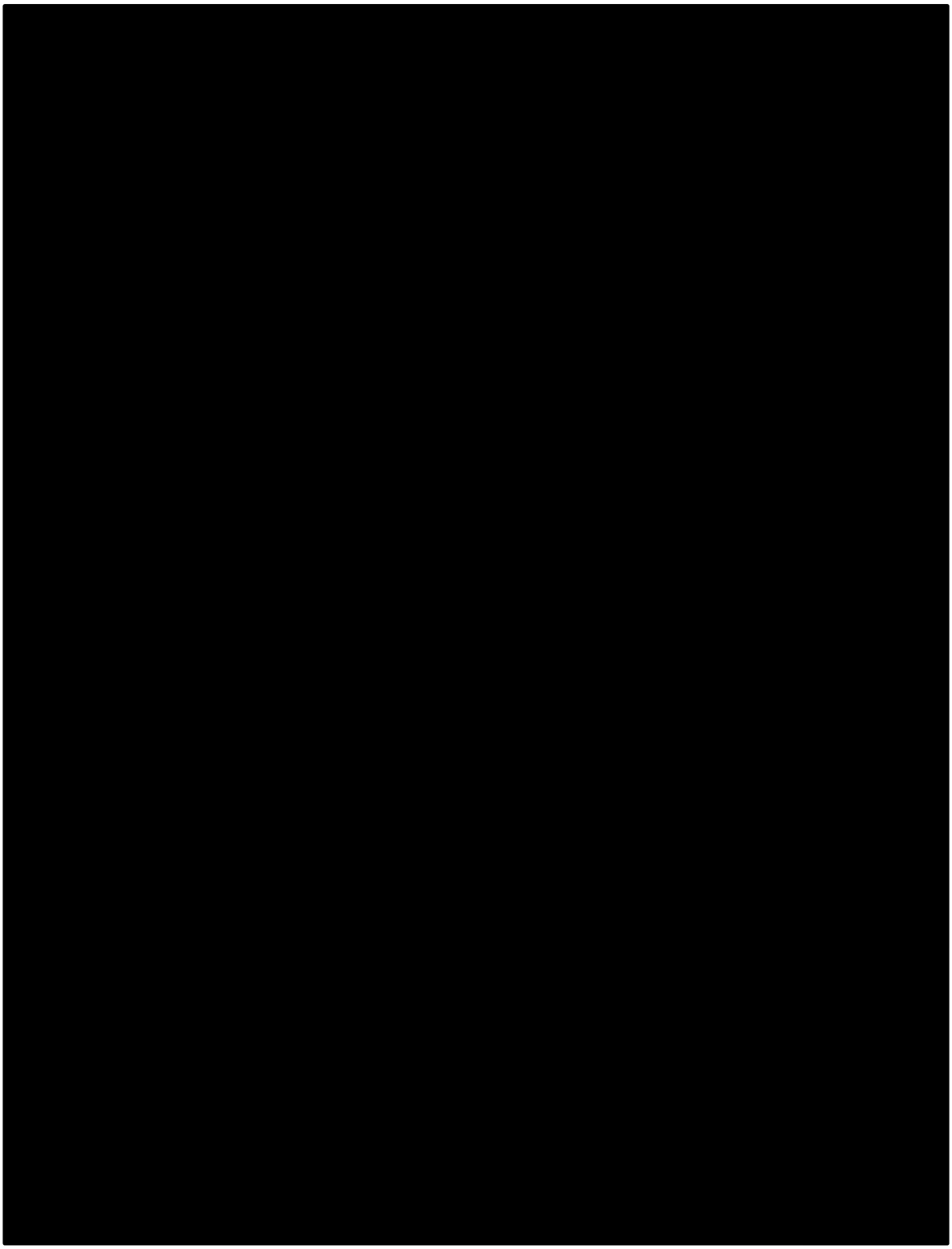


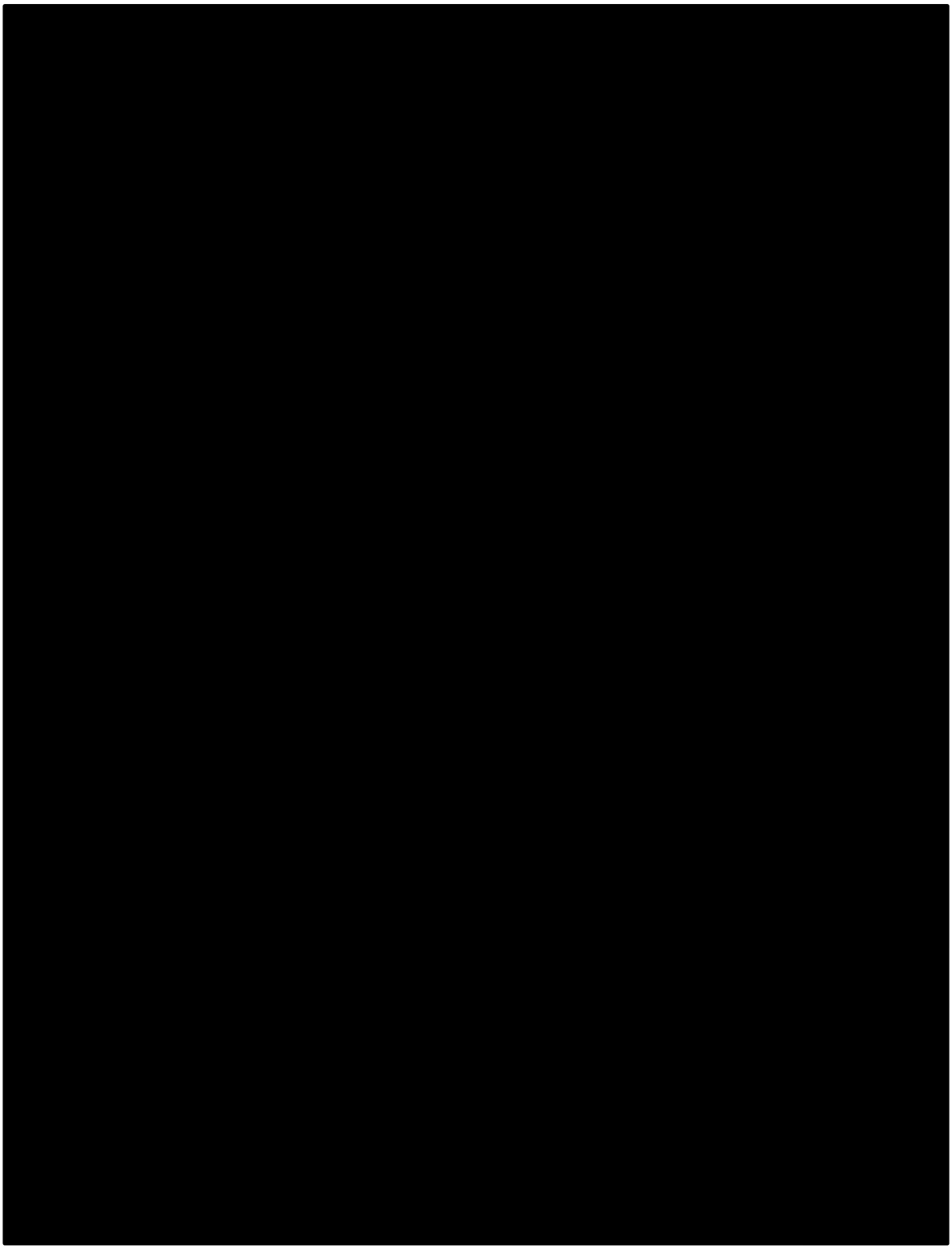


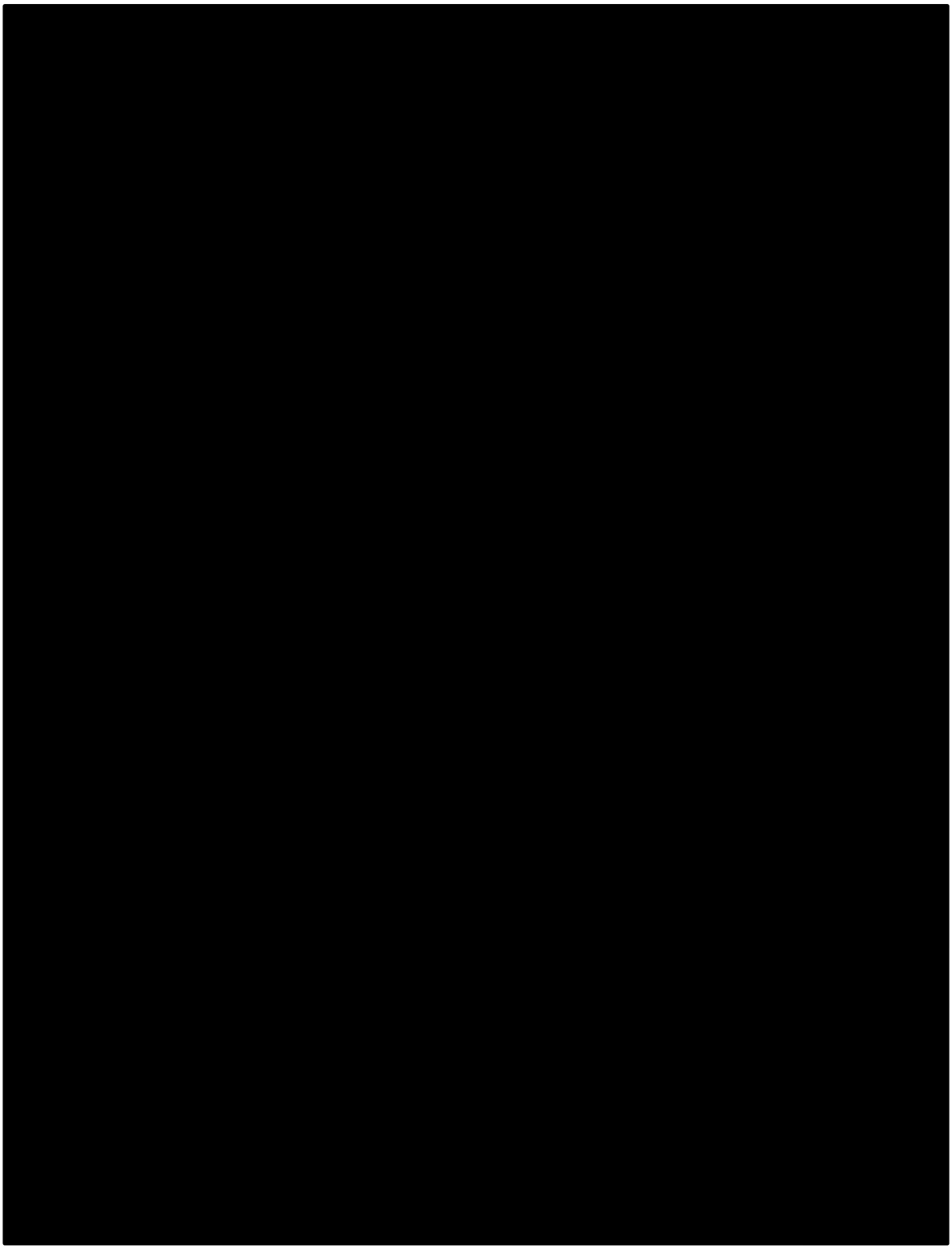




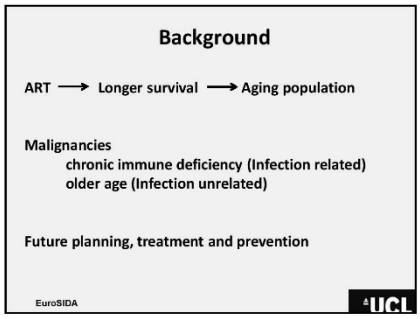


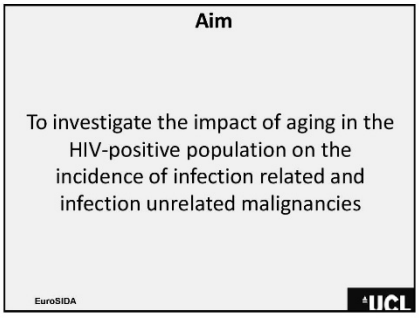


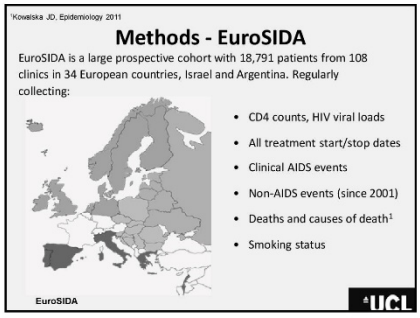


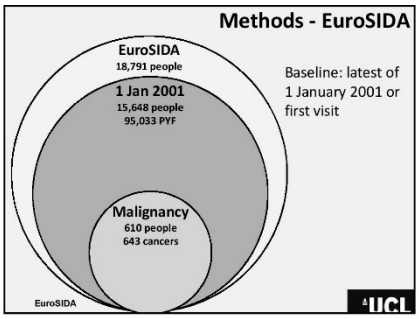


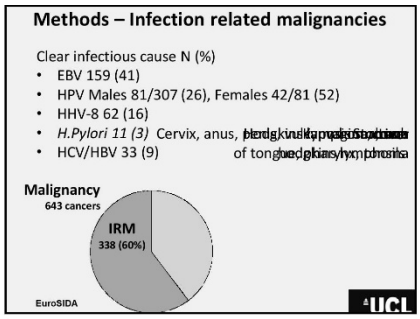
Appendix IV. EACS 2013 presentation entitled "Infection related and unrelated malignancies, HIV and the aging population"







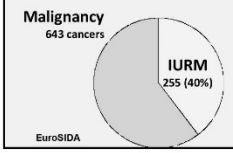




Methods – Infection unrelated malignancies

No clear infectious cause N (%)

- Lung cancer 55 (22)
- Prostate cancer males 28/202 (14)
- Breast cancer females 26/53 (49)
- Colon and rectal cancer 23 (9)



EuroSIDA

UCL

Methods - Statistics

Annual incidence rates, stratified by age

Poisson regression

Linear exponential smoothing models (LESMs)

EuroSIDA

UCL

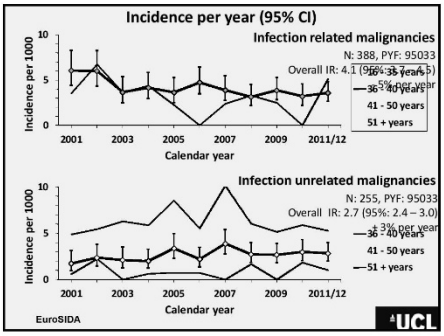
Baseline Characteristics (latest of 1 January 2001 or first visit)

	N/Median	%/IQR
Total	15,648	100
Aged 51 or older	2,502	16
Prior malignancy	931	6
Male	11,356	73
White ethnicity	13,821	88
Region		
Argentina	597	4
East	4,774	30
West	3,220	21
North	3,332	21
South	3,725	24
Risk group		
Heterosexual	5,008	32
IDU	3,381	22
Homosexual	6,124	39
Current or previous smokers	5,454	35
CD4 (cells/mm)	410	265-588
HIV Viral Load (copies/ml)	123	49-5200

EuroSIDA

UCL

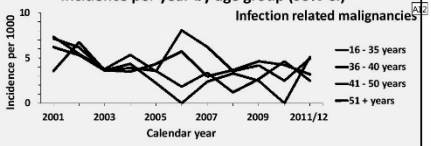
Incidence per year (95% CI)

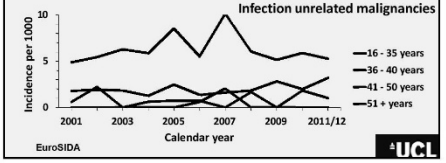


EuroSIDA

UCL

Incidence per year by age group (95% CI)

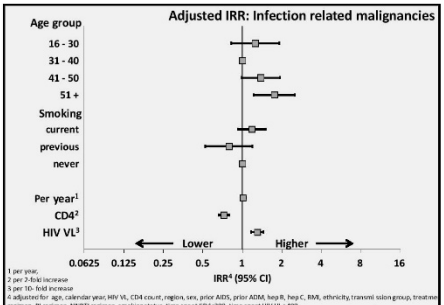




EuroSIDA

UCL

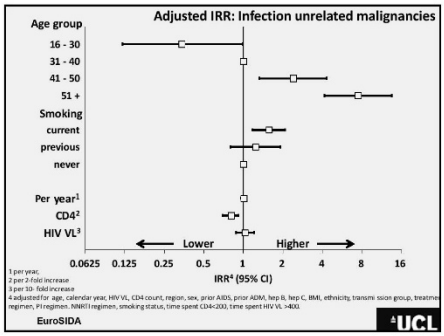
Adjusted IRR: Infection related malignancies

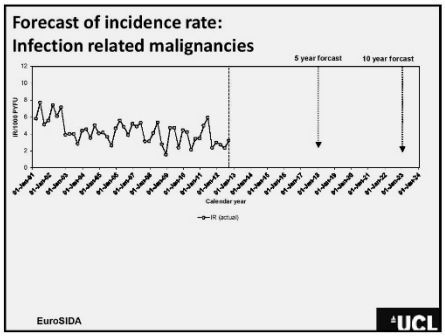


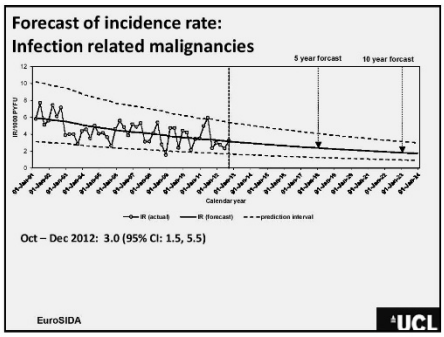
1 per year;
2 per 2-fold increase
3 per 10- fold increase
4 adjusted for: age, calendar year, HIV VL, CD4 count, region, sex, prior AIDS, prior ADM, hep B, hep C, BMI, ethnicity, transmi sion group, treatme regimen, PI regimen, NNRTI regimen, smoking status, time spent CD4<200, time spent HIV VL >400.

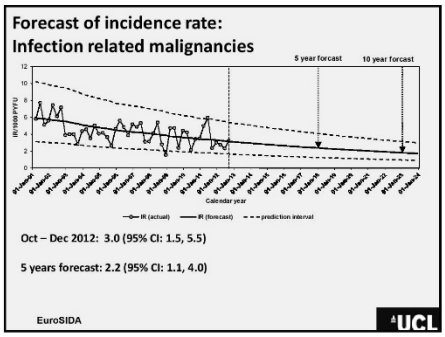
EuroSIDA

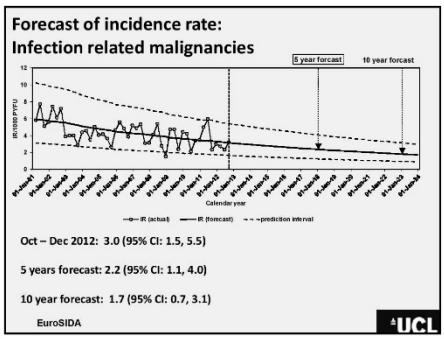
UCL

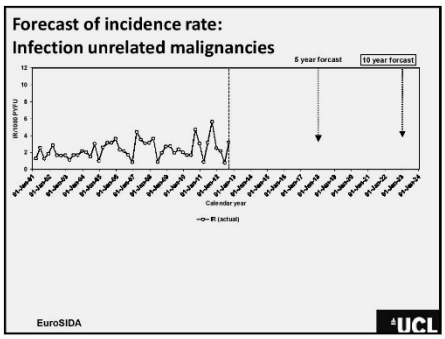

Adjusted IRR: Infection unrelated malignancies
Age group
16 - 30
31 - 40
41 - 50
51 +
Smoking
current
previous
never
Per year[1]
CD4[2]
HIV VL[3]
Lower
Higher
0.0625 0.125 0.25 0.5 1 2 4 8 16
IRR[4] (95% CI)
1 per year,
2 per 2-fold increase
3 per 10- fold increase
4 adjusted for age, calendar year, HIV VL, CD4 count, region, sex, prior AIDS, prior ADM, hep B, hep C, BMI, ethnicity, transmission group, treatment regimen, PI regimen, NNRTI regimen, smoking status, time spent CD4<200, time spent HIV VL >400.
EuroSIDA
UCL


Forecast of incidence rate:
Infection related malignancies
5 year forcast
10 year forcast
IR/1000 PYFU
Calendar year
IR (actual)
EuroSIDA
UCL

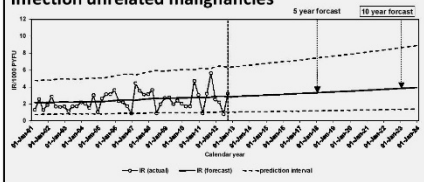

Forecast of incidence rate:
Infection related malignancies
5 year forcast
10 year forcast
IR/1000 PYFU
Calendar year
IR (actual)
IR (forecast)
prediction interval
Oct – Dec 2012: 3.0 (95% CI: 1.5, 5.5)
EuroSIDA
UCL


Forecast of incidence rate:
Infection related malignancies
5 year forcast
10 year forcast
IR/1000 PYFU
Calendar year
IR (actual)
IR (forecast)
prediction interval
Oct – Dec 2012: 3.0 (95% CI: 1.5, 5.5)
5 years forecast: 2.2 (95% CI: 1.1, 4.0)
EuroSIDA
UCL


Forecast of incidence rate:
Infection related malignancies
5 year forcast
10 year forcast
IR/1000 PYFU
Calendar year
IR (actual)
IR (forecast)
prediction interval
Oct – Dec 2012: 3.0 (95% CI: 1.5, 5.5)
5 years forecast: 2.2 (95% CI: 1.1, 4.0)
10 year forecast: 1.7 (95% CI: 0.7, 3.1)
EuroSIDA
UCL


Forecast of incidence rate:
Infection unrelated malignancies
5 year forcast
10 year forcast
IR/1000 PYFU
Calendar year
IR (actual)
EuroSIDA
UCL

Forecast of incidence rate: Infection unrelated malignancies

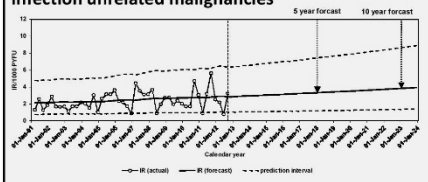


Oct – Dec 2012: 3.1 (95% CI: 1.2, 6.7)

EuroSIDA

UCL

Forecast of incidence rate: Infection unrelated malignancies



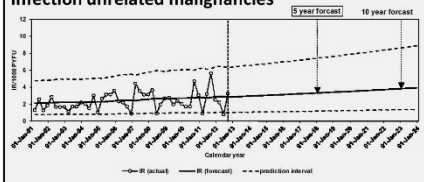
Oct – Dec 2012: 3.1 (95% CI: 1.2, 6.7)

5 years forecast: 3.6 (95% CI: 1.4, 7.7)

EuroSIDA

UCL

Forecast of incidence rate: Infection unrelated malignancies



Oct – Dec 2012: 3.1 (95% CI: 1.2, 6.7)

5 years forecast: 3.6 (95% CI: 1.4, 7.7)

10 year forecast: 4.1 (95% CI: 1.6, 8.8)

EuroSIDA

UCL

Forecast of incidence rate (95% CI)

GROUP	Infection related malignancies		Infection unrelated malignancies	
	Oct – Dec 2012	5 years	0ct-Dec 2012	5 years
Overall	3.0 (1.5, 5.5)	2.2 (1.1, 4.0)	3.1 (1.2, 6.7)	3.6 (1.4, 7.7)
BL age >50	2.7 (0.6, 7.3)	1.5 (0.1, 4.7)	7.8 (3.4, 16.9)	9.1 (4.0, 19.6)
MSM	3.6 (1.7, 6.7)	2.5 (1.1, 4.8)	3.5 (1.6, 7.0)	4.2 (1.9, 8.1)
CD4<350	4.6 (1.8, 9.8)	2.7 (0.9, 6.3)	3.5 (1.4, 7.7)	4.4 (1.84, 9.41)
Current smokers	2.8 (0.5, 8.4)	2.3 (0.8, 5.0)	3.4 (1.4, 7.3)	5.8 (1.8, 15.5)

All decreasing

EuroSIDA

UCL

Forecast of incidence rate (95% CI)

GROUP	Infection related malignancies		Infection unrelated malignancies	
	Oct – Dec 2012	5 years	0ct-Dec 2012	5 years
Overall	3.0 (1.5, 5.5)	2.2 (1.1, 4.0)	3.1 (1.2, 6.7)	3.6 (1.4, 7.7)
BL age >50	2.7 (0.6, 7.3)	1.5 (0.1, 4.7)	7.8 (3.4, 16.9)	9.1 (4.0, 19.6)
MSM	3.6 (1.7, 6.7)	2.5 (1.1, 4.8)	3.5 (1.6, 7.0)	4.2 (1.9, 8.1)
CD4<350	4.6 (1.8, 9.8)	2.7 (0.9, 6.3)	3.5 (1.4, 7.7)	4.4 (1.84, 9.41)
Current smokers	2.8 (0.5, 8.4)	2.3 (0.8, 5.0)	3.4 (1.4, 7.3)	5.8 (1.8, 15.5)

EuroSIDA

UCL

Forecast of incidence rate (95% CI)

GROUP	Infection related malignancies		Infection unrelated malignancies	
	Oct – Dec 2012	5 years	0ct-Dec 2012	5 years
Overall	3.0 (1.5, 5.5)	2.2 (1.1, 4.0)	3.1 (1.2, 6.7)	3.6 (1.4, 7.7)
BL age >50	2.7 (0.6, 7.3)	1.5 (0.1, 4.7)	7.8 (3.4, 16.9)	9.1 (4.0, 19.6)
MSM	3.6 (1.7, 6.7)	2.5 (1.1, 4.8)	3.5 (1.6, 7.0)	4.2 (1.9, 8.1)
CD4<350	4.6 (1.8, 9.8)	2.7 (0.9, 6.3)	3.5 (1.4, 7.7)	4.4 (1.84, 9.41)
Current smokers	2.8 (0.5, 8.4)	2.3 (0.8, 5.0)	3.4 (1.4, 7.3)	5.8 (1.8, 15.5)

All increasing

EuroSIDA

UCL

Forecast of incidence rate (95% CI)

GROUP	Infection related malignancies		Infection unrelated malignancies	
	Oct – Dec 2012	5 years	0ct-Dec 2012	5 years
Overall	3.0 (1.5, 5.5)	2.2 (1.1, 4.0)	3.1 (1.2, 6.7)	3.6 (1.4, 7.7)
BL age >50	2.7 (0.6, 7.3)	1.5 (0.1, 4.7)	7.8 (3.4, 16.9)	9.1 (4.0, 19.6)
MSM	3.6 (1.7, 6.7)	2.5 (1.1, 4.8)	3.5 (1.6, 7.0)	4.2 (1.9, 8.1)
CD4<350	4.6 (1.8, 9.8)	2.7 (0.9, 6.3)	3.5 (1.4, 7.7)	4.4 (1.84, 9.41)
Current smokers	2.8 (0.5, 8.4)	2.3 (0.8, 5.0)	3.4 (1.4, 7.3)	5.8 (1.8, 15.5)

EuroSIDA

Limitations

Observational study

Follow-up from 2001

Small counts

Forecasts

Lack of population projections

EuroSIDA

Conclusions

Infection related malignancy incidence is decreasing

Infection unrelated malignancy incidence is stable.

Older age is associated with infection related and unrelated cancers.

Aging population will lead to increasing proprotion of infection unrelated malignancies.

Targeted preventive measures and studies evaluating the cost-benefit of screening should be considered

EuroSIDA

The EuroSIDA Study Group

The multi-centre study group of EuroSIDA (national coordinators in parenthesis).

[illegible]

Steering Committee: [illegible]

Additional voting members: [illegible]

Coordinating Centre Staff: [illegible]

EuroSIDA representatives to EuroCoord: [illegible]

Statement of Funding:
[illegible]

EuroSIDA

Appendix V. CROI 2017 presentation entitled "Cessation of cigarette smoking and the impact on cancer incidence in the D:A:D study"

Email: leah.shepherd@ucl.ac.uk

CROI 2017

Cessation of cigarette smoking and the impact on cancer incidence in the D:A:D Study

Leah Shepherd[1], Lene Ryom[2], Kathy Petoumenos[3], Camilla Ingrid Hatleberg[2], Antonella d'Arminio Monforte[4], Caroline Sabin[1], Fabrice Bonnet[5], Peter Reiss[6], Stephane de Wit[7], Christian Pradier[8], Rainer Weber[9], Wafaa el-Sadr[10], Jens Lundgren[2], Amanda Mocroft[1]

On behalf of the D:A:D Study group

[1]Research Department of Infection and Population Health, UCL, London, United Kingdom; [2]CHIP, Department of Infectious Diseases, Section 2100, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [3]The Kirby Institute, UNSW Australia, Sydney, Australia; [4]Dipartimento di Scienze della Salute, Clinica di Malattie Infettive, 1° Ospedale, Azienda Ospedaliera/Polo Universitario San Paolo, Milan, Italy; [5]CHU de Bordeaux and INSERM U897 - Université de Bordeaux, Service, Pierre; [6]Academic Medical Center, Dept. of Global Health and Div. of Infectious Diseases, University of Amsterdam, and HIV Monitoring Foundation, Amsterdam, The Netherlands; [7]Division of Infectious Diseases, Saint-Pierre University Hospital, Université Libre de Bruxelles, Brussels, Belgium; [8]Department of Infectious and Tropical Diseases, Nice Hospital, Nice, France; [9]Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland; [10]CHP, Columbia University and Harlem Hospital, New York, USA

D:A:D

UCL

Disclosure

Ms Leah Shepherd has no financial relationships with commercial entities to disclose.

Background

- Cancers are a major source of morbidity and mortality in HIV-positive [HIV+] persons in the context of available treatment, due to longer life expectancy, reduced immune function and behavioural factors [1]
- HIV+ persons often have higher smoking rates than similar HIV- persons[2]
- The incidence of most cancers, including lung, increase with older age. Therefore, as the HIV+ population ages, smoking cessation is a critically important evidence-based modifiable risk factor for cancer [3]
- The decline in cancer incidence with longer time since cessation is well established in the HIV-negative population [4]
- The clinical benefits of smoking cessation on cancer risk have not been reported for HIV+ persons

1. Smith et al 2014, Lancet 2. Reekie et al 2010, Clin Infect Dis 3. Jose et al 2011, CID 4. Fry et al 2013, Respiratory Pathology and Pharmacology

Study objective

To estimate cancer rates after smoking cessation in HIV+ persons from the D:A:D study.

Methods

- All persons with no reported history of cancer at baseline were included
- Baseline: latest of study entry or 1 January 2004
- Persons were followed from baseline until earliest of
  - First cancer diagnosis
  - Death
  - Last visit plus 6 months
  - 1 February 2015 (administrative censoring date)

Smoking status[1]

- **Current smoker**
- **Never smoker**
- **Ex smoker at baseline:** those who stopped smoking prior to baseline
- **Ex smoker during follow up**: those who stopped smoking during follow-up

[1] Smoking status represents current smoking behaviour and is time updated

Smoking status[1]

- **Current smoker**
- **Never smoker**
- **Ex smoker at baseline:** those who stopped smoking prior to baseline
- **Ex smoker during follow up**: those who stopped smoking during follow-up

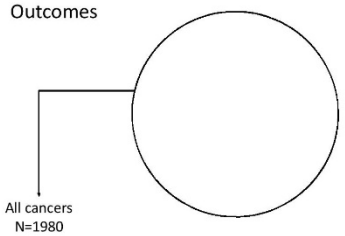
[1] Smoking status represents current smoking behaviour and is time updated

Smoking status[1]

- **Current smoker**
- **Never smoker**
- **Ex smoker at baseline:** those who stopped smoking prior to baseline
- **Ex smoker during follow up**: those who stopped smoking during follow-up
  - < 1 year since cessation
  - 1 – 2 years
  - 2 – 3 years
  - 3 – 5 years
  - > 5 years

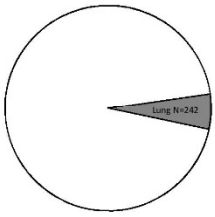
[1] Smoking status represents current smoking behaviour and is time updated

Outcomes



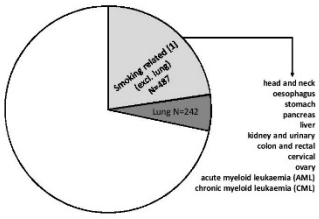
All cancers
N=1980

Outcomes



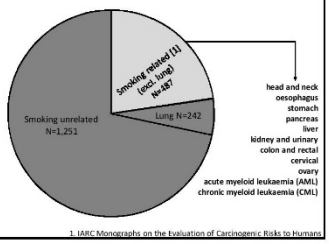
1. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

Outcomes



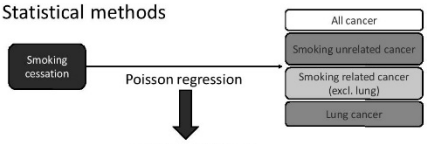
1. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

Outcomes



1. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

Statistical methods



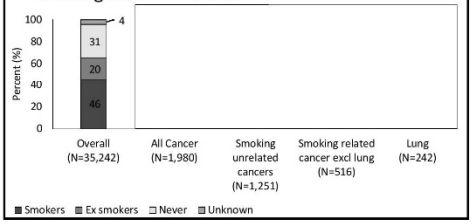
Models were adjusted for
age, gender, transmission group, race, BMI, calendar year, cART use, CD4, HIV viral-load, hepatitis B and C status, AIDS defining events (excluding cancers), anaemia, hypertension, diabetes, cardiovascular disease and duration of smoking in D:A:D

Characteristics at baseline

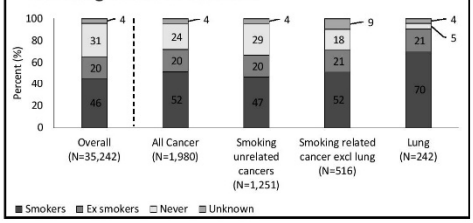
35,424 people contributed 285,103 person years of follow-up with a median of 9 (IQR: 6 – 11) years per person

Factors	All persons (N=35,424)
N %	
Male	25,689 (72.5)
Transmission mode	
Sex between men	14,875 (42.0)
Injecting drug use	5,658 (16.0)
Prior AIDS diagnosis	7,371 (20.8)
HIV Viral load < 500 cps/mL	18,659 (52.7)
Median IQR	
Age (years)	40 (34-46)
CD4 (cells/mm^3)	444 (295-632)

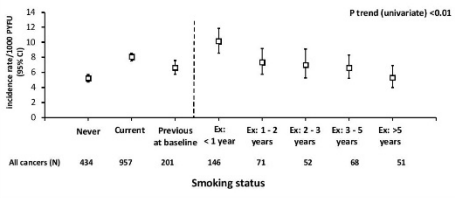
Smoking status at baseline



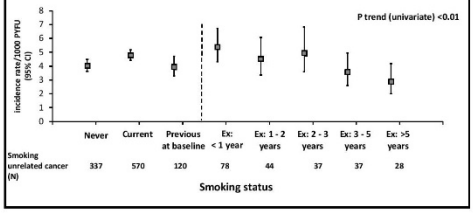
Smoking status at baseline



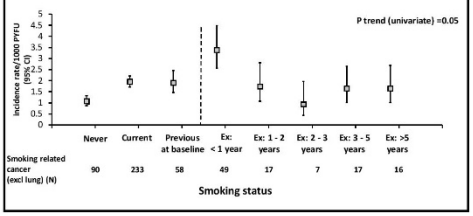
Crude incidence: All cancers



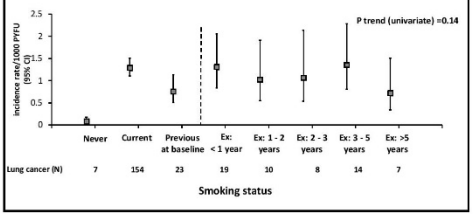
Crude incidence: Smoking unrelated cancer



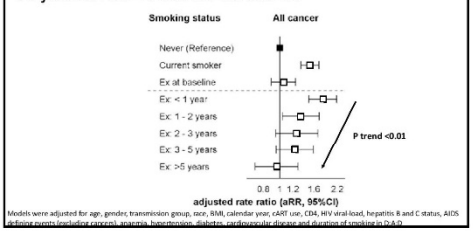
Crude incidence: Smoking related cancer (excl lung)



Crude incidence: Lung cancer

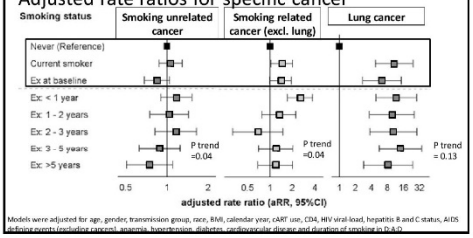


Adjusted rate ratios for all cancer



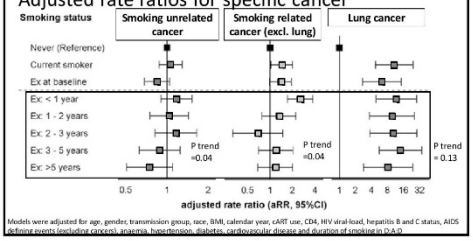
Models were adjusted for age, gender, transmission group, race, BMI, calendar year, cART use, CD4, HIV viral-load, hepatitis B and C status, AIDS defining events (excluding cancers), anaemia, hypertension, diabetes, cardiovascular disease and duration of smoking in D:A:D

Adjusted rate ratios for specific cancer



Models were adjusted for age, gender, transmission group, race, BMI, calendar year, cART use, CD4, HIV viral-load, hepatitis B and C status, AIDS defining events (excluding cancers), anaemia, hypertension, diabetes, cardiovascular disease and duration of smoking in D:A:D

Adjusted rate ratios for specific cancer



Models were adjusted for age, gender, transmission group, race, BMI, calendar year, cART use, CD4, HIV viral-load, hepatitis B and C status, AIDS defining events (excluding cancers), anaemia, hypertension, diabetes, cardiovascular disease and duration of smoking in D:A:D

Limitations

- Smoking data collected at each clinic visit. No information on exact start/stop dates, intensity, duration or pack years
- Smoking status is collected inconsistently on some patients. Sensitivity analysis excluding persons with no smoking update in the last 2 years had similar results
- Observational study

Conclusions 1

- Incidence of smoking related cancers excluding lung rapidly declined following cessation
- Lung cancer incidence appears to remain elevated in HIV+ persons several years after cessation. This suggests that the oncogenic potential for smoking is not reversed for lung cancer in the time frame that we have investigated
- This is in contrast with similar studies in HIV negative persons, which show a consistent decline in lung cancer incidence with increasing time since cessation

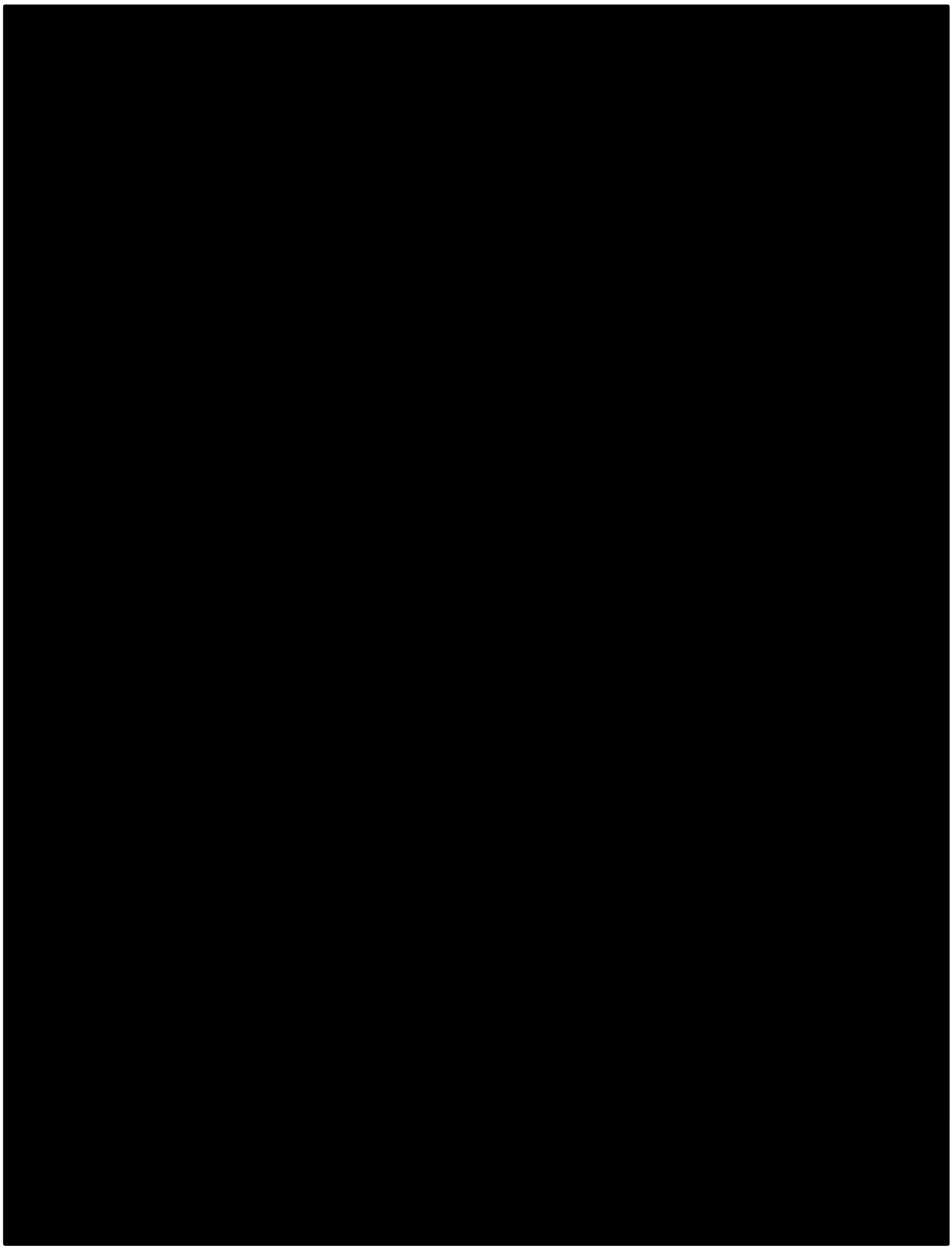
Conclusions 2

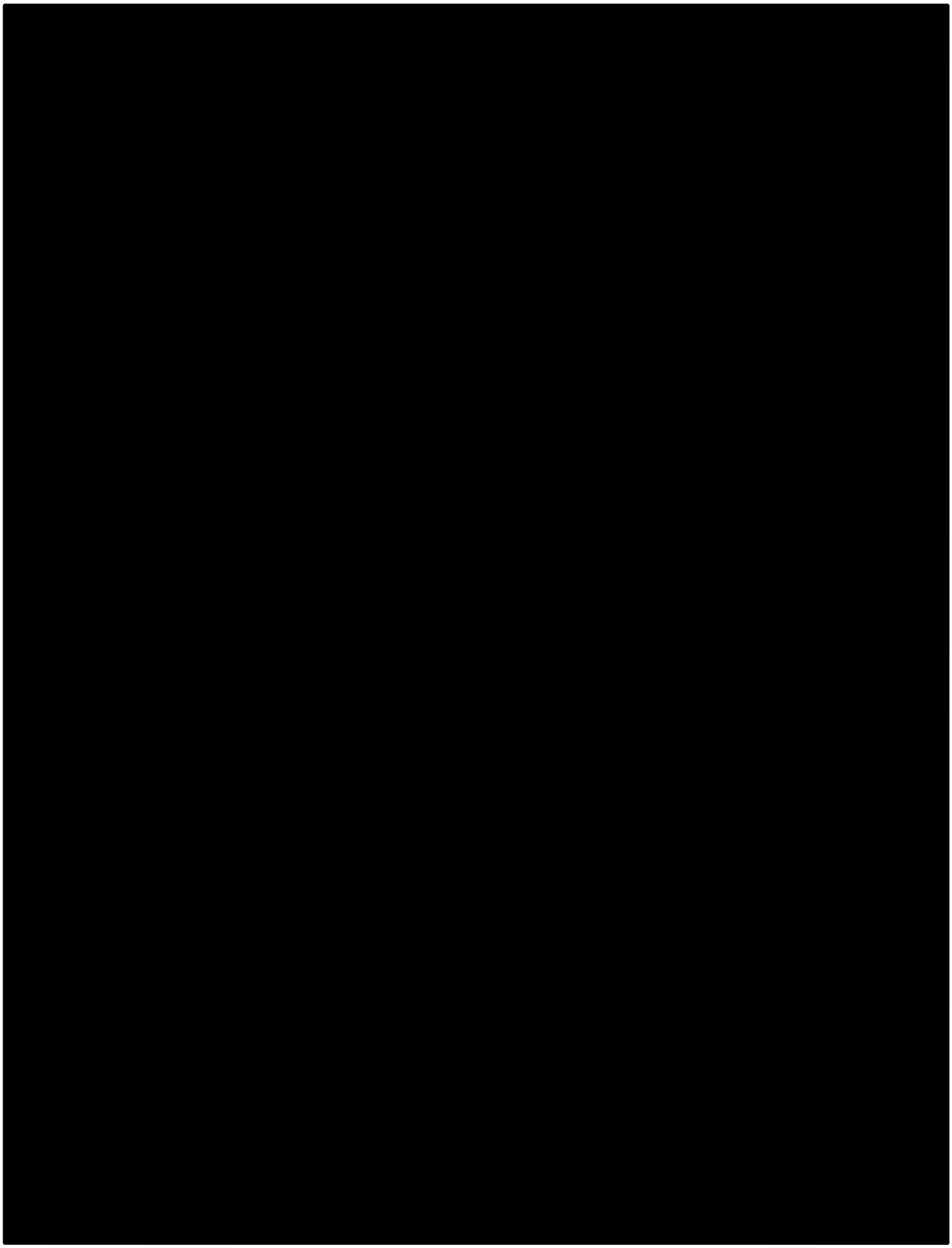
- Deterring uptake of smoking and smoking cessation efforts should be a priority to reduce the risk of cancer, however, monitoring and awareness of lung cancer should continue in those who stop smoking
- Our study followed persons for a median of 9 years, however studies in the HIV- population follow people for as long as 30 years
- Studies with long follow-up as HIV+ persons age are needed to identify whether and when lung cancer incidence declines

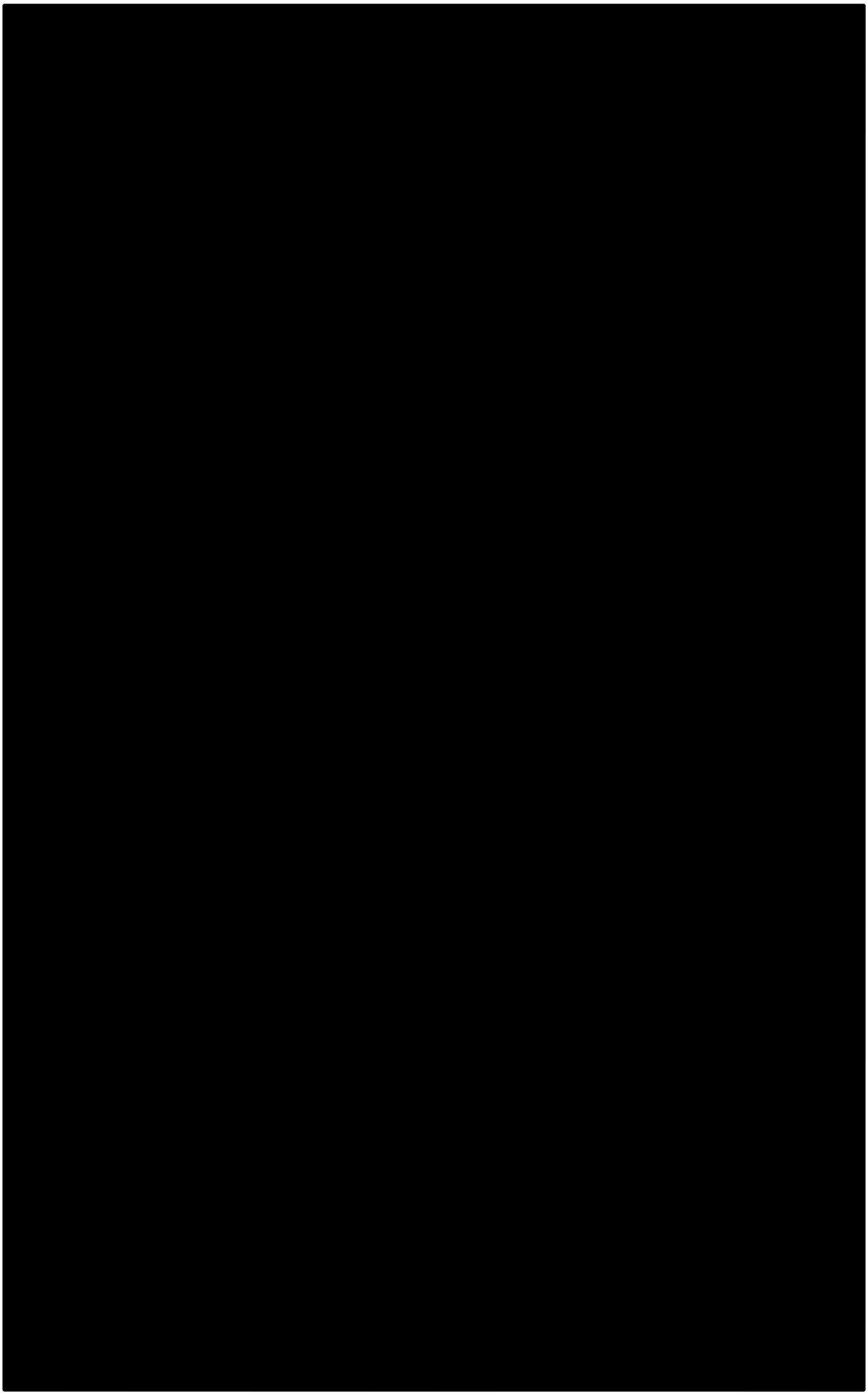
Acknowledgements

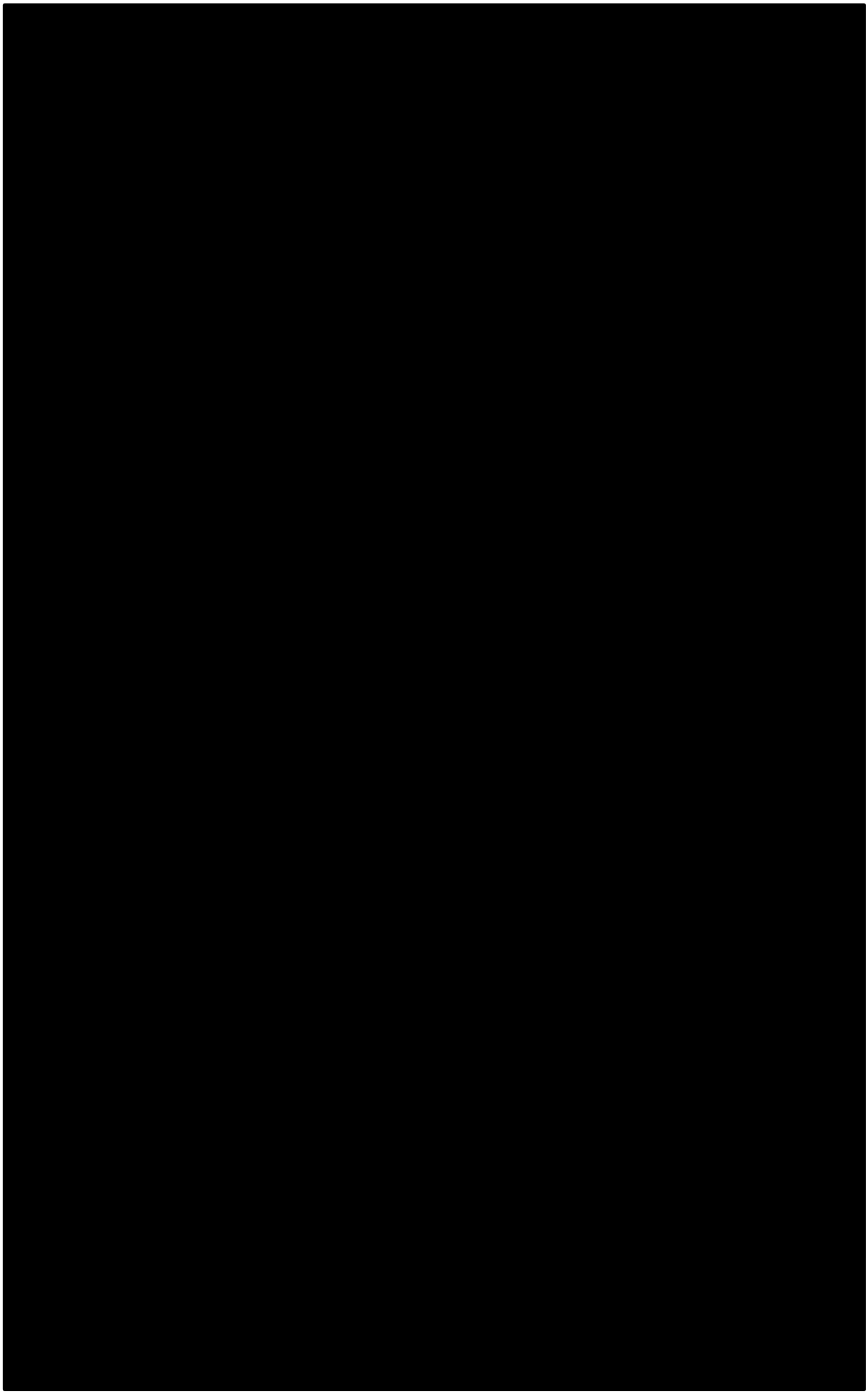
Steering Committee: Members indicated w/ *; ċ chair;
Cohort PIs: W El-Sadr* (CPCRA), G Calvo* (BASS), F Bonnet/F Dabis* (Aquitaine), O Kirk/ A Mocroft* (EuroSIDA), M Law* (AHOD), A d'Arminio Monforte* (ICONA), L Morfeldt* (HIV@IVUS), C Pradier* (Nice), P Reiss* (ATHENA), R Weber* (SHCS), S De Wit* (Brussels)
Cohort coordinators & data managers: A Lind-Thomsen (coordinator), R Saibel Brandt, M Hildebeight, S Zahmi, FWNM Wit (ATHENA), A Scherrer, F Schöni-Affolter, M Rickenbach (SHCS), A Travelli, I Fanti (ICONA), O Leleux, E Boerg, J Mourali, F Le Marec, E Boerg (Aquitaine), E Thulin, A Sundström (HIV@IVUS), G Bartsch, G Thompsen (CPCRA), C Necsoi, M Delforge (Brussels), E Fontas, C Caissotti, K Dallet (Nice), S Mateu, F Torres (BASS), K Petoumenos A Blance, R Puhr (AHOD), K Grønborg Laut, D Kristensen (EuroSIDA)
Statisticians: CA Sabin*, AN Phillips*, DA Kamara, CJ Smith, A Mocroft*
D:A:D coordinating office: CI Hatleberg, L Ryom, A Lind-Thomsen, RS Brandt, D Raben, C Matthews, A Bojesen, AL Grevsen, JD Lundgren*ţ
Member of the D:A:D Oversight Committee: B Powderly*, N Shortman*, C Moecklinghoff*, G Reilly*, X Franquet*
D:A:D working group experts:
Kidney: L Ryom, A Mocroft*, O Kirk*, P Reiss*, C Smit, M Ross, CA Fux, P Morlat, E Fontas, DA Kamara, CJ Smith, JD Lundgren*ţ
Mortality: CJ Smith, L Ryom, CI Hatleberg, AN Phillips*, R Weber*, P Morlat, C Pradier*, P Reiss*, FWNM Wit, N Friis-Møller, J Kowalska, JD Lundgren*ţ
Cancer: CA Sabin*, L Ryom, CI Hatleberg, M Law*, A d'Arminio Monforte*, F Dabis*, F Bonnet, P Reiss*, FWNM Wit, CJ Smith, DA Kamara, J Bohlius, M Bower, G Fätkenheuer, A Grulich, JD Lundgren*ţ
External endpoint reviewer: A Sjøl (CVD), P Meidahl (oncology), JS Iversen (nephrology)
Funding: Oversight Committee for The Evaluation of Metabolic Complications of HAART with representatives from academia, patient community, FDA, EMA and a consortium of AbbVie, Bristol-Myers Squibb, Gilead Sciences, ViiV Healthcare, Merck and Janssen Pharmaceuticals. Also supported by a grant (grant number DHSC 20) from the Dutch National Research Foundation.

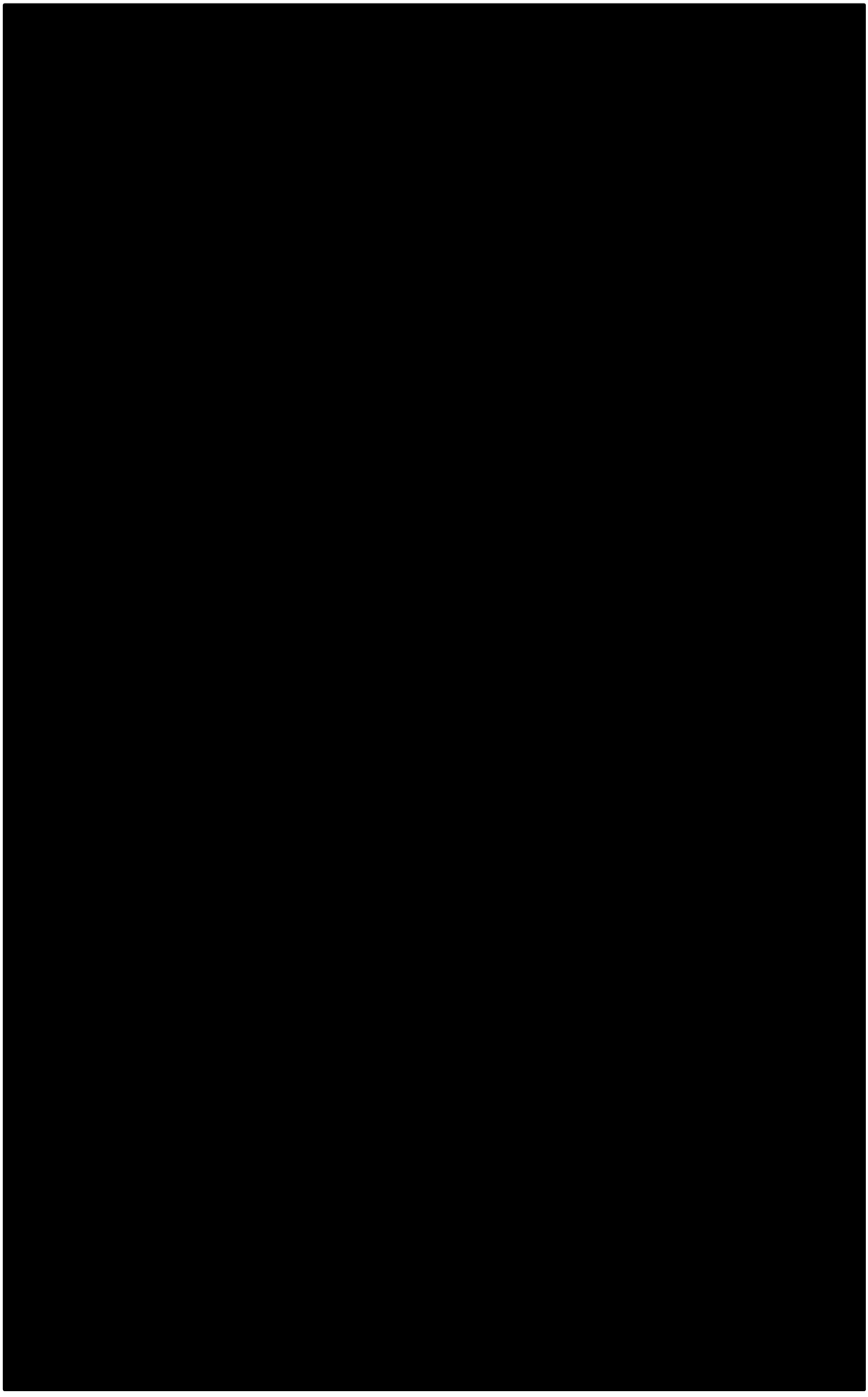
Appendix VI. Submitted draft manuscript "Differences in virological and immunological risk factors for non-Hodgkin and Hodgkin lymphoma: The D:A:D Study"

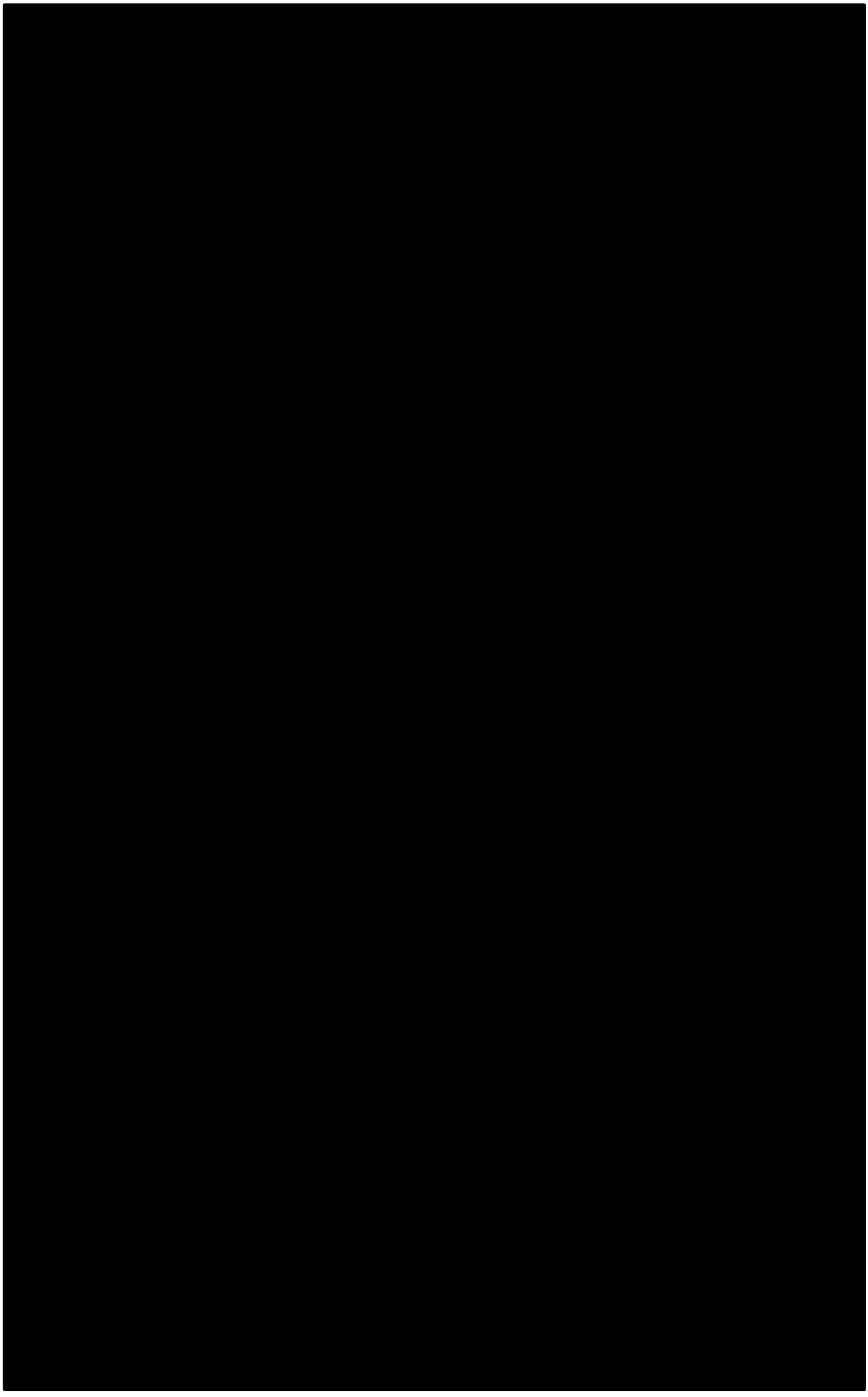


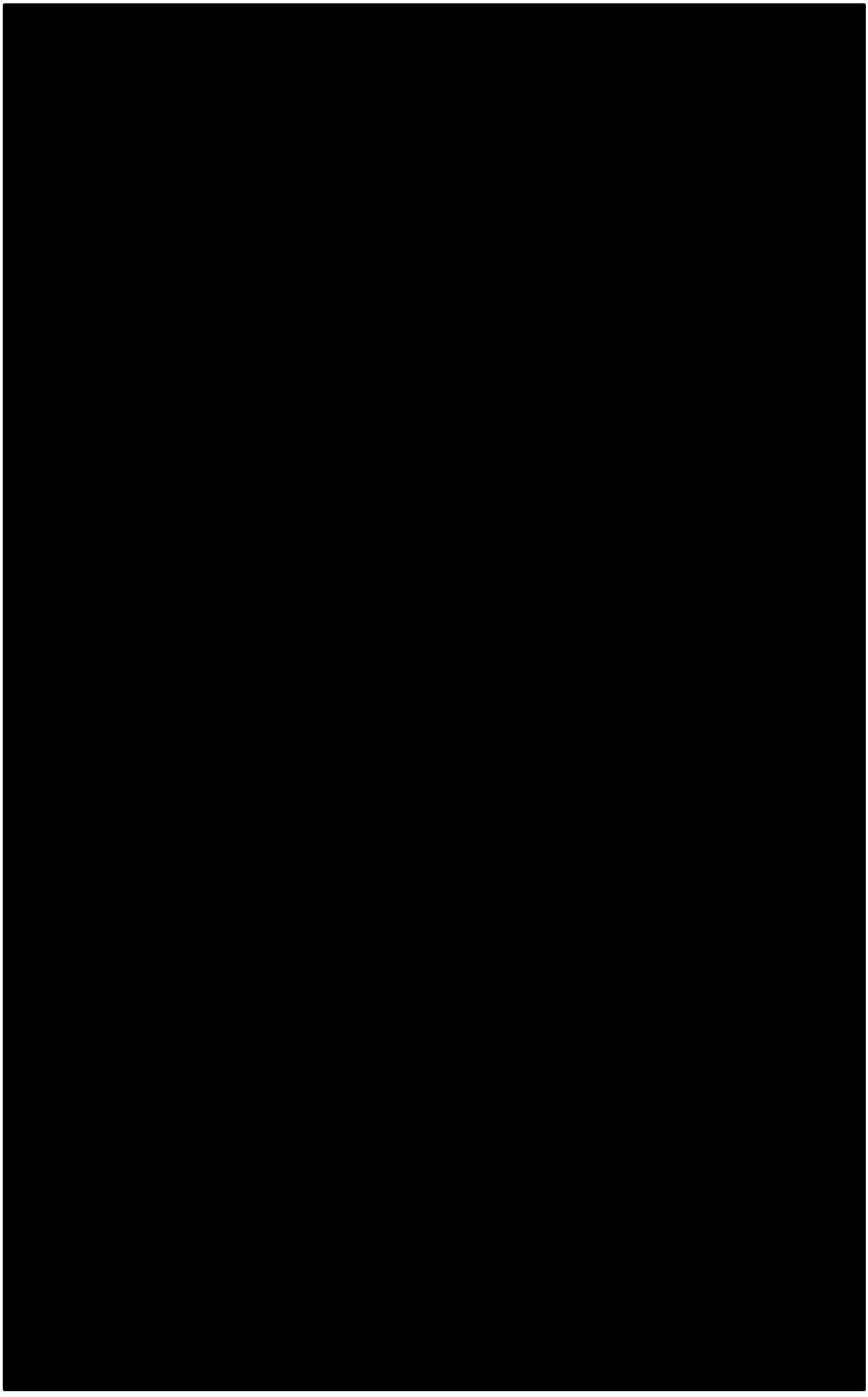


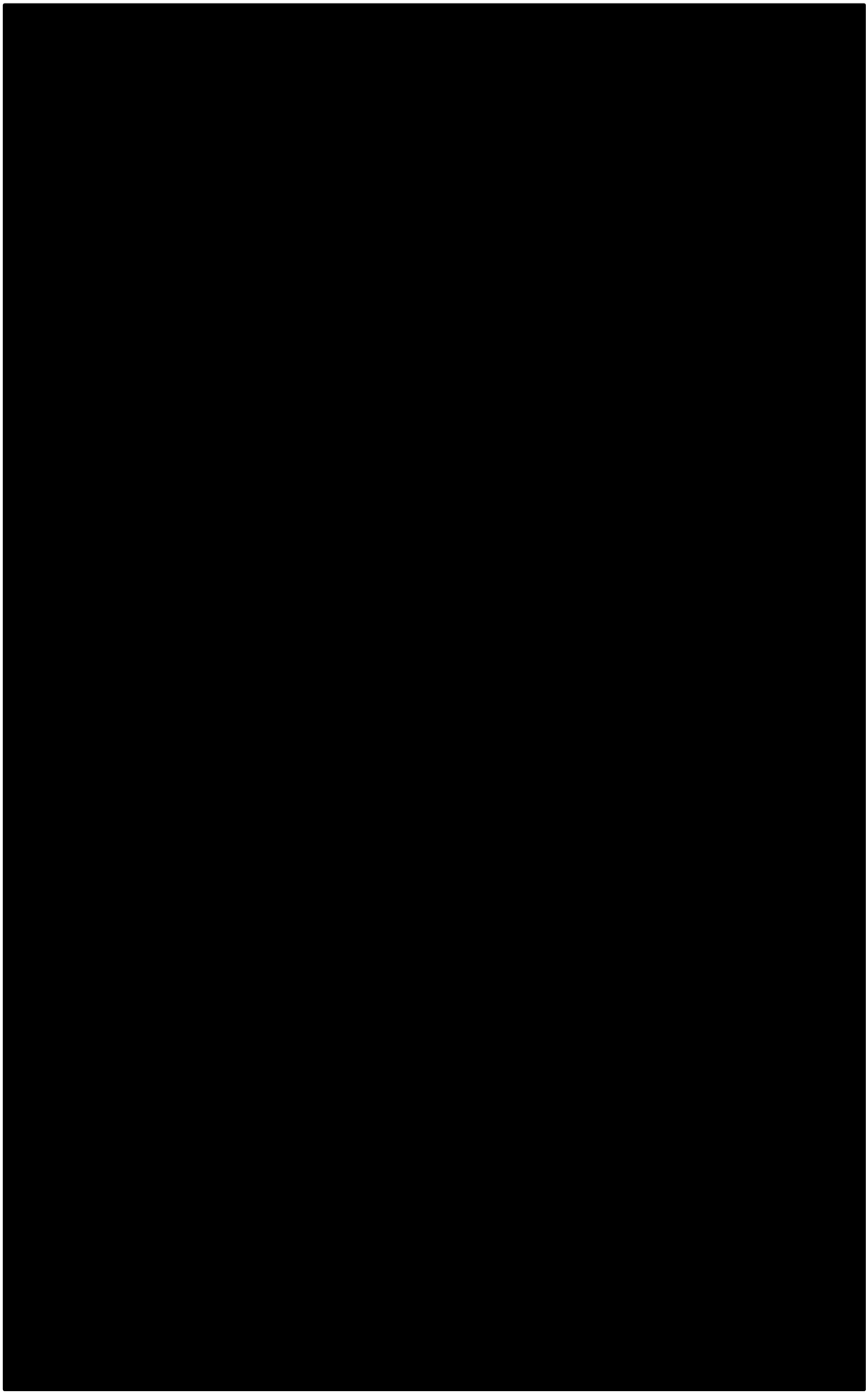


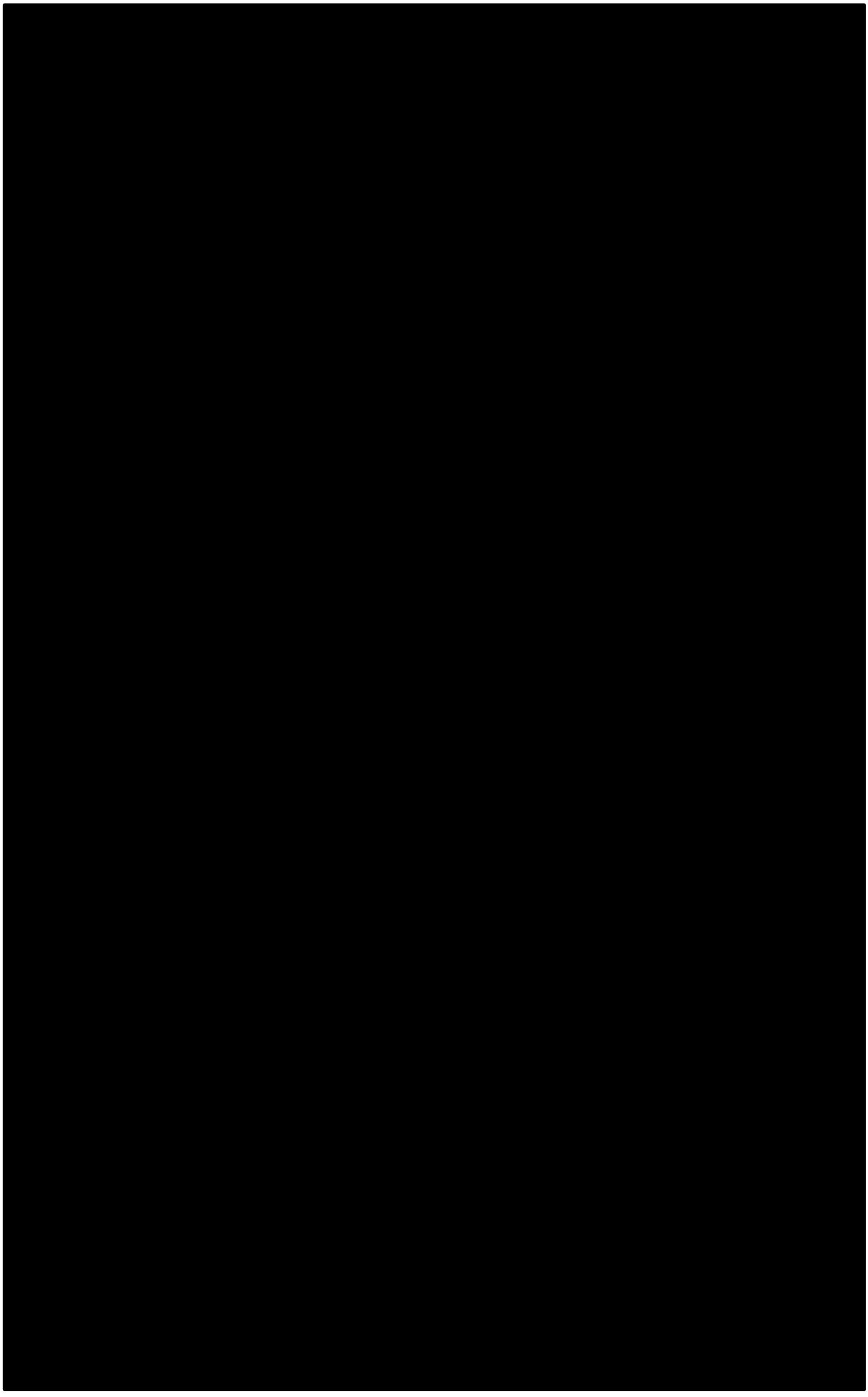


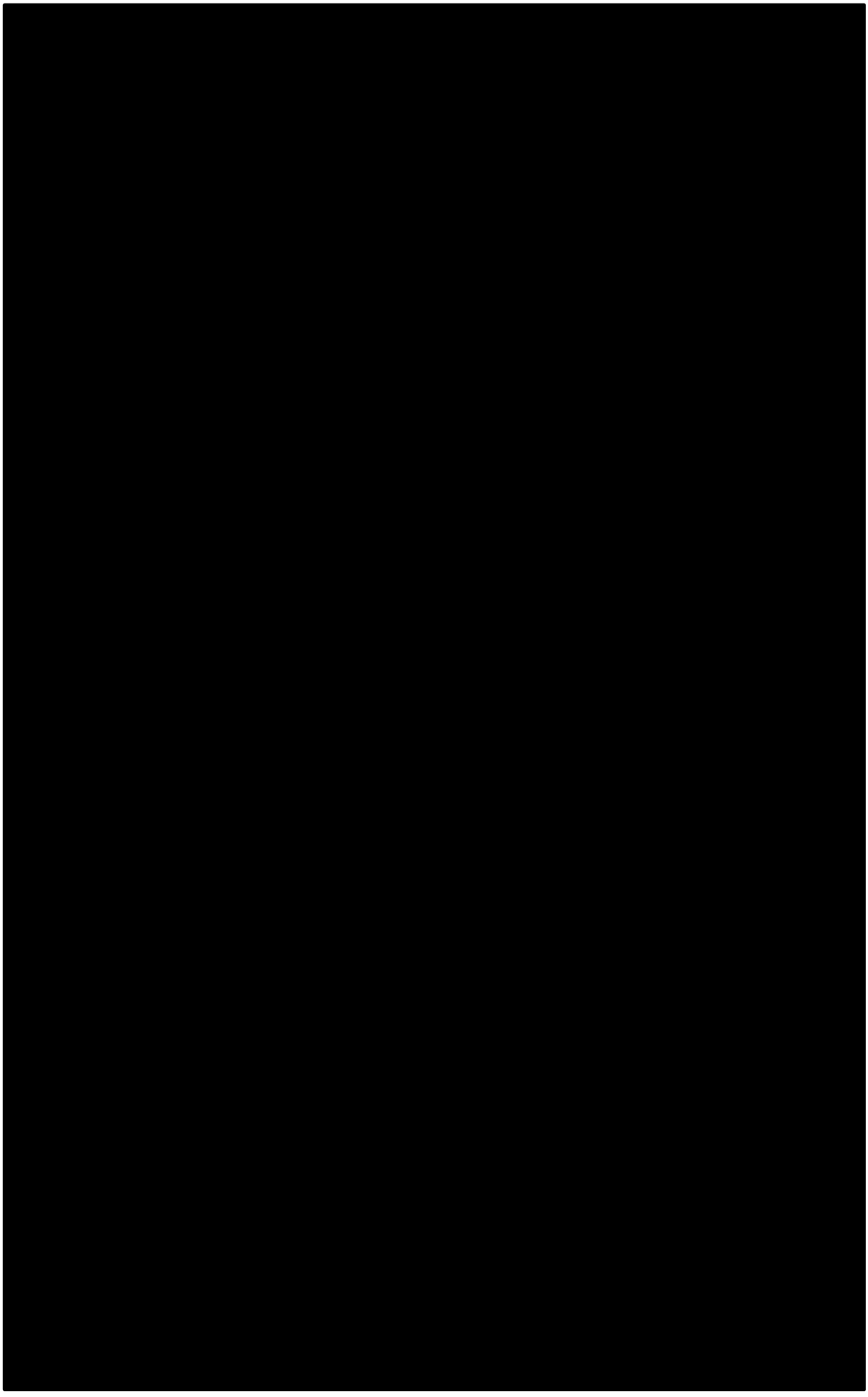


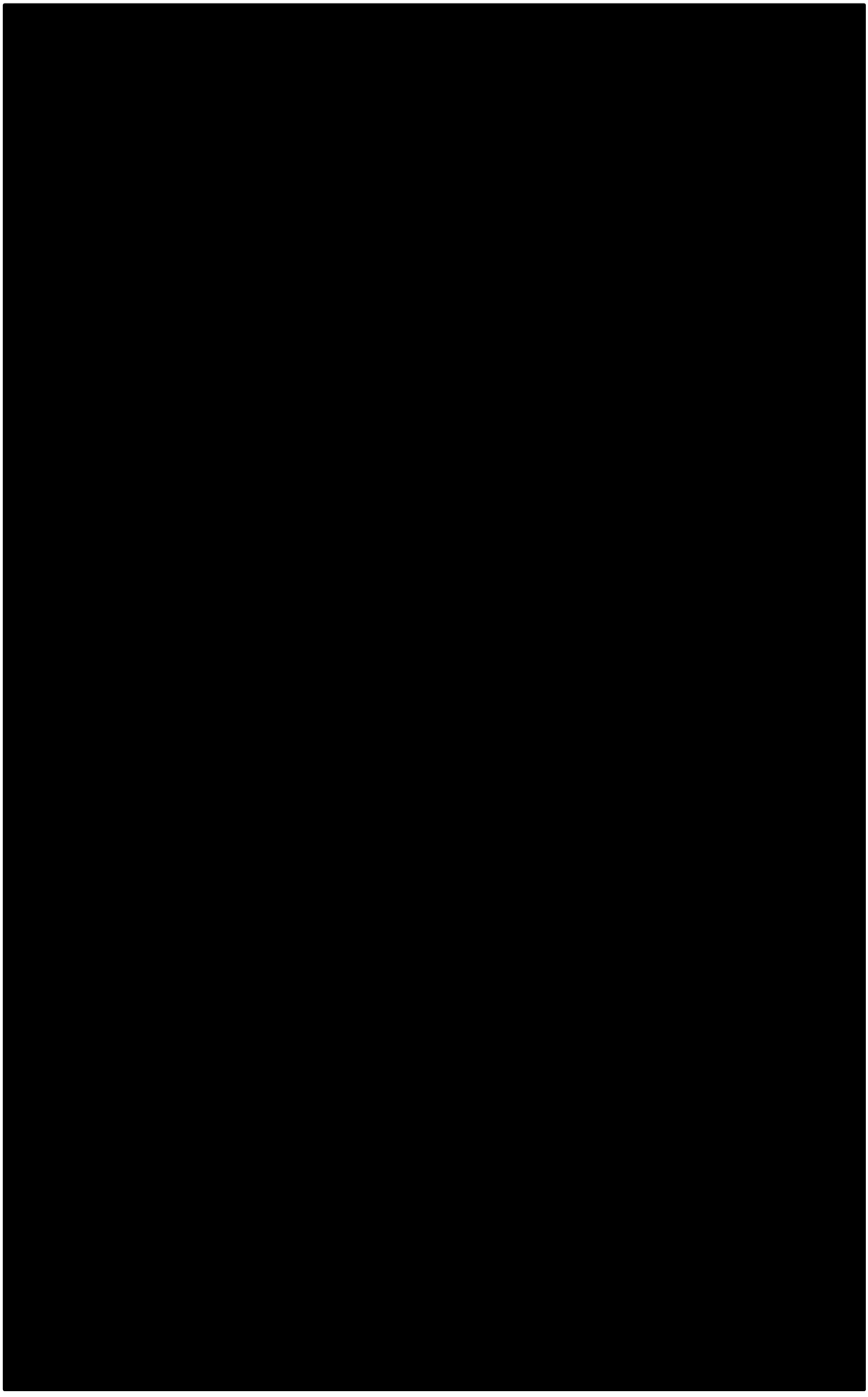


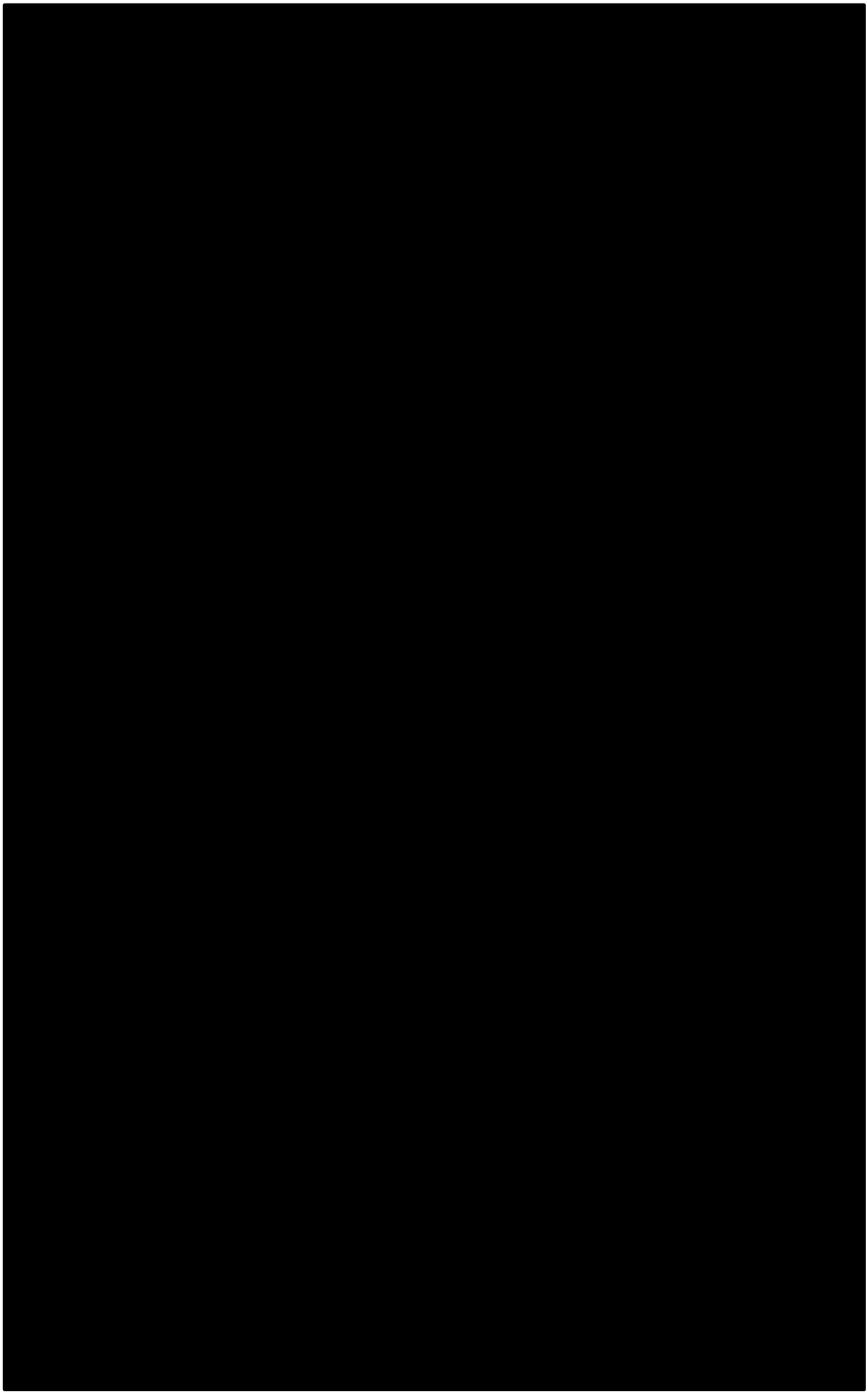


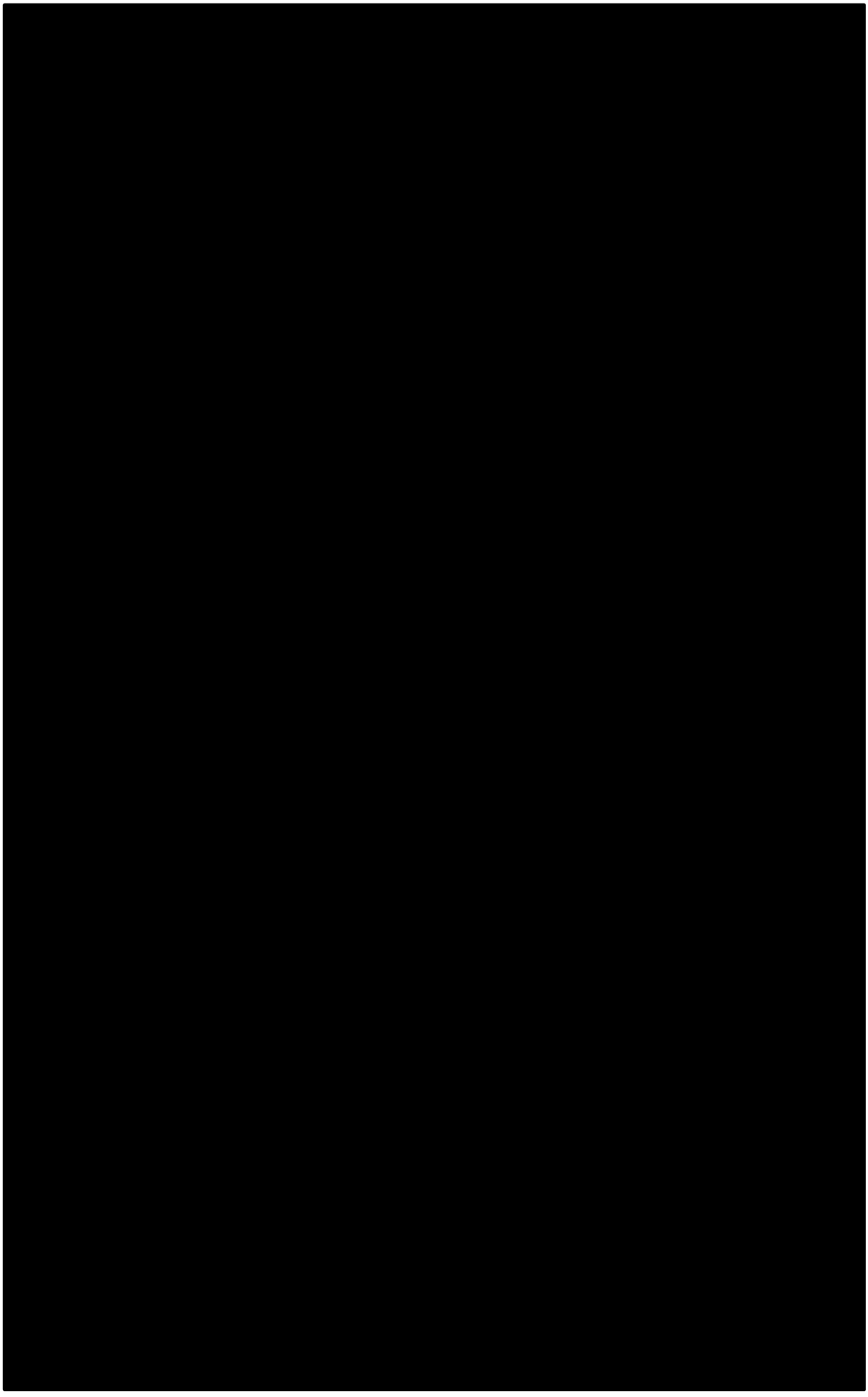


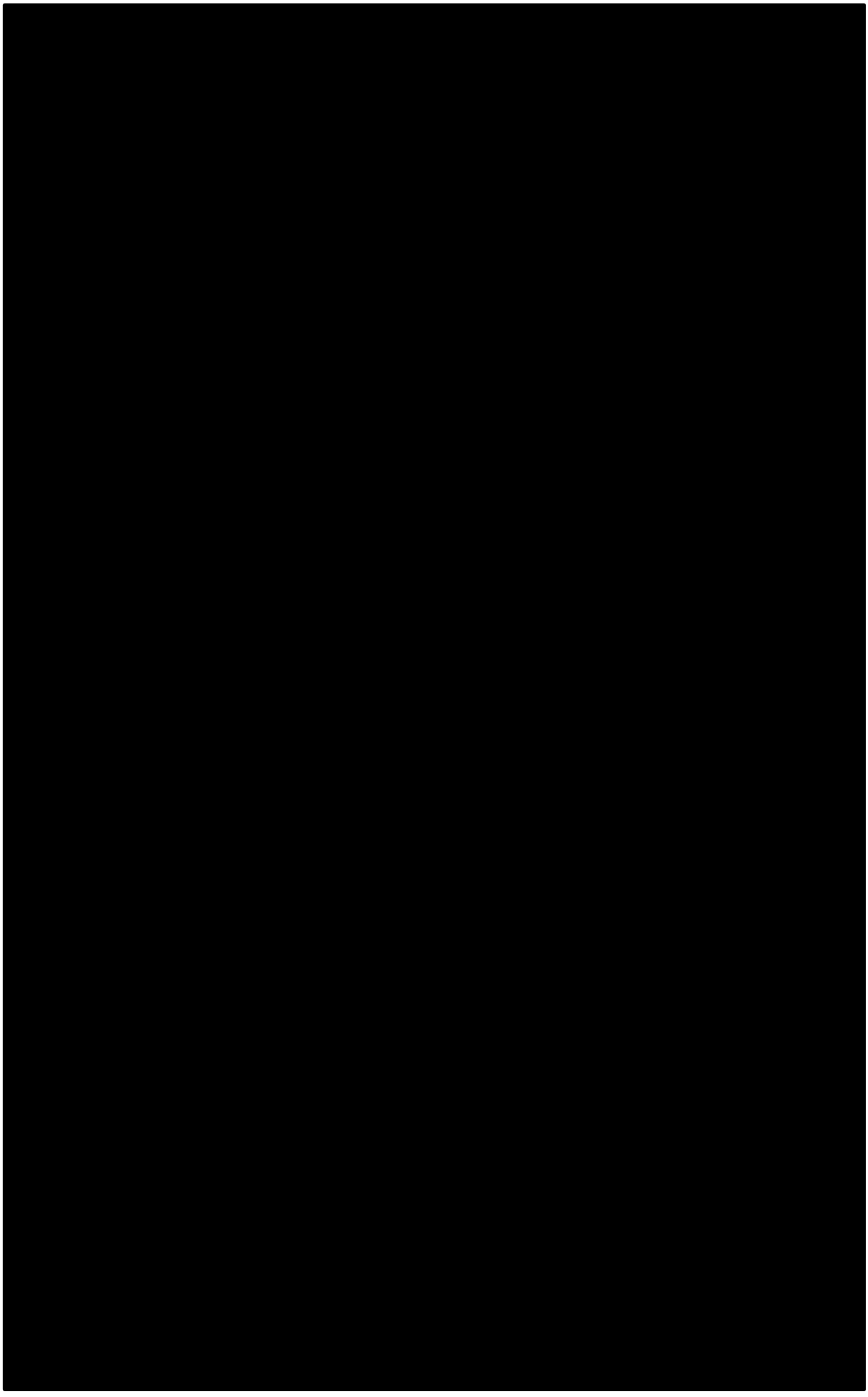


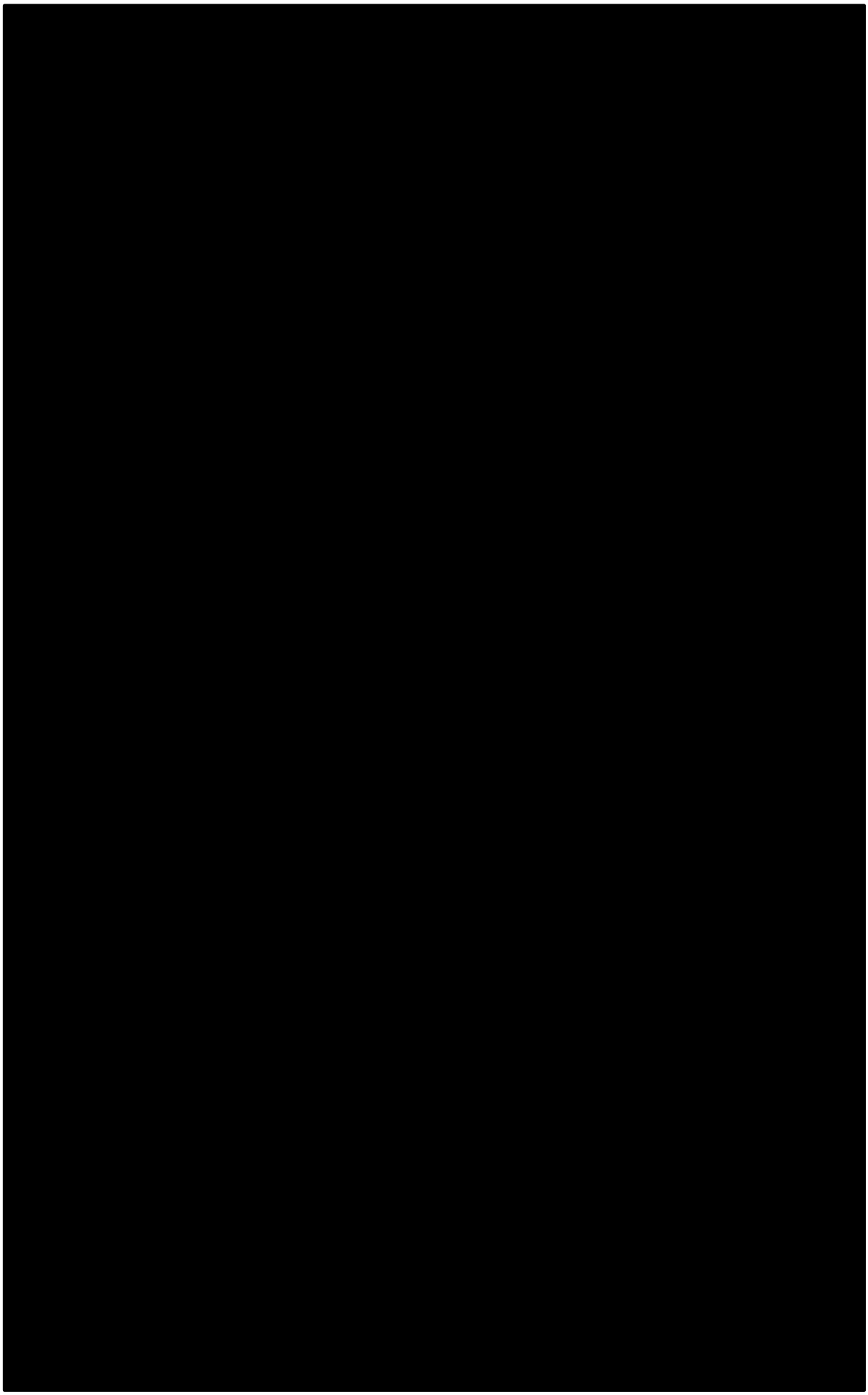


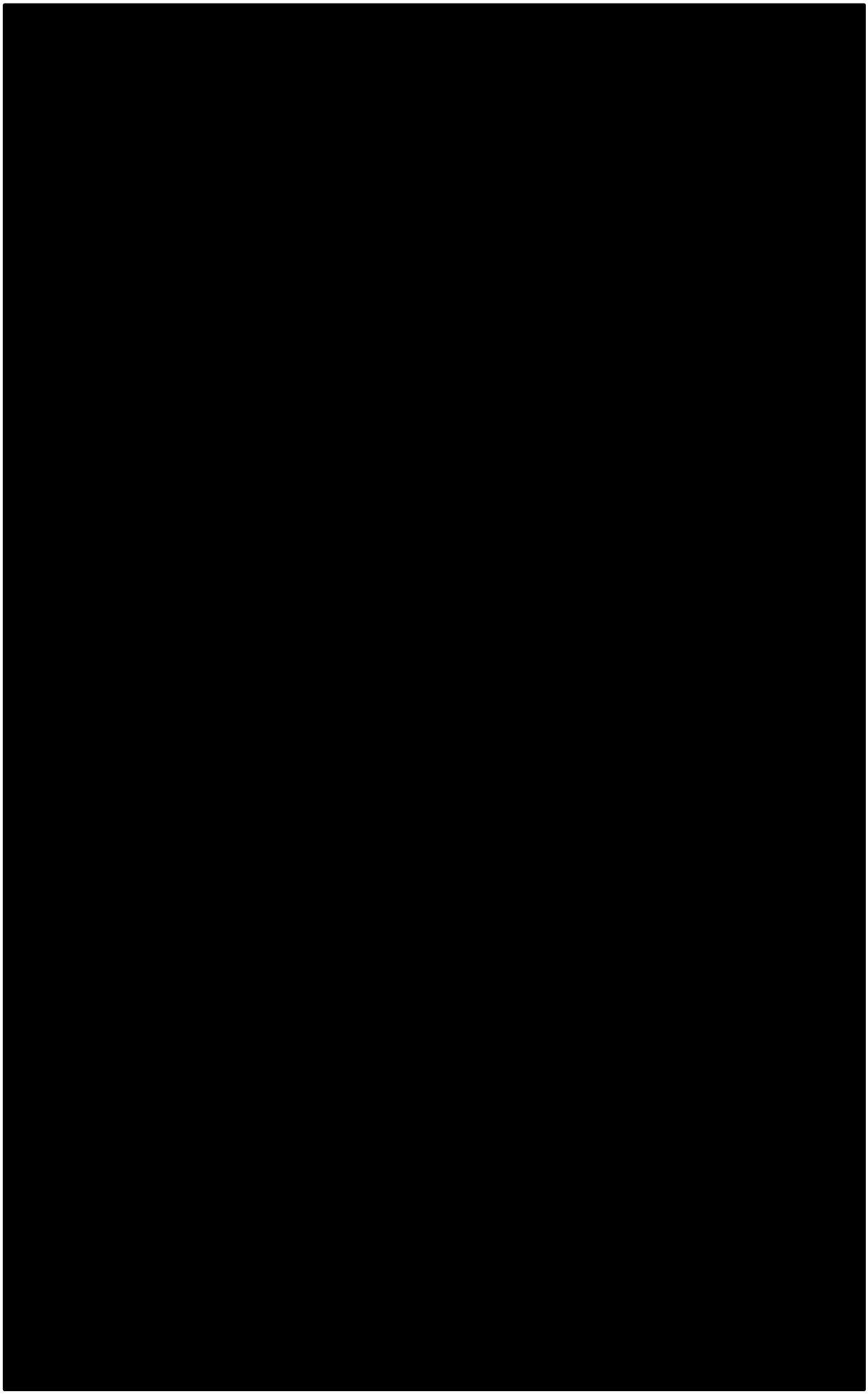


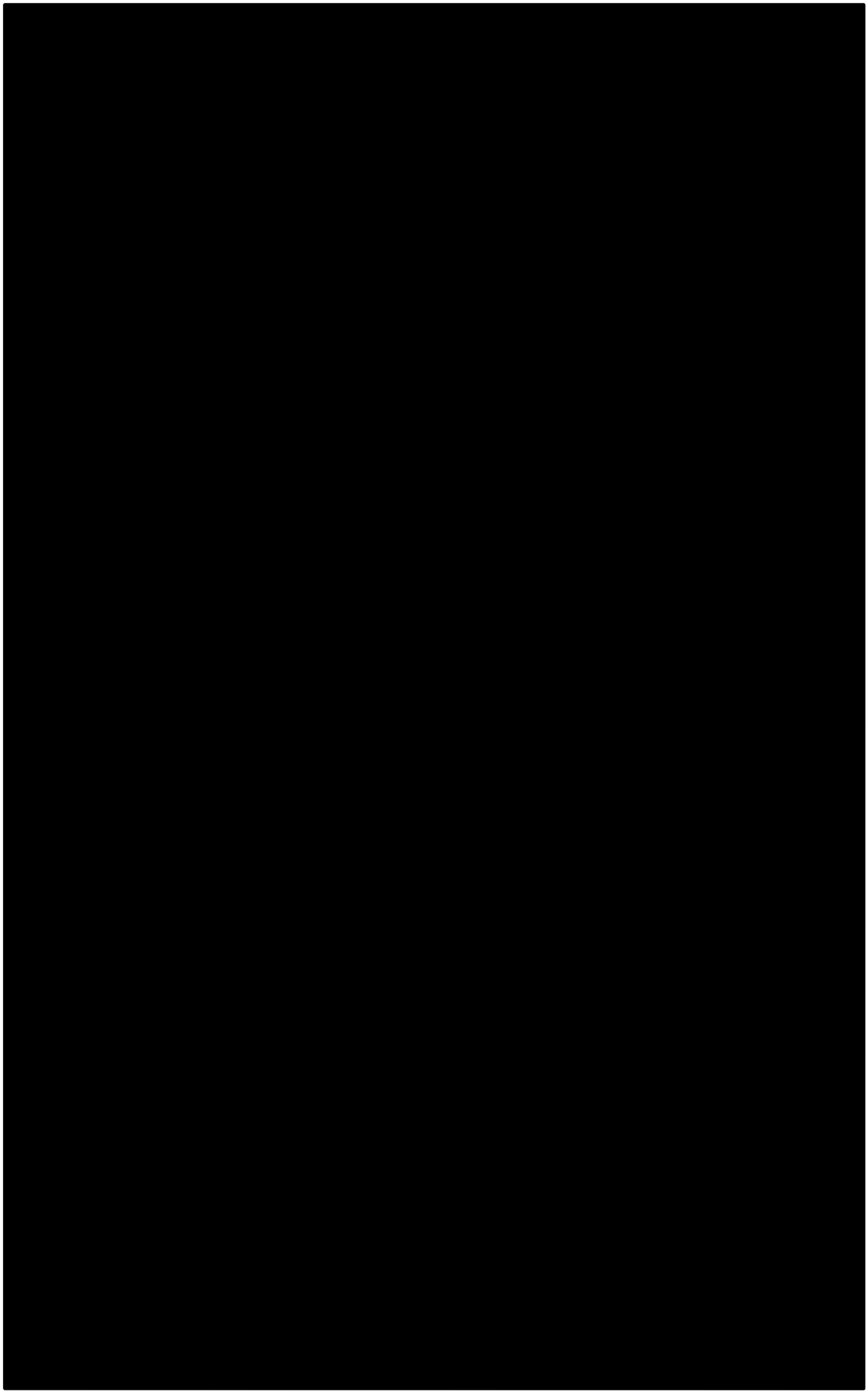


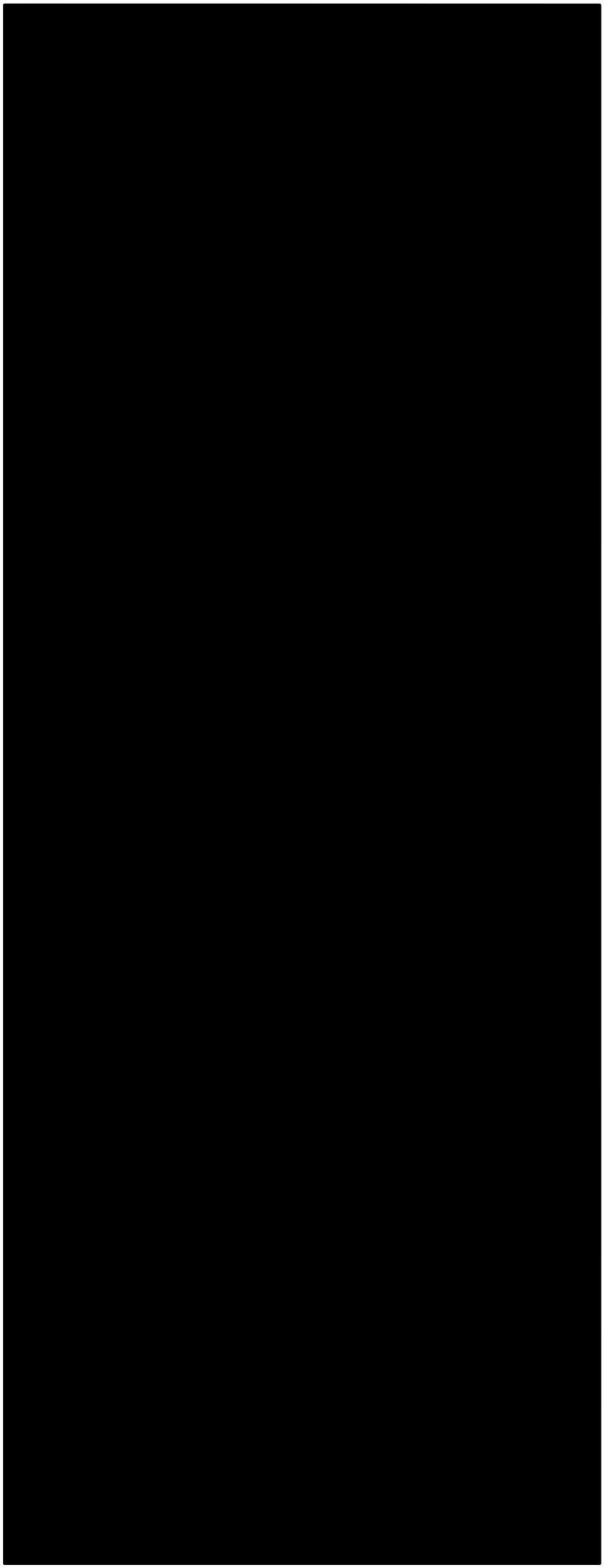


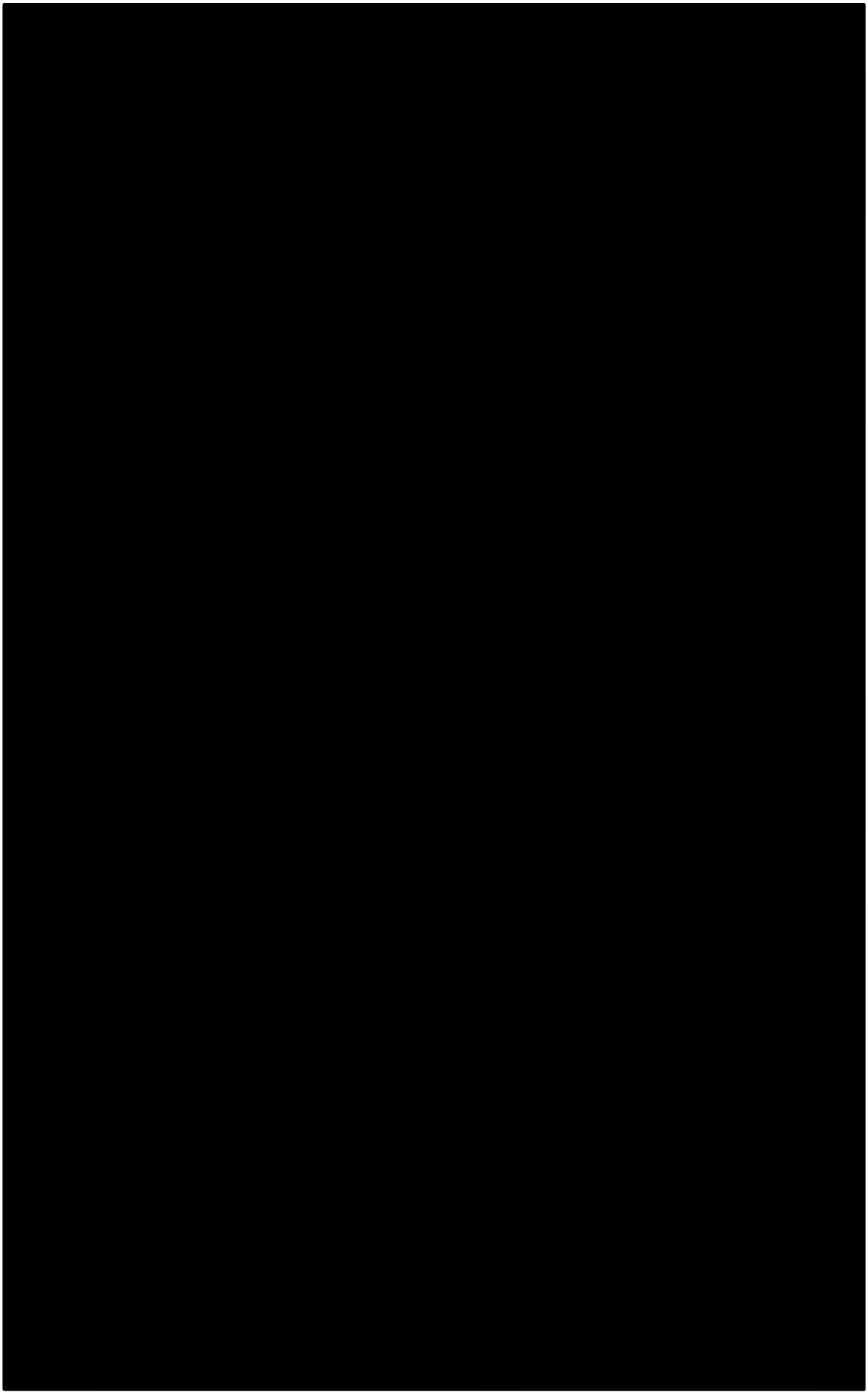


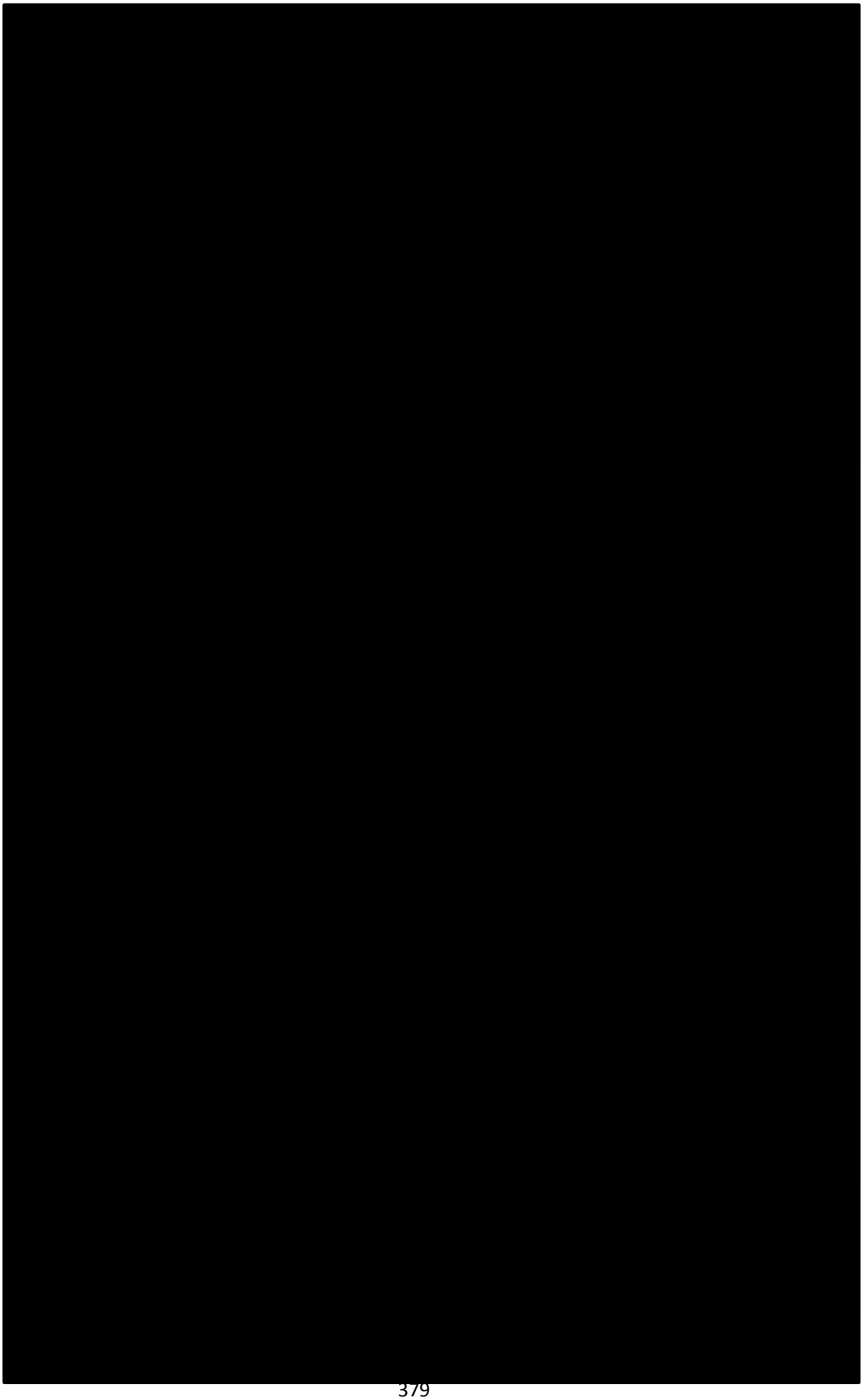












Appendix VII. HIV Glasgow Drug therapy 2016 presentation entitled "5 Differences in virological and immunological risk factors for non-Hodgkin and Hodgkin lymphoma in the D:A:D study"

Email: leah.shepherd@ucl.ac.uk

HIV Glasgow Drug Therapy 2016

Differences in virological and immunological risk factors for non-Hodgkin and Hodgkin lymphoma: The D:A:D study

Leah Shepherd, Lene Ryom, Matthew Law, Camilla Ingrid Hatleberg, Stephane de Wit, Antonella d'Arminio Monforte, Manuel Battegay, Andrew Phillips, Fabrice Bonnet, Peter Reiss, Christian Pradier, Andrew Grulich, Caroline Sabin, Jens Lundgren, Amanda Mocroft

On behalf of the D:A:D Study group

D:A:D

UCL

Background

- HIV positive individuals are at higher risk of infection-related malignancies due to immune deficiency [1].
- Since the introduction of combination antiretroviral treatment (cART), a decline in non-Hodgkin lymphoma (NHL) but not Hodgkin lymphoma (HL) incidence has been observed [2].
- Despite this progress for NHL, incidence of NHL and HL remain approximately 10 fold higher than in the HIV- population [1, 3].
- Previous studies suggest that risk factors affecting for NHL and HL may differ amoung HIV+ persons.

1. Grulich et al. 2007. The Lancet, 370(9581).
2. Patel et al. 2008. Annals of Internal Medicine, 148(10).
3. Robbins, et al. 2014. AIDS, 28(6).

Objective

To identify risk factors associated with developing NHL or HL in HIV+ people in the D:A:D study.

Methods

- **Two independent outcomes**
  - HL (collected and validated since 2004)
  - NHL (AIDS defining event [1])
- **Baseline:** Latest of study entry, first CD4 cell count, or 1/1/2004
- **D:A:D participants followed from baseline until earliest of**
  - NHL or HL diagnosis
  - Last visit + 6 months
  - Death
  - 1 February 2015
- **Exclusions**
  - History of NHL or HL prior to baseline
  - No CD4 available at baseline

[1] Centers for Disease Control and Prevention. MMWR Recomm Rep 1992;18(41(RR-17)).

Methods

Independent risk factors for NHL and HL were identified using Poisson regression

Demographic
- Age
- Gender
- Race
- Mode of HIV infection
- Smoking status
- BMI
- Calendar year

HIV related
- HIV treatment Regimen,Duration
- HIV Viral load (VL) Current HIV-Viral load (HIV-VL)
- Area under the curve (AUC) HIV-VL (from first follow-up)
- CD4 Current CD4

Comorbidities
- AIDS diagnosis (other than cancer)
- AIDS defining malignancy (ADM)
- Non-AIDS defining malignancy (NADM)
- HCV and HBV status
- Hypertension
- Anaemia
- Diabetes
- Cardiovascular events

Area under the curve (AUC)

- A time varying measure of cumulative exposure to HIV replication.
- Calculated since entry into D:A:D.
- Same idea as pack years for smoking.
- Divided into quintiles

1st quintile: Lowest AUC of HIV-VL
2
3
4
5th quintile: Highest AUC of HIV-VL

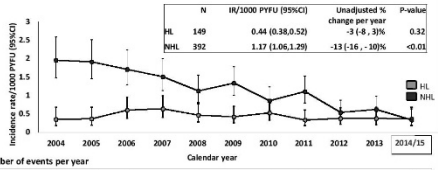
Higher AUC-HIV-VL level = More exposure to HIV viral replication

Baseline characteristics

Factor	Total	
	N	%
Median age (years, IQR)	40	34 - 47
Median year of baseline (IQR)	2004	2004 - 2006
Male gender	30214	73
~~White ethnicity~~	~~20658~~	~~50~~
MSM transmission mode	18124	44
Prior AIDS (other than cancer)	8748	21
On cART	21310	51
CD4>350 cells/mm^3	25563	62
HIV-VL <500 copies/mL	21171	51

IQR: Interquartile range, baseline: latest of study entry or 1/1/2004

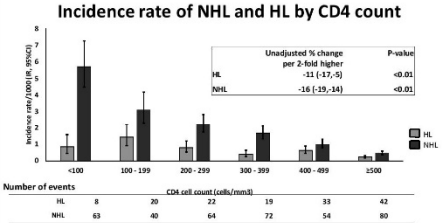
Incidence rate of NHL and HL by year



Number of events per year

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014/15
HL	9	10	17	21	15	15	18	11	12	11	10
NHL	50	52	50	48	36	45	29	34	17	18	8

Incidence rate of NHL and HL by CD4 count

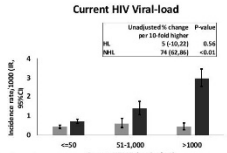


Number of events

	<100	100 - 199	200 - 299	300 - 399	400 - 499	≥500
HL	8	20	22	19	33	42
NHL	63	40	64	72	54	80

Incidence rate of NHL and HL by current and AUC of HIV-VL

Current HIV Viral-load



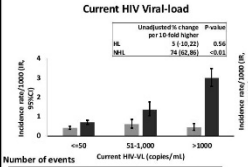
Number of events

	<=50	51-1,000	>1000
HL[1]	98	26	24
NHL[2]	164	60	166

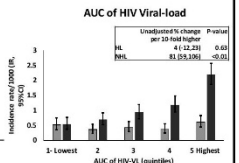
Missing: [1]N=1, [2]N=2, [3]N=2, [4]N=29

Incidence rate of NHL and HL by current and AUC of HIV-VL

Current HIV Viral-load



AUC of HIV Viral-load

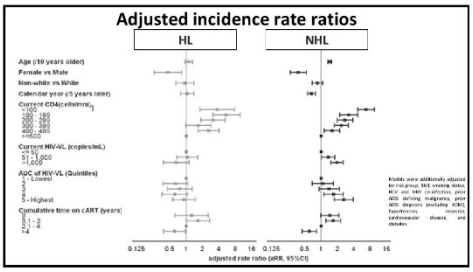


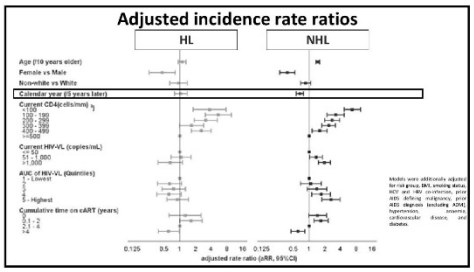
Number of events

	<=50	51-1,000	>1000	1- Lowest	2	3	4	5 Highest
HL[3]	98	26	24	28	24	29	25	41
NHL[4]	164	60	166	29	46	62	79	147

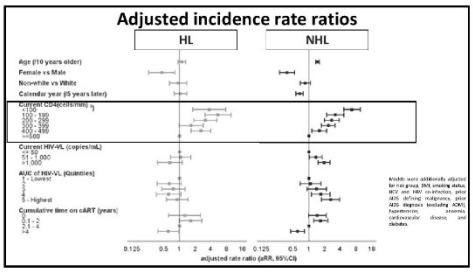
Missing: [1]N=1, [2]N=2, [3]N=2, [4]N=29

Adjusted incidence rate ratios

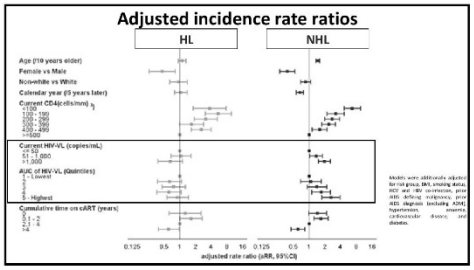



Adjusted incidence rate ratios

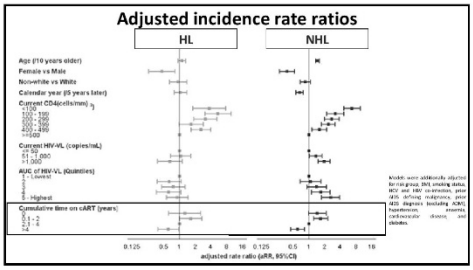
Models were additionally adjusted for risk group, BMI, smoking status, HCV and HBV co-infection, prior AIDS defining malignancy, prior AIDS diagnosis (excluding ADM), hypertension, anaemia, cardiovascular disease, and diabetes.


Adjusted incidence rate ratios

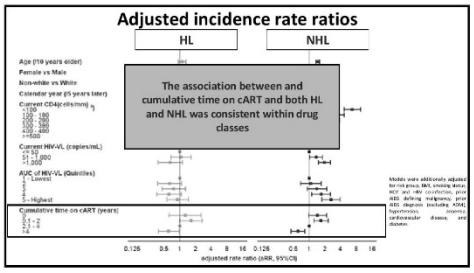
Models were additionally adjusted for risk group, BMI, smoking status, HCV and HBV co-infection, prior AIDS defining malignancy, prior AIDS diagnosis (excluding ADM), hypertension, anaemia, cardiovascular disease, and diabetes.


Adjusted incidence rate ratios

Models were additionally adjusted for risk group, BMI, smoking status, HCV and HBV co-infection, prior AIDS defining malignancy, prior AIDS diagnosis (excluding ADM), hypertension, anaemia, cardiovascular disease, and diabetes.

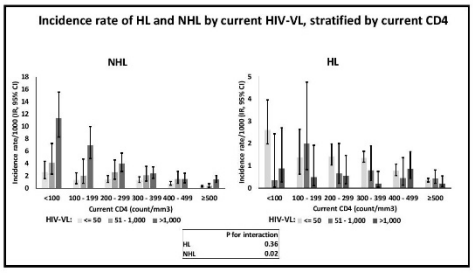

Adjusted incidence rate ratios

Models were additionally adjusted for risk group, BMI, smoking status, HCV and HBV co-infection, prior AIDS defining malignancy, prior AIDS diagnosis (excluding ADM), hypertension, anaemia, cardiovascular disease, and diabetes.


Adjusted incidence rate ratios

The association between and cumulative time on cART and both HL and NHL was consistent within drug classes

Models were additionally adjusted for risk group, BMI, smoking status, HCV and HBV co-infection, prior AIDS defining malignancy, prior AIDS diagnosis (excluding ADM), hypertension, anaemia, cardiovascular disease, and diabetes.


Incidence rate of HL and NHL by current HIV-VL, stratified by current CD4

	P for interaction
HL	0.36
NHL	0.02

Strengths and limitations

Strengths

- Large dataset > 40,000 HIV positive individuals
- Large number of NHL and HL
- HL events centrally validated, NHL diagnosed AS ADE (1993) standard AIDs defining events.
- Detailed data collection on several important and specific HIV-related risk factors

Limitations

- HL collected and centrally validated from 2004 only
- Observational study
- Residual confounding my remain and reverse causality cannot be ruled out
- Limited data on dissemination/stage of disease

Conclusions

- NHL incidence was associated with lower current CD4 and higher current and historical exposure to viral replication.
- This indicates that ongoing viral replication may play a part in NHL development along side current-immunodeficiency.
- HL incidence was elevated in those with current-immunodeficiency, but current and historical exposure to uncontrolled HIV replication were not associated.
- Factors involved in the pathology of HL are less clear.

Conclusions

- Preventive measures should include identification and management of persons with HIV to minimise exposure to uncontrolled viremia and advanced immunodeficiency. Our results highlight the importance of early diagnosis and early Cart initiation.

Acknowledgements

Steering Committee: Members indicated w/ *; ¢ chair;
Cohort PIs: W El-Sadr* (CPCRA), G Calvo* (BASS), F Dabis* (Aquitaine), O Kirk* (EuroSIDA), M Law* (AHOD), A d'Arminio Monforte* (ICONA), L Morfeldt* (HivBIVUS), C Pradier* (Nice), P Reiss* (ATHENA), R Weber* (SHCS), S De Wit* (Brussels)
Cohort coordinators and data managers: A Lind-Thomsen (coordinator), R Sabel Brandt, M Hillebregt, S Zahed, FWNM Wit (ATHENA), F Schöni-Affolter, M Rickenbach (SHCS), A Travelli, I Fanti (ICONA), O Leleux, J Mourali (Aquitaine), E Thulin, A Sundström (HIVBIVUS), G Bartsch, G Thompsen (CPCRA), M Delforge (Brussels), E Fontas, C Caissotti, K Dollet (Nice), S Mateu, F Torres (BASS), A Blance, R Puhr (AHOD), D Kristensen (EuroSIDA)
Statisticians: CA Sabin*, AN Phillips*, DA Kamara, CJ Smith, A Mocroft*
D:A:D coordinating office: CI Hatleberg, L Ryom, A Lind-Thomsen, RS Brandt, D Raben, C Matthews, A Boesen, AL Grevsen, JD Lundgren*¶
Member of the D:A:D Oversight Committee: B Powderly*, N Shortman*, C Moecklinghoff*.
S Reiny*, X Franquet*
D:A:D working group experts: Kidney: L Ryom, A Mocroft, O Kirk *, P Reiss*, C Smit, M Ross, CA Fux, P Morlat, E Fontas, DA Kamara, CJ Smith, JD Lundgren *¢
Mortality: CJ Smith, L Ryom, CI Hatleberg, AN Phillips*, R Weber*, P Morlat, C Pradier*, P Reiss*, FWNM Wit, N Friis-Møller, J Kowalska, JD Lundgren*¶
Cancer: CA Sabin*, L Ryom, CI Hatleberg, M Law*, A d'Arminio Monforte*, F Dabis*, F Bonnet*, P Reiss*, FWNM Wit, CJ Smith, DA Kamara, J Bohlius, M Bower, G Fätkenheuer, A Grulich, JD Lundgren*¶
External endpoint reviewer: A Sjøl (CVD), P Meidahl (oncology), JS Iversen (nephrology)
Funding: 'Oversight Committee for The Evaluation of Metabolic Complications of HAART' with representatives from academia, patient community, FDA, EMA and a consortium of AbbVie, Bristol-Myers Squibb, Gilead Sciences, ViiV Healthcare, Merck and Janssen Pharmaceuticals

Appendix VIII. CROI poster entitled “Risk Factors for Hodgkin (HL) and non-Hodgkin lymphoma (NHL) in Europe”

Poster No. 630

CROI 2016

Risk Factors for Hodgkin (HL) and Non-Hodgkin Lymphoma (NHL) in Europe

L Shepherd[1], ÁH Borges[2], J Bogner[3], A Horban[4], E Kuzovatova[5], M Battegay[6], M Losso[7], S Edwards[8], C Duvivier[9], S Moreno[10], JD Lundgren[2], A Mocroft[1] on behalf of EuroSIDA in EuroCOORD

[1]University College London, London, UK; [2]CHIP, Department of Infectious Diseases, Section 2100, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [3]Medizinische Poliklinik, Munich, Germany; [4]Warsaw Medical University, Hospital of Infectious Diseases, Warsaw, Poland; [5]Academician I.N. Blokhina Nizhny Novgorod Research Institute of Epidemiology and Microbiology, Russian Inspectorate for the Protection of Consumer Rights and Human Welfare, Nizhny Novgorod, Russia; [6]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland; [7]Hospital Ramos Mejia, Buenos Aires, Argentina; [8]Mortimer Market Centre, Central and North West London Community Foundation Trust, London, United Kingdom; [9]Infectious Diseases, AP-HP, Necker-Enfants Malades Hospital, Necker-Pasteur Infectious Diseases Center, Descartes University, Sorbonne Paris Cité, EA7327, IHU Imagine, Paris, France; [10] Department of Infectious Diseases, University Hospital Ramón y Cajal, IRYCIS, Madrid, Spain

Leah Shepherd
UCL, Department of Infection and Population Health,
Royal Free Hospital, Pond Street, London, NW3 2QG
+44 20 7794 0500 ext 34570
Leah.shepherd@ucl.ac.uk

BACKGROUND

Non-Hodgkin (NHL) and Hodgkin lymphomas (HL) are common in HIV+ people[1]. Previous research has described significant declines in NHL[2] but not HL[3] after cART, however data on individual risk factors is limited.

OBJECTIVE

Determine the role of demographic, immunological and HIV-related factors on NHL and HL incidence across Europe.

METHODS

EuroSIDA participants with follow-up after 1/1/2001 and without a history of NHL or HL were included. Crude incidence of NHL and HL were calculated and stratified by patient characteristics. Separate Poisson regression models were used to identify risk factors for NHL and HL. Both current and historical measures of HIV Viral-load (HIV-VL) (% of time with HIV-VL <400 copies/ml) and immunosuppression (Nadir CD4, % of time with CD4<200 cells/mm^3) were considered.

RESULTS

Baseline characteristics (Table 1)

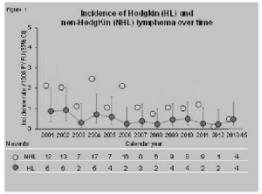
- A total of 14,820 people were included contributing 97,220 person years of follow-up (PYFU), of which 14,679 (95,804 PYFU) were included in analyses for NHL and 14,785 (96,824PYFU) were included for analyses of HL. **Table 1.**
- The cohort was mainly male (73%), white (87%) with a median age of 39 years at baseline. At baseline, 80% had ever used cART, 59% had a CD4 > 350 cells/mm^3 and 54% had a HIV-VL <400 copies/mL. **Table 1.**

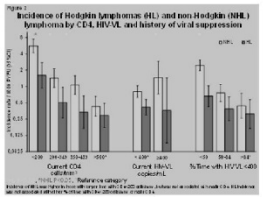
Incidence of NHL and HL (Figure 1)

- In total, 117 developed NHL (incidence rate 1.2/1000 PYFU, 95%CI 1.0-1.5) and 45 developed HL (0.5/1000 PYFU, 95%CI 0.3–0.6). Incidence of NHL and HL declined over time. NHL incidence declined by 12 (95%CI: 7–16)% from 2.1 (1.2,3.7) /1000 PYFU in 2001 to 0.5 (0.2,1.3) /1000 PYFU in 2013/14/15 and incidence of HL declined by 9 (1–15)% per year from 0.9 (0.4,2.1)/1000 PYFU to 0.5 (0.2,1.3) /1000 PYFU in unadjusted analyses. **Figure 1.**

Table 1: Characteristics at baseline[1]

Factor	Total N (%)	Non-Hodgkin lymphoma N (%)	Hodgkin lymphoma N (%)
Total	14,820 (100)	117 (100)	45 (100)
Median age (years, IQR)	39.1 (33.1,46.3)	41.8 (37.7,50.5)	40.4 (34.2,44.5)
Median Year of baseline (IQR)	2003 (2001,2006)	2001 (2001,2004)	2001 (2001,2003)
Male	10,894 (73.5)	101 (87.2)	39 (86.8)
White	12,954 (87.4)	101 (86.8)	37 (82.2)
HIV transmission mode: Homosexual	6,819 (46.0)	62 (53.0)	24 (53.3)
Prior AIDS (Not NHL)	4,096 (27.6)	35 (29.9)	15 (33.3)
Prior non-AIDS (Not HL)	460 (3.1)	6 (4.3)	3 (6.7)
History of cART use	11,838 (79.8)	89 (76.1)	39 (86.7)
CD4> 350 cells/mm^3	8,803 (59.4)	63 (45.3)	29 (64.2)
HIV Viral Load <400 copies/mL	7,949 (53.6)	44 (38.0)	19 (42.2)





Incidence of NHL and HL Continued (Figures 2 and 3)

- Of the 117 NHL, 48 (41%) were immunoblastic, 14 (12%) were Burkitt, 5 (4%) were primary brain, and 46 (43%) were of unknown or other subtype.
- In unadjusted analysis, incidence of NHL increased with lower current CD4 category, HIV-VL≥400 copies/mL, and was higher in those who spent less than half of follow – up with HIV-VL <400 copies/mL. **Figure 2.** Those with a prior AIDS defining malignancy (ADM, excluding NHL) also had higher incidence. **Figure 3.**
- Incidence of HL was higher in those with lower current CD4 category, however this was less marked than for NHL. **Figure 2**.Incidence of HL was not found to be associated with current HIV-VL, % follow-up spent with HIV-VL <400 copies/mL or a prior ADM (excluding NHL). **Figure 2 and Figure 3.**
- Neither NHL or HL incidence was associated with age, cART use, or transmission mode.

Adjusted analysis (Figure 4)

After adjustment for factors displayed and listed in the footnote of **Figure 4**:

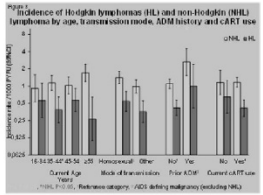
- Historical HIV-related variables, such as a prior diagnosis of ADM or less time with controlled HIV RNA, were more strongly associated with NHL incidence than current HIV-VL. **Figure 4.**
- The association between lower CD4 cell count and increased incidence remained for both HL and NHL, however no time trend in either NHL or HL incidence was evident after adjustment. **Figure 4.**
- Older age was associated with higher NHL but not HL incidence. **Figure 4.**

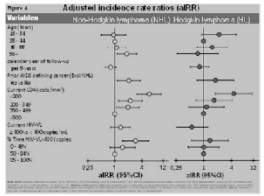
CONCLUSION

NHL development was associated with current immunodeficiency and history of uncontrolled viral replication, suggesting that exposure to uncontrolled viral replication may play a part in NHL development in addition to current immunodeficiency. Conversely, HL incidence was elevated in those with current severe immunodeficiency (CD4<200 cells/mm^3), but cumulative exposure to uncontrolled HIV replication was not a risk factor.

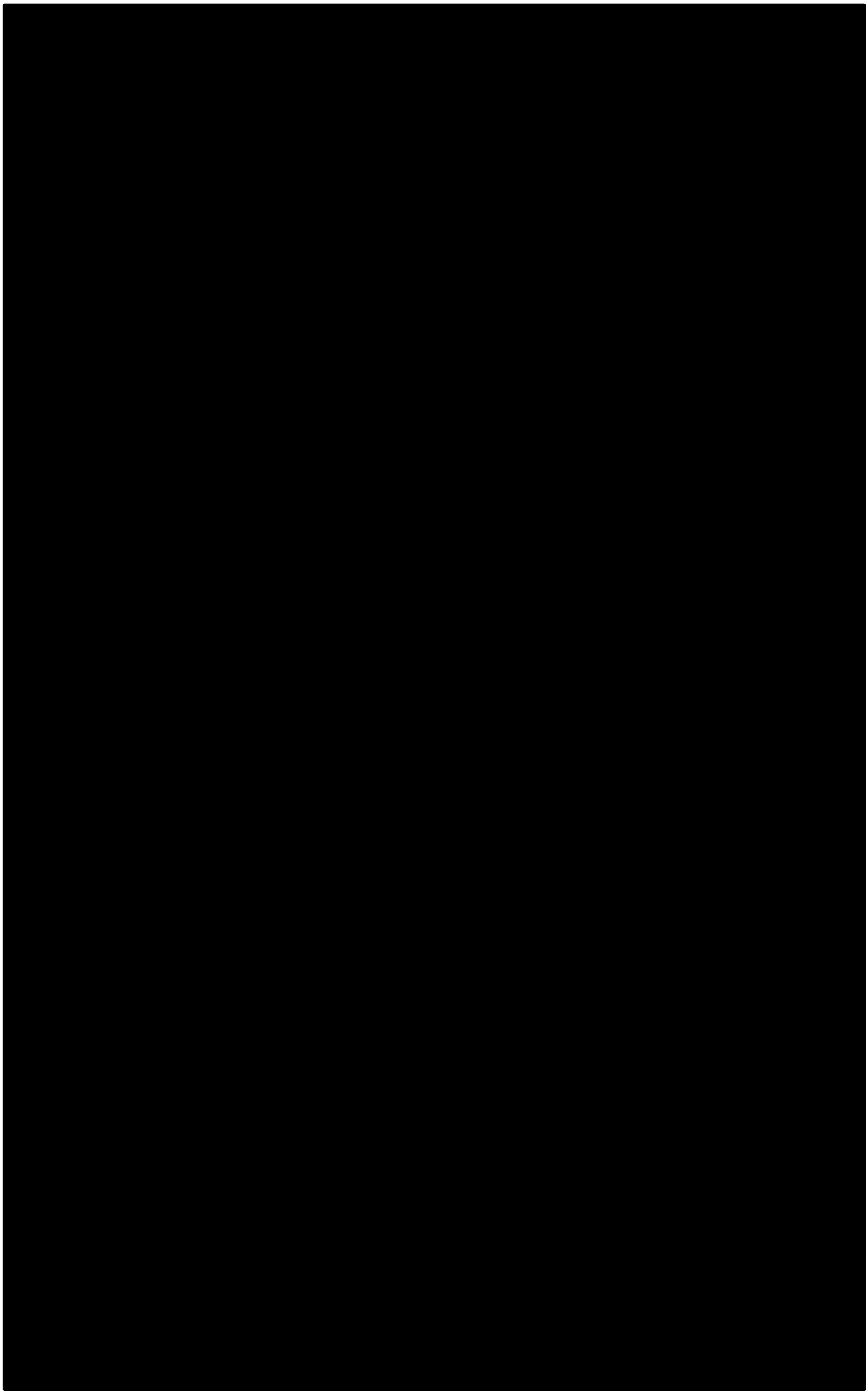
References

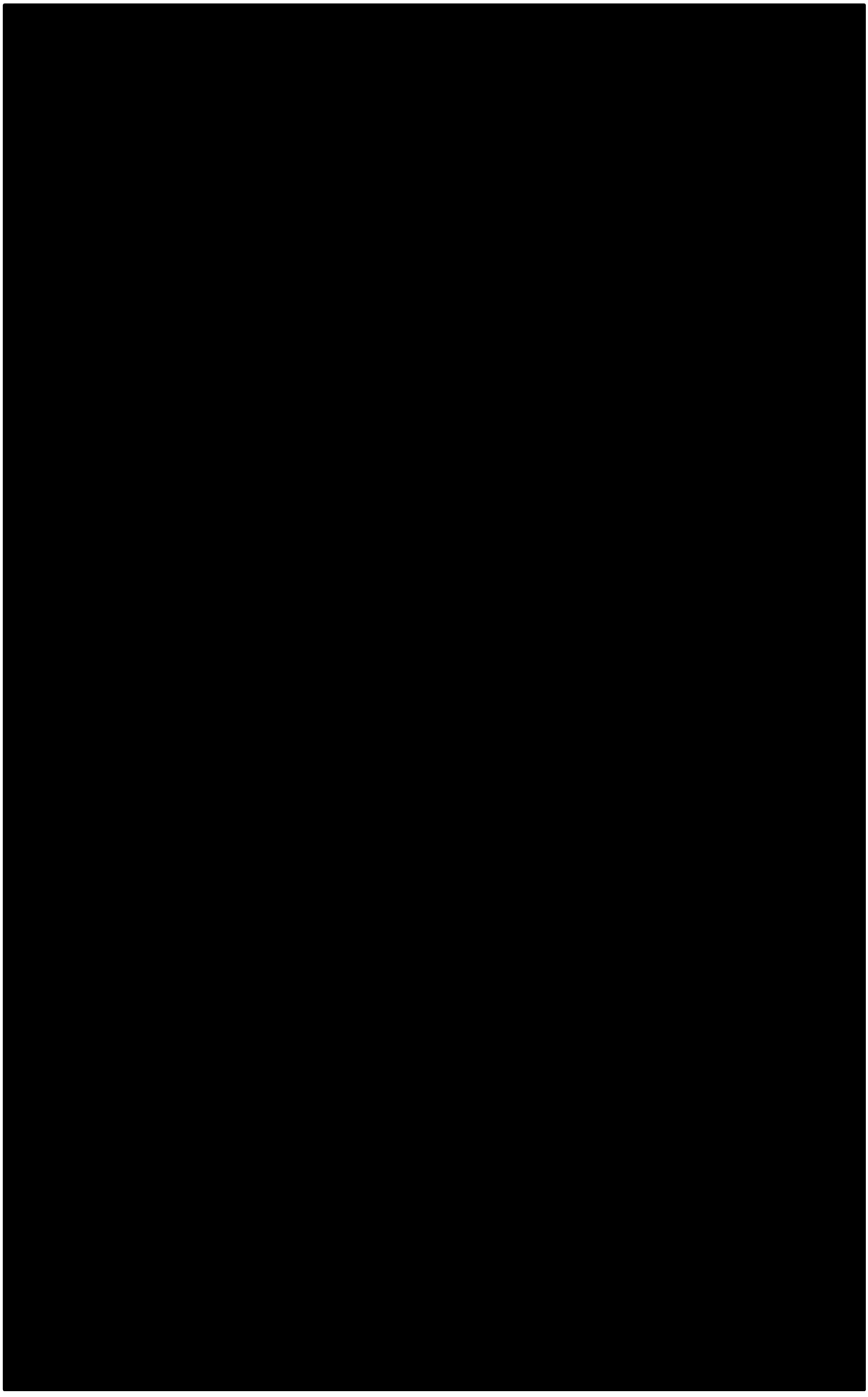
1. Silverberg, M. J., et al. (2011). "HIV Infection, Immunodeficiency, Viral Replication, and the Risk of Cancer." Cancer Epidemiol Biomarkers Prev 20(12): 2551-2559.
2. Clifford, G. M., et al. (2005). "Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy." J Natl Cancer Inst 97(6): 425-432.
3. Patel, P., et al. (2008). "Incidence of Types of Cancer among HIV-Infected Persons Compared with the General Population in the United States, 1992–2003." Ann Intern Med 148(10): 728-736.

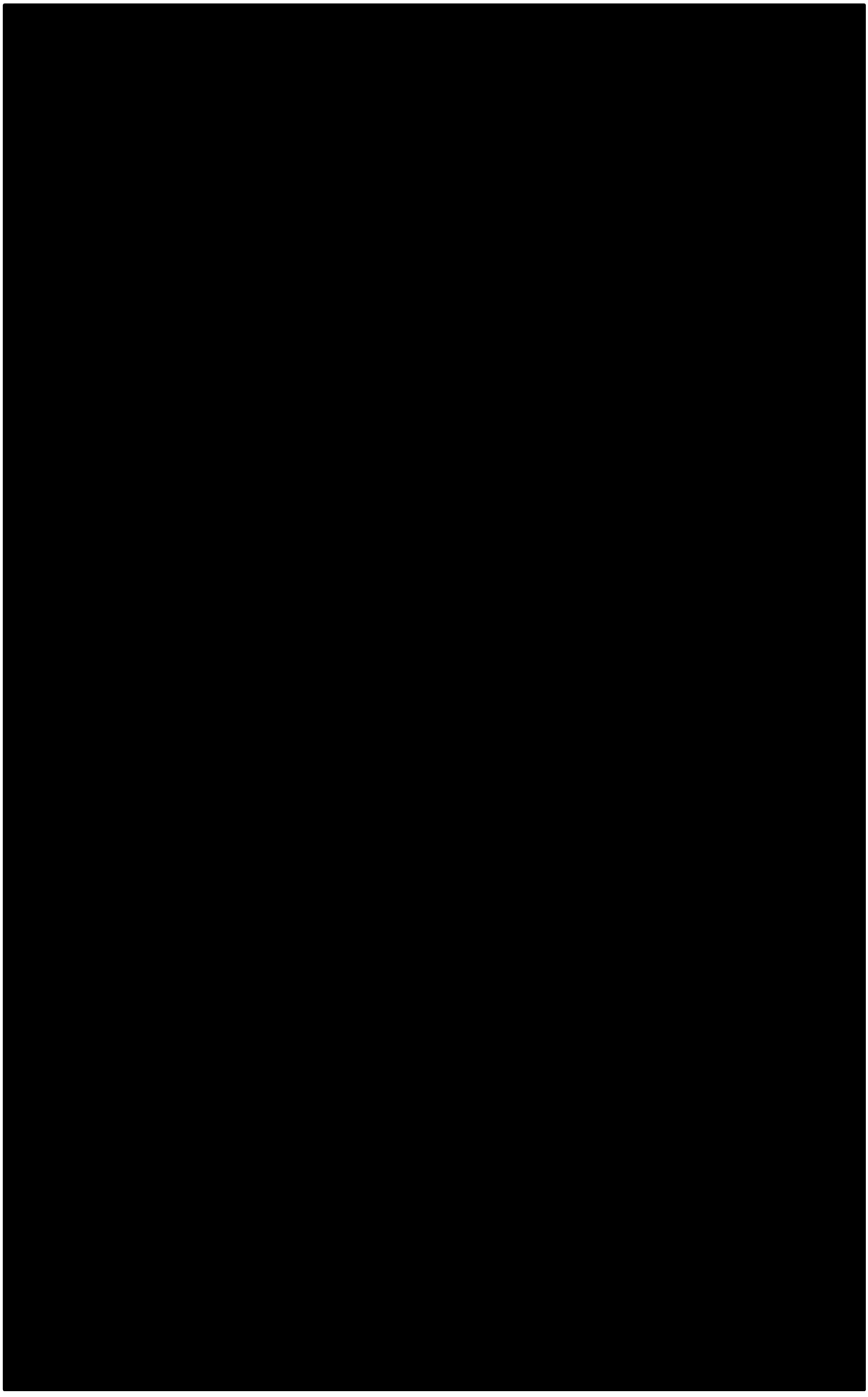


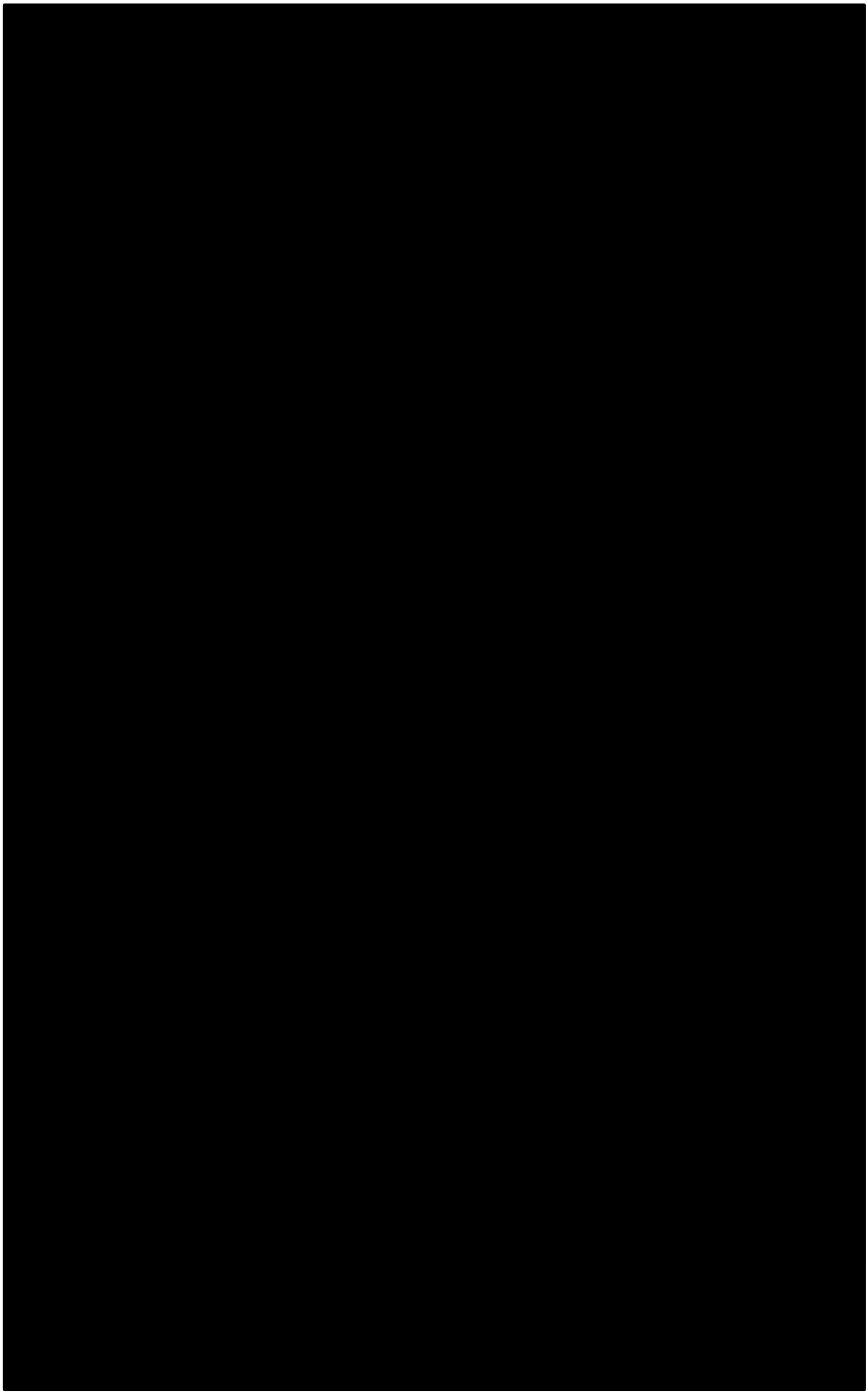


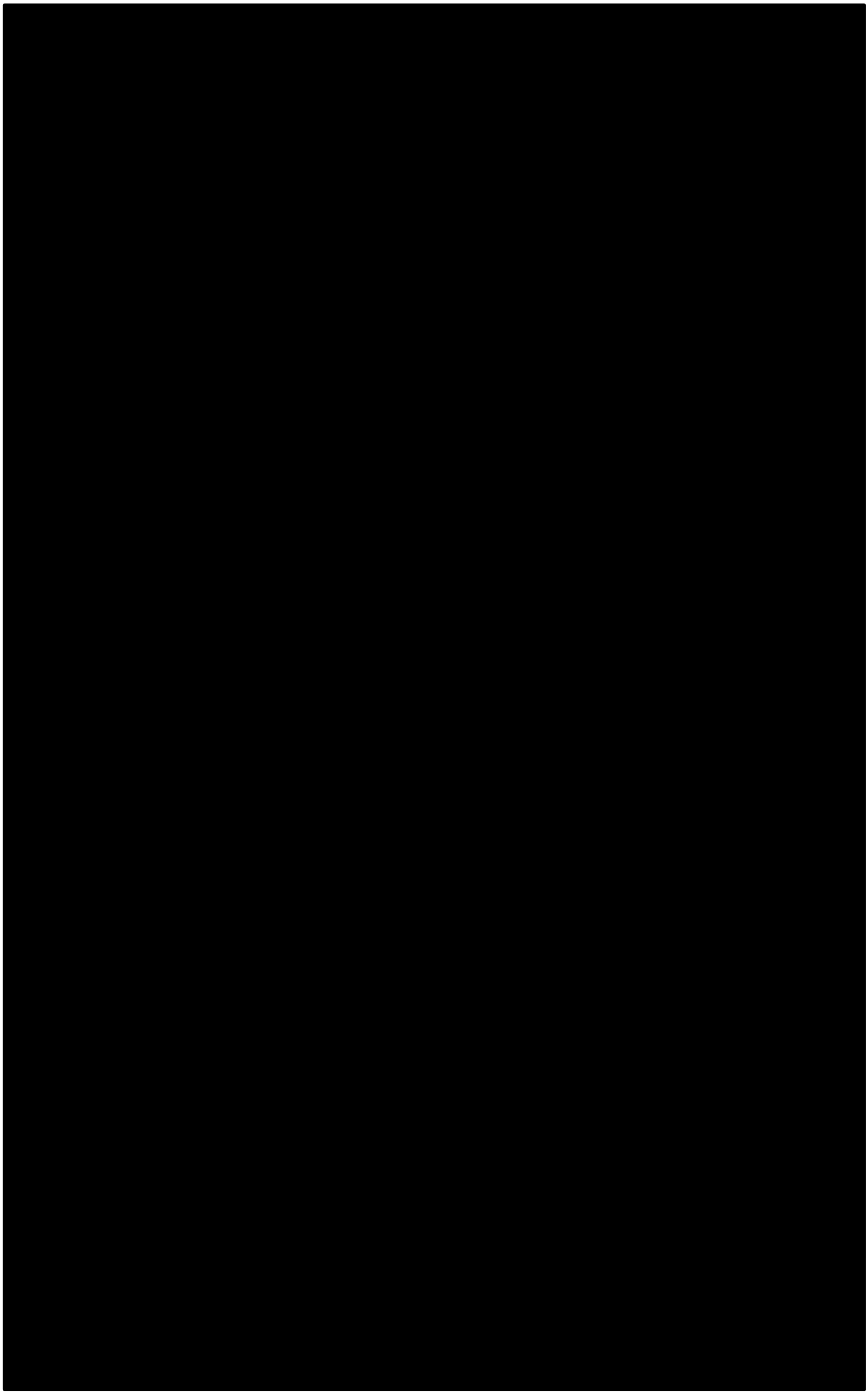
Download poster at: www.cphiv.dk

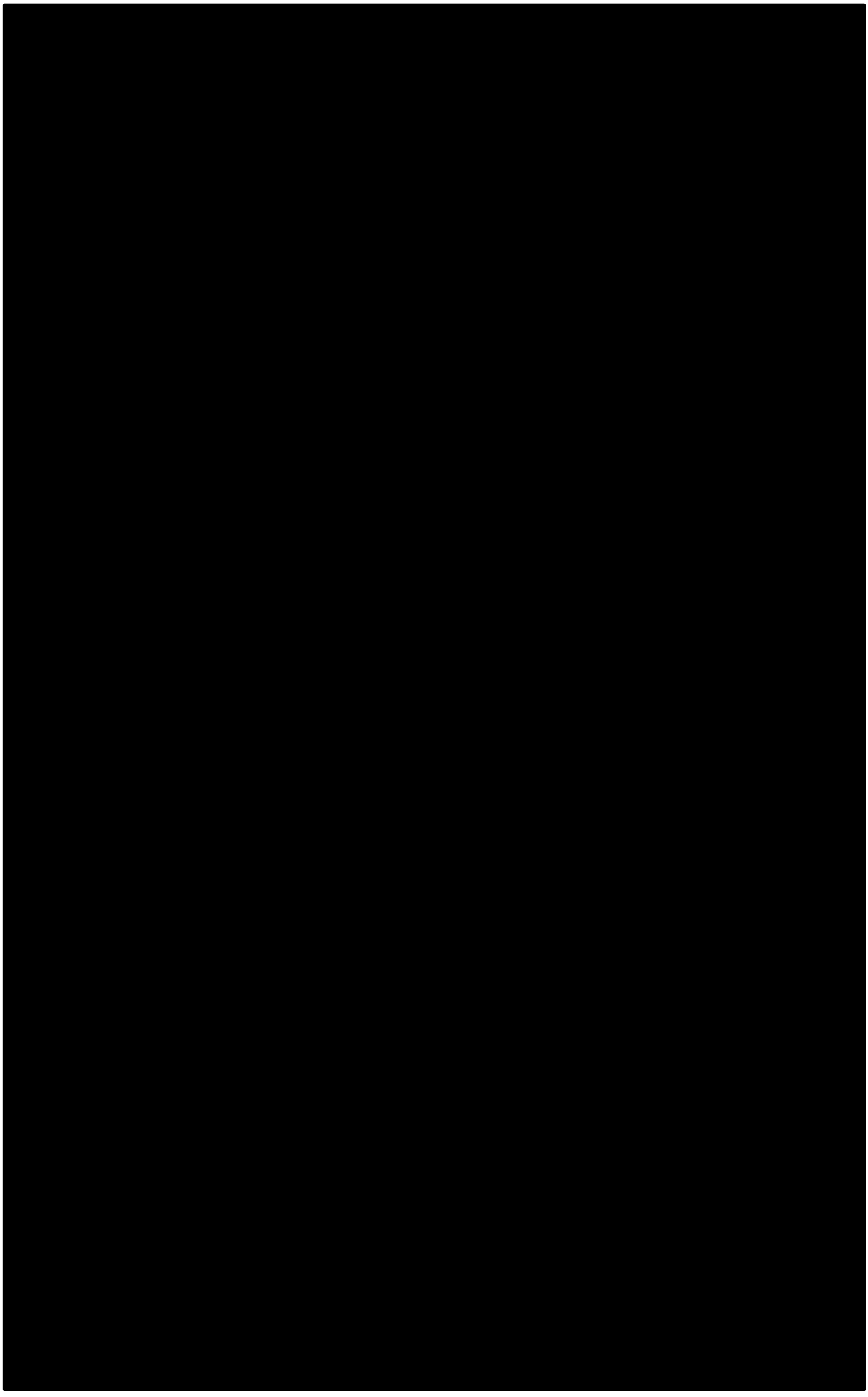


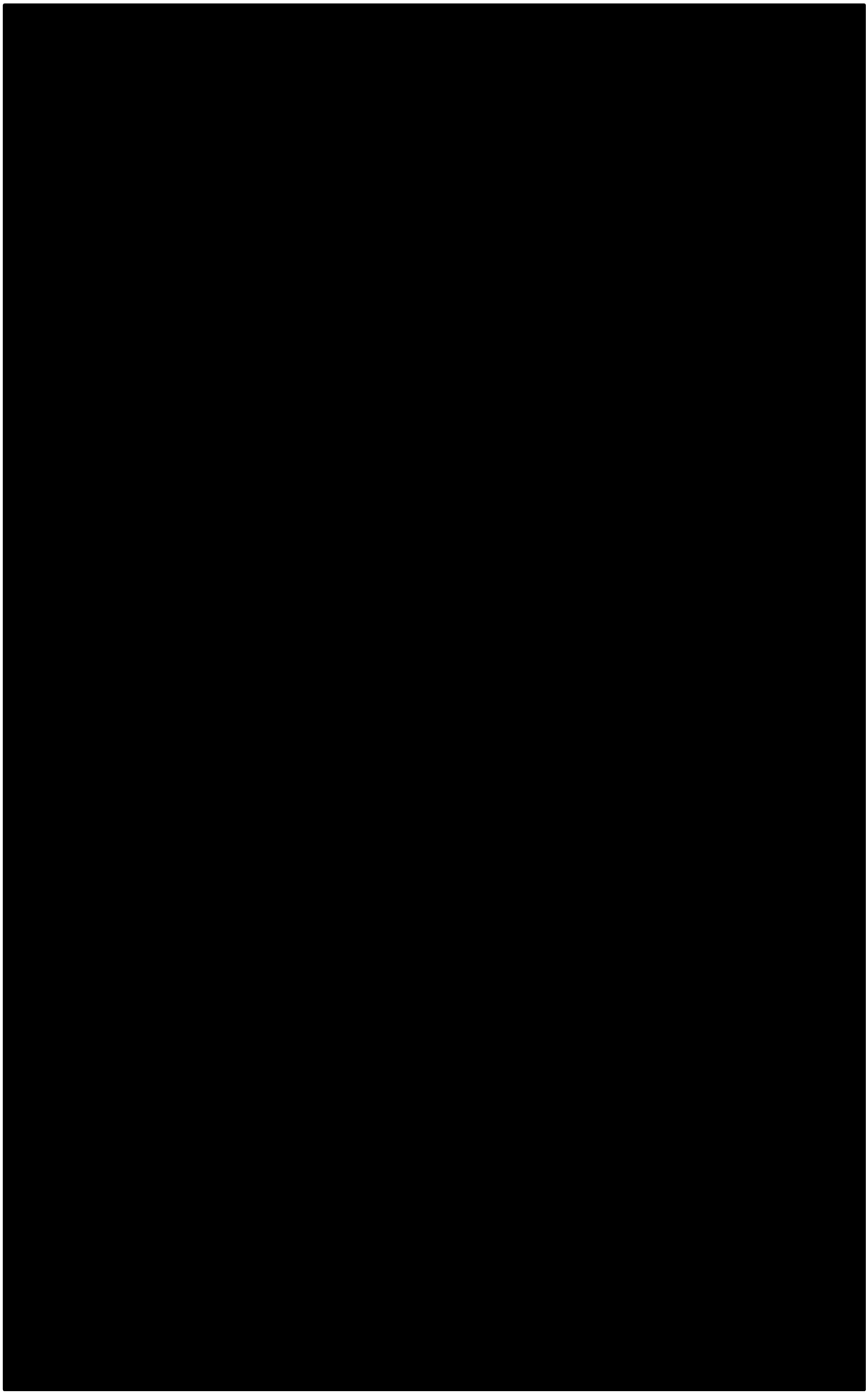


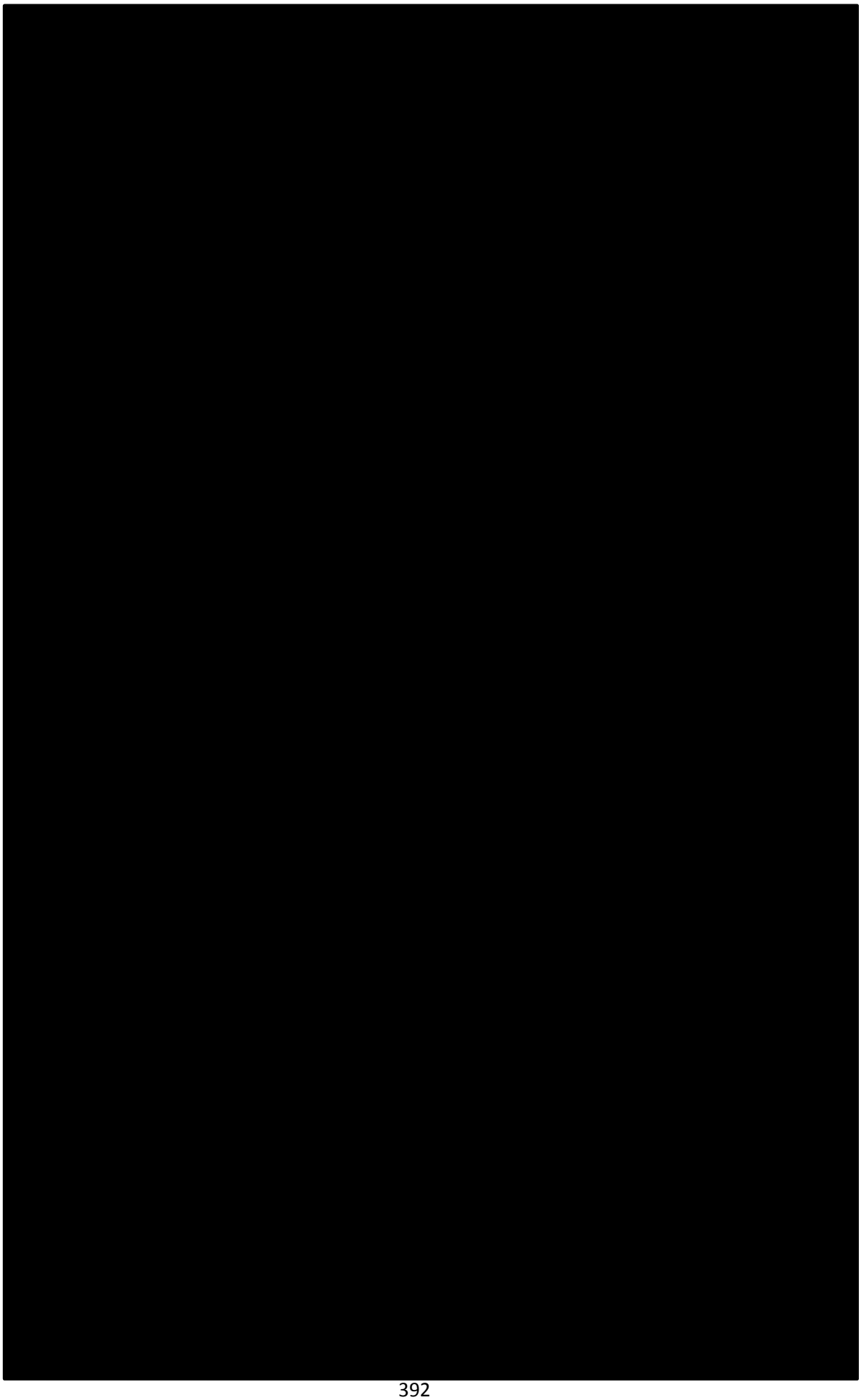


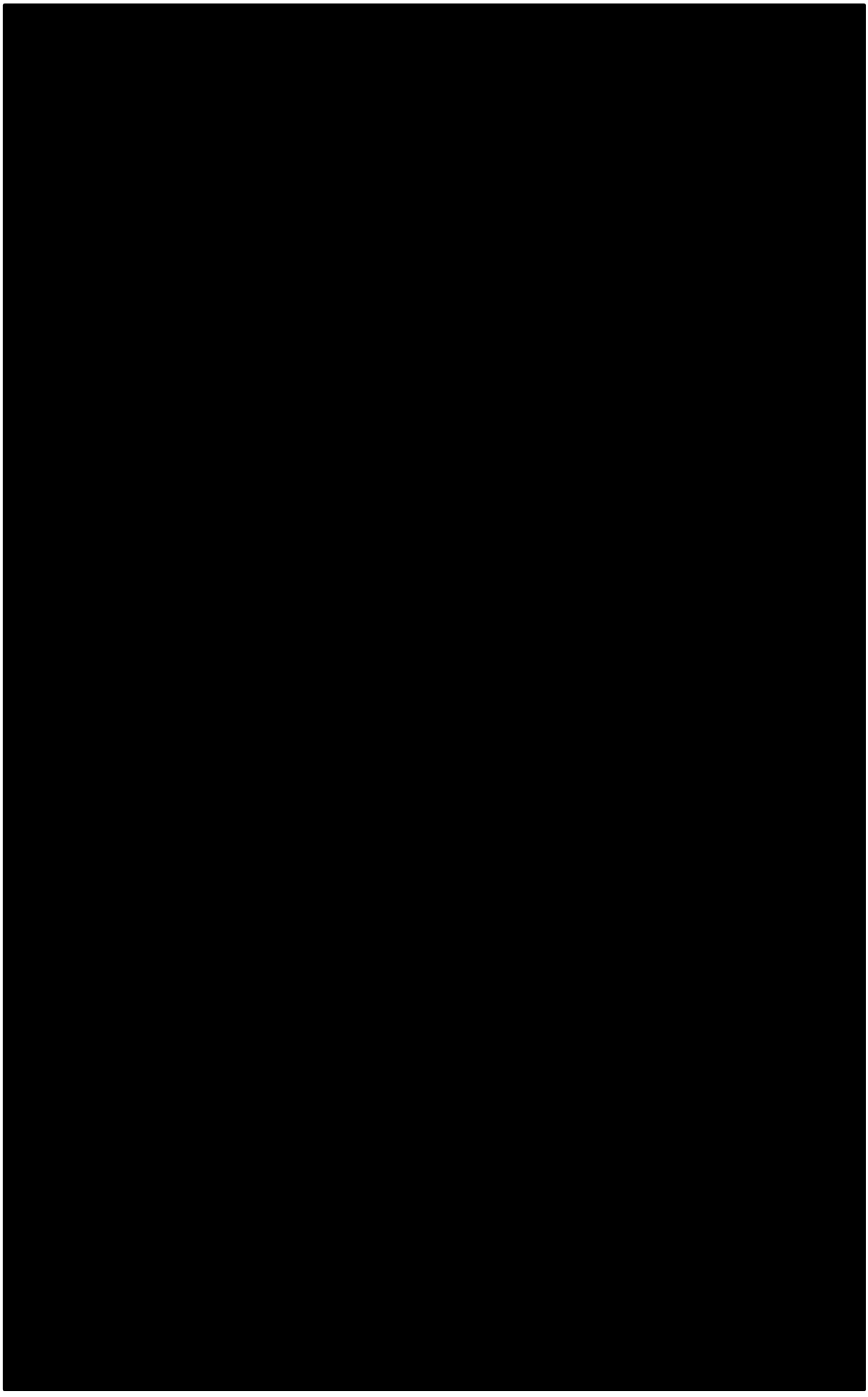


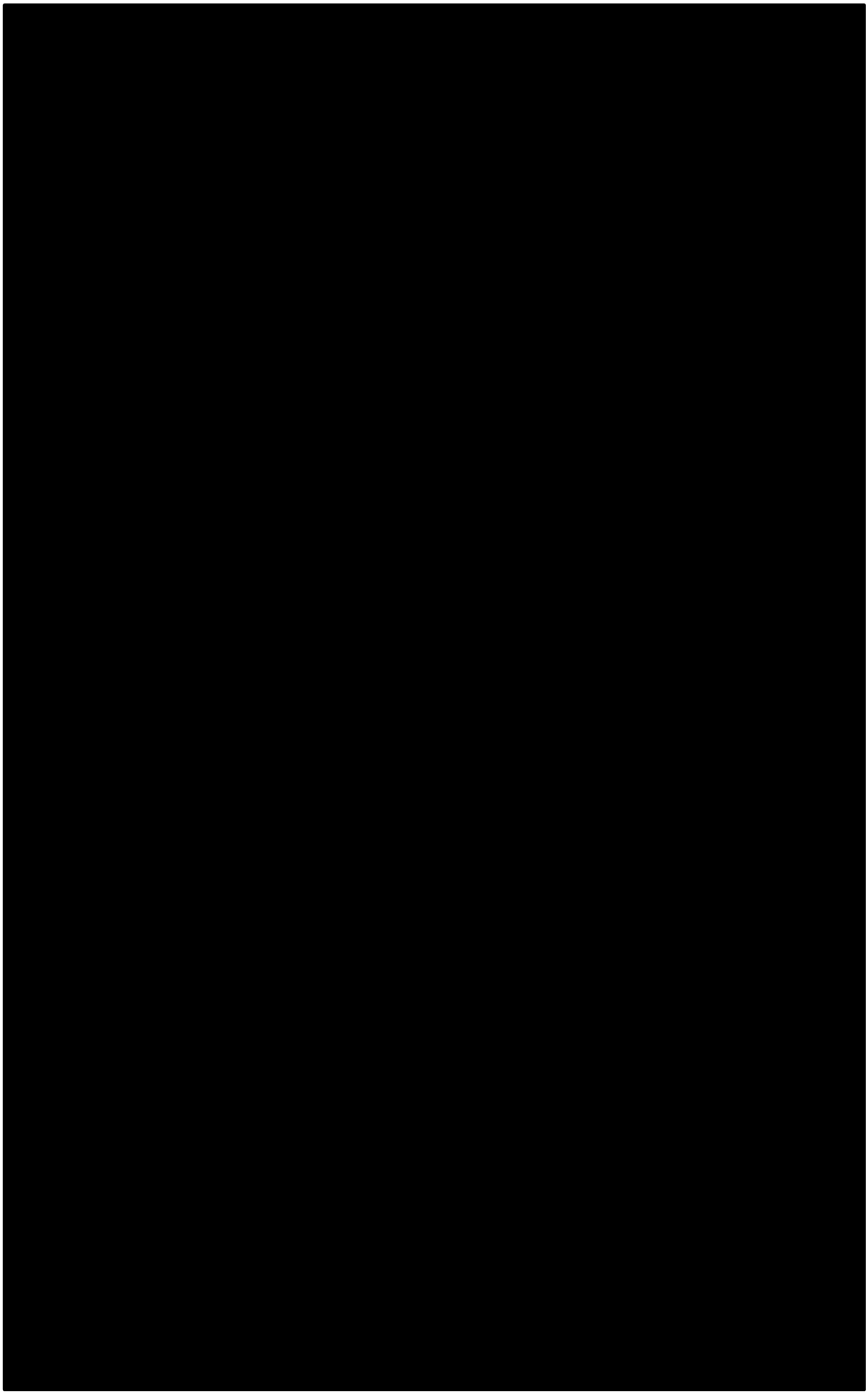


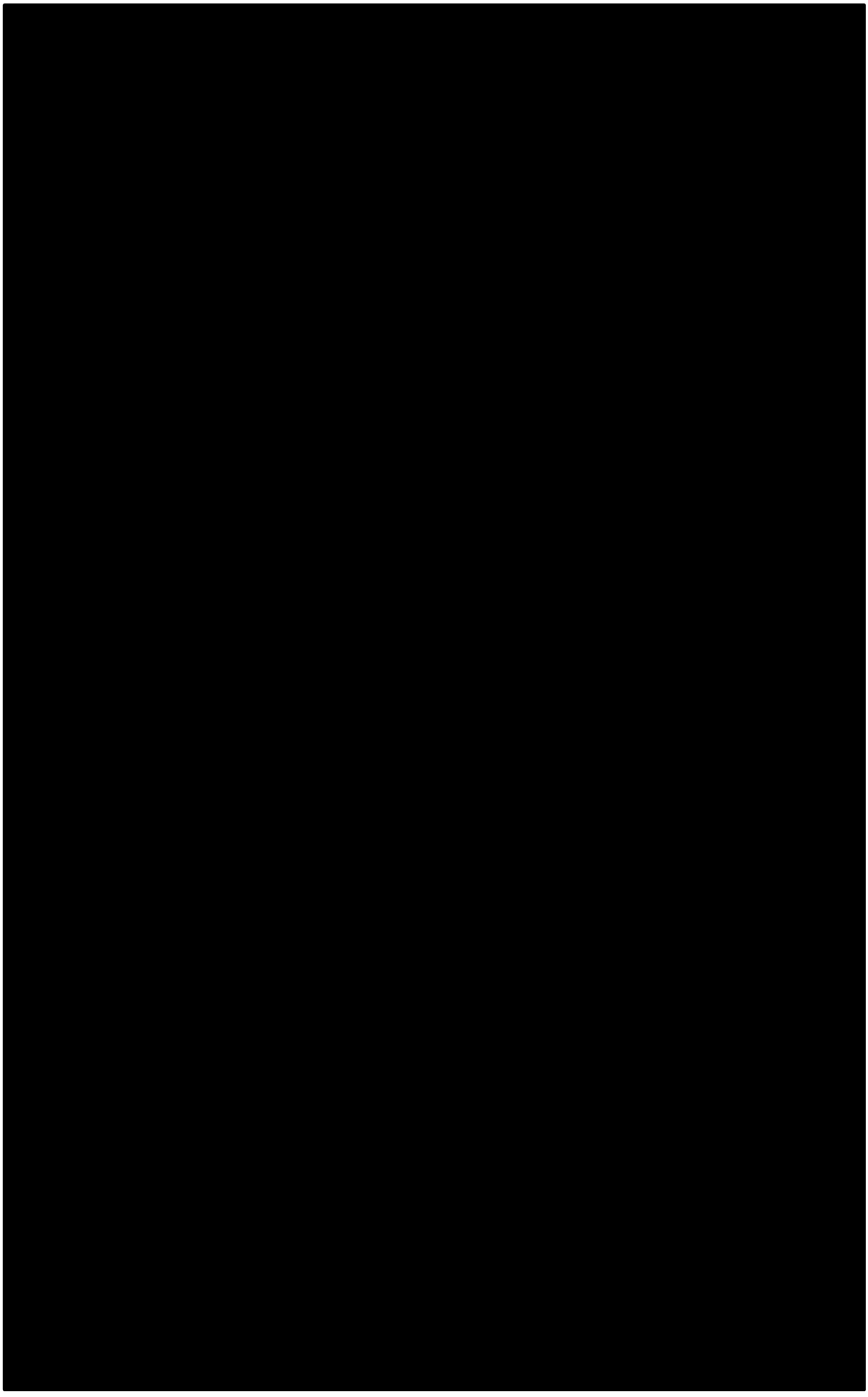


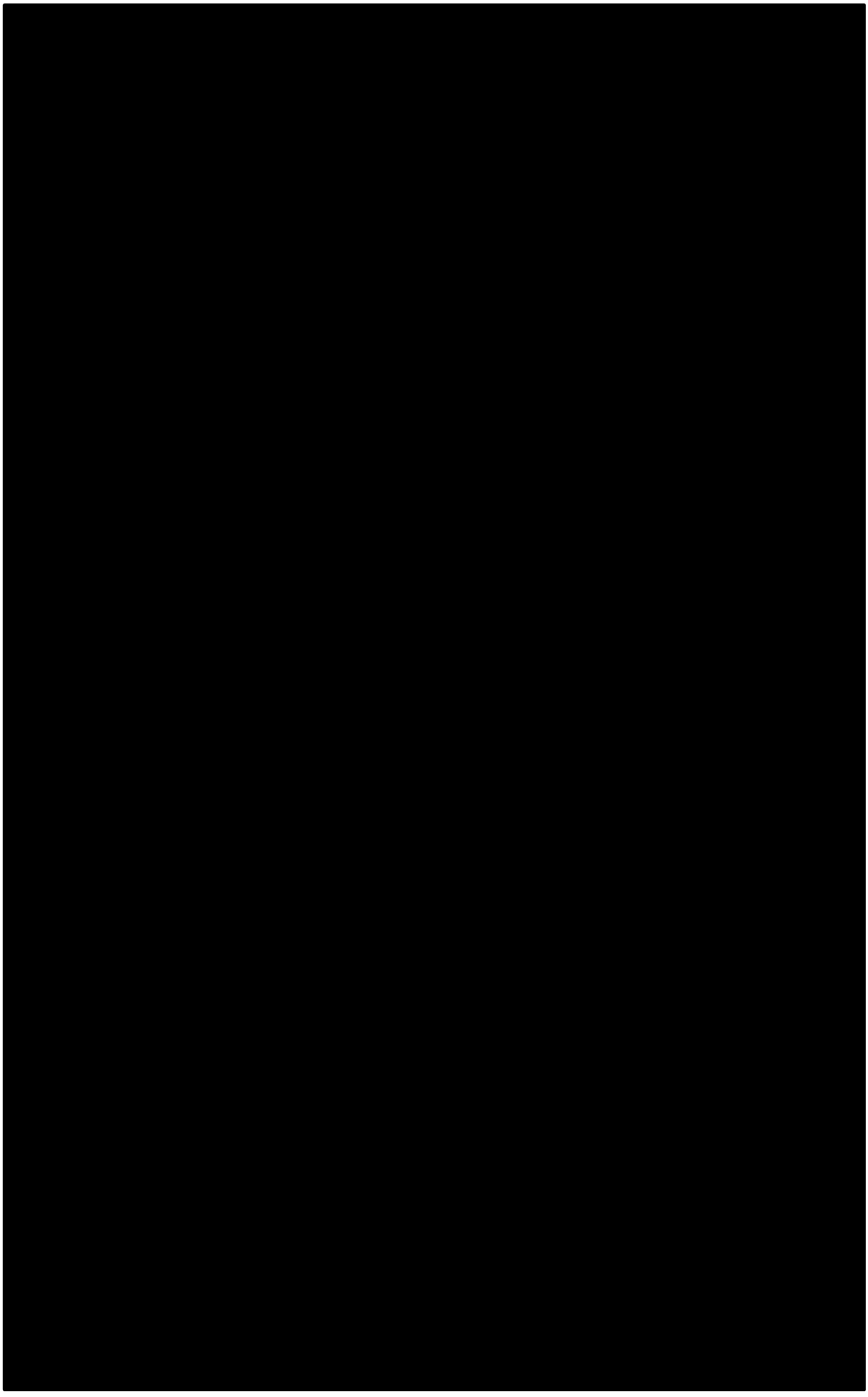


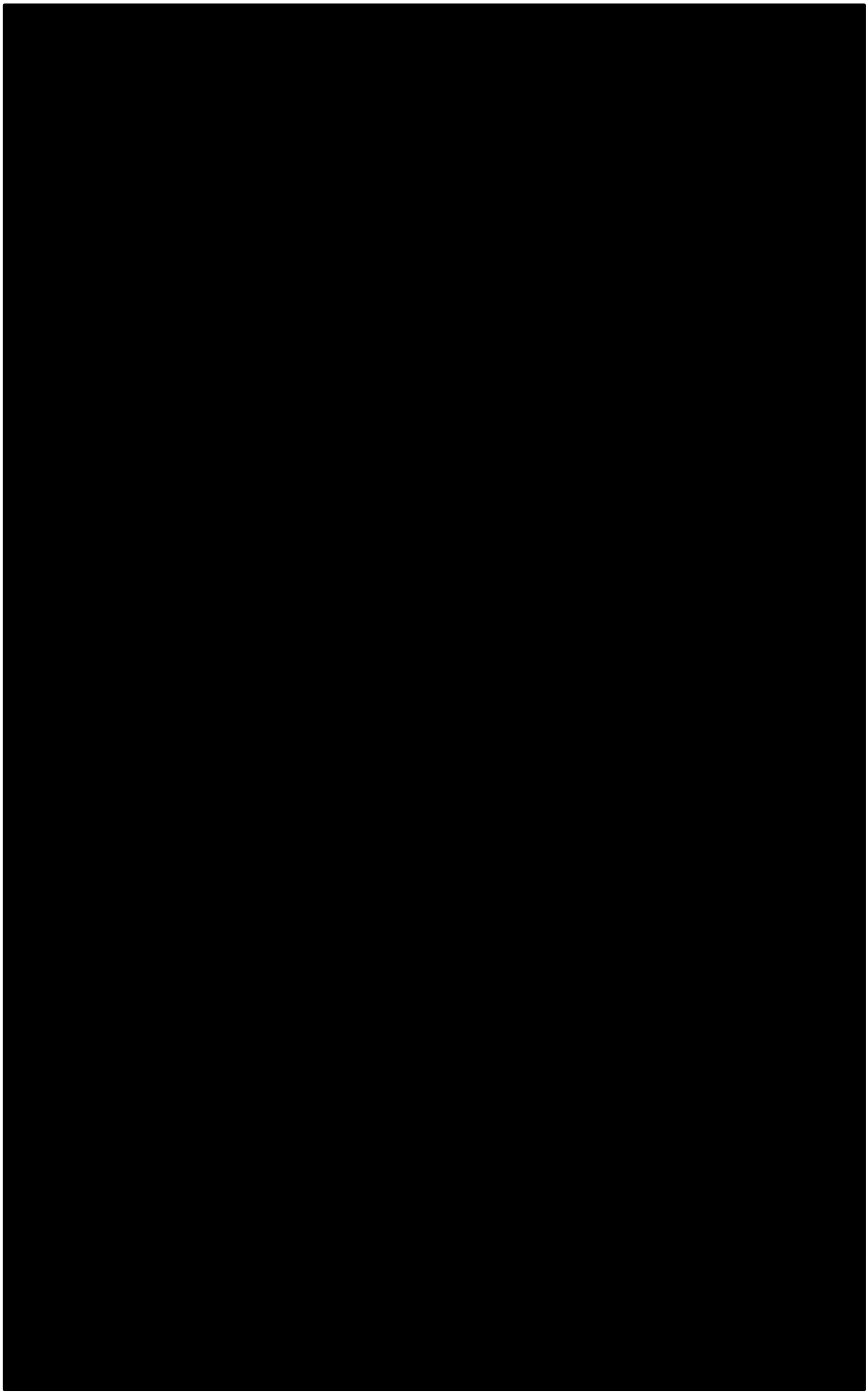


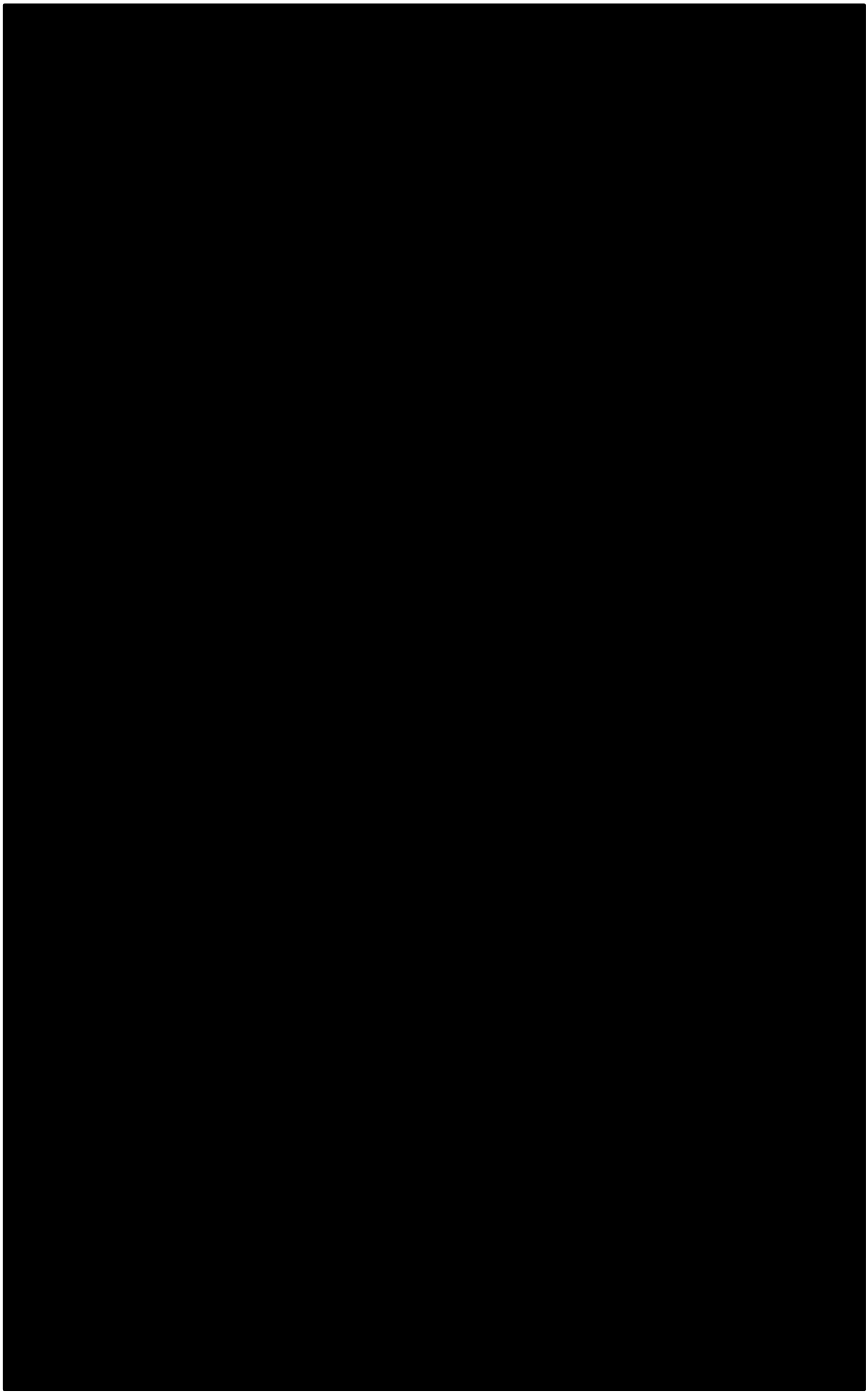


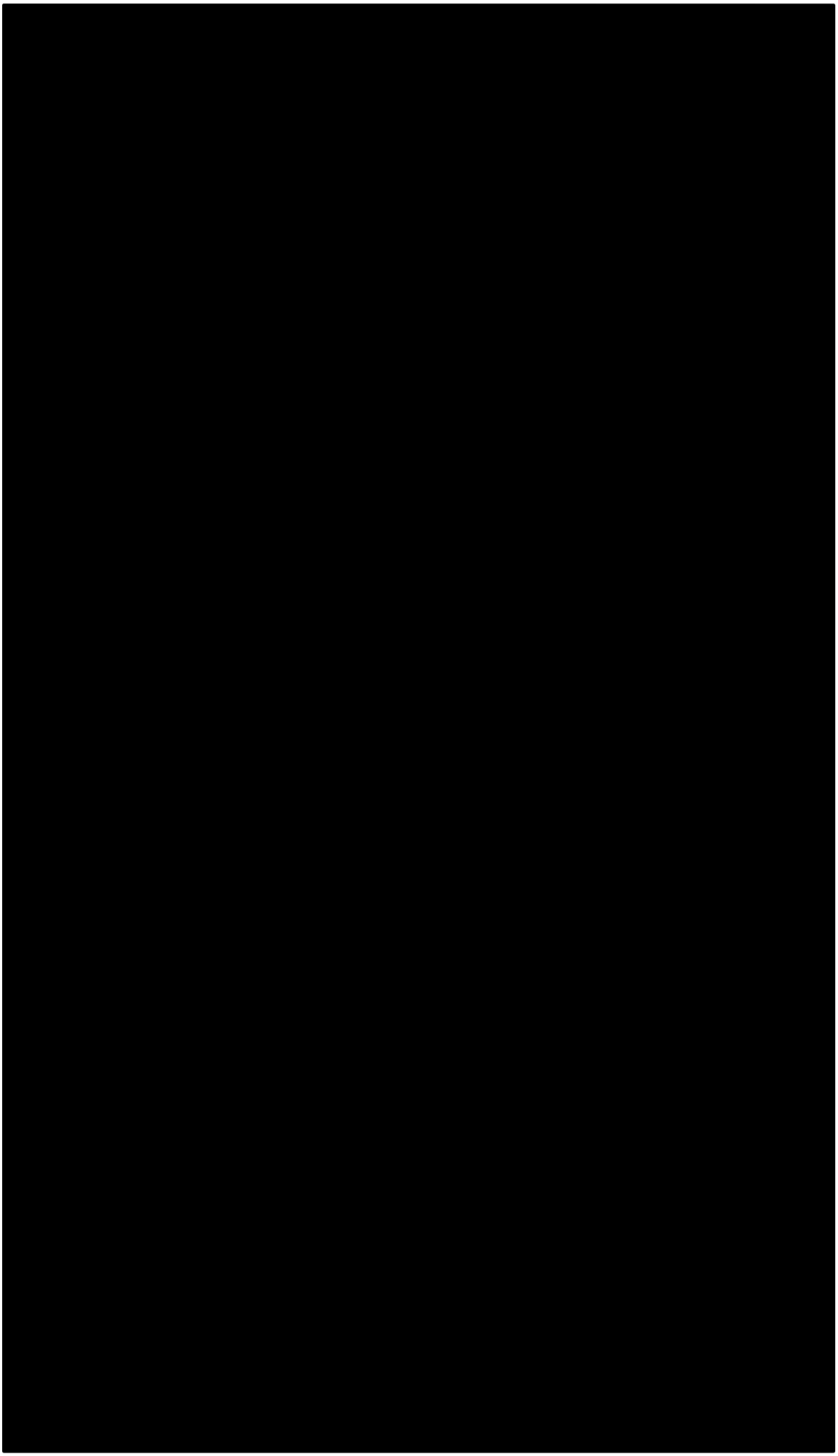


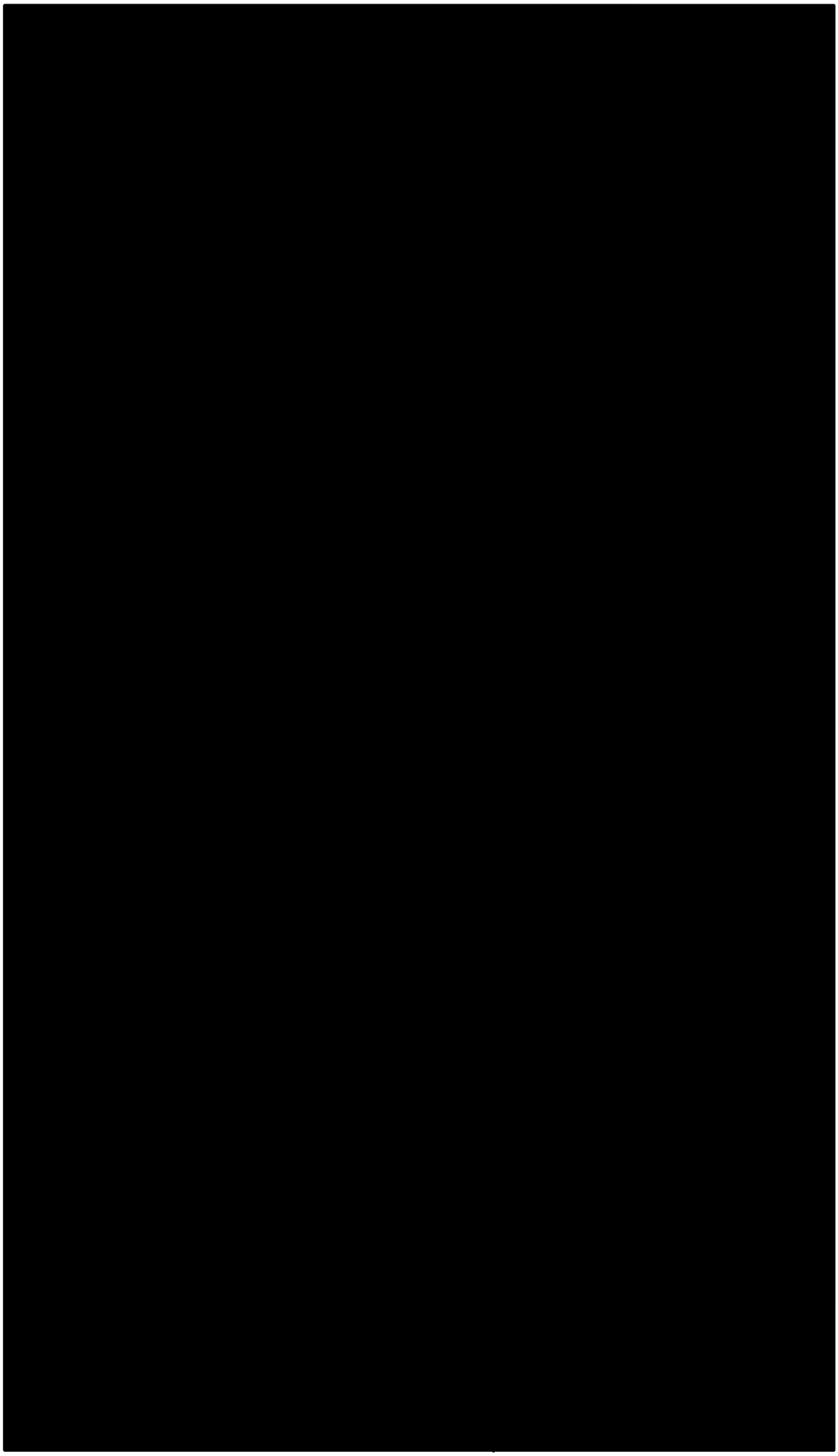




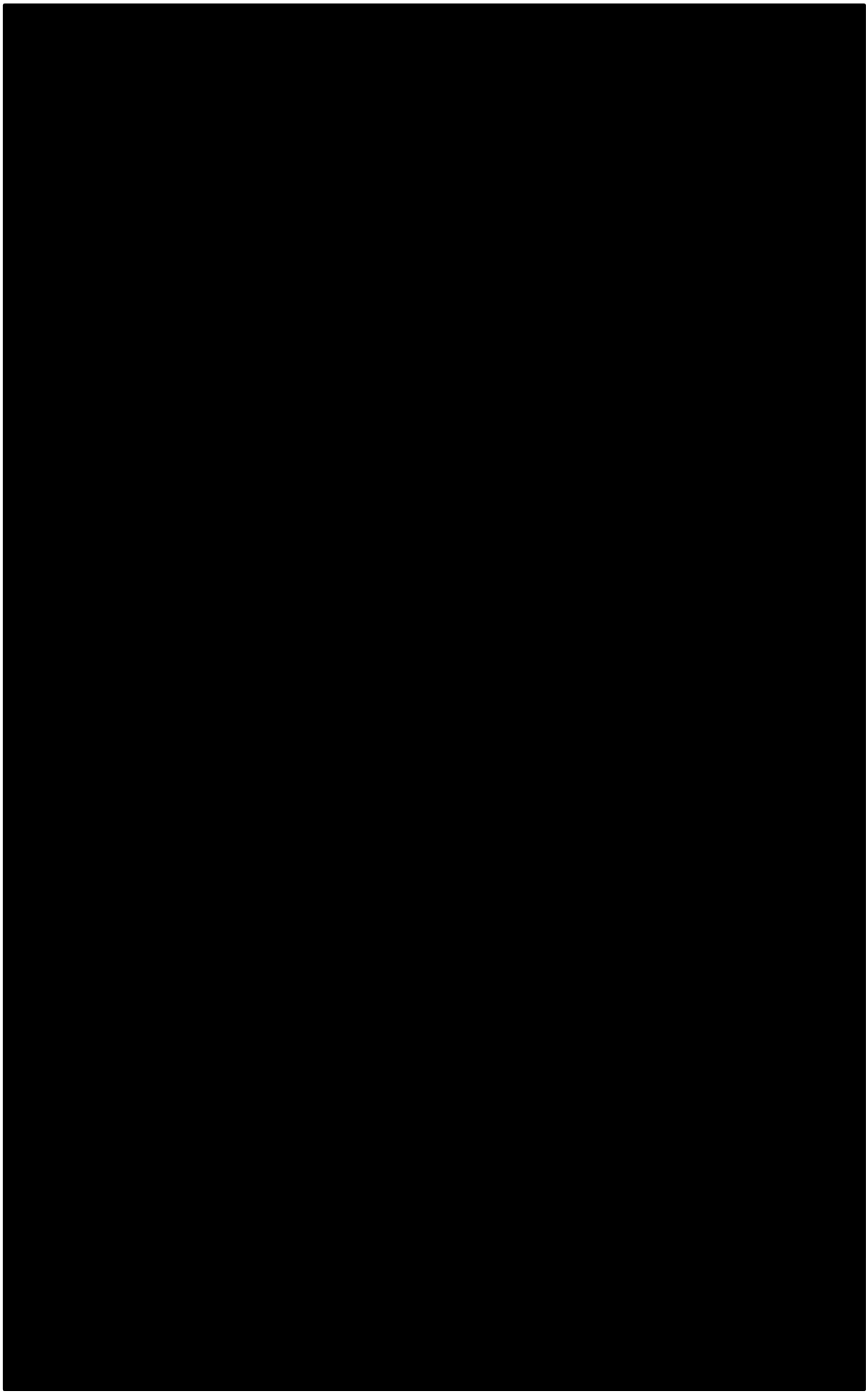


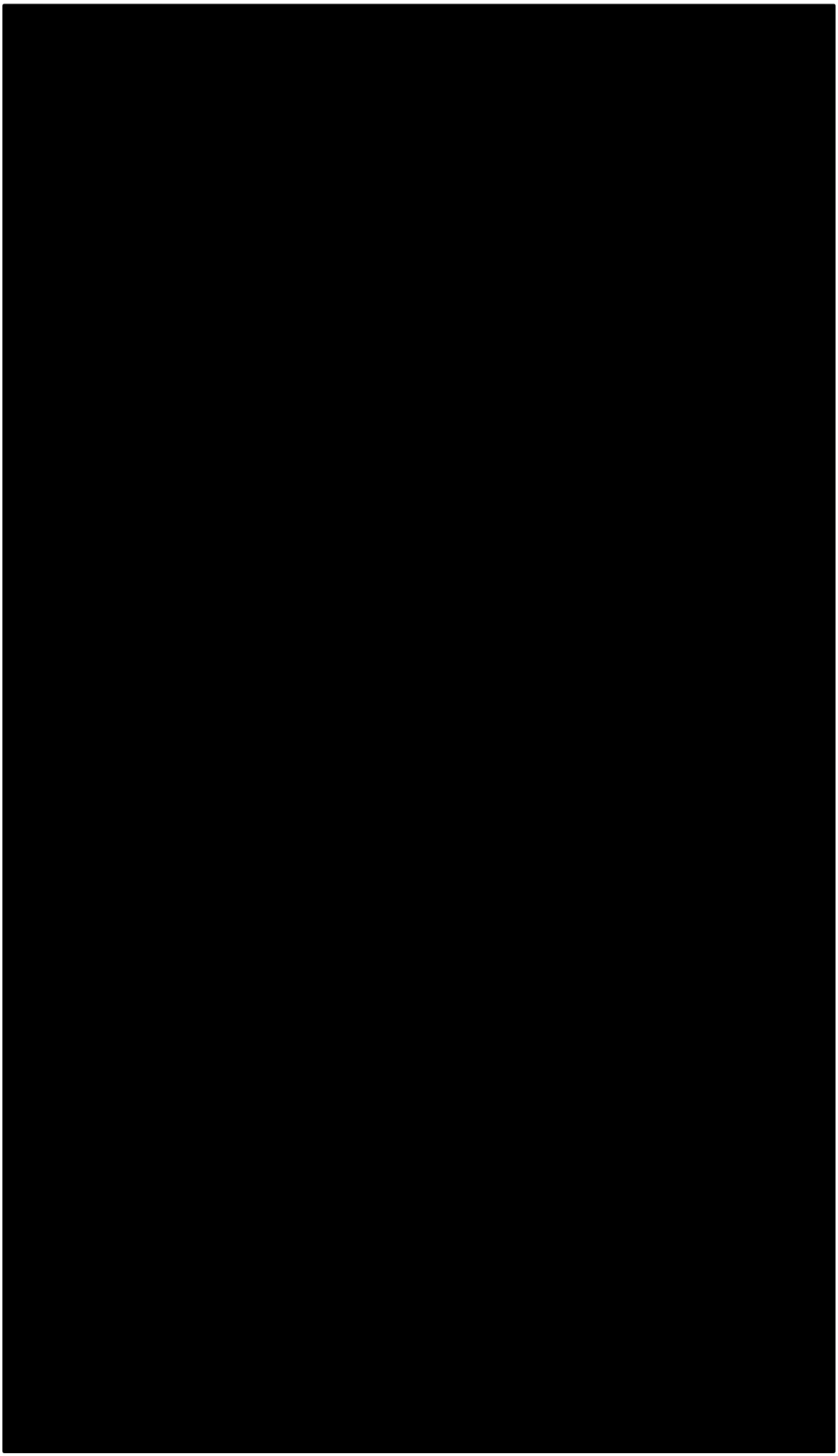


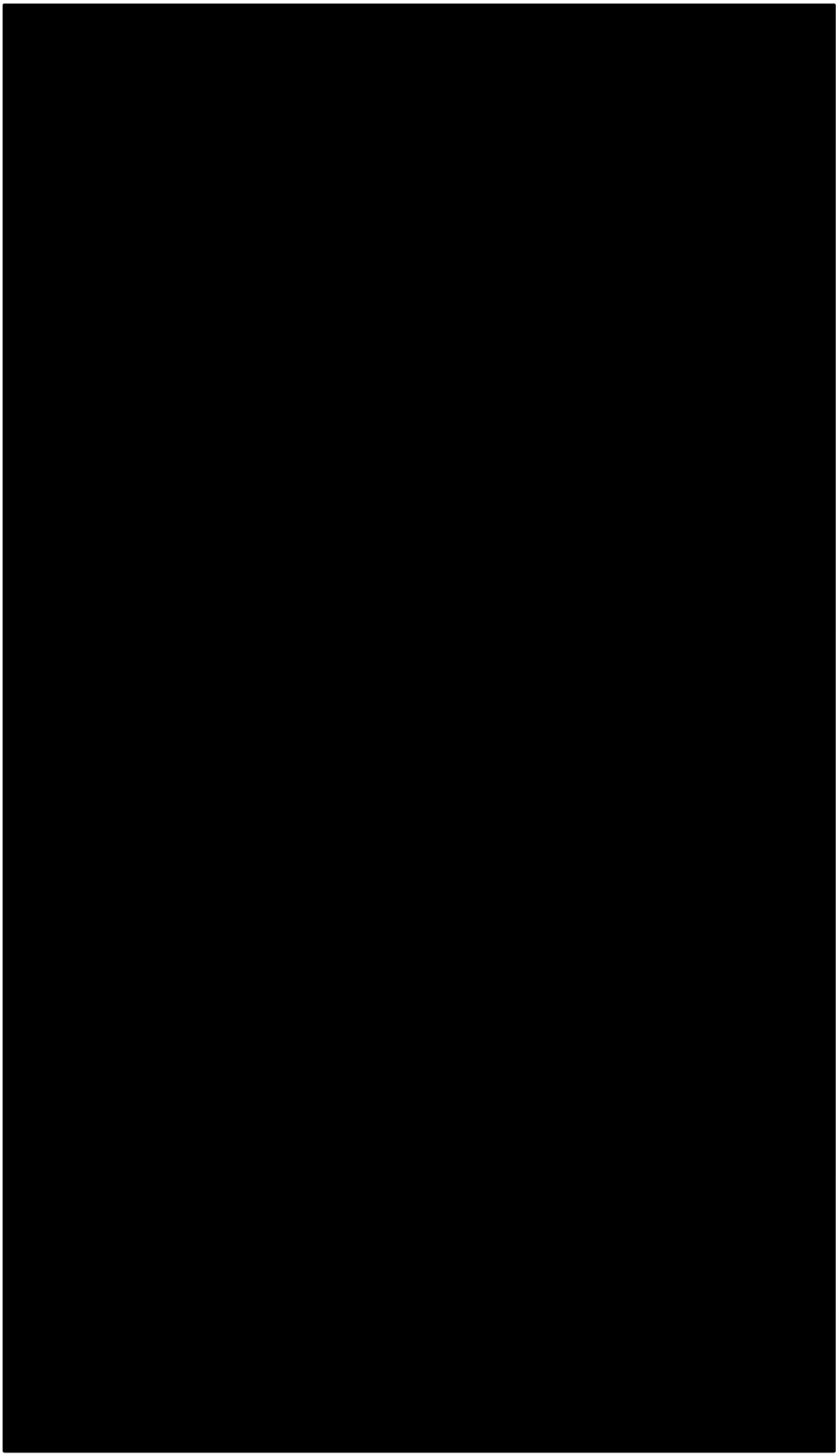


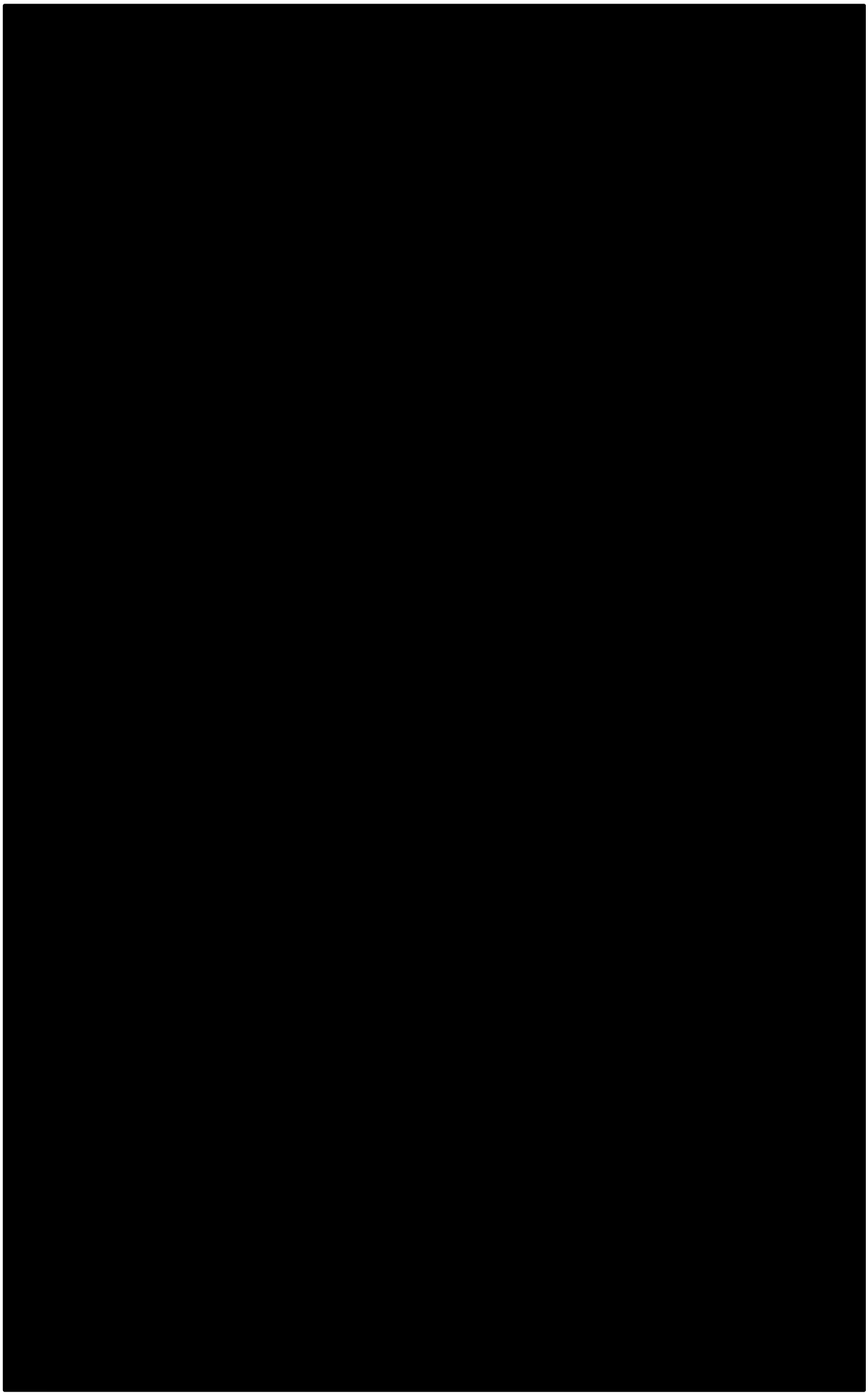


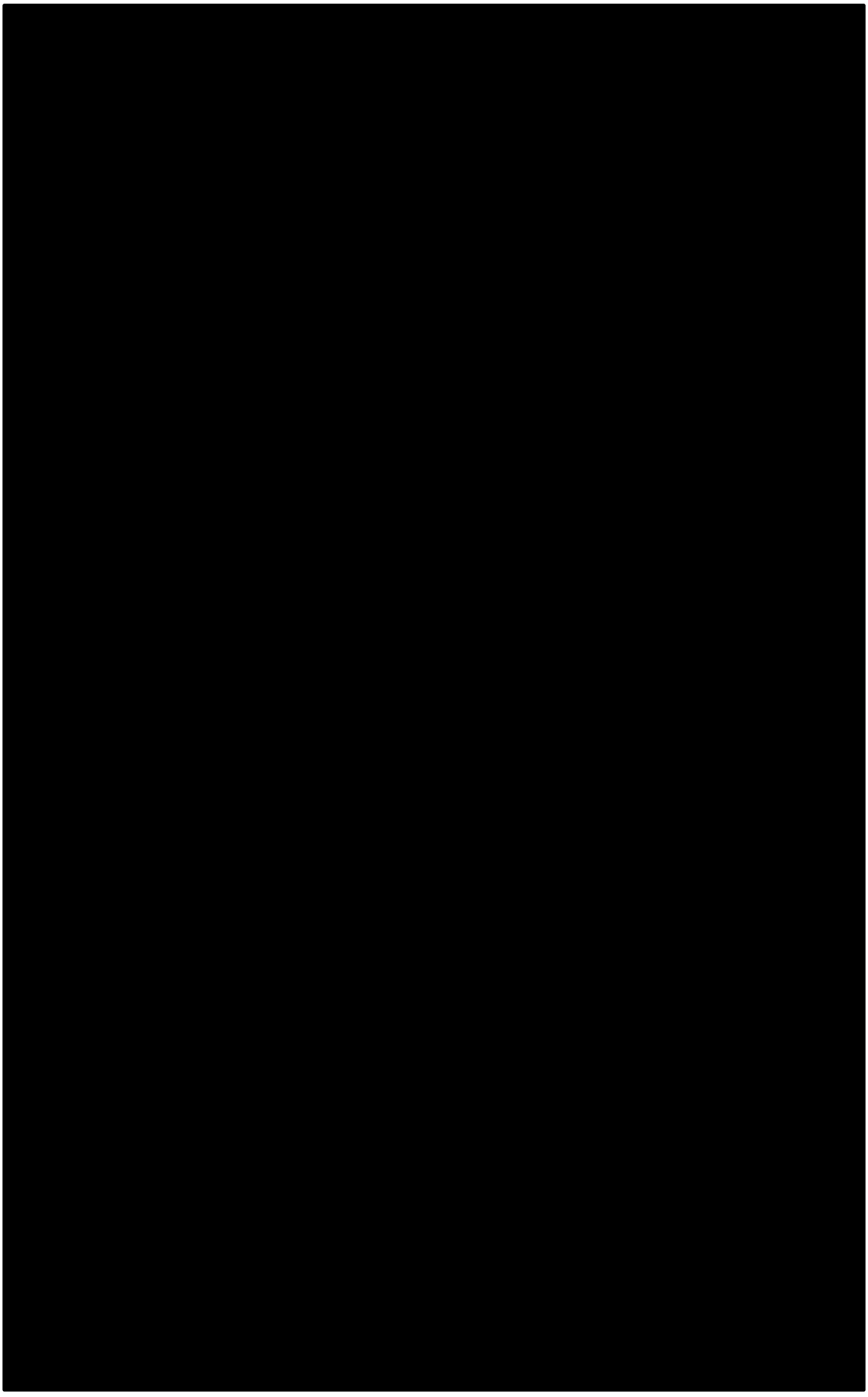
[redacted]

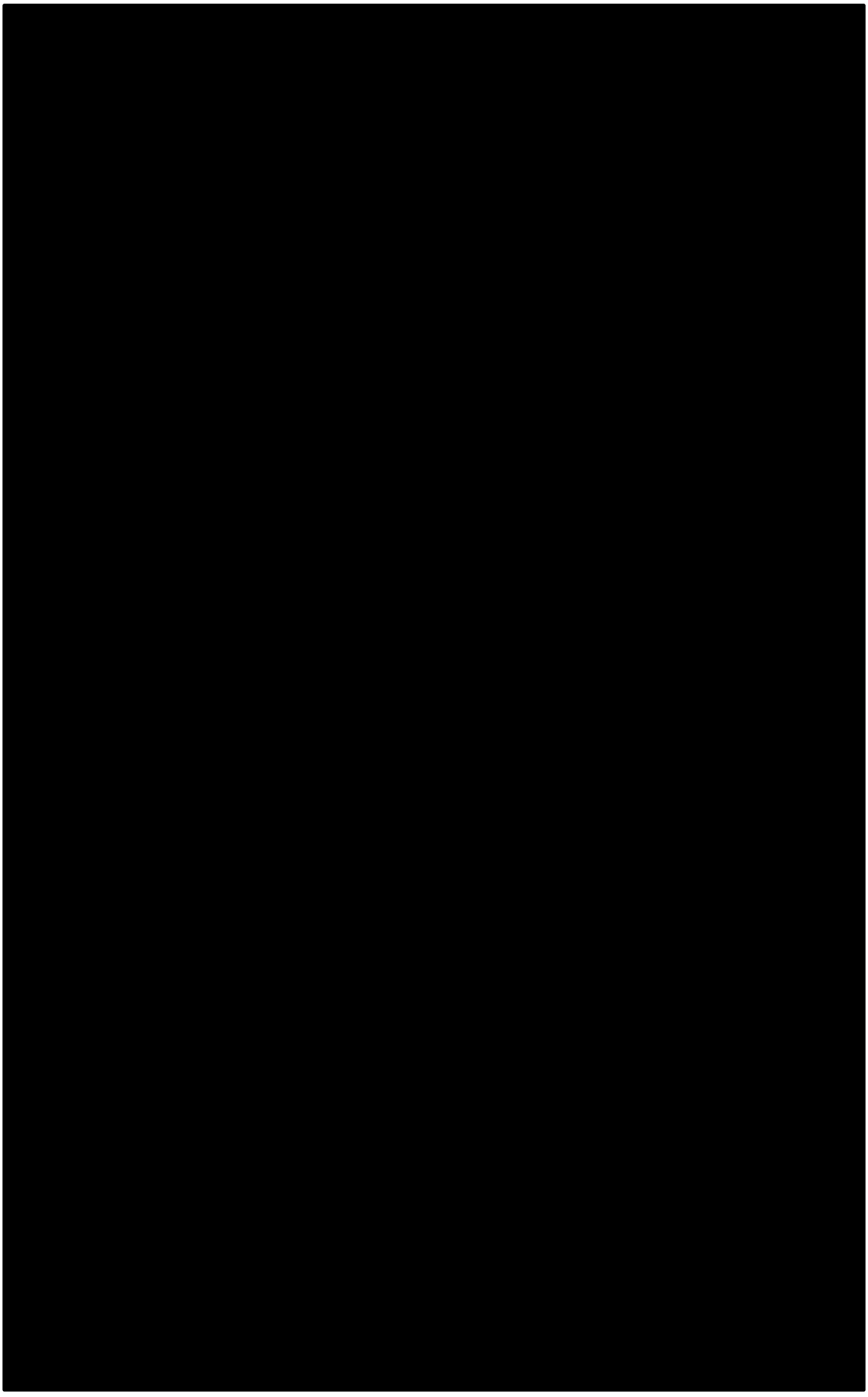




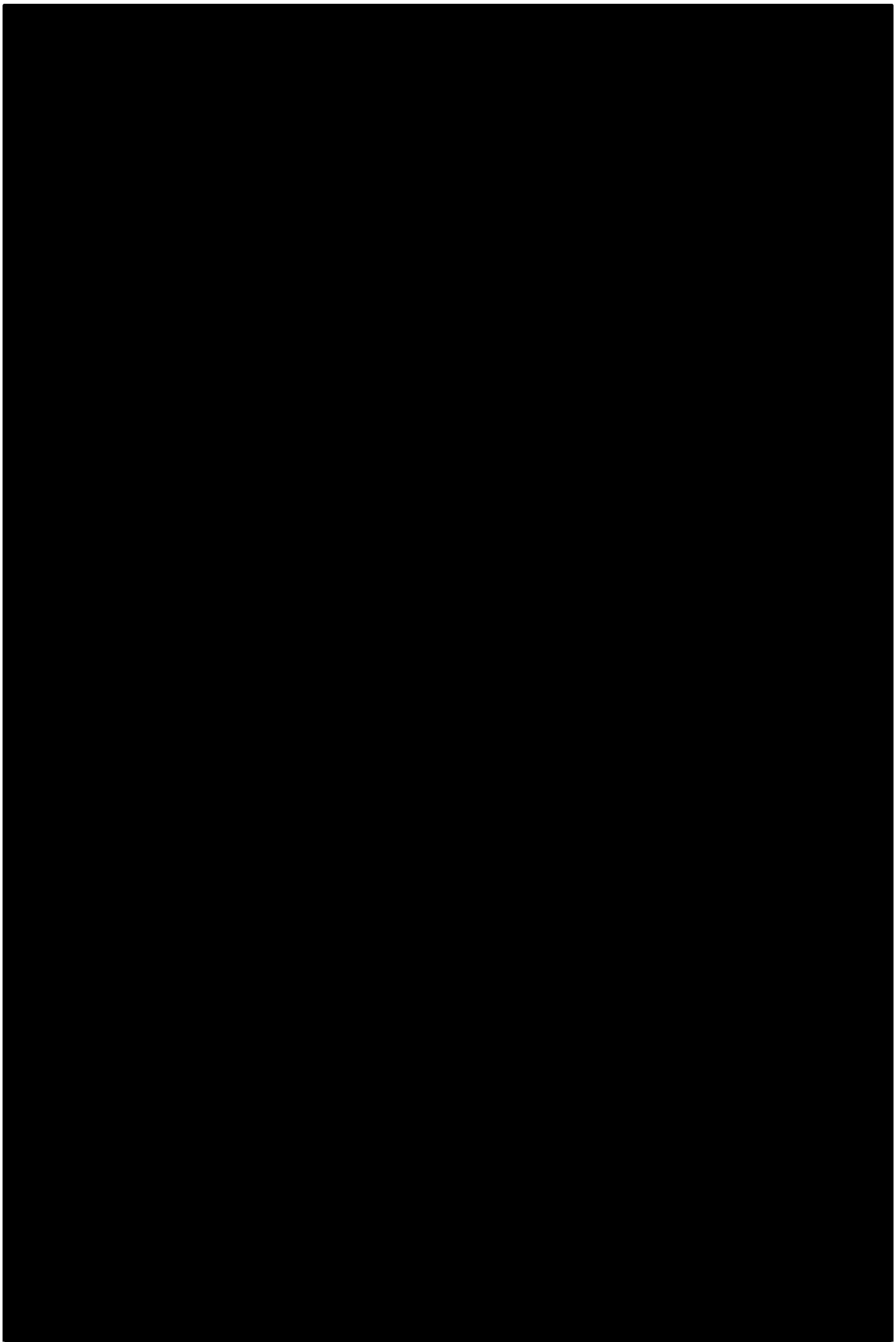


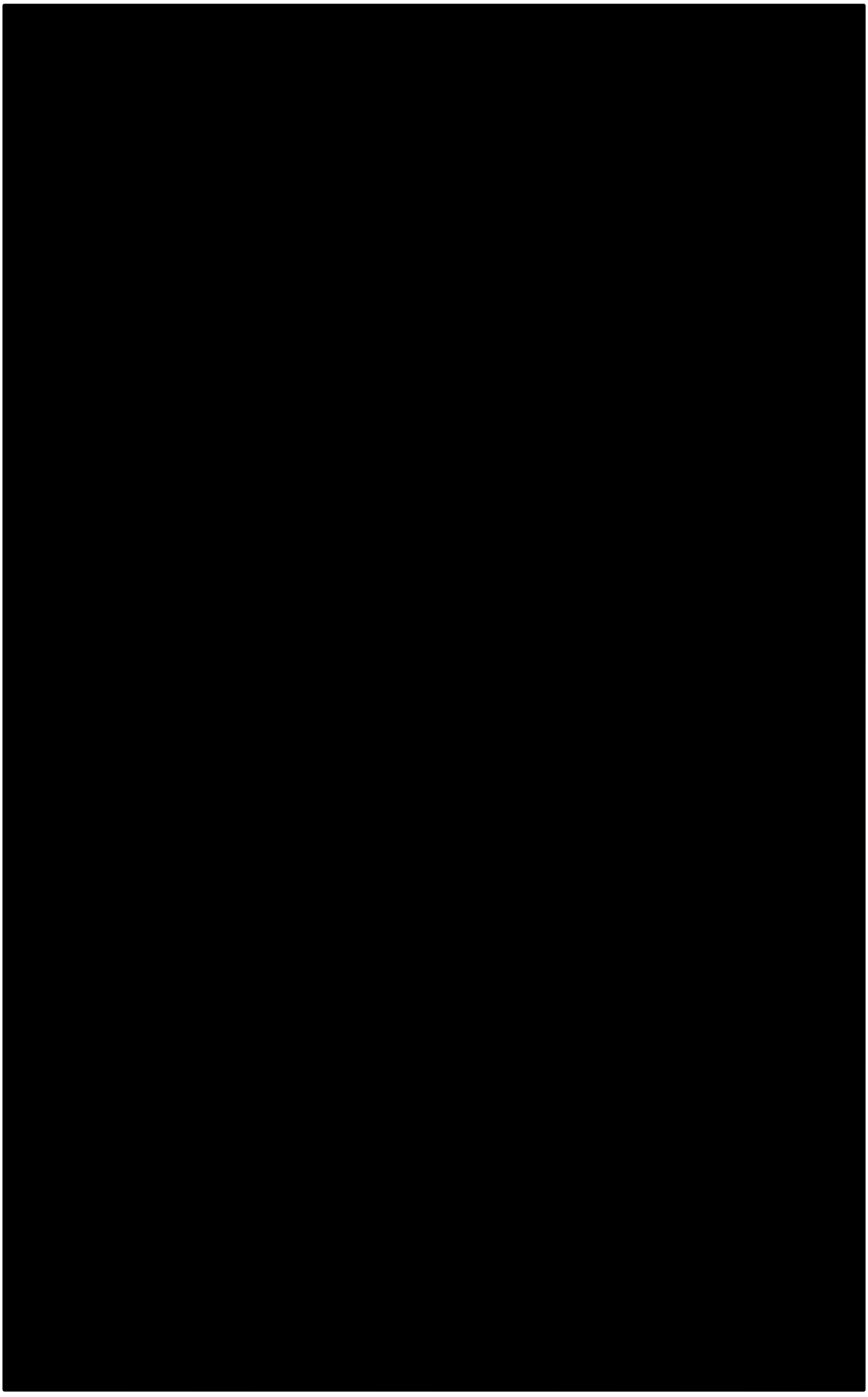


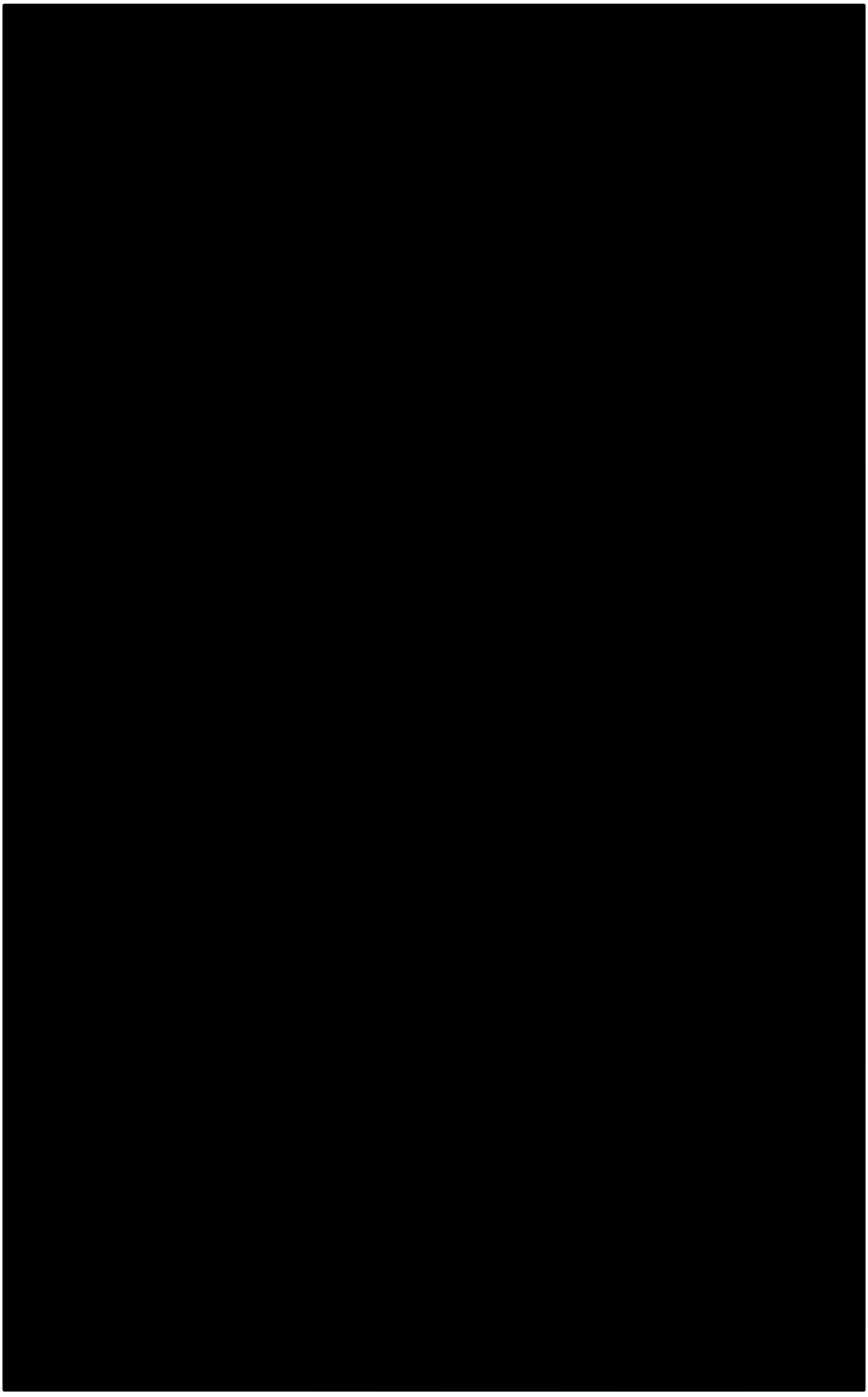


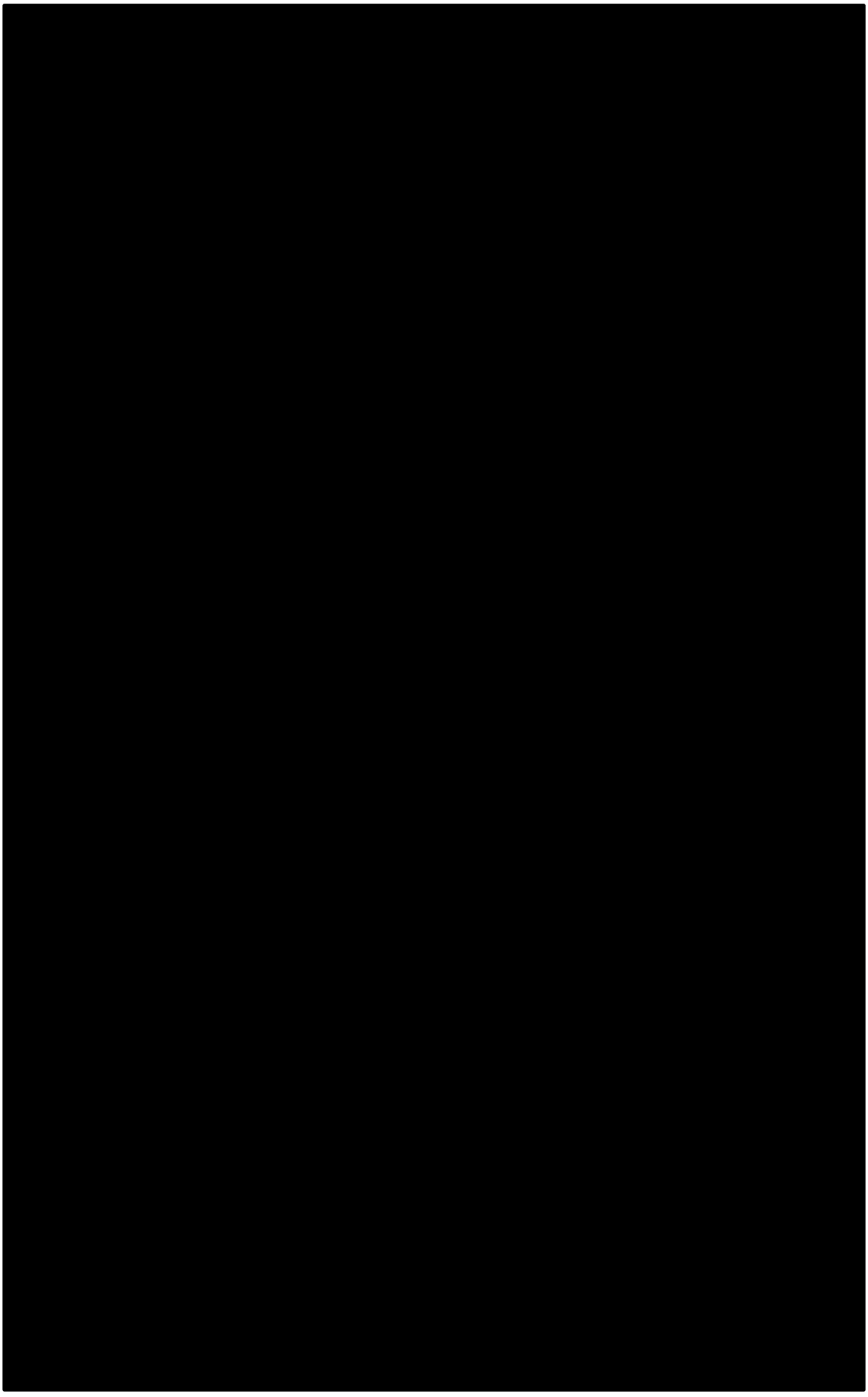


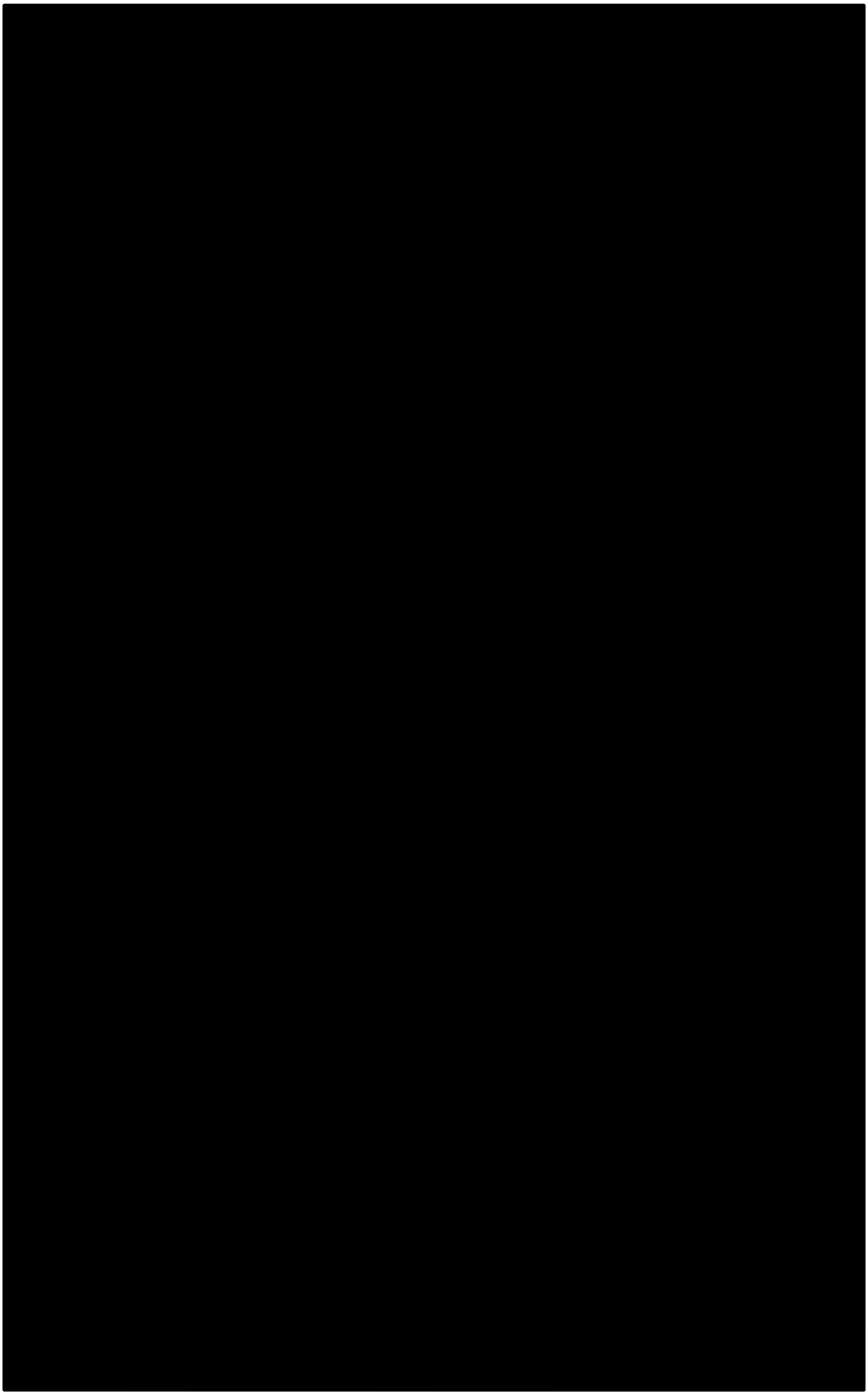
Appendix XI. Published manuscript “Predictive value of prostate specific antigen in a European HIV-positive cohort: does one size fit all?”

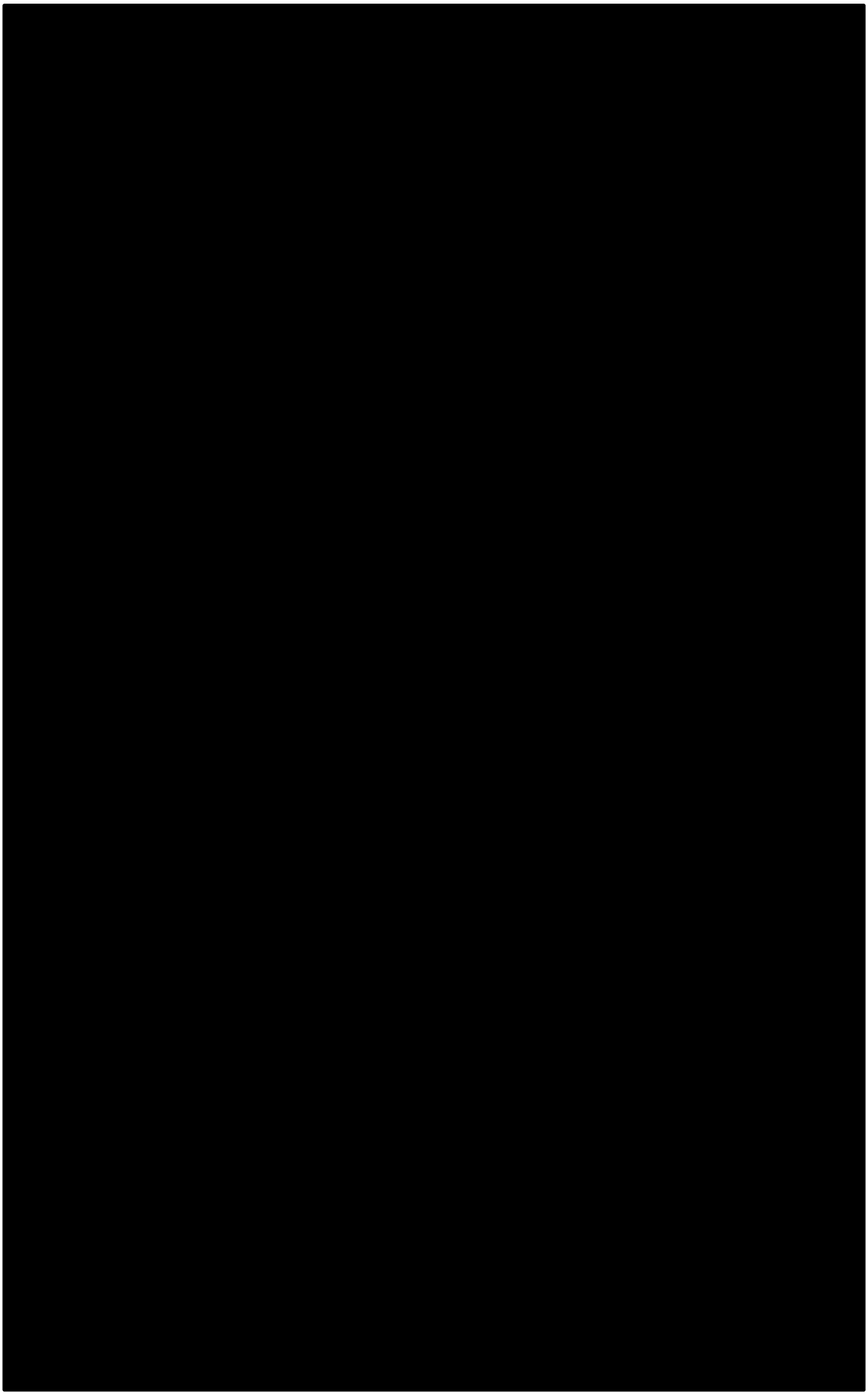




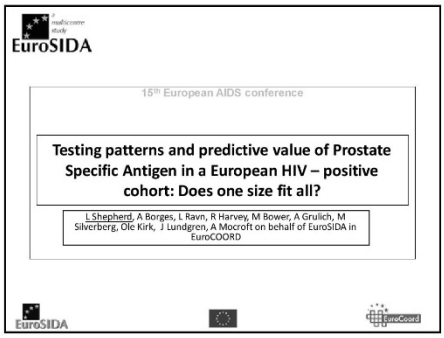


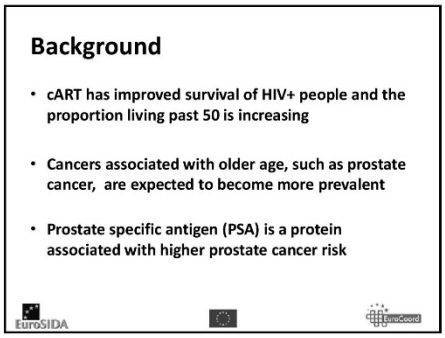


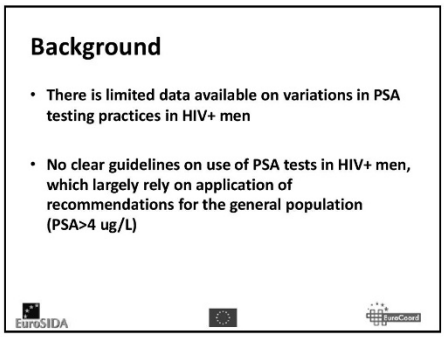


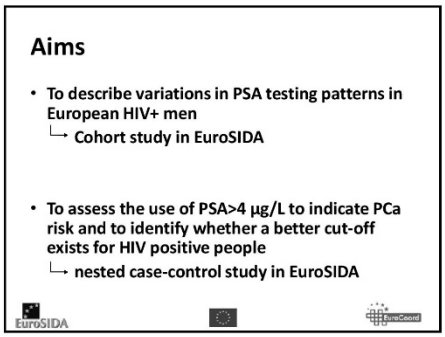


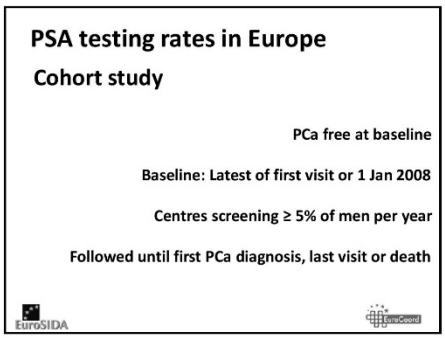
Appendix XII. EACS 2015 presentation entitled "Testing patterns and predictive value of prostate specific antigen in a European HIV-positive cohort: Does one size fit all?"

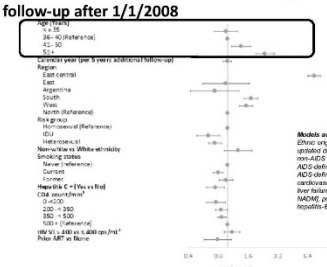












PSA testing rates: Baseline characteristics

Baseline N(%)/Median (IQR)	All Men	≥1 PSA test
Overall	4,482 (100)	1,318 (100)
Age (years)	41 (35,48)	44 (38,52)
Region		
East	694 (15)	302 (23)
Argentina	262 (6)	27 (2)
South	1,389 (31)	393 (30)
West	814 (18)	284 (22)
North	1,323 (30)	312 (24)
Risk group		
Homosexual	2,701 (60)	860 (65)
Heterosexual	561 (13)	141 (11)
IDU	925 (21)	242 (18)
Non-white ethnicity	348 (8)	78 (6)
Prior AIDS event	1,202 (27)	413 (31)
Prior Non-AIDS event*	198 (4)	66 (5)
Prior ART	3,917 (87)	1,205 (91)
CD4 cells/mm^3	510 (360,702)	519 (368,720)
HIV-viral load copies/ml	<49 (<39,<59)	<49 (<39,<49)

*Non-AIDS defining events: pancreatitis, grade 3 or 4 hepatic encephalopathy or liver-related death, myocardial infarction, stroke, coronary artery bypass graft, coronary angioplasty, carotid endarterectomy (grouped together as serious CV events), and end stage renal disease

Adjusted incidence rate ratios of receiving PSA testing durin follow-up after 1/1/2008

Models additionally adjusted for:
Ethnic origin, BMI at baseline, and time-updated diagnoses of AIDS defining and non-AIDS malignancies [ADM], NADM, AIDS-defining [excluding ADM], and non-AIDS-defining events [defined as cardiovascular, end-stage renal disease, liver failure and pancreatitis, excluding NADM], prior hypertension, prior and hepatitis-B infection

Adjusted incidence rate ratios of receiving PSA testing durin follow-up after 1/1/2008

Models additionally adjusted for:
Ethnic origin, BMI at baseline, and time-updated diagnoses of AIDS defining and non-AIDS malignancies [ADM], NADM, AIDS-defining [excluding ADM], and non-AIDS-defining events [defined as cardiovascular, end-stage renal disease, liver failure and pancreatitis, excluding NADM], prior hypertension, prior and hepatitis-B infection

Adjusted incidence rate ratios of receiving PSA testing durin follow-up after 1/1/2008

Models additionally adjusted for:
Ethnic origin, BMI at baseline, and time-updated diagnoses of AIDS defining and non-AIDS malignancies [ADM], NADM, AIDS-defining [excluding ADM], and non-AIDS-defining events [defined as cardiovascular, end-stage renal disease, liver failure and pancreatitis, excluding NADM], prior hypertension, prior and hepatitis-B infection

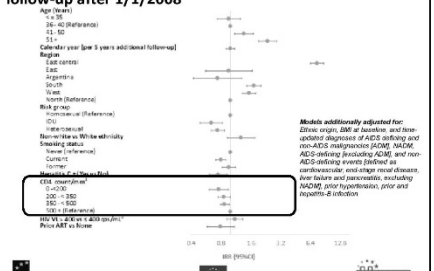
Adjusted incidence rate ratios of receiving PSA testing durin follow-up after 1/1/2008

Models additionally adjusted for:
Ethnic origin, BMI at baseline, and time-updated diagnoses of AIDS defining and non-AIDS malignancies [ADM], NADM, AIDS-defining [excluding ADM], and non-AIDS-defining events [defined as cardiovascular, end-stage renal disease, liver failure and pancreatitis, excluding NADM], prior hypertension, prior and hepatitis-B infection

Adjusted incidence rate ratios of receiving PSA testing durin follow-up after 1/1/2008

Models additionally adjusted for:
Ethnic origin, BMI at baseline, and time-updated diagnoses of AIDS defining and non-AIDS malignancies [ADM], NADM, AIDS-defining [excluding ADM], and non-AIDS-defining events [defined as cardiovascular, end-stage renal disease, liver failure and pancreatitis, excluding NADM], prior hypertension, prior and hepatitis-B infection

Adjusted incidence rate ratios of receiving PSA testing during follow-up after 1/1/2008



2. To assess the use of PSA>4 µg/L to indicate PCa risk and to identify whether a better cut-off exists for HIV positive people

Optimal PSA cut off

Nested case control study

Optimal PSA cut off

Nested case control study

Cases

Prostate cancer

After 1 Jan 2001

Prior plasma sample

Optimal PSA cut off

Nested case control study

Cases/ Controls

No prostate cancer

After 1 Jan 2001

Prior plasma sample

Optimal PSA cut off

Nested case control study

Cases/controls

1st sample date ± 2years

Last sample date ± 2years

Matched

Age (1st sample) ± 10 years

CD4 (1st sample) ±200 cells/mm^3

Region of Europe

Optimal PSA cut off

Nested case control study

Cases/controls — **Total PSA (tPSA)**

Matched

Samples

Optimal PSA cut off

EuroSIDA
Men with follow-up >1 January 2001
9,112

Nested case control study

Optimal PSA cut off

EuroSIDA
Men with follow-up >1 January 2001
9,112

Controls N=40

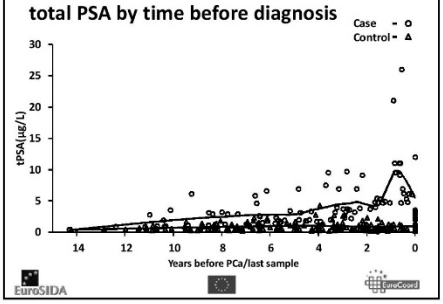
Prostate cancers N=21

Nested case control study

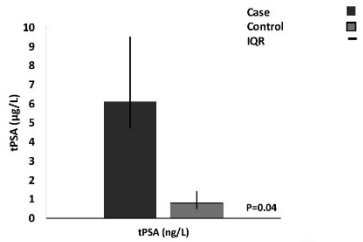
Baseline Characteristics (first sample)

Factors	Total	Prostate cancer		P
		Cases	Controls	
	N (%)			
Total	61 (100.0)	21 (100.0)	40 (100.0)	-
Risk group				
Homosexual	47 (77.0)	17 (81.0)	30 (75.0)	0.98
Heterosexual	7 (11.5)	2 (9.5)	5 (12.5)	
IDU	2 (3.3)	0 (0.0)	2 (5.0)	
Non White ethnicity	4 (6.6)	0(0.0)	4(10.0)	0.99
Prior NADM	2 (3.3)	2 (9.5)	0(0.0)	0.99
Priorr ADM	6 (9.8)	0(0.0)	6 (15.0)	0.99
On cART	58 (95.1)	20 (95.2)	38 (95.0)	1.00
	Median (IQR)			
Age	51 (48,57)	52 (49,57)	51 (47,56)	0.18
CD4 count (cells/mm^3)	437 (243,610)	460 (260,610)	426 (230,595)	0.07
log_{10} HIV VL (copies/ml)	1.9 (1.6,2.6)	1.9 (1.6,2.6)	2.0 (1.6,2.6)	0.40

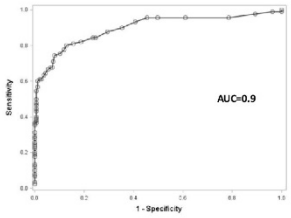
total PSA by time before diagnosis



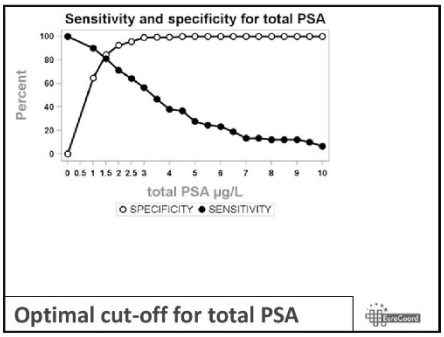
Median latest total PSA in cases and controls



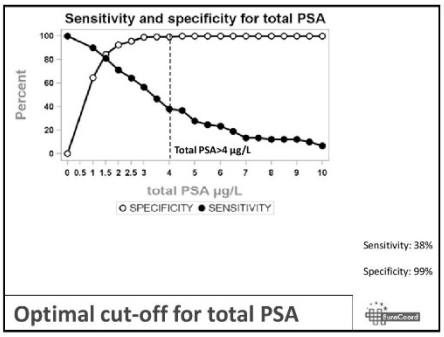
ROC curve and Area Under the Curve for total PSA



EuroSIDA



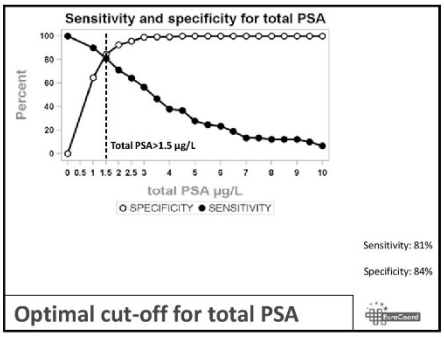
Optimal cut-off for total PSA



Sensitivity: 38%

Specificity: 99%

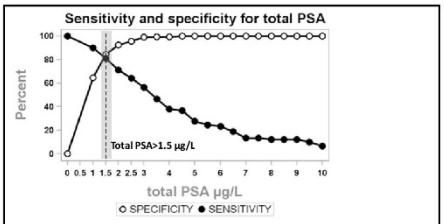
Optimal cut-off for total PSA



Sensitivity: 81%

Specificity: 84%

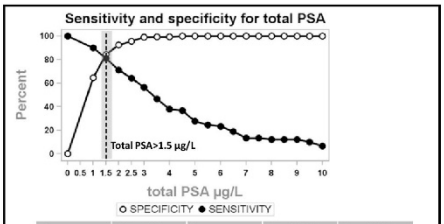
Optimal cut-off for total PSA



Sensitivity: 81%

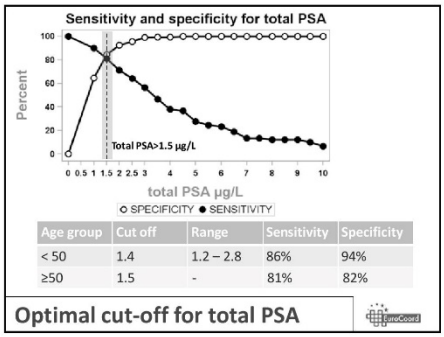
Specificity: 84%

Optimal cut-off for total PSA



Age group	Cut off	Range	Sensitivity	Specificity
< 50	1.4	1.2 – 2.8	86%	94%

Optimal cut-off for total PSA



Age group	Cut off	Range	Sensitivity	Specificity
< 50	1.4	1.2 – 2.8	86%	94%
≥50	1.5	-	81%	82%

Optimal cut-off for total PSA

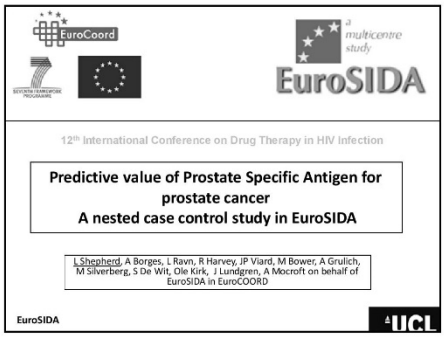
Limitations

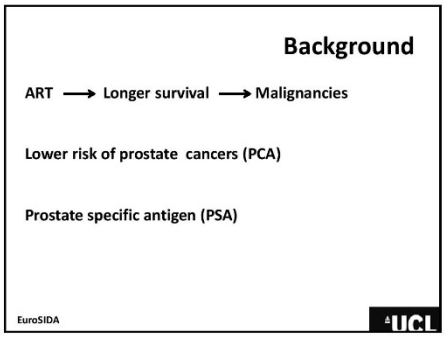
- PSA testing not reported by all centres and under reported
- Reason for PSA testing unknown
- Small number of prostate cancers
- Observational study

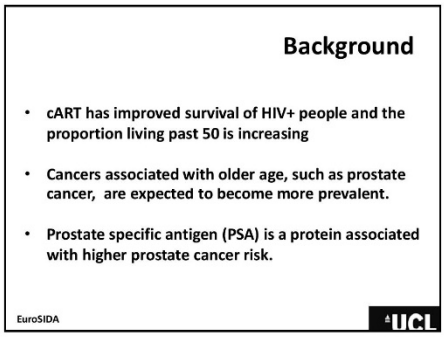
Conclusions

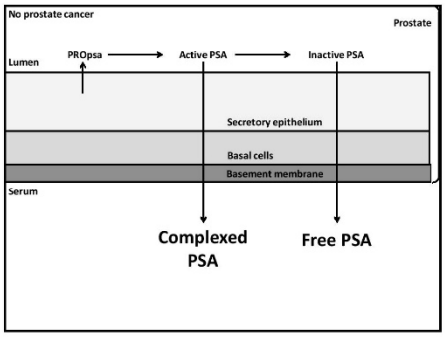
- PSA testing in HIV+ men varied in clinics across Europe, and was particularly high in older men
- Total PSA>4µg/L to indicate high PCa risk was not sensitive in HIV+ men
- Use of the lower cut-off of PSA>1.5µg/L should be considered
- Clear guidelines on the role of PSA in PCa screening and management for HIV+ men are needed

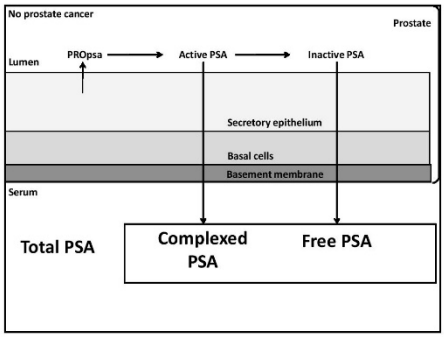
Appendix XIII. Internatinal confrerence on drug therapy in HIV infection presentation entitled “Predictive value of prostate specific antigen for prostate cancer: A nested case control study in EuroSIDA”

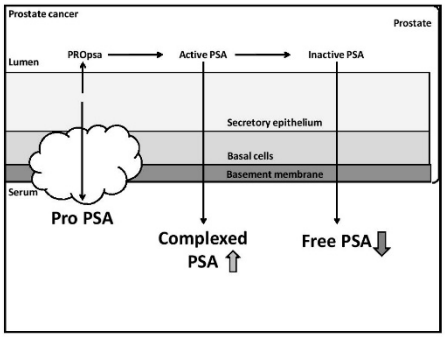












Background

ART ⟶ Longer survival ⟶ Malignancies

Lower risk of prostate cancers (PCA)

Prostate specific antigen (PSA)

EuroSIDA

UCL

Aims

"What is the predictive value of PSA in HIV+ men?"

Changes in markers prior to prostate diagnosis

How well does elevated PSA predict future prostate cancer?

Appropriateness of PSA>4 µg/mL

EuroSIDA

UCL

Methods - EuroSIDA

EuroSIDA is a large prospective cohort with 18,794 patients from 108 clinics in 34 European countries, Israel and Argentina. Regularly collecting:

- CD4 counts, HIV viral loads
- Non-AIDS events (since 2001)
- Prospectively stored plasma samples.

EuroSIDA

UCL

UCL

Methods – Study design

Nested case control study

EuroSIDA

UCL

Methods – Study design

Nested case control study

Cases

EuroSIDA

UCL

Methods – Study design

Nested case control study

Cases

Prostate cancer

After 1 Jan 2001

Prior plasma sample

EuroSIDA

Methods – Study design

Nested case control study

Cases

No prostate cancer

Controls

After 1 Jan 2001

Prior plasma sample

EuroSIDA

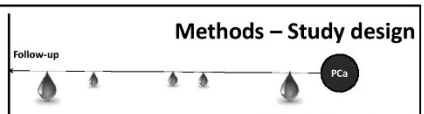
Methods – Study design

Nested case control study

Cases	1st sample date ± 2years
	Last sample date ± 2years
Controls	Age (1st sample) ± 10 years
Matched	CD4 (1st sample) ±200 cells/mm^3
	Region of Europe

EuroSIDA

Methods – Study design



Prostate cancer

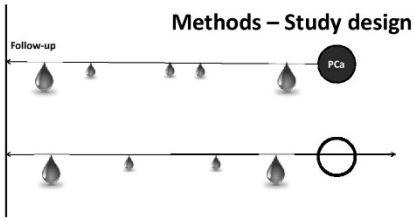
After 1 Jan 2001

Prior plasma sample

Nested case control study

EuroSIDA

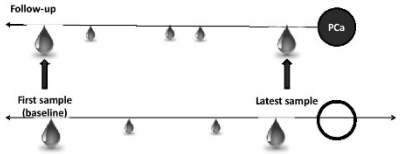
Methods – Study design



Nested case control study

EuroSIDA

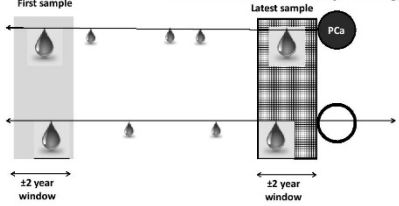
Methods – Study design



Nested case control study

EuroSIDA

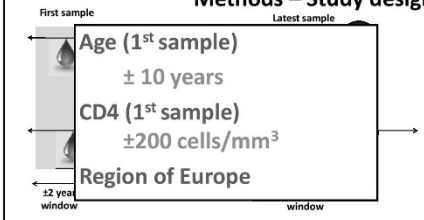
Methods – Study design



Nested case control study

EuroSIDA

Methods – Study design



Nested case control study

UCL EuroSIDA

Methods – Samples

All samples prior to diagnosis (or equivalent date in controls)

Total PSA

Free PSA

Testosterone

Sex hormone binding globulin (SHBG)

UCL EuroSIDA

Methods – Study design

EuroSIDA
9692 people
After 2001

Nested case control study

UCL EuroSIDA

Methods – Study design

EuroSIDA
4978 men
After 2001

Nested case control study

UCL EuroSIDA

Methods – Study design

EuroSIDA
4978 men
After 2001

Controls N=40

Prostate cancers N=21

Nested case control study

UCL EuroSIDA

Methods – Study design

Baseline: First sample date

Follow-up
Median: 6 IQR: 2 – 9 years

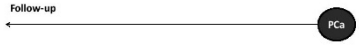
Last sample - Prostate cancer
Median: 7 IQR: 4-11 months

Controls N=40

Nested case control study

UCL EuroSIDA

Methods – Study design



Nested case control study

UCL EuroSIDA

Methods – Study design

Also matched on:

1st sample date	**± 2years**
Last sample date	**± 2years**
Age (1st sample)	**± 10 years**
CD4 (1st sample)	**±200 cells/mm^3**
Region of Europe	

UCL EuroSIDA

UCL

Mixed models

Conditional logistic regression

Area under the curve (AUC)

Sensitivity and specificity

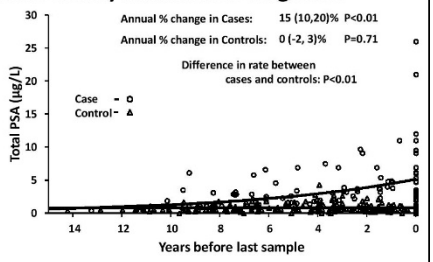
Statistical methods

EuroSIDA

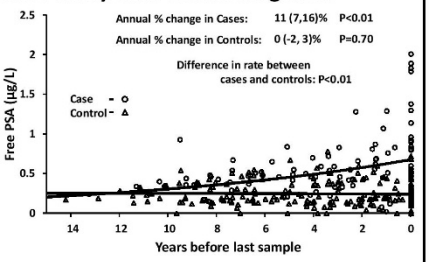
Baseline characteristics (first sample)

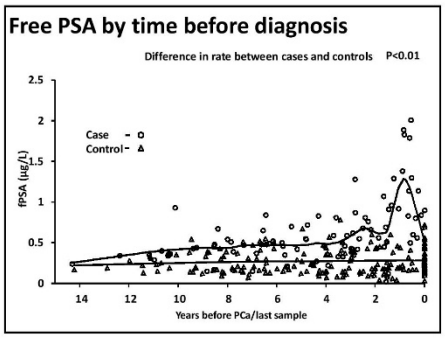
Factors	Total	Prostate cancer		P
		Cases	Controls	
	N (%)			
Total	61 (100.0)	21 (100.0)	40 (100.0)	-
Risk group				
Homosexual	47 (77.0)	17 (81.0)	30 (75.0)	0.98
Heterosexual	7 (11.5)	2 (9.5)	5 (12.5)	
IDU	2 (3.3)	0 (0.0)	2 (5.0)	
White ethnicity	57 (93.4)	21 (100.0)	36 (90.0)	0.99
No prior NADM	59 (96.7)	19 (90.5)	40 (100.0)	0.99
No prior ADM	55 (90.2)	21 (100.0)	34 (85.0)	0.99
On cART	58 (95.1)	20 (95.2)	38 (95.0)	1.00
	Median (IQR)			
Age	51 (48,57)	52 (49,57)	51 (47,56)	0.18
CD4 count (cells/mm^3)	437 (243,610)	460 (260,610)	426 (230,595)	0.07
log$_{10}$ HIV VL (copies/ml)	1.9 (1.6,2.6)	1.9 (1.6,2.6)	2.0 (1.6,2.6)	0.40

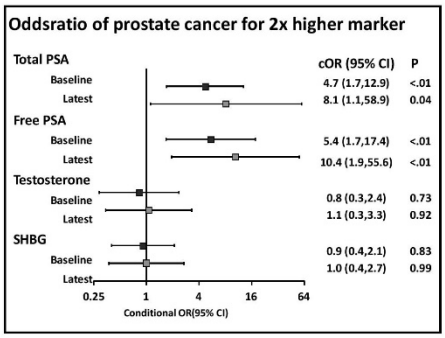
total PSA by time before diagnosis

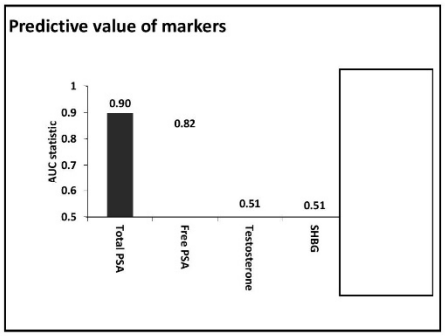


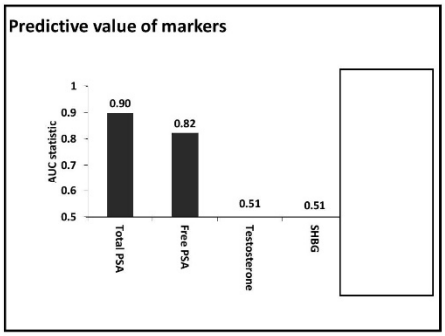
Free PSA by time before diagnosis

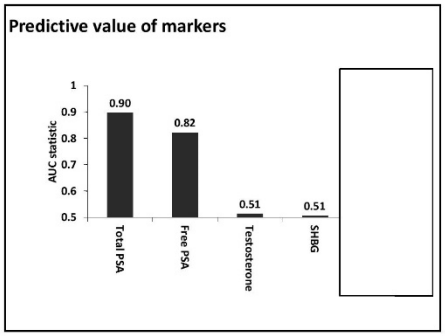


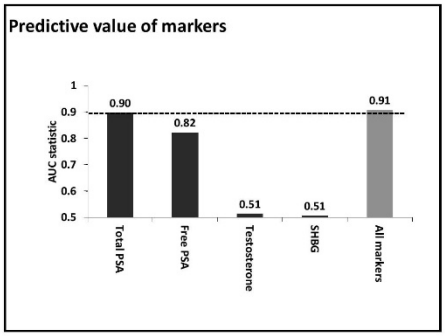

Free PSA by time before diagnosis
Difference in rate between cases and controls P<0.01
Case
Control
fPSA (µg/L)
Years before PCa/last sample


Oddsratio of prostate cancer for 2x higher marker
Total PSA
Baseline
Latest
Free PSA
Baseline
Latest
Testosterone
Baseline
Latest
SHBG
Baseline
Latest
cOR (95% CI)
4.7 (1.7,12.9)
8.1 (1.1,58.9)
5.4 (1.7,17.4)
10.4 (1.9,55.6)
0.8 (0.3,2.4)
1.1 (0.3,3.3)
0.9 (0.4,2.1)
1.0 (0.4,2.7)
P
<.01
0.04
<.01
<.01
0.73
0.92
0.83
0.99
Conditional OR(95% CI)

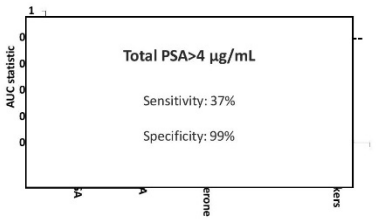

Predictive value of markers
AUC statistic
0.90
0.82
0.51
0.51
Total PSA
Free PSA
Testosterone
SHBG


Predictive value of markers
AUC statistic
0.90
0.82
0.51
0.51
Total PSA
Free PSA
Testosterone
SHBG


Predictive value of markers
AUC statistic
0.90
0.82
0.51
0.51
Total PSA
Free PSA
Testosterone
SHBG


Predictive value of markers
AUC statistic
0.90
0.82
0.51
0.51
0.91
Total PSA
Free PSA
Testosterone
SHBG
All markers

Predictive value of markers



Limitations

- Small number of prostate cancers
- Other markers: ProPSA
- No information on cancer treatment

Strengths

- Many prostate cancers (relatively)
- Prospective and independent plasma samples

Conclusions

PSA highly predictive of PCa in HIV+ men

PSA is elevated more than 5 years before prostate cancer

Further analysis to identify age-specific PSA cut-offs for maximum sensitivity and specificity for identifying high risk patients for further testing


EuroSIDA study group

1. Nakagawa, F., et al., *Projected life expectancy of people with HIV according to timing of diagnosis.* AIDS, 2012. **26**(3): p. 335-343 10.1097/QAD.0b013e32834dcec9.

2. Nakagawa, F., M. May, and A. Phillips, *Life expectancy living with HIV: recent estimates and future implications.* Current Opinion in Infectious Diseases, 2013. **26**(1): p. 17-25 10.1097/QCO.0b013e32835ba6b1.

3. Deeks, S.G. and A.N. Phillips, *HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity.* BMJ, 2009. **338**.

4. IARC, *Human Immunodeficiency Viruses and Human T-Cell Lymphotropic Viruses.* IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 1996. **67**: p. 1-424.

5. Mocroft, A., et al., *Changes in the cause of death among HIV positive subjects across Europe: results from the EuroSIDA study.* AIDS, 2002. **16**(12): p. 1663-1671.

6. Shiels, M.S., J.J. Goedert, and E.A. Engels, *Recent trends and future directions in human immunodeficiency virus-associated cancer.* Cancer, 2010. **116**(23): p. 5344-5347.

7. Shiels, M.S., et al., *Cancer Burden in the HIV-Infected Population in the United States.* Journal of the National Cancer Institute, 2011. **103**(9): p. 753-762.

8. Silverberg, M.J., et al., *HIV infection and the risk of cancers with and without a known infectious cause.* AIDS, 2009. **23**(17): p. 2337-2345.

9. Silverberg, M.J. and D.I. Abrams, *AIDS-defining and non-AIDS-defining malignancies: cancer occurrence in the antiretroviral therapy era.* Current Opinion in Oncology, 2007. **19**(5): p. 446-451.

10. Holmes, E.C., *On the origin and evolution of the human immunodeficiency virus (HIV).* Biological Reviews, 2001. **76**(2): p. 239-254.

11. McCune, J.M., *The dynamics of CD4+ T-cell depletion in HIV disease.* Nature, 2001. **410**(6831): p. 974-9.

12. Mocroft, A., et al., *Decline in the AIDS and death rates in the EuroSIDA study: an observational study.* The Lancet, 2003. **362**(9377): p. 22-29.

13. Antiretroviral Therapy Cohort Collaboration, *Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies.* Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 2010. **50**(10): p. 1387-1396.

14. Shiels, M.S., et al., *A meta-analysis of the incidence of non-AIDS cancers in HIV-infected individuals.* Journal of acquired immune deficiency syndromes (1999), 2009. **52**(5): p. 611-622.

15. Grulich, A.E., et al., *Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis.* The Lancet, 2007. **370**(9581): p. 59-67.

16. Silverberg, M.J., et al., *HIV Infection, Immunodeficiency, Viral Replication, and the Risk of Cancer.* Cancer Epidemiology Biomarkers & Prevention, 2011. **20**(12): p. 2551-2559.

17. Clifford, G.M. and S. Franceschi, *Cancer risk in HIV-infected persons: influence of CD4+ count.* Future Oncology, 2009. **5**(5): p. 669-678.

18. Borges, A.H., R. Dubrow, and M.J. Silverberg, *Factors contributing to risk for cancer among HIV-infected individuals, and evidence that earlier combination antiretroviral therapy will alter this risk.* Curr Opin HIV AIDS, 2014. **9**(1): p. 34-40.

19. Seaberg, E.C., et al., *Cancer Incidence in the Multicenter AIDS Cohort Study Before and During the HAART Era.* Cancer, 2010. **116**(23): p. 5507-5516.

20. Clifford, G.M., et al., *Cancer Risk in the Swiss HIV Cohort Study: Associations With Immunodeficiency, Smoking, and Highly Active Antiretroviral Therapy.* Journal of the National Cancer Institute, 2005. **97**(6): p. 425-432.

21. Engels, E.A., et al., *Trends in cancer risk among people with AIDS in the United States 1980–2002.* AIDS, 2006. **20**(12): p. 1645-1654.

22. Gottlieb, M., et al., *Pneumocystis pneumonia--Los Angeles.* MMWR. Morbidity and Mortality Weekly Report, 1981. **30**(21): p. 250-2.

23. Friedman-Kien, A., et al., *Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California.* MMWR Morb Mortal Wkly Rep, 1981. **30**(25): p. 305-8.

24. Ehrenkranz, N., et al., *Pneumocystis carinii pneumonia among persons with hemophilia A.* MMWR. Morbidity and Mortality Weekly Report, 1982. **31**(2): p. 365-7.

25. Jaffe, H.W., D.J. Bregman, and R.M. Selik, *Acquired Immune Deficiency Syndrome in the United States: The First 1,000 Cases.* Journal of Infectious Diseases, 1983. **148**(2): p. 339-345.

26. Ammann, A., et al., *Possible transfusion-associated acquired immune deficiency syndrome (AIDS)--California.* MMWR. Morbidity and Mortality Weekly Report, 1982. **31**(48): p. 652-4.

27. Barré-Sinoussi, F., et al., *Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS).* Science, 1983. **220**(4599): p. 868-871.

28. Wain-Hobson, S., et al., *Nucleotide sequence of the AIDS virus, LAV.* Cell, 1985. **40**(1): p. 9-17.

29. Gallo, R.C., et al., *Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS).* Science, 1983. **220**(4599): p. 865-7.

30. Gallo, R.C., et al., *Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS.* Science, 1984. **224**(4648): p. 500-503.

31. Popovic, M., et al., *Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS.* Science, 1984. **224**(4648): p. 497-500.

32. Sarngadharan, M., et al., *Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS.* Science, 1984. **224**(4648): p. 506-508.

33. Schupbach, J., et al., *Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS.* Science, 1984. **224**(4648): p. 503-505.

34. Levy, J., et al., *Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS.* Science, 1984. **225**(4664): p. 840-842.

35. Ratner, L., R.C. Gallo, and F. Wong-Staal, *HTLV-III, LAV, ARV are variants of same AIDS virus.* Nature, 1985. **313**(6004): p. 636-637.

36. Coffin, J., et al., *What to Call the AIDS Virus?* Nature, 1986. **321**(6065): p. 10-10.

37. Sharp, P.M. and B.H. Hahn, *Origins of HIV and the AIDS Pandemic.* Cold Spring Harbor Perspectives in Medicine, 2011. **1**(1).

38. Ancelle, R., et al., *Long Incubation Period for HIV-2 Infection.* Lancet, 1987. **1**(8534): p. 688-689.

39. Marlink, R., et al., *Reduced rate of disease development after HIV-2 infection as compared to HIV-1.* Science, 1994. **265**(5178): p. 1587-90.

40. Simon, F., et al., *Cellular and Plasma Viral Load in Patients Infected with HIV-2.* AIDS, 1993. **7**(11): p. 1411-1417.

41. Hirsch, V.M., et al., *An African primate lentivirus (SIVsm) closely related to HIV-2.* Nature, 1989. **339**(6223): p. 389-92.

42. Chen, Z., et al., *Human immunodeficiency virus type 2 (HIV-2) seroprevalence and characterization of a distinct HIV-2 genetic subtype from the natural range of simian immunodeficiency virus-infected sooty mangabeys.* J Virol, 1997. **71**(5): p. 3953-60.

43. Chen, Z., et al., *Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop.* J Virol, 1996. **70**(6): p. 3617-27.

44. Gao, F., et al., *Genetic diversity of human immunodeficiency virus type 2: evidence for distinct sequence subtypes with differences in virus biology.* J Virol, 1994. **68**(11): p. 7433-47.

45. Gao, F., et al., *Human infection by genetically diverse SIVSM-related HIV-2 in west Africa.* Nature, 1992. **358**(6386): p. 495-9.

46. Lemey, P., et al., *Tracing the origin and history of the HIV-2 epidemic.* Proc Natl Acad Sci U S A, 2003. **100**(11): p. 6588-92.

47. Keele, B.F., et al., *Chimpanzee reservoirs of pandemic and nonpandemic HIV-1.* Science, 2006. **313**(5786): p. 523-6.

48. Gao, F., et al., *Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes.* Nature, 1999. **397**(6718): p. 436-41.

49. Santiago, M.L., et al., *SIVcpz in wild chimpanzees.* Science, 2002. **295**(5554): p. 465.

50. Peeters, M., et al., *Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon.* AIDS, 1989. **3**(10): p. 625-30.

51. Huet, T., et al., *Genetic organization of a chimpanzee lentivirus related to HIV-1.* Nature, 1990. **345**(6273): p. 356-9.

52. Sharp, P.M., D.L. Robertson, and B.H. Hahn, *Cross-Species Transmission and Recombination of AIDS Viruses.* Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 1995. **349**(1327): p. 41-47.

53. Peeters, M., et al., *Risk to human health from a plethora of Simian immunodeficiency viruses in primate bushmeat.* Emerging Infectious Diseases, 2002. **8**(5): p. 451-457.

54. Van Heuverswyn, F. and M. Peeters, *The origins of HIV and implications for the global epidemic.* Current Infectious Disease Reports, 2007. **9**(4): p. 338-346.

55. Worobey, M., et al., *Origin of AIDS: Contaminated polio vaccine theory refuted.* Nature, 2004. **428**(6985): p. 820-820.

56. Blancou, P., et al., *Polio vaccine samples not linked to AIDS.* Nature, 2001. **410**(6832): p. 1045-1046.

57. Curtis, T., *The origin of AIDS*, in *Rolling Stone*. 1992. p. 54-59.

58. Kyle, W., *Simian retroviruses, poliovaccine, and origin of AIDS.* The Lancet, 1992. **339**(8793): p. 600-601.

59. Hooper, E., *The river: a journey back to the source of HIV and AIDS. London: Allen Lane*. 1999: The Penguin Press.

60. Woodman, Z. and C. Williamson, *HIV molecular epidemiology: transmission and adaptation to human populations.* Curr Opin HIV AIDS, 2009. **4**(4): p. 247-52.

61. Bannister, W.P., et al., *HIV-1 subtypes and response to combination antiretroviral therapy in Europe.* Antiviral Therapy, 2006. **11**(6): p. 707-15.

62. Cohen, M.S., et al., *The spread, treatment, and prevention of HIV-1: evolution of a global pandemic.* Journal of Clinical Investigation, 2008. **118**(4): p. 1244-54.

63. Worobey, M., et al., *Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960.* Nature, 2008. **455**(7213): p. 661-U57.

64. Korber, B., et al., *Timing the ancestor of the HIV-1 pandemic strains.* Science, 2000. **288**(5472): p. 1789-96.

65. Lemey, P., et al., *The molecular population genetics of HIV-1 group O.* Genetics, 2004. **167**(3): p. 1059-68.

66. Sonnet, J., et al., *Early AIDS cases originating from Zaire and Burundi (1962-1976).* Scand J Infect Dis, 1987. **19**(5): p. 511-7.

67. Zhu, T., et al., *An African HIV-1 sequence from 1959 and implications for the origin of the epidemic.* Nature, 1998. **391**(6667): p. 594-7.

68. Nahmias, A.J., et al., *Evidence for human infection with an HTLV-III/LAV like virus in central Africa,1959.* The Lancet, 1986. **327**(8492): p. 1279-1280.

69. Williams, G., T.B. Stretton, and J.C. Leonard, *AIDS IN 1959?* The Lancet, 1983. **322**(8359): p. 1136.

70. Corbitt, G. and A. Bailey, *AIDS in Manchester, 1959?* The Lancet, 1995. **345**(8956): p. 1058.

71. Zhu, T. and D.D. Ho, *Was HIV present in 1959?* Nature, 1995. **374**(6522): p. 503-4.

72. Gilbert, M.T., et al., *The emergence of HIV/AIDS in the Americas and beyond.* Proc Natl Acad Sci U S A, 2007. **104**(47): p. 18566-70.

73. Lukashov, V.V. and J. Goudsmit, *Recent Evolutionary History of Human Immunodeficiency Virus Type 1 Subtype B: Reconstruction of Epidemic Onset Based on Sequence Distances to the Common Ancestor.* Journal of Molecular Evolution, 2002. **54**(5): p. 680-691.

74. Jaffe, H.W., et al., *The acquired immunodeficiency syndrome in a cohort of homosexual men. A six-year follow-up study.* Ann Intern Med, 1985. **103**(2): p. 210-4.

75. Robbins, K.E., et al., *US human immunodeficiency virus type 1 epidemic: Date of origin, population history, and characterization of early strains.* Journal of Virology, 2003. **77**(11): p. 6359-6366.

76. Li, W.H., M. Tanimura, and P.M. Sharp, *Rates and Dates of Divergence between AIDS Virus Nucleotide-Sequences.* Molecular Biology and Evolution, 1988. **5**(4): p. 313-330.

77. Pitchenik, A.E., et al., *Opportunistic infections and Kaposi's sarcoma among Haitians: evidence of a new acquired immunodeficiency state.* Ann Intern Med, 1983. **98**(3): p. 277-84.

78. Johnson, W.D., Jr. and J.W. Pape, *AIDS in Haiti.* Immunol Ser, 1989. **44**: p. 65-78.

79. Cohen, J., *HIV/AIDS: Latin America & Caribbean. HAITI: making headway under hellacious circumstances.* Science, 2006. **313**(5786): p. 470-3.

80. Brunet, J.B., E. Bouvet, and V. Massari, *Epidemiological aspects of acquired immune deficiency syndrome in France.* Ann N Y Acad Sci, 1984. **437**: p. 334-9.

81. Glauser, M.P. and P. Francioli, *Clinical and epidemiological survey of acquired immune deficiency syndrome in Europe.* Eur J Clin Microbiol, 1984. **3**(1): p. 55-8.

82. Clumeck, N., et al., *Acquired Immunodeficiency Syndrome in African Patients.* New England Journal of Medicine, 1984. **310**(8): p. 492-497.

83. Melbye, M., et al., *Seroepidemiology of HTLV-III antibody in Danish homosexual men: prevalence, transmission, and disease outcome.* Br Med J (Clin Res Ed), 1984. **289**(6445): p. 573-5.

84. Lukashov, V.V., et al., *Evidence for HIV type 1 strains of U.S. intravenous drug users as founders of AIDS epidemic among intravenous drug users in northern Europe.* AIDS Res Hum Retroviruses, 1996. **12**(12): p. 1179-83.

85. Marx, P.A., P.G. Alcabes, and E. Drucker, *Serial human passage of simian immunodeficiency virus by unsterile injections and the emergence of epidemic human immunodeficiency virus in Africa.* Philos Trans R Soc Lond B Biol Sci, 2001. **356**(1410): p. 911-20.

86. Chitnis, A., D. Rawls, and J. Moore, *Origin of HIV type 1 in colonial French Equatorial Africa?* AIDS Res Hum Retroviruses, 2000. **16**(1): p. 5-8.

87. Sousa, J.D.d., et al., *High GUD Incidence in the Early 20th Century Created a Particularly Permissive Time Window for the Origin and Initial Spread of Epidemic HIV Strains.* PLoS ONE, 2010. **5**(4): p. e9936.

88. Weiss, R.A., *Gulliver's travels in HIVland.* Nature, 2001. **410**(6831): p. 963-7.

89. Tatem, A.J., D.J. Rogers, and S.I. Hay, *Global transport networks and infectious disease spread.* Adv Parasitol, 2006. **62**: p. 293-343.

90. Perrin, L., L. Kaiser, and S. Yerly, *Travel and the spread of HIV-1 genetic variants.* Lancet Infect Dis, 2003. **3**(1): p. 22-7.

91. Goto, T., M. Nakai, and K. Ikuta, *The life-cycle of human immunodeficiency virus type 1.* Micron, 1998. **29**(2-3): p. 123-38.

92. Klimas, N., A.O. Koneru, and M.A. Fletcher, *Overview of HIV.* Psychosom Med, 2008. **70**(5): p. 523-30.

93. Compton, A.A., H.S. Malik, and M. Emerman, *Host gene evolution traces the evolutionary history of ancient primate lentiviruses.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2013. **368**(1626).

94. Berger, E.A., P.M. Murphy, and J.M. Farber, *Chemokine receptors as HIV-1 coreceptors: Roles in viral entry, tropism, and disease.* Annual Review of Immunology, 1999. **17**: p. 657-700.

95. Smith, J.A. and R. Daniel, *Following the path of the virus: the exploitation of host DNA repair mechanisms by retroviruses.* ACS Chem Biol, 2006. **1**(4): p. 217-26.

96. Doitsh, G., et al., *Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection.* Nature, 2013. **advance online publication**.

97. Cooper, A., et al., *HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration.* Nature, 2013. **498**(7454): p. 376-9.

98. Chun, T.-W., et al., *Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy.* Proceedings of the National Academy of Sciences, 1997. **94**(24): p. 13193-13197.

99. Potter, S.J., et al., *Preserved central memory and activated effector memory CD4(+) T-cell subsets in human immunodeficiency virus controllers: An ANRS EP36 study.* Journal of Virology, 2007. **81**(24): p. 13904-13915.

100. Blankson, J.N., D. Persaud, and R.F. Siliciano, *The challenge of viral reservoirs in HIV-1 infection.* Annu Rev Med, 2002. **53**: p. 557-93.

101. Zhang, Z., et al., *Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells.* Science, 1999. **286**(5443): p. 1353-7.

102. Pantaleo, G., C. Graziosi, and A.S. Fauci, *New concepts in the immunopathogenesis of human immunodeficiency virus infection.* N Engl J Med, 1993. **328**(5): p. 327-35.

103. Mellors, J.W., et al., *Prognosis in HIV-1 infection predicted by the quantity of virus in plasma.* Science, 1996. **272**(5265): p. 1167-1170.

104. Mindel, A. and M. Tenant-Flowers, *ABC of AIDS: Natural history and management of early HIV infection.* BMJ, 2001. **322**(7297): p. 1290-3.

105. Daar, E.S., et al., *Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection.* N Engl J Med, 1991. **324**(14): p. 961-4.

106. Schacker, T.W., et al., *Biological and virologic characteristics of primary HIV infection.* Ann Intern Med, 1998. **128**(8): p. 613-20.

107. Haase, A.T., *Population biology of HIV-1 infection: viral and CD4+ T cell demographics and dynamics in lymphatic tissues.* Annual Review of Immunology, 1999. **17**: p. 625-56.

108. Bacchetti, P. and A.R. Moss, *Incubation period of AIDS in San Francisco.* Nature, 1989. **338**(6212): p. 251-3.

109. Bailey, N.T., *A revised assessment of the HIV/AIDS incubation period, assuming a very short early period of high infectivity and using only San Francisco public health data on prevalence and incidence.* Stat Med, 1997. **16**(21): p. 2447-58.

110. Prins, M., et al., *Do gender differences in CD4 cell counts matter?* AIDS, 1999. **13**(17): p. 2361-2364.

111. Phillips, A.N., *CD4 Lymphocyte Depletion Prior to the Development of AIDS.* AIDS, 1992. **6**(7): p. 735-736.

112. Koot, M., et al., *Prognostic Value of HIV-1 Syncytium-Inducing Phenotype for Rate of CD4+ Cell Depletion and Progression to AIDS.* Annals of Internal Medicine, 1993. **118**(9): p. 681-688.

113. Schellekens, P.T.A., et al., *Biphasic Rate of CD4+ Cell Count Decline during Progression to AIDS Correlates with HIV-1 Phenotype.* AIDS, 1992. **6**(7): p. 665-669.

114. Malone, J.L., et al., *Sources of variability in repeated T-helper lymphocyte counts from human immunodeficiency virus type 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important.* J Acquir Immune Defic Syndr, 1990. **3**(2): p. 144-51.

115. Mocroft, A.J., et al., *Survival of AIDS patients according to type of AIDS-defining event.* International Journal of Epidemiology, 1997. **26**(2): p. 400-407.

116. Hollingsworth, T.D., R.M. Anderson, and C. Fraser, *HIV-1 transmission, by stage of infection.* Journal of Infectious Diseases, 2008. **198**(5): p. 687-93.

117. Luo, K., et al., *The role of initial AIDS-defining illness in survival following AIDS.* Aids, 1995. **9**(1): p. 57-63.

118. Chequer, P., et al., *Determinants of survival in adult Brazilian AIDS patients, 1982-1989. The Brazilian State AIDS Program Co-Ordinators.* Aids, 1992. **6**(5): p. 483-7.

119. Turner, B.J., et al., *The AIDS-defining diagnosis and subsequent complications: a survival-based severity index.* J Acquir Immune Defic Syndr, 1991. **4**(10): p. 1059-71.

120. Babiker, A., et al., *Time from HIV-1 seroconversion to AIDS and death before widespread use of highly-active antiretroviral therapy: a collaborative re-analysis.* Lancet, 2000. **355**(9210): p. 1131-1137.

121. Gail, M.H., et al., *Survival after AIDS diagnosis in a cohort of hemophilia patients. Multicenter Hemophilia Cohort Study.* Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, 1997. **15**(5): p. 363-9.

122. Mills, G.D. and P.D. Jones, *Relationship between CD4 Lymphocyte Count and AIDS Mortality, 1986-1991.* AIDS, 1993. **7**(10): p. 1383-1386.

123. Phillips, A.N., et al., *Serial Lymphocyte-CD4 Counts and Development of AIDS.* Lancet, 1991. **337**(8738): p. 389-392.

124. Yarchoan, R., et al., *CD4 Count and the Risk for Death in Patients Infected with HIV Receiving Antiretroviral Therapy.* Annals of Internal Medicine, 1991. **115**(3): p. 184-189.

125. Pirzada, Y., S. Khuder, and H. Donabedian, *Predicting AIDS-related events using CD4 percentage or CD4 absolute counts.* AIDS research and therapy, 2006. **3**: p. 20.

126. OBrien, W.A., et al., *Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS.* New England Journal of Medicine, 1996. **334**(7): p. 426-431.

127. Loveday, C. and A. Hill, *Prediction of progression to AIDS with serum HIV-1 RNA and CD4 count.* Lancet, 1995. **345**(8952): p. 790-1.

128. Welles, S.L., et al., *Prognostic value of plasma human immunodeficiency virus type 1 (HIV-1) RNA levels in patients with advanced HIV-1 disease and with little or no prior zidovudine therapy. AIDS Clinical Trials Group Protocol 116A/116B/117 Team.* Journal of Infectious Diseases, 1996. **174**(4): p. 696-703.

129. Phillips, A.N., et al., *HIV-1 RNA levels and the development of clinical disease.* AIDS, 1996. **10**(8): p. 859-865.

130. Carre, N., et al., *Predictive value of viral load and other markers for progression to clinical AIDS after CD4+ cell count falls below 200/mu L.* International Journal of Epidemiology, 1998. **27**(5): p. 897-903.

131. Yerly, S., et al., *A critical assessment of the prognostic value of HIV-1 RNA levels and CD4(+) cell counts in HIV-infected patients.* Archives of Internal Medicine, 1998. **158**(3): p. 247-252.

132. Hogg, R.S., et al., *Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy.* JAMA, 2001. **286**(20): p. 2568-77.

133. Centers for Disease Control and Prevention, *1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults.* MMWR Recomm Rep, 1992. **18**(41(RR-17)): p. 1-19.

134. US Deparment of Health and Human Services. *HSRA Guide for HIV/AIDS Clinical Care*. 2014 April 2014 [cited 2014 2 June 2014]; Available from: http://www.aidsetc.org/sites/default/files/resources_files/Clinical_Manual_4-30-2014_0.pdf.

135. WHO, *WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children.* 2007.

136. EACS, *EACS Guidelines Version 7.1*. 2014.

137. British HIV Association (BHIVA), *British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012 (2013 update).* HIV Medicine, 2013. **15**(Suppl. 1): p. 1 - 85.

138. WHO, *Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV*. 2015.

139. (BHIVA), B.H.A., *British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015 (2016 interim update)*. 2016.

140. EACS, *EACS Guidelines version 8.2*. 2017.

141. Insight Start Study Group, et al., *Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection.* New England Journal of Medicine, 2015. **373**(9): p. 795-807.

142. MRC clinical trials unit. *Strategic Timing of AntiRetroviral Treatment (START)*. 2015 [cited 2015 2/3/2015]; Available from: http://www.ctu.mrc.ac.uk/our_research/research_areas/hiv/studies/start/.

143. Maini, M.K., et al., *Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men.* Genitourinary Medicine, 1996. **72**(1): p. 27-31.

144. Bofill, M., et al., *Laboratory control values for CD4 and CD8 T lymphocytes. Implications for HIV-1 diagnosis.* Clinical and Experimental Immunology, 1992. **88**(2): p. 243-52.

145. Yu, L.M., P.J. Easterbrook, and T. Marshall, *Relationship between CD4 count and CD4% in HIV-infected people.* International Journal of Epidemiology, 1997. **26**(6): p. 1367-1372.

146. Kartikeyan, S., et al., ***HIV and AIDS: Basic elements and priorities***. 2007, Netherlands: Springer.

147. Seay, J.S., et al., *Self-reported sleep disturbance is associated with lower CD4 count and 24-h urinary dopamine levels in ethnic minority women living with HIV.* Psychoneuroendocrinology, 2013. **38**(11): p. 2647-2653.

148. Ironson, G., et al., *Psychosocial factors predict CD4 and viral load change in men and women with human immunodeficiency virus in the era of highly active antiretroviral treatment.* Psychosom Med, 2005. **67**(6): p. 1013-21.

149. Pedersen, B.K. and L. Hoffman-Goetz, *Exercise and the Immune System: Regulation, Integration, and Adaptation.* Physiological Reviews, 2000. **80**(3): p. 1055-1081.

150. Darbyshire, J., D.A. Schoenfeld, and I.V. Weller, *Clinical trials in HIV infection: current problems and future directions.* AIDS, 1991. **5 Suppl 2**: p. S167-73.

151. Behr, T.M., et al., *Radioimmunotherapy of small volume disease of colorectal cancer metastatic to the liver: preclinical evaluation in comparison to standard chemotherapy and initial results of a phase I clinical study.* Clinical Cancer Research, 1999. **5**(10 Suppl): p. 3232s-3242s.

152. Lodi, S., et al., *Time From Human Immunodeficiency Virus Seroconversion to Reaching CD4+ Cell Count Thresholds < 200, < 350, and < 500 Cells/mm(3): Assessment of Need Following Changes in Treatment Guidelines.* Clinical infectious diseases, 2011. **53**(8): p. 817-825.

153. Tollerud, D.J., et al., *The influence of age, race, and gender on peripheral blood mononuclear-cell subsets in healthy nonsmokers.* Journal of Clinical Immunology, 1989. **9**(3): p. 214-22.

154. Phillips, A.N., et al., *More rapid progression to AIDS in older HIV-infected people: the role of CD4+ T-cell counts.* J Acquir Immune Defic Syndr, 1991. **4**(10): p. 970-5.

155. Murphy, D.G., et al., *Multicenter Comparison of Roche COBAS AMPLICOR MONITOR Version 1.5, Organon Teknika NucliSens QT with Extractor, and Bayer Quantiplex Version 3.0 for Quantification of Human Immunodeficiency Virus Type 1 RNA in Plasma.* Journal of Clinical Microbiology, 2000. **38**(11): p. 4034-4041.

156. Schutten, M., et al., *Evaluation of the analytical performance of the new Abbott RealTime RT-PCRs for the quantitative detection of HCV and HIV-1 RNA.* Journal of Clinical Virology, 2007. **40**(2): p. 99-104.

157. Palmer, S., et al., *New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma.* Journal of Clinical Microbiology, 2003. **41**(10): p. 4531-4536.

158. Pascual-Pareja, J.F., et al., *Detection of HIV-1 at between 20 and 49 Copies per Milliliter by the Cobas TaqMan HIV-1 v2.0 Assay Is Associated with Higher Pretherapy Viral Load and Less Time on Antiretroviral Therapy.* Journal of Clinical Microbiology, 2010. **48**(5): p. 1911-1912.

159. Mellors, J.W., et al., *Plasma viral load and CD4(+) lymphocytes as prognostic markers of HIV-1 infection.* Annals of Internal Medicine, 1997. **126**(12): p. 946-954.

160. Mellors, J.W., et al., *Quantitation of HIV-1 RNA in Plasma Predicts Outcome after Seroconversion.* Annals of Internal Medicine, 1995. **122**(8): p. 573-579.

161. Phillips, A. and P. Pezzotti, *Short-term risk of AIDS according to current CD4 cell count and viral load in antiretroviral drug-naive individuals and those treated in the monotherapy era.* AIDS, 2004. **18**(1): p. 51-8.

162. Lyles, C.M., et al., *Longitudinal human immunodeficiency virus type 1 load in the italian seroconversion study: correlates and temporal trends of virus load.* Journal of Infectious Diseases, 1999. **180**(4): p. 1018-24.

163. de Wolf, F., et al., *AIDS prognosis based on HIV-1 RNA, CD4+ T-cell count and function: markers with reciprocal predictive value over time after seroconversion.* AIDS, 1997. **11**(15): p. 1799-806.

164. Spijkerman, I.J.B., et al., *Early and late HIV-1 RNA level and its association with other markers and disease progression in long-term AIDS-free homosexual men.* AIDS, 1997. **11**(11): p. 1383-1388.

165. Cozzi Lepri, A., et al., *The relative prognostic value of plasma HIV RNA levels and CD4 lymphocyte counts in advanced HIV infection.* AIDS, 1998. **12**(13): p. 1639-1643.

166. Sabin, C.A., et al., *Immune markers and viral load after HIV-1 seroconversion as predictors of disease progression in a cohort of haemophilic men.* AIDS, 1998. **12**(11): p. 1347-1352.

167. Ledergerber, B., et al., *Predictors of trend in CD4-positive T-cell count and mortality among HIV-1-infected individuals with virological failure to all three antiretroviral-drug classes.* Lancet, 2004. **364**(9428): p. 51-62.

168. Lyles, R.H., et al., *Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men.* Journal of Infectious Diseases, 2000. **181**(3): p. 872-880.

169. Ruiz, L., et al., *Quantitative HIV-1 RNA as a marker of clinical stability and survival in a cohort of 302 patients with a mean CD4 cell count of 300x10(6)/l.* AIDS, 1996. **10**(11): p. F39-F44.

170. Hubert, J.B., et al., *Natural history of serum HIV-1 RNA levels in 330 patients with a known date of infection. The SEROCO Study Group.* AIDS, 2000. **14**(2): p. 123-31.

171. Engels, E.A., et al., *Plasma HIV viral load in patients with hemophilia and late-stage HIV disease: a measure of current immune suppression. Multicenter Hemophilia Cohort Study.* Ann Intern Med, 1999. **131**(4): p. 256-64.

172. Lyles, C.M., et al., *Cell-associated infectious HIV-1 viral load as a predictor of clinical progression and survival among HIV-1 infected injection drug users and homosexual men.* European Journal of Epidemiology, 1999. **15**(2): p. 99-108.

173. Cao, Y., et al., *Virologic and Immunologic Characterization of Long-Term Survivors of Human Immunodeficiency Virus Type 1 Infection.* New England Journal of Medicine, 1995. **332**(4): p. 201-208.

174. Pantaleo, G., et al., *Studies in subjects with long-term nonprogressive human immunodeficiency virus infection.* N Engl J Med, 1995. **332**(4): p. 209-16.

175. UNAIDS, *AIDS DATA*. 2016.

176. UNAIDS WHO, *Global report: UNAIDS report on the global AIDS epidemic 2013*. 2013.

177. Adler, M.W., *ABC of AIDS Development of the epidemic.* BMJ, 2001. **322**(7296): p. 1226-1229.

178. Chitwood, D.D., et al., *HIV seropositivity of needles from shooting galleries in South Florida.* American Journal of Public Health, 1990. **80**(2): p. 150-152.

179. Varghese, B., et al., *Reducing the Risk of Sexual HIV Transmission: Quantifying the Per-Act Risk for HIV on the Basis of Choice of Partner, Sex Act, and Condom Use.* Sexually Transmitted Diseases, 2002. **29**(1): p. 38-43.

180. Mortimer, P.P., *ABC of AIDS. The virus and the tests.* Br Med J (Clin Res Ed), 1987. **294**(6587): p. 1602-5.

181. Rezza, G., et al., *Needle sharing and other behaviours related to HIV spread among intravenous drug users.* AIDS, 1989. **3**(4): p. 247-8.

182. Donegan, E., et al., *Infection with Human Immunodeficiency Virus Type 1 (HIV-1) among Recipients of Antibody-Positive Blood Donations.* Annals of Internal Medicine, 1990. **113**(10): p. 733-739.

183. Baggaley, R.F., et al., *Risk of HIV-1 transmission for parenteral exposure and blood transfusion: a systematic review and meta-analysis.* Aids, 2006. **20**(6): p. 805-12.

184. Atkins, M.C., et al., *Fluctuations of HIV load in semen of HIV positive patients with newly acquired sexually transmitted diseases.* BMJ, 1996. **313**(7053): p. 341-2.

185. Quinn, T.C., et al., *Viral load and heterosexual transmission of human immunodeficiency virus type 1.* New England Journal of Medicine, 2000. **342**(13): p. 921-929.

186. Adler, D.H., *The impact of HAART on HPV-related cervical disease.* Curr HIV Res, 2010. **8**(7): p. 493-7.

187. Centers for Disease Control and Prevention. *HIV Transmission Risk*. 2014 [cited 2015 20 Mar 2015]; Available from: http://www.cdc.gov/hiv/policies/law/risk.html.

188. Patel, P., et al., *Estimating per-act HIV transmission risk: a systematic review.* AIDS, 2014. **28**(10): p. 1509-19.

189. European Centre for Disease Prevention and Control, *Surveillance Report: HIV/AIDS surveillance in Europe 2012*. 2012.

190. Centers for Disease Control and Prevention. *Statistics Overview: Diagnoses of HIV infection, by transmission category*. 2015 [cited 2015 20 Mar 2015]; Available from: http://www.cdc.gov/hiv/statistics/basics/.

191. Rodger, A.J., et al., *Sexual Activity Without Condoms and Risk of HIV Transmission in Serodifferent Couples When the HIV-Positive Partner Is Using Suppressive Antiretroviral Therapy.* Jama, 2016. **316**(2): p. 171-81.

192. McCormack, S., et al., *Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial.* The Lancet, 2016. **387**(10013): p. 53-60.

193. Auvert, B., et al., *Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial.* PLoS Med, 2005. **2**(11): p. e298.

194. Gray, R.H., et al., *Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial.* Lancet, 2007. **369**(9562): p. 657-66.

195. Bailey, R.C., et al., *Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial.* Lancet, 2007. **369**(9562): p. 643-56.

196. WHO. *Male circumcision for HIV prevention*. 2013 [cited 2015 20 Mar 2015]; Available from: http://www.who.int/hiv/topics/malecircumcision/en/.

197. Johnson, K.E. and T.C. Quinn, *Update on male circumcision: prevention success and challenges ahead.* Curr Infect Dis Rep, 2008. **10**(3): p. 243-51.

198. Templeton, D.J., et al., *Circumcision and risk of HIV infection in Australian homosexual men.* AIDS, 2009. **23**(17): p. 2347-51.

199. Bell, D.M., *Occupational risk of human immunodeficiency virus infection in healthcare workers: an overview.* Am J Med, 1997. **102**(5b): p. 9-15.

200. Cardo, D.M., et al., *A case-control study of HIV seroconversion in health care workers after percutaneous exposure. Centers for Disease Control and Prevention Needlestick Surveillance Group.* N Engl J Med, 1997. **337**(21): p. 1485-90.

201. Busch, M.P., et al., *Factors influencing human immunodeficiency virus type 1 transmission by blood transfusion. Transfusion Safety Study Group.* J Infect Dis, 1996. **174**(1): p. 26-33.

202. Sloand, E.M., E. Pitt, and H.G. Klein, *Safety of the blood supply.* Jama, 1995. **274**(17): p. 1368-73.

203. Buchanan, A.M. and C.K. Cunningham, *Advances and Failures in Preventing Perinatal Human Immunodeficiency Virus Infection.* Clinical Microbiology Reviews, 2009. **22**(3): p. 493-507.

204. Mayaux, M.J., et al., *Maternal factors associated with perinatal HIV-1 transmission: the French Cohort Study: 7 years of follow-up observation. The French Pediatric HIV Infection Study Group.* J Acquir Immune Defic Syndr Hum Retrovirol, 1995. **8**(2): p. 188-94.

205. Newell, M.L., et al., *Vertical transmission of HIV-1: maternal immune status and obstetric factors. The European Collaborative Study.* Aids, 1996. **10**(14): p. 1675-81.

206. Thorne, C. and M.L. Newell, *Mother-to-child transmission of HIV infection and its prevention.* Curr HIV Res, 2003. **1**(4): p. 447-62.

207. Cavarelli, M. and G. Scarlatti, *Human immunodeficiency virus type 1 mother-to-child transmission and prevention: successes and controversies.* J Intern Med, 2011. **270**(6): p. 561-79.

208. Connor, E.M., et al., *Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group.* N Engl J Med, 1994. **331**(18): p. 1173-80.

209. Mofenson, L.M., *Mother-child HIV-1 transmission: Timing and determinants.* Obstet Gynecol Clin North Am, 1997. **24**(4): p. 759-84.

210. Dao, H., et al., *International recommendations on antiretroviral drugs for treatment of HIV-infected women and prevention of mother-to-child HIV transmission in resource-limited settings: 2006 update.* Am J Obstet Gynecol, 2007. **197**(3 Suppl): p. S42-55.

211. Dickover, R.E., et al., *Identification of levels of maternal hiv-1 rna associated with risk of perinatal transmission: Effect of maternal zidovudine treatment on viral load.* JAMA, 1996. **275**(8): p. 599-605.

212. Cooper, E.R., et al., *Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission.* J Acquir Immune Defic Syndr, 2002. **29**(5): p. 484-94.

213. UNAIDS WHO, *Global report: UNAIDS report on the global AIDS epidemic 2012*. 2012.

214. WHO, *Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: executive summary*. 2012.

215. Rodger, A.J., et al., *Mortality in well controlled HIV in the continuous antiretroviral therapy arms of the SMART and ESPRIT trials compared with the general population.* AIDS, 2013. **27**(6): p. 973-979.

216. Mocroft, A., et al., *AIDS across Europe, 1994-98: the EuroSIDA study.* Lancet, 2000. **356**(9226): p. 291-6.

217. Mocroft, A., et al., *Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group.* Lancet, 1998. **352**(9142): p. 1725-30.

218. Palella, F.J., Jr., et al., *Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators.* N Engl J Med, 1998. **338**(13): p. 853-60.

219. Antiretroviral Therapy Cohort Collaboration, *Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies.* Lancet, 2008. **372**(9635): p. 293-299.

220. Smith, C.J., et al., *Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration.* Lancet, 2014. **384**(9939): p. 241-8.

221. Mocroft, A., et al., *Serious Fatal and Nonfatal Non-AIDS-Defining Illnesses in Europe.* J Acquir Immune Defic Syndr, 2010. **55**(2): p. 262-270.

222. Palmisano, L. and S. Vella, *A brief history of antiretroviral therapy of HIV infection: success and challenges.* Ann Ist Super Sanita, 2011. **47**(1): p. 44-8.

223. Hirsch, M.S. and J.C. Kaplan, *Treatment of human immunodeficiency virus infections.* Antimicrobial Agents and Chemotherapy, 1987. **31**(6): p. 839-843.

224. Wright, K., *AIDS therapy. First tentative signs of therapeutic promise.* Nature, 1986. **323**(6086): p. 283.

225. Richman, D.D., et al., *The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial.* N Engl J Med, 1987. **317**(4): p. 192-7.

226. U.S. Food and Drug Administration. *Antiretroviral drugs used in the treatment of HIV infection*. 2014 [cited 2015 28/Jan/2015]; Available from: http://www.fda.gov/forpatients/illness/hivaids/treatment/ucm118915.htm.

227. de Bethune, M.P., *Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989-2009).* Antiviral Res, 2010. **85**(1): p. 75-90.

228. Larder, B.A., G. Darby, and D.D. Richman, *HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy.* Science, 1989. **243**(4899): p. 1731-4.

229. Larder, B.A. and S.D. Kemp, *Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT).* Science, 1989. **246**(4934): p. 1155-8.

230. Rooke, R., et al., *Isolation of drug-resistant variants of HIV-1 from patients on long-term zidovudine therapy. Canadian Zidovudine Multi-Centre Study Group.* AIDS, 1989. **3**(7): p. 411-5.

231. Eron, J.J., Jr. and M.S. Hirsch, *New anti-HIV-1 therapies and combinations: current data and prospects.* Aids, 1990. **4 Suppl 1**: p. S193-200.

232. De Clercq, E., *Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV.* Int J Antimicrob Agents, 2009. **33**(4): p. 307-20.

233. Wensing, A.M., N.M. van Maarseveen, and M. Nijhuis, *Fifteen years of HIV Protease Inhibitors: raising the barrier to resistance.* Antiviral Res, 2010. **85**(1): p. 59-74.

234. Collier, A.C., et al., *Combination therapy with zidovudine, didanosine and saquinavir.* Antiviral Research, 1996. **29**(1): p. 99.

235. Revicki, D.A., et al., *Quality of life outcomes of combination zalcitabine-zidovudine, saquinavir-zidovudine, and saquinavir-zalcitabine-zidovudine therapy for HIV-infected adults with CD4 cell counts between 50 and 350 per cubic millimeter.* AIDS, 1999. **13**(7): p. 851-858.

236. Collier, A.C., et al., *Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine.* New England Journal of Medicine, 1996. **334**(16): p. 1011-1017.

237. Carr, A., et al., *A randomised, open-label comparison of three highly active antiretroviral therapy regimens including two nucleoside analogues and indinavir for previously untreated HIV-1 infection: the OzCombo1 study.* Aids, 2000. **14**(9): p. 1171-80.

238. Hammer, S.M., et al., *A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team.* N Engl J Med, 1997. **337**(11): p. 725-33.

239. Floridia, M., et al., *A randomized, double-blind trial on the use of a triple combination including nevirapine, a nonnucleoside reverse transcriptase HIV inhibitor, in antiretroviral-naive patients with advanced disease.* J Acquir Immune Defic Syndr Hum Retrovirol, 1999. **20**(1): p. 11-9.

240. Henry, K., et al., *A randomized, controlled, double-blind study comparing the survival benefit of four different reverse transcriptase inhibitor therapies (three-drug, two-drug, and alternating drug) for the treatment of advanced AIDS. AIDS Clinical Trial Group 193A Study Team.* J Acquir Immune Defic Syndr Hum Retrovirol, 1998. **19**(4): p. 339-49.

241. Montaner, J.S., et al., *A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study.* Jama, 1998. **279**(12): p. 930-7.

242. D'Aquila, R.T., et al., *Nevirapine, zidovudine, and didanosine compared with zidovudine and didanosine in patients with HIV-1 infection. A randomized, double-blind, placebo-controlled trial. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators.* Ann Intern Med, 1996. **124**(12): p. 1019-30.

243. Food and Drug Administration, *Human Immunodeficiency Virus-1 Infection: Developing Antiretroviral Drugs for Treatment. Guidance for Industry* 2015.

244. HIV iBASE, *Introduction to combination therapy* 2014: HIV i-Base.

245. Chao, S.H., *The HIV landscape in a managed care environment: current challenges and potential solutions.* J Manag Care Pharm, 2006. **12**(7 Suppl B): p. S2-5.

246. Monforte, A.d.A., et al., *Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naïve patients.* AIDS, 2000. **14**(5): p. 499-507.

247. Parienti, J.J., et al., *Predictors of virologic failure and resistance in HIV-infected patients treated with nevirapine- or efavirenz-based antiretroviral therapy.* Clin Infect Dis, 2004. **38**(9): p. 1311-6.

248. Parienti, J.J., et al., *Average adherence to boosted protease inhibitor therapy, rather than the pattern of missed doses, as a predictor of HIV RNA replication.* Clin Infect Dis, 2010. **50**(8): p. 1192-7.

249. Parienti, J.-J., et al., *Not All Missed Doses Are the Same: Sustained NNRTI Treatment Interruptions Predict HIV Rebound at Low-to-Moderate Adherence Levels.* PLoS ONE, 2008. **3**(7): p. e2783.

250. The Strategies for Management of Antiretroviral Therapy (SMART) Study Group, *CD4+ Count–Guided Interruption of Antiretroviral Treatment.* New England Journal of Medicine, 2006. **355**(22): p. 2283-2296.

251. Group, T.S.f.M.o.A.T.S., *Inferior Clinical Outcome of the CD4+ Cell Count–Guided Antiretroviral Treatment Interruption Strategy in the SMART Study: Role of CD4+ Cell Counts and HIV RNA Levels during Follow-up.* Journal of Infectious Diseases, 2008. **197**(8): p. 1145-1155.

252. Mocroft, A., et al., *Reasons for modification and discontinuation of antiretrovirals: results from a single treatment centre.* AIDS, 2001. **15**(2): p. 185-194.

253. Margolis, A.M., et al., *A review of the toxicity of HIV medications.* J Med Toxicol, 2014. **10**(1): p. 26-39.

254. U.S. Department of Health and Human Services, N.I.o.H., National Institute of Allergy and Infectious Diseases, Division of AIDS,. *Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0.* . 2014 November 2014; Available from:

http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf.

255. Lampe, F.C., et al., *Changes over time in risk of initial virological failure of combination antiretroviral therapy: a multicohort analysis, 1996 to 2002.* Arch Intern Med, 2006. **166**(5): p. 521-8.

256. Moore, R.D., et al., *An improvement in virologic response to highly active antiretroviral therapy in clinical practice from 1996 through 2002.* J Acquir Immune Defic Syndr, 2005. **39**(2): p. 195-8.

257. Phillips, A.N., et al., *When should antiretroviral therapy for HIV be started?* Vol. 334. 2007. 76-78.

258. Bannister, W.P., et al., *Regional changes over time in initial virologic response rates to combination antiretroviral therapy across Europe.* J Acquir Immune Defic Syndr, 2006. **42**(2): p. 229-37.

259. AIDSinfo. *Clinical Guidelines Portal*. 2016 [cited 2016 25 May 2016]; Available from: https://aidsinfo.nih.gov/guidelines.

260. Fischl, M.A., et al., *The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial.* N Engl J Med, 1987. **317**(4): p. 185-91.

261. Connolly, K.J., et al., *Phase I study of 2'-3'-dideoxyinosine administered orally twice daily to patients with AIDS or AIDS-related complex and hematologic intolerance to zidovudine.* The American Journal of Medicine, 1991. **91**(5): p. 471-478.

262. Cooley, T.P., et al., *Once-Daily Administration of 2',3'-Dideoxyinosine (ddI) in Patients with the Acquired Immunodeficiency Syndrome or AIDS-Related Complex.* New England Journal of Medicine, 1990. **322**(19): p. 1340-1345.

263. Dolin, R., et al., *Zidovudine compared with didanosine in patients with advanced HIV type 1 infection and little or no previous experience with zidovudine. AIDS Clinical Trials Group.* Arch Intern Med, 1995. **155**(9): p. 961-74.

264. Kahn, J.O., et al., *A controlled trial comparing continued zidovudine with didanosine in human immunodeficiency virus infection. The NIAID AIDS Clinical Trials Group.* N Engl J Med, 1992. **327**(9): p. 581-7.

265. Lambert, J.S., et al., *2',3'-Dideoxyinosine (ddI) in Patients with the Acquired Immunodeficiency Syndrome or AIDS-Related Complex.* New England Journal of Medicine, 1990. **322**(19): p. 1333-1340.

266. Meng, T.C., et al., *Combination therapy with zidovudine and dideoxycytidine in patients with advanced human immunodeficiency virus infection. A phase I/II study.* Ann Intern Med, 1992. **116**(1): p. 13-20.

267. Fischl, M.A., et al., *Zalcitabine compared with zidovudine in patients with advanced HIV-1 infection who received previous zidovudine therapy.* Ann Intern Med, 1993. **118**(10): p. 762-9.

268. Follansbee, S., et al. *The efficacy of zalcitabine (ddC, HIVID) versus zidovudine (ZDV) as monotherapy in ZDV-naive patients with advanced HIV disease; a randomized, double-blind, comparative trial (ACTG 114; N3300)*. in *IXth International Conference on AIDS/IV STD World Congress, Berlin, Germany*. 1993.

269. Abrams, D.I., et al., *A comparative trial of didanosine or zalcitabine after treatment with zidovudine in patients with human immunodeficiency virus infection. The Terry Beirn Community Programs for Clinical Research on AIDS.* N Engl J Med, 1994. **330**(10): p. 657-62.

270. Fischl, M.A., et al., *Combination and monotherapy with zidovudine and zalcitabine in patients with advanced HIV disease. The NIAID AIDS Clinical Trials Group.* Ann Intern Med, 1995. **122**(1): p. 24-32.

271. Saravolatz, L.D., et al., *Zidovudine alone or in combination with didanosine or zalcitabine in HIV-infected patients with the acquired immunodeficiency syndrome or fewer than 200 CD4 cells per cubic millimeter. Investigators for the Terry Beirn Community Programs for Clinical Research on AIDS.* N Engl J Med, 1996. **335**(15): p. 1099-106.

272. Moyle, G.J., et al., *Zidovudine monotherapy versus zidovudine plus zalcitabine combination therapy in HIV-positive persons with CD4 cell counts 300-500 cells/mm3: a double-blind controlled trial. The M50003 Study Group Coordinating and Writing Committee.* Antivir Ther, 1997. **2**(4): p. 229-36.

273. Spruance, S.L., et al., *Clinical efficacy of monotherapy with stavudine compared with zidovudine in HIV-infected, zidovudine-experienced patients. A randomized, double-blind, controlled trial. Bristol-Myers Squibb Stavudine/019 Study Group.* Ann Intern Med, 1997. **126**(5): p. 355-63.

274. Bartlett, J.A., et al., *Lamivudine plus zidovudine compared with zalcitabine plus zidovudine in patients with HIV infection. A randomized, double-blind, placebo-controlled trial. North American HIV Working Party.* Ann Intern Med, 1996. **125**(3): p. 161-72.

275. Eron, J.J., et al., *Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party.* N Engl J Med, 1995. **333**(25): p. 1662-9.

276. Ingrand, D., et al., *Phase I/II study of 3TC (lamivudine) in HIV-positive, asymptomatic or mild AIDS-related complex patients: sustained reduction in viral markers. The Lamivudine European HIV Working Group.* Aids, 1995. **9**(12): p. 1323-9.

277. Katlama, C., et al., *Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naive patients. A randomized controlled comparison with zidovudine monotherapy. Lamivudine European HIV Working Group.* Jama, 1996. **276**(2): p. 118-25.

278. Staszewski, S., et al., *Safety and efficacy of lamivudine-zidovudine combination therapy in zidovudine-experienced patients. A randomized controlled comparison with zidovudine monotherapy. Lamivudine European HIV Working Group.* Jama, 1996. **276**(2): p. 111-7.

279. van Leeuwen, R., et al., *Evaluation of safety and efficacy of 3TC (lamivudine) in patients with asymptomatic or mildly symptomatic human immunodeficiency virus infection: a phase I/II study.* J Infect Dis, 1995. **171**(5): p. 1166-71.

280. Kuritzkes, D.R., et al., *Continued lamivudine versus delavirdine in combination with indinavir and zidovudine or stavudine in lamivudine-experienced patients: results of Adult AIDS Clinical Trials Group protocol 370.* Aids, 2000. **14**(11): p. 1553-61.

281. Katlama, C., et al., *The role of abacavir (ABC, 1592) in antiretroviral therapy-experienced patients: results from a randomized, double-blind, trial. CNA3002 European Study Team.* Aids, 2000. **14**(7): p. 781-9.

282. Saag, M.S., et al., *Antiretroviral effect and safety of abacavir alone and in combination with zidovudine in HIV-infected adults. Abacavir Phase 2 Clinical Team.* AIDS, 1998. **12**(16): p. F203-9.

283. Staszewski, S., et al., *A dose-ranging study to evaluate the safety and efficacy of abacavir alone or in combination with zidovudine and lamivudine in antiretroviral treatment-naive subjects.* AIDS, 1998. **12**(16): p. F197-202.

284. Gallant, J.E., et al., *Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial.* Jama, 2004. **292**(2): p. 191-201.

285. Schooley, R.T., et al., *Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study.* AIDS, 2002. **16**(9): p. 1257-63.

286. Sax, P.E., et al., *Tenofovir alafenamide versus tenofovir disoproxil fumarate, coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomised, double-blind, phase 3, non-inferiority trials.* Lancet, 2015. **385**(9987): p. 2606-15.

287. Benson, C.A., et al., *A randomized study of emtricitabine and lamivudine in stably suppressed patients with HIV.* Aids, 2004. **18**(17): p. 2269-76.

288. Saag, M.S., et al., *Efficacy and safety of emtricitabine vs stavudine in combination therapy in antiretroviral-naive patients: a randomized trial.* Jama, 2004. **292**(2): p. 180-9.

289. Sax, P.E., et al., *Abacavir/lamivudine versus tenofovir DF/emtricitabine as part of combination regimens for initial treatment of HIV: final results.* J Infect Dis, 2011. **204**(8): p. 1191-201.

290. Cameron, D.W., et al., *Randomised placebo-controlled trial of ritonavir in advanced HIV-1 disease. The Advanced HIV Disease Ritonavir Study Group.* Lancet, 1998. **351**(9102): p. 543-9.

291. Gulick, R.M., et al., *Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy.* N Engl J Med, 1997. **337**(11): p. 734-9.

292. Hirsch, M.S., et al., *Long-term efficacy, safety, and tolerability of indinavir-based therapy in protease inhibitor-naive adults with advanced HIV infection.* Clin Infect Dis, 2003. **37**(8): p. 1119-24.

293. Lewi, D.S., et al., *Randomized, double-blind trial comparing indinavir alone, zidovudine alone and indinavir plus zidovudine in antiretroviral therapy-naive HIV-infected individuals with CD4 cell counts between 50 and 250/mm3.* Rev Inst Med Trop Sao Paulo, 2000. **42**(1): p. 27-36.

294. Kirk, O., et al., *Combination therapy containing ritonavir plus saquinavir has superior short-term antiretroviral efficacy: a randomized trial.* Aids, 1999. **13**(1): p. F9-16.

295. Michelet, C., et al., *Ritonavir-saquinavir dual protease inhibitor compared to ritonavir alone in human immunodeficiency virus-infected patients.* Antimicrob Agents Chemother, 2001. **45**(12): p. 3393-402.

296. Vella, S., et al., *A randomized controlled trial of a protease inhibitor (saquinavir) in combination with zidovudine in previously untreated patients with advanced HIV infection.* Antivir Ther, 1996. **1**(3): p. 129-40.

297. Roca, B., C.J. Gomez, and A. Arnedo, *A randomized, comparative study of lamivudine plus stavudine, with indinavir or nelfinavir, in treatment-experienced HIV-infected patients.* AIDS, 2000. **14**(2): p. 157-61.

298. Goodgame, J.C., et al., *Amprenavir in combination with lamivudine and zidovudine versus lamivudine and zidovudine alone in HIV-1-infected antiretroviral-naive adults. Amprenavir PROAB3001 International Study Team.* Antivir Ther, 2000. **5**(3): p. 215-25.

299. Murphy, R.L., et al., *Treatment with amprenavir alone or amprenavir with zidovudine and lamivudine in adults with human immunodeficiency virus infection. AIDS Clinical Trials Group 347 Study Team.* J Infect Dis, 1999. **179**(4): p. 808-16.

300. Murphy, R.L., et al., *ABT-378/ritonavir plus stavudine and lamivudine for the treatment of antiretroviral-naive adults with HIV-1 infection: 48-week results.* Aids, 2001. **15**(1): p. F1-9.

301. Murphy, R.L., et al., *Seven-year efficacy of a lopinavir/ritonavir-based regimen in antiretroviral-naive HIV-1-infected patients.* HIV Clin Trials, 2008. **9**(1): p. 1-10.

302. Walmsley, S., et al., *Lopinavir-ritonavir versus nelfinavir for the initial treatment of HIV infection.* New England Journal of Medicine, 2002. **346**(26): p. 2039-2046.

303. Johnson, M., et al., *Atazanavir plus ritonavir or saquinavir, and lopinavir/ritonavir in patients experiencing multiple virological failures.* Aids, 2005. **19**(7): p. 685-94.

304. Murphy, R.L., et al., *Dose-ranging, randomized, clinical trial of atazanavir with lamivudine and stavudine in antiretroviral-naive subjects: 48-week results.* Aids, 2003. **17**(18): p. 2603-14.

305. Sanne, I., et al., *Results of a phase 2 clinical trial at 48 weeks (AI424-007): a dose-ranging, safety, and efficacy comparative trial of atazanavir at three doses in combination with didanosine and stavudine in antiretroviral-naive subjects.* J Acquir Immune Defic Syndr, 2003. **32**(1): p. 18-29.

306. Squires, K., et al., *Comparison of once-daily atazanavir with efavirenz, each in combination with fixed-dose zidovudine and lamivudine, as initial therapy for patients infected with HIV.* J Acquir Immune Defic Syndr, 2004. **36**(5): p. 1011-9.

307. Wood, R., et al., *Long-term efficacy and safety of atazanavir with stavudine and lamivudine in patients previously treated with nelfinavir or atazanavir.* J Acquir Immune Defic Syndr, 2004. **36**(2): p. 684-92.

308. Haas, D.W., et al., *Therapy with atazanavir plus saquinavir in patients failing highly active antiretroviral therapy: a randomized comparative pilot trial.* Aids, 2003. **17**(9): p. 1339-49.

309. Gathe, J.C., Jr., et al., *SOLO: 48-week efficacy and safety comparison of once-daily fosamprenavir /ritonavir versus twice-daily nelfinavir in naive HIV-1-infected patients.* Aids, 2004. **18**(11): p. 1529-37.

310. Gathe, J.C., Jr., et al., *Long-term (120-Week) antiviral efficacy and tolerability of fosamprenavir/ritonavir once daily in therapy-naive patients with HIV-1 infection: an uncontrolled, open-label, single-arm follow-on study.* Clin Ther, 2006. **28**(5): p. 745-54.

311. Rodriguez-French, A., et al., *The NEAT study: a 48-week open-label study to compare the antiviral efficacy and safety of GW433908 versus nelfinavir in antiretroviral therapy-naive HIV-1-infected patients.* J Acquir Immune Defic Syndr, 2004. **35**(1): p. 22-32.

312. Eron, J., Jr., et al., *The KLEAN study of fosamprenavir-ritonavir versus lopinavir-ritonavir, each in combination with abacavir-lamivudine, for initial treatment of HIV infection over 48 weeks: a randomised non-inferiority trial.* Lancet, 2006. **368**(9534): p. 476-82.

313. Cahn, P., et al., *Ritonavir-boosted tipranavir demonstrates superior efficacy to ritonavir-boosted protease inhibitors in treatment-experienced HIV-infected patients: 24-week results of the RESIST-2 trial.* Clin Infect Dis, 2006. **43**(10): p. 1347-56.

314. Gathe, J., et al., *Efficacy of the protease inhibitors tipranavir plus ritonavir in treatment-experienced patients: 24-week analysis from the RESIST-1 trial.* Clin Infect Dis, 2006. **43**(10): p. 1337-46.

315. Arasteh, K., et al., *Efficacy and safety of darunavir/ritonavir in treatment-experienced HIV type-1 patients in the POWER 1, 2 and 3 trials at week 96.* Antivir Ther, 2009. **14**(6): p. 859-64.

316. Clotet, B., et al., *Efficacy and safety of darunavir-ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: a pooled subgroup analysis of data from two randomised trials.* Lancet, 2007. **369**(9568): p. 1169-78.

317. De Meyer, S.M., et al., *Efficacy of once-daily darunavir/ritonavir 800/100 mg in HIV-infected, treatment-experienced patients with no baseline resistance-associated mutations to darunavir.* J Acquir Immune Defic Syndr, 2008. **49**(2): p. 179-82.

318. Madruga, J.V., et al., *Efficacy and safety of darunavir-ritonavir compared with that of lopinavir-ritonavir at 48 weeks in treatment-experienced, HIV-infected patients in TITAN: a randomised controlled phase III trial.* Lancet, 2007. **370**(9581): p. 49-58.

319. Mills, A.M., et al., *Once-daily darunavir/ritonavir vs. lopinavir/ritonavir in treatment-naive, HIV-1-infected patients: 96-week analysis.* Aids, 2009. **23**(13): p. 1679-88.

320. Molina, J.M., et al., *Safety and efficacy of darunavir (TMC114) with low-dose ritonavir in treatment-experienced patients: 24-week results of POWER 3.* J Acquir Immune Defic Syndr, 2007. **46**(1): p. 24-31.

321. Ortiz, R., et al., *Efficacy and safety of once-daily darunavir/ritonavir versus lopinavir/ritonavir in treatment-naive HIV-1-infected patients at week 48.* AIDS, 2008. **22**(12): p. 1389-97.

322. Katlama, C., et al., *Efficacy and safety of TMC114/ritonavir in treatment-experienced HIV patients: 24-week results of POWER 1.* Aids, 2007. **21**(4): p. 395-402.

323. Been-Tiktak, A.M., et al., *Efficacy and safety of combination therapy with delavirdine and zidovudine: a European/Australian phase II trial.* Int J Antimicrob Agents, 1999. **11**(1): p. 13-21.

324. Friedland, G.H., et al., *Efficacy and safety of delavirdine mesylate with zidovudine and didanosine compared with two-drug combinations of these agents in persons with HIV disease with CD4 counts of 100 to 500 cells/mm3 (ACTG 261). ACTG 261 Team.* J Acquir Immune Defic Syndr, 1999. **21**(4): p. 281-92.

325. Joly, V., et al., *Delavirdine in combination with zidovudine in treatment of human immunodeficiency virus type 1-infected patients: evaluation of efficacy and emergence of viral resistance in a randomized, comparative phase III trial. The M/3331/0013B Study Group.* Antimicrob Agents Chemother, 2000. **44**(11): p. 3155-7.

326. Para, M.F., et al., *ACTG 260: a randomized, phase I-II, dose-ranging trial of the anti-human immunodeficiency virus activity of delavirdine monotherapy. The AIDS Clinical Trials Group Protocol 260 Team.* Antimicrob Agents Chemother, 1999. **43**(6): p. 1373-8.

327. van Leth, F., et al., *Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study.* Lancet, 2004. **363**(9417): p. 1253-63.

328. Podzamczer, D., et al., *A randomized clinical trial comparing nelfinavir or nevirapine associated to zidovudine/lamivudine in HIV-infected naive patients (the Combine Study).* Antivir Ther, 2002. **7**(2): p. 81-90.

329. Staszewski, S., et al., *Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team.* N Engl J Med, 1999. **341**(25): p. 1865-73.

330. Haas, D.W., et al., *Therapy with efavirenz plus indinavir in patients with extensive prior nucleoside reverse-transcriptase inhibitor experience: a randomized, double-blind, placebo-controlled trial.* J Infect Dis, 2001. **183**(3): p. 392-400.

331. Lazzarin, A., et al., *Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-2: 24-week results from a randomised, double-blind, placebo-controlled trial.* Lancet, 2007. **370**(9581): p. 39-48.

332. Madruga, J.V., et al., *Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial.* Lancet, 2007. **370**(9581): p. 29-38.

333. Cohen, C.J., et al., *Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naive adults infected with HIV-1 (THRIVE): a phase 3, randomised, non-inferiority trial.* Lancet, 2011. **378**(9787): p. 229-37.

334. Molina, J.M., et al., *Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial.* Lancet, 2011. **378**(9787): p. 238-46.

335. Kilby, J.M., et al., *The safety, plasma pharmacokinetics, and antiviral activity of subcutaneous enfuvirtide (T-20), a peptide inhibitor of gp41-mediated virus fusion, in HIV-infected adults.* AIDS Res Hum Retroviruses, 2002. **18**(10): p. 685-93.

336. Lalezari, J.P., et al., *A controlled Phase II trial assessing three doses of enfuvirtide (T-20) in combination with abacavir, amprenavir, ritonavir and efavirenz in non-nucleoside reverse transcriptase inhibitor-naive HIV-infected adults.* Antivir Ther, 2003. **8**(4): p. 279-87.

337. Lazzarin, A., et al., *Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia.* New England Journal of Medicine, 2003. **348**(22): p. 2186-2195.

338. Gulick, R.M., et al., *Maraviroc for previously treated patients with R5 HIV-1 infection.* N Engl J Med, 2008. **359**(14): p. 1429-41.

339. Saag, M., et al., *A double-blind, placebo-controlled trial of maraviroc in treatment-experienced patients infected with non-R5 HIV-1.* J Infect Dis, 2009. **199**(11): p. 1638-47.

340. Sierra-Madero, J., et al., *Efficacy and safety of maraviroc versus efavirenz, both with zidovudine/lamivudine: 96-week results from the MERIT study.* HIV Clin Trials, 2010. **11**(3): p. 125-32.

341. Cooper, D.A., et al., *Efficacy and safety of maraviroc vs. efavirenz in treatment-naive patients with HIV-1: 5-year findings.* Aids, 2014. **28**(5): p. 717-25.

342. De Castro, N., et al., *Switch from enfuvirtide to raltegravir in virologically suppressed multidrug-resistant HIV-1-infected patients: a randomized open-label trial.* Clin Infect Dis, 2009. **49**(8): p. 1259-67.

343. Eron, J.J., et al., *Switch to a raltegravir-based regimen versus continuation of a lopinavir-ritonavir-based regimen in stable HIV-infected patients with suppressed viraemia (SWITCHMRK 1 and 2): two multicentre, double-blind, randomised controlled trials.* Lancet, 2010. **375**(9712): p. 396-407.

344. Grinsztejn, B., et al., *Safety and efficacy of the HIV-1 integrase inhibitor raltegravir (MK-0518) in treatment-experienced patients with multidrug-resistant virus: a phase II randomised controlled trial.* Lancet, 2007. **369**(9569): p. 1261-9.

345. Lennox, J.L., et al., *Safety and efficacy of raltegravir-based versus efavirenz-based combination therapy in treatment-naive patients with HIV-1 infection: a multicentre, double-blind randomised controlled trial.* Lancet, 2009. **374**(9692): p. 796-806.

346. Markowitz, M., et al., *Sustained antiretroviral effect of raltegravir after 96 weeks of combination therapy in treatment-naive patients with HIV-1 infection.* J Acquir Immune Defic Syndr, 2009. **52**(3): p. 350-6.

347. Markowitz, M., et al., *Rapid and durable antiretroviral effect of the HIV-1 Integrase inhibitor raltegravir as part of combination therapy in treatment-naive patients with HIV-1 infection: results of a 48-week controlled study.* J Acquir Immune Defic Syndr, 2007. **46**(2): p. 125-33.

348. Yazdanpanah, Y., et al., *High rate of virologic suppression with raltegravir plus etravirine and darunavir/ritonavir among treatment-experienced patients infected with multidrug-resistant HIV: results of the ANRS 139 TRIO trial.* Clin Infect Dis, 2009. **49**(9): p. 1441-9.

349. Molina, J.M., et al., *Efficacy and safety of once daily elvitegravir versus twice daily raltegravir in treatment-experienced patients with HIV-1 receiving a ritonavir-boosted protease inhibitor: randomised, double-blind, phase 3, non-inferiority study.* Lancet Infect Dis, 2012. **12**(1): p. 27-35.

350. Zolopa, A.R., et al., *Activity of elvitegravir, a once-daily integrase inhibitor, against resistant HIV Type 1: results of a phase 2, randomized, controlled, dose-ranging clinical trial.* J Infect Dis, 2010. **201**(6): p. 814-22.

351. Zolopa, A., et al., *A randomized double-blind comparison of coformulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate versus efavirenz/emtricitabine/tenofovir disoproxil fumarate for initial treatment of HIV-1 infection: analysis of week 96 results.* J Acquir Immune Defic Syndr, 2013. **63**(1): p. 96-100.

352. Rockstroh, J.K., et al., *A randomized, double-blind comparison of coformulated elvitegravir/cobicistat/emtricitabine/tenofovir DF vs ritonavir-boosted atazanavir plus coformulated emtricitabine and tenofovir DF for initial treatment of HIV-1 infection: analysis of week 96 results.* J Acquir Immune Defic Syndr, 2013. **62**(5): p. 483-6.

353. Castagna, A., et al., *Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study.* J Infect Dis, 2014. **210**(3): p. 354-62.

354. Eron, J.J., et al., *Safety and efficacy of dolutegravir in treatment-experienced subjects with raltegravir-resistant HIV type 1 infection: 24-week results of the VIKING Study.* J Infect Dis, 2013. **207**(5): p. 740-8.

355. Molina, J.-M., et al., *Once-daily dolutegravir is superior to once-daily darunavir/ritonavir in treatment-naïve HIV-1-positive individuals: 96 week results from FLAMINGO.* Journal of the International AIDS Society, 2014. **17**(4Suppl 3): p. 19490.

356. van Lunzen, J., et al., *Once daily dolutegravir (S/GSK1349572) in combination therapy in antiretroviral-naive adults with HIV: planned interim 48 week results from SPRING-1, a dose-ranging, randomised, phase 2b trial.* Lancet Infect Dis, 2012. **12**(2): p. 111-8.

357. Cahn, P., et al., *Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study.* Lancet, 2013. **382**(9893): p. 700-8.

358. Raffi, F., et al., *Once-daily dolutegravir versus twice-daily raltegravir in antiretroviral-naive adults with HIV-1 infection (SPRING-2 study): 96 week results from a randomised, double-blind, non-inferiority trial.* The Lancet Infectious Diseases, 2013. **13**(11): p. 927-935.

359. Walmsley, S., et al., *Brief Report: Dolutegravir Plus Abacavir/Lamivudine for the Treatment of HIV-1 Infection in Antiretroviral Therapy-Naive Patients: Week 96 and Week 144 Results From the SINGLE Randomized Clinical Trial.* J Acquir Immune Defic Syndr, 2015. **70**(5): p. 515-9.

360. Walmsley, S.L., et al., *Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection.* N Engl J Med, 2013. **369**(19): p. 1807-18.

361. Gallant, J.E., et al., *Brief Report: Cobicistat Compared With Ritonavir as a Pharmacoenhancer for Atazanavir in Combination With Emtricitabine/Tenofovir Disoproxil Fumarate: Week 144 Results.* J Acquir Immune Defic Syndr, 2015. **69**(3): p. 338-40.

362. Gallant, J.E., et al., *Cobicistat versus ritonavir as a pharmacoenhancer of atazanavir plus emtricitabine/tenofovir disoproxil fumarate in treatment-naive HIV type 1-infected patients: week 48 results.* J Infect Dis, 2013. **208**(1): p. 32-9.

363. Arts, E.J. and D.J. Hazuda, *HIV-1 Antiretroviral Drug Therapy.* Cold Spring Harbor Perspectives in Medicine, 2012. **2**(4).

364. NIH. *Types of HIV/AIDS Antiretroviral Drugs*. 2013 [cited 2015 28/1/2015]; Available from: http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Treatment/Pages/arvDrugClasses.aspx.

365. The body. *How HIV Drugs Work*. 2014 [cited 2015 28/1/2015]; Available from: http://www.thebody.com/content/art968.html.

366. AIDSMAP. *How NRTIs and NtRTIs work*. 2017 [cited 2015 28/1/2015]; Available from: http://www.aidsmap.com/How-NRTIs-and-NtRTIs-work/page/1729427/.

367. Koczor, C.A. and W. Lewis, *Nucleoside reverse transcriptase inhibitor toxicity and mitochondrial DNA.* Expert Opin Drug Metab Toxicol, 2010. **6**(12): p. 1493-504.

368. British HIV Association (BHIVA), *British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012.* HIV Medicine, 2012. **13**(Suppl. 2): p. 1-85.

369. Sabin, C.A., et al., *Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration.* Lancet, 2008. **371**(9622): p. 1417-26.

370. Obel, N., et al., *Abacavir and risk of myocardial infarction in HIV-infected patients on highly active antiretroviral therapy: a population-based nationwide cohort study.* HIV Med, 2010. **11**(2): p. 130-6.

371. Worm, S.W., et al., *Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study.* Journal of Infectious Diseases, 2010. **201**(3): p. 318-30.

372. Bedimo, R.J., et al., *Abacavir use and risk of acute myocardial infarction and cerebrovascular events in the highly active antiretroviral therapy era.* Clin Infect Dis, 2011. **53**(1): p. 84-91.

373. Mocroft, A., et al., *Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients.* Aids, 2010. **24**(11): p. 1667-78.

374. WHO, *Rapid advice: Antiretroviral therapy for HIV infection in adults and adolescents* 2009.

375. WHO, *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection* 2013.

376. AIDSMAP. *Protease inhibitors*. 2017 [cited 2017; Available from: http://www.aidsmap.com/Protease-inhibitors/page/1729414/.

377. Zhang, X., et al., *Thorough QT/QTc study of ritonavir-boosted saquinavir following multiple-dose administration of therapeutic and supratherapeutic doses in healthy participants.* J Clin Pharmacol, 2012. **52**(4): p. 520-9.

378. Mathez, D., et al., *Reductions in viral load and increases in T lymphocyte numbers in treatment-naive patients with advanced HIV-1 infection treated with ritonavir, zidovudine and zalcitabine triple therapy.* Antivir Ther, 1997. **2**(3): p. 175-83.

379. Notermans, D.W., et al., *Decrease of HIV-1 RNA levels in lymphoid tissue and peripheral blood during treatment with ritonavir, lamivudine and zidovudine. Ritonavir/3TC/ZDV Study Group.* Aids, 1998. **12**(2): p. 167-73.

380. García, F., et al., *A randomized study comparing triple versus double antiretroviral therapy or no treatment in HIV-1-infected patients in very early stage disease: the Spanish Earth-1 study.* AIDS, 1999. **13**(17): p. 2377-2388.

381. Lederman, M.M., et al., *Immunologic Responses Associated with 12 Weeks of Combination Antiretroviral Therapy Consisting of Zidovudine, Lamivudine, and Ritonavir: Results of AIDS Clinical Trials Group Protocol 315.* Journal of Infectious Diseases, 1998. **178**(1): p. 70-79.

382. Maguire, M., et al., *Absence of zidovudine resistance in antiretroviral-naive patients following zidovudine/lamivudine/protease inhibitor combination therapy: virological evaluation of the AVANTI 2 and AVANTI 3 studies.* AIDS, 2000. **14**(9): p. 1195-201.

383. Zeldin, R.K. and R.A. Petruschke, *Pharmacological and therapeutic properties of ritonavir-boosted protease inhibitor therapy in HIV-infected patients.* J Antimicrob Chemother, 2004. **53**(1): p. 4-9.

384. Kempf, D.J., et al., *Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir.* Antimicrob Agents Chemother, 1997. **41**(3): p. 654-60.

385. Wynn, G.H., et al., *Antiretrovirals, part 1: overview, history, and focus on protease inhibitors.* Psychosomatics, 2004. **45**(3): p. 262-70.

386. Pomerantz, R.J. and D.L. Horn, *Twenty years of therapy for HIV-1 infection.* Nat Med, 2003. **9**(7): p. 867-873.

387. Drake, S.M., *NNRTIs—a new class of drugs for HIV.* Journal of Antimicrobial Chemotherapy, 2000. **45**(4): p. 417-420.

388. Cohen, C., et al., *Pooled week 48 safety and efficacy results from the ECHO and THRIVE phase III trials comparing TMC278 vs EFV in treatment-naïve, HIV-1-infected patients.* Journal of the International AIDS Society, 2010. **13**(Suppl 4): p. O48-O48.

389. Pommier, Y., A.A. Johnson, and C. Marchand, *Integrase inhibitors to treat HIV/AIDS.* Nat Rev Drug Discov, 2005. **4**(3): p. 236-48.

390. Hicks, C. and R.M. Gulick, *Raltegravir: The First HIV Type 1 Integrase Inhibitor.* Clinical Infectious Diseases, 2009. **48**(7): p. 931-939.

391. Garrido, C., et al., *Broad Phenotypic Cross-Resistance to Elvitegravir in HIV-Infected Patients Failing on Raltegravir-Containing Regimens.* Antimicrobial Agents and Chemotherapy, 2012. **56**(6): p. 2873-2878.

392. Briz, V., E. Poveda, and V. Soriano, *HIV entry inhibitors: mechanisms of action and resistance pathways.* Journal of Antimicrobial Chemotherapy, 2006. **57**(4): p. 619-627.

393. Esté, J.A. and A. Telenti, *HIV entry inhibitors.* The Lancet, 2007. **370**(9581): p. 81-88.

394. Lalezari, J.P., et al., *Enfuvirtide, an HIV-1 Fusion Inhibitor, for Drug-Resistant HIV Infection in North and South America.* New England Journal of Medicine, 2003. **348**(22): p. 2175-2185.

395. Matthews, T., et al., *Enfuvirtide: the first therapy to inhibit the entry of HIV-1 into host CD4 lymphocytes.* Nat Rev Drug Discov, 2004. **3**(3): p. 215-225.

396. MacArthur, R.D. and R.M. Novak, *Maraviroc: The First of a New Class of Antiretroviral Agents.* Clinical Infectious Diseases, 2008. **47**(2): p. 236-241.

397. Xu, G.G., J. Guo, and Y. Wu, *Chemokine Receptor CCR5 Antagonist Maraviroc: Medicinal Chemistry and Clinical Applications.* Current topics in medicinal chemistry, 2014. **14**(13): p. 1504-1514.

398. Naggie, S. and C. Hicks, *Protease inhibitor-based antiretroviral therapy in treatment-naive HIV-1-infected patients: the evidence behind the options.* J Antimicrob Chemother, 2010. **65**(6): p. 1094-9.

399. Bangalore, S., et al., *Fixed-Dose Combinations Improve Medication Compliance: A Meta-Analysis.* The American Journal of Medicine, 2007. **120**(8): p. 713-719.

400. (BHIVA), B.H.A., *British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015*. 2015.

401. Flexner, C., *HIV drug development: the next 25 years.* Nat Rev Drug Discov, 2007. **6**(12): p. 959-966.

402. UNAIDS, *90-90-90: An ambitious treatment target to help end the AIDS epidemic*. 2014.

403. European Centre for Disease Prevention and Control, *Thematic report: HIV continuum of care. Monitoring implementation of the Dublin declaration on Partnership to Fight HIV/AIDS in Europe and Central Asia: 2014 progress report*. 2015, ECDC: Stockholm.

404. Centers for Disease Control and Prevention (CDC), *Advancing HIV prevention: new strategies for a changing epidemic--United States, 2003.* MMWR Morb Mortal Wkly Rep, 2003. **52**(15): p. 329-32.

405. UNAIDS, *The gap report*. 2014.

406. UNAIDS, *Global AIDS update 2016*. 2016.

407. UNAIDS. *AIDSinfo*. 2016; Available from: http://aidsinfo.unaids.org/#.

408. European Centre for Disease Prevention and Control/WHO Regional Office for Europe, *HIV/AIDS surveillance in Europe 2015*. 2016.

409. DeHovitz, J., A. Uuskula, and N. El-Bassel, *The HIV Epidemic in Eastern Europe and Central Asia.* Current HIV/AIDS Reports, 2014. **11**(2): p. 168-176.

410. WHO Europe, *HIV/AIDS In Europe: Moving from death sentence to chronic disease management*. 2006.

411. UNAIDS, *HIV - Related Stigma, Discrimination and Human Rights Violations: Case studies of successful programmes* 2005.

412. Bailey, H., A. Turkova, and C. Thorne, *Syphilis, hepatitis C and HIV in Eastern Europe.* Curr Opin Infect Dis, 2017. **30**(1): p. 93-100.

413. Kazatchkine, M., *Drug use, HIV, HCV and TB: major interlinked challenges in Eastern Europe and Central Asia.* J Int AIDS Soc, 2014. **17**(4 Suppl 3): p. 19501.

414. Sulkowski, M.S. and D.L. Thomas, *Hepatitis C in the HIV-Infected Person.* Annals of Internal Medicine, 2003. **138**(3): p. 197-207.

415. Podlekareva, D.N., et al., *Mortality from HIV and TB coinfections is higher in Eastern Europe than in Western Europe and Argentina.* AIDS (London, England), 2009. **23**(18): p. 2485-2495.

416. Mathers, B.M., et al., *HIV prevention, treatment, and care services for people who inject drugs: a systematic review of global, regional, and national coverage.* The Lancet, 2010. **375**(9719): p. 1014-1028.

417. Sarang, A., T. Rhodes, and N. Sheon, *Systemic barriers accessing HIV treatment among people who inject drugs in Russia: a qualitative study.* Health Policy and Planning, 2013. **28**(7): p. 681-691.

418. Spina, M., et al., *Neoplastic complications of HIV infection.* Ann Oncol, 1999. **10**(11): p. 1271-86.

419. UNAIDS, *HIV and aging: A special supplement to the UNAIDS report on the global AIDS epidemic* 2013.

420. Lifson, A.R. and H.A. Lando, *Smoking and HIV: Prevalence, Health Risks, and Cessation Strategies.* Current HIV/AIDS Reports, 2012. **9**(3): p. 223-230.

421. Browning, K.K., et al., *Tobacco use and cessation in HIV-infected individuals.* Clinics in chest medicine, 2013. **34**(2): p. 181-90.

422. Galvan, F.H., et al., *The prevalence of alcohol consumption and heavy drinking among people with HIV in the United States: results from the HIV Cost and Services Utilization Study.* J Stud Alcohol, 2002. **63**(2): p. 179-86.

423. Green, T.C., et al., *Patterns of drug use and abuse among aging adults with and without HIV: A latent class analysis of a US Veteran cohort.* Drug and Alcohol Dependence, 2010. **110**(3): p. 208-220.

424. Kahn, J.A., et al., *Prevalence and Risk Factors for HPV in HIV-Positive Young Women Receiving Their First HPV Vaccination.* JAIDS Journal of Acquired Immune Deficiency Syndromes, 2012.

425. van Kesteren, N., *Sexual risk behavior among HIV-positive men who have sex with men: a literature review.* Patient education and counseling, 2007. **65**(1): p. 5.

426. Grulich, A.E., et al., *HIV, cancer, and aging.* Sex Health, 2011. **8**(4): p. 521-5.

427. Reekie, J., et al., *Relationship between current level of immunodeficiency and non-acquired immunodeficiency syndrome-defining malignancies.* Cancer, 2010. **116**(22): p. 5306-5315.

428. Borges, Á.H., et al., *Predicting risk of cancer during HIV infection: the role of inflammatory and coagulation biomarkers.* AIDS, 2013. **27**(9): p. 1433-1441.

429. Bruyand, M., et al., *Role of Uncontrolled HIV RNA Level and Immunodeficiency in the Occurrence of Malignancy in HIV-Infected Patients during the Combination Antiretroviral Therapy Era: Agence Nationale de Recherche sur le Sida (ANRS) CO3 Aquitaine Cohort.* Clinical infectious diseases, 2009. **49**(7): p. 1109-1116.

430. Franceschi, S., et al., *Changing patterns of cancer incidence in the early- and late-HAART periods: the Swiss HIV Cohort Study.* Br J Cancer, 2010. **103**(3): p. 416-22.

431. Marcus, J.L., et al., *Prostate cancer incidence and prostate-specific antigen testing among HIV-positive and HIV-negative men.* J Acquir Immune Defic Syndr, 2014. **66**(5): p. 495-502.

432. Crum-Cianflone, N., et al., *Trends in the incidence of cancers among HIV-infected persons and the impact of antiretroviral therapy: a 20-year cohort study.* AIDS, 2009. **23**(1): p. 41-50.

433. Guiguet, M., et al., *Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study.* Lancet Oncology, 2009. **10**(12): p. 1152-1159.

434. Chiu, C.G., et al., *Overview of cancer incidence and mortality among people living with HIV/AIDS in British Columbia, Canada: Implications for HAART use and NADM development.* BMC Cancer, 2017. **17**(270).

435. Bohlius, J., et al., *Incidence and risk factors of HIV-related non-Hodgkin's lymphoma in the era of combination antiretroviral therapy: a European multicohort study.* Antivir Ther, 2009. **14**(8): p. 1065-74.

436. Robbins, H.A., et al., *Epidemiologic contributions to recent cancer trends among HIV-infected people in the United States.* AIDS, 2014. **28**(6): p. 881-890.

437. Park, L.S., et al., *Time trends in cancer incidence in persons living with HIV/AIDS in the antiretroviral therapy era: 1997-2012.* AIDS, 2016. **30**(11): p. 1795-806.

438. Patel, P., et al., *Incidence of Types of Cancer among HIV-Infected Persons Compared with the General Population in the United States, 1992–2003.* Annals of Internal Medicine, 2008. **148**(10): p. 728-736.

439. Grulich, A.E., et al., *Decreasing rates of Kaposi's sarcoma and non-Hodgkin's lymphoma in the era of potent combination anti-retroviral therapy.* AIDS, 2001. **15**(5): p. 629-33.

440. Kirk, O., et al., *Non-Hodgkin lymphoma in HIV-infected patients in the era of highly active antiretroviral therapy.* Blood, 2001. **98**(12): p. 3406-3412.

441. Biggar, R.J., et al., *AIDS-related cancer and severity of immunosuppression in persons with AIDS.* J Natl Cancer Inst, 2007. **99**(12): p. 962-72.

442. Spano, J.P., et al., *AIDS and non-AIDS-related malignancies: a new vexing challenge in HIV-positive patients. Part I: Kaposi's sarcoma, non-Hodgkin's lymphoma, and Hodgkin's lymphoma.* European journal of internal medicine, 2002. **13**(3): p. 170-179.

443. Howlader, N., et al., *Contributions of HIV to Non-Hodgkin Lymphoma Mortality Trends in the United States.* Cancer Epidemiology Biomarkers & Prevention, 2016. **25**(9): p. 1289-1296.

444. Simard, E.P., R.M. Pfeiffer, and E.A. Engels, *Spectrum of cancer risk late after AIDS onset in the United States.* Arch Intern Med, 2010. **170**(15): p. 1337-45.

445. Grulich, A.E. and C.M. Vajdic, *The Epidemiology of Cancers in Human Immunodeficiency Virus Infection and After Organ Transplantation.* Seminars in Oncology, 2015. **42**(2): p. 247-257.

446. Tirelli, U., S. Franceschi, and A. Carbone, *Malignant tumours in patients with HIV infection.* BMJ, 1994. **308**(6937): p. 1148-53.

447. Beral, V., et al., *AIDS-associated non-Hodgkin lymphoma.* Lancet, 1991. **337**(8745): p. 805-9.

448. Stevens, P.L. and N.M. Reddy, *Immune surveillance and lymphoid malignancy in immunocompromised host.* American Journal of Blood Research, 2013. **3**(2): p. 91-101.

449. Silverberg, M.J., et al., *Cumulative Incidence of Cancer among HIV-infected Individuals in North America.* Annals of Internal Medicine, 2015. **163**(7): p. 507-518.

450. Bower, M., et al., *CD4 counts and the risk of systemic non-Hodgkin's lymphoma in individuals with HIV in the UK.* Haematologica, 2009. **94**(6): p. 875-880.

451. Kirk, O., et al., *Non-Hodgkin lymphoma in HIV-infected patients in the era of highly active antiretroviral therapy.* Blood, 2001. **98**(12): p. 3406-3412.

452. Cesarman, E., *Pathology of lymphoma in HIV.* Current opinion in oncology, 2013. **25**(5): p. 487-494.

453. Ensoli, B., et al., *Biology of Kaposi's sarcoma.* European Journal of Cancer, 2001. **37**(10): p. 1251-1269.

454. InfoNet, A. *Kaposi's Sarcoma*. 2016 [cited 2017; Available from: http://www.aidsinfonet.org/uploaded/factsheets/98_eng_511.pdf.

455. Gallafent, J.H., et al., *Profile of Patients With Kaposi's Sarcoma in the Era of Highly Active Antiretroviral Therapy.* Journal of Clinical Oncology, 2005. **23**(6): p. 1253-1260.

456. Stebbing, J., et al., *A prognostic index for AIDS-associated Kaposi's sarcoma in the era of highly active antiretroviral therapy.* The Lancet, 2006. **367**(9521): p. 1495-1502.

457. Ledergerber, B., et al., *AIDS-related opportunistic illnesses occurring after initiation of potent antiretroviral therapy: the Swiss HIV Cohort Study.* JAMA, 1999. **282**(23): p. 2220-6.

458. Jones, J.L., et al., *Incidence and trends in Kaposi's sarcoma in the era of effective antiretroviral therapy.* J Acquir Immune Defic Syndr, 2000. **24**(3): p. 270-4.

459. Mocroft, A., et al., *The changing pattern of Kaposi sarcoma in patients with HIV, 1994–2003.* Cancer, 2004. **100**(12): p. 2644-2654.

460. International Collaboration on HIV and Cancer, *Highly active antiretroviral therapy and incidence of cancer in human immunodeficiency virus-infected adults.* J Natl Cancer Inst, 2000. **92**(22): p. 1823-30.

461. Walboomers, J.M.M., et al., *Human papillomavirus is a necessary cause of invasive cervical cancer worldwide.* The Journal of Pathology, 1999. **189**(1): p. 12-19.

462. Clifford, G.M., et al., *Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis.* British Journal of Cancer, 2003. **88**(1): p. 63-73.

463. Bosch, F.X., et al., *Prevalence of Human Papillomavirus in Cervical Cancer: a Worldwide Perspective.* Journal of the National Cancer Institute, 1995. **87**(11): p. 796-802.

464. Hleyhel, M., et al., *Risk of AIDS-defining cancers among HIV-1-infected patients in France between 1992 and 2009: results from the FHDH-ANRS CO4 cohort.* Clinical Infectious Diseases, 2013. **57**(11): p. 1638-1647.

465. Engels, E.A., et al., *Cancer risk in people infected with human immunodeficiency virus in the United States.* Int J Cancer, 2008. **123**(1): p. 187-94.

466. Bräuninger, A., et al., *Molecular biology of Hodgkin's and Reed/Sternberg cells in Hodgkin's lymphoma.* International Journal of Cancer, 2006. **118**(8): p. 1853-1861.

467. Powles, T., et al., *Highly Active Antiretroviral Therapy and the Incidence of Non–AIDS-Defining Cancers in People With HIV Infection.* Journal of Clinical Oncology, 2009. **27**(6): p. 884-890.

468. Bohlius, J., et al., *HIV-1-related Hodgkin lymphoma in the era of combination antiretroviral therapy: incidence and evolution of CD4(+) T-cell lymphocytes.* Blood, 2011. **117**(23): p. 6100-6108.

469. Frisch, M., et al., *Association of cancer with AIDS-related immunosuppression in adults.* JAMA, 2001. **285**(13): p. 1736-45.

470. Biggar, R.J., et al., *Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS.* Blood, 2006. **108**(12): p. 3786-3791.

471. Ambinder, R.F., *Epstein-barr virus and hodgkin lymphoma.* Hematology Am Soc Hematol Educ Program, 2007: p. 204-9.

472. Ryerson, A.B., et al., *Burden of potentially human papillomavirus-associated cancers of the oropharynx and oral cavity in the US, 1998–2003.* Cancer, 2008. **113**(S10): p. 2901-2909.

473. Kurdgelashvili, G., et al., *Incidence of potentially human papillomavirus–related neoplasms in the United States, 1978 to 2007.* Cancer, 2013. **119**(12): p. 2291-2299.

474. Bouvard, V., et al., *A review of human carcinogens—Part B: biological agents.* The Lancet Oncology, 2009. **10**(4): p. 321-322.

475. Dubrow, R., et al., *HIV infection, aging, and immune function: implications for cancer risk and prevention.* Current opinion in oncology, 2012. **24**(5): p. 506-16.

476. IARC, *Human Papillomaviruses.* IARC monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, 2007. **2017**(90).

477. IARC, *A review of human carcinogens—Part B: biological agents.* IARC monographs on the Evaluation of Carcinogenic Risk of Chemicals to humans, 2012. **100B**.

478. Chaturvedi, A.K., et al., *Risk of Human Papillomavirus-Associated Cancers Among Persons With AIDS.* Journal of the National Cancer Institute, 2009. **101**(16): p. 1120-1130.

479. Silverberg, M.J., et al., *Risk of Anal Cancer in HIV-Infected and HIV-Uninfected Individuals in North America.* Clinical infectious diseases, 2012. **54**(7): p. 1026-1034.

480. Bower, M., et al., *HIV-associated anal cancer: has highly active antiretroviral therapy reduced the incidence or improved the outcome?* J Acquir Immune Defic Syndr, 2004. **37**(5): p. 1563-5.

481. Piketty, C., et al., *Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy.* AIDS, 2008. **22**(10): p. 1203-11.

482. Wang, C.-c.J., M.J. Silverberg, and D.I. Abrams, *Non-AIDS-Defining Malignancies in the HIV-Infected Population.* Current infectious disease reports, 2014. **16**(6): p. 406-406.

483. Hessol, N.A., et al., *Cancer risk among participants in the women's interagency HIV study.* J Acquir Immune Defic Syndr, 2004. **36**(4): p. 978-85.

484. Rosenthal, E., et al., *Mortality due to hepatitis C-related liver disease in HIV-infected patients in France (Mortavic 2001 study).* AIDS, 2003. **17**(12): p. 1803-1809.

485. Konopnicki, D., et al., *Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort.* AIDS, 2005. **19**(6): p. 593-601.

486. Shiels, M.S., et al., *Reduced Risk of Prostate Cancer in U.S. Men with AIDS.* Cancer Epidemiology Biomarkers & Prevention, 2010. **19**(11): p. 2910-2915.

487. Mbulaiteye, S.M., et al., *Immune deficiency and risk for malignancy among persons with AIDS.* J Acquir Immune Defic Syndr, 2003. **32**(5): p. 527-33.

488. Biggar, R.J., et al., *Cancer risk in elderly persons with HIV/AIDS.* J Acquir Immune Defic Syndr, 2004. **36**(3): p. 861-868.

489. Mdodo, R., et al., *Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: cross-sectional surveys.* Annals of internal medicine, 2015. **162**(5): p. 335-344.

490. Grierson, J., et al., *HIV Futures 6: Making Positive Lives Count*. monograph series number 74. 2009, Melbourne : Australian Research Centre in Sex, Health and Society, La Trobe University: The Australian Research Centre in Sex, Health and Society, Latrobe University, Melbourne, Australia

491. Benard, A., et al., *Tobacco addiction and HIV infection: toward the implementation of cessation programs. ANRS CO3 Aquitaine Cohort.* AIDS Patient Care STDS, 2007. **21**(7): p. 458-68.

492. Duval, X., et al., *Living with HIV, antiretroviral treatment experience and tobacco smoking: results from a multisite cross-sectional study.* Antivir Ther, 2008. **13**(3): p. 389-97.

493. Tesoriero, J.M., et al., *Smoking among HIV positive New Yorkers: prevalence, frequency, and opportunities for cessation.* AIDS Behav, 2010. **14**(4): p. 824-35.

494. Mamary, E.M., D. Bahrs, and S. Martinez, *Cigarette smoking and the desire to quit among individuals living with HIV.* AIDS Patient Care STDS, 2002. **16**(1): p. 39-42.

495. Crothers, K., et al., *Decreased awareness of current smoking among health care providers of HIV-positive compared to HIV-negative veterans.* J Gen Intern Med, 2007. **22**(6): p. 749-54.

496. Helleberg, M., et al., *Smoking and life expectancy among HIV-infected individuals on antiretroviral therapy in Europe and North America.* Aids, 2015. **29**(2): p. 221-9.

497. Moore, P.S. and Y. Chang, *Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection.* N Engl J Med, 1995. **332**(18): p. 1181-5.

498. Moore, P.S., et al., *Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma.* AIDS, 1996. **10**(2): p. 175-80.

499. Ambinder, R.F., *Epstein–Barr virus associated lymphoproliferations in the AIDS setting.* European Journal of Cancer, 2001. **37**(10): p. 1209-1216.

500. Carbone, A., et al., *HIV-associated lymphomas and gamma-herpesviruses.* Blood, 2009. **113**(6): p. 1213-1224.

501. Parkin, D.M., *The global health burden of infection-associated cancers in the year 2002.* International Journal of Cancer, 2006. **118**(12): p. 3030-3044.

502. Fukayama, M., R. Hino, and H. Uozaki, *Epstein-Barr virus and gastric carcinoma: virus-host interactions leading to carcinoma.* Cancer Science, 2008. **99**(9): p. 1726-1733.

503. Joint United Nations Programme on HIV/AIDS (UNAIDS). *HIV AND AGING: A special supplement to the UNAIDS report on the global AIDS epidemic 2013*. 2013; Available from: http://www.unaids.org/sites/default/files/media_asset/20131101_JC2563_hiv-and-aging_en_0.pdf.

504. Mills, E.J., T. Barnighausen, and J. Negin, *HIV and aging--preparing for the challenges ahead.* N Engl J Med, 2012. **366**(14): p. 1270-3.

505. Deeks, S.G., *HIV infection, inflammation, immunosenescence, and aging.* Annu Rev Med, 2011. **62**: p. 141-55.

506. Desai, S. and A. Landay, *Early Immune Senescence in HIV Disease.* Current HIV/AIDS reports, 2010. **7**(1): p. 4-10.

507. Appay, V. and D. Sauce, *Immune activation and inflammation in HIV-1 infection: causes and consequences.* J Pathol, 2008. **214**(2): p. 231-41.

508. Ginaldi, L., et al., *Chronic antigenic load and apoptosis in immunosenescence.* Trends Immunol, 2005. **26**(2): p. 79-84.

509. Bhaskaran, K., et al., *Changes in the risk of death after HIV seroconversion compared with mortality in the general population.* Jama, 2008. **300**(1): p. 51-9.

510. Lohse, N., et al., *Survival of persons with and without HIV infection in Denmark, 1995-2005.* Ann Intern Med, 2007. **146**(2): p. 87-95.

511. Gueler, A., et al., *Life expectancy in HIV-positive persons in Switzerland: matched comparison with general population.* AIDS, 2017. **31**(3): p. 427-436.

512. Legarth, R.A., et al., *Long-Term Mortality in HIV-Infected Individuals 50 Years or Older: A Nationwide, Population-Based Cohort Study.* J Acquir Immune Defic Syndr, 2016. **71**(2): p. 213-8.

513. May, M., et al., *Impact of late diagnosis and treatment on life expectancy in people with HIV-1: UK Collaborative HIV Cohort (UK CHIC) Study.* Bmj, 2011. **343**: p. d6016.

514. Scutellari, P.N., C. Orzincolo, and E.M. Guarnelli, *Periodontal disease in patients with HIV infection. Radiographic study.* Radiol Med, 1996. **92**(5): p. 562-8.

515. Rasmussen, L.D., et al., *Risk of cataract surgery in HIV-infected individuals: a Danish Nationwide Population-based cohort study.* Clin Infect Dis, 2011. **53**(11): p. 1156-63.

516. Baker, J.V., et al., *HIV replication, inflammation, and the effect of starting antiretroviral therapy on plasma asymmetric dimethylarginine, a novel marker of endothelial dysfunction.* J Acquir Immune Defic Syndr, 2012. **60**(2): p. 128-34.

517. Kuller, L.H., et al., *Inflammatory and coagulation biomarkers and mortality in patients with HIV infection.* PLoS Med, 2008. **5**(10): p. e203.

518. Neuhaus, J., et al., *Markers of Inflammation, Coagulation, and Renal Function Are Elevated in Adults with HIV Infection.* Journal of Infectious Diseases, 2010. **201**(12): p. 1788-1795.

519. Fumaz, C.R., et al., *Increased peripheral proinflammatory cytokines in HIV-1-infected patients with prolonged viral suppression suffering from high psychological stress.* J Acquir Immune Defic Syndr, 2009. **52**(3): p. 427-8.

520. Rodger, A.J., et al., *Activation and Coagulation Biomarkers Are Independent Predictors of the Development of Opportunistic Disease in Patients with HIV Infection.* The Journal of Infectious Diseases, 2009. **200**(6): p. 973-983.

521. Park, L.S., et al., *Prevalence of non-HIV cancer risk factors in persons living with HIV/AIDS: a meta-analysis.* AIDS, 2016. **30**(2): p. 273-91.

522. Okada, F., *Inflammation-Related Carcinogenesis: Current Findings in Epidemiological Trends, Causes and Mechanisms.* Yonago Acta Medica, 2014. **57**(2): p. 65-72.

523. IARC, *Body Fatness.* IARC Handbooks of Cancer Prevention, 2016. **16**.

524. Vineis, P. and C.P. Wild, *Global cancer patterns: causes and prevention.* The Lancet, 2014. **383**(9916): p. 549-557.

525. IARC, *Solar and ultraviolet radiation.* IARC monographs on the evaluation of carcinogenic risks to humans, 2012. **100D**.

526. Cogliano, V.J., et al., *Preventable exposures associated with human cancers.* J Natl Cancer Inst, 2011. **103**(24): p. 1827-39.

527. IARC, *Air pollution and cancer.* IARC Scientific Publications, 2013. **161**.

528. Chlebowski, R.T., et al., *Breast cancer after use of estrogen plus progestin and estrogen alone: Analyses of data from 2 women's health initiative randomized clinical trials.* JAMA Oncology, 2015. **1**(3): p. 296-305.

529. Bower, M., et al., *British HIV Association guidelines for HIV-associated malignancies 2014.* HIV medicine, 2014. **15**(S2): p. 1-92.

530. Spano, J.-P., et al., *AIDS-Related Malignancies: State of the Art and Therapeutic Challenges.* Journal of Clinical Oncology, 2008. **26**(29): p. 4834-4842.

531. Biggar, R.J., et al., *Survival after cancer diagnosis in persons with AIDS.* J Acquir Immune Defic Syndr, 2005. **39**(3): p. 293-9.

532. Rengan, R., et al., *Effect of HIV on survival in patients with non-small-cell lung cancer in the era of highly active antiretroviral therapy: a population-based study.* Lancet Oncol, 2012. **13**(12): p. 1203-9.

533. Alfa-Wali, M., et al., *Chemoradiotherapy for anal cancer in HIV patients causes prolonged CD4 cell count suppression.* Ann Oncol, 2012. **23**(1): p. 141-7.

534. Gotti, D., et al., *Survival in HIV-Infected Patients after a Cancer Diagnosis in the cART Era: Results of an Italian Multicenter Study.* PLoS ONE, 2014. **9**(4): p. e94768.

535. Marcus, J.L., et al., *Survival among HIV-Infected and HIV-Uninfected Individuals with Common Non–AIDS-Defining Cancers.* Cancer Epidemiology Biomarkers and Prevention, 2015. **24**(8): p. 1167-1173.

536. Vaccher, E., et al., *Improvement of systemic human immunodeficiency virus-related non-Hodgkin lymphoma outcome in the era of highly active antiretroviral therapy.* Clin Infect Dis, 2003. **37**(11): p. 1556-64.

537. Lim, S.T., et al., *AIDS-related Burkitt's lymphoma versus diffuse large-cell lymphoma in the pre-highly active antiretroviral therapy (HAART) and HAART eras: significant differences in survival with standard chemotherapy.* J Clin Oncol, 2005. **23**(19): p. 4430-8.

538. Hentrich, M., et al., *Highly active antiretroviral therapy (HAART) improves survival in HIV-associated Hodgkin's disease: results of a multicenter study.* Annals of Oncology, 2006. **17**(6): p. 914-9.

539. Berretta, M., et al., *Drug–Drug Interactions Based on Pharmacogenetic Profile between Highly Active Antiretroviral Therapy and Antiblastic Chemotherapy in Cancer Patients with HIV Infection.* Frontiers in Pharmacology, 2016. **7**: p. 71.

540. Rackal, J.M., et al., *Provider training and experience for people living with HIV/AIDS.* Cochrane Database Syst Rev, 2011(6): p. Cd003938.

541. Handford, C.D., et al., *The association of hospital, clinic and provider volume with HIV/AIDS care and mortality: systematic review and meta-analysis.* AIDS Care, 2012. **24**(3): p. 267-82.

542. Curtis, J.R., et al., *Variations in intensive care unit utilization for patients with human immunodeficiency virus-related Pneumocystis carinii pneumonia: importance of hospital characteristics and geographic location.* Crit Care Med, 1998. **26**(4): p. 668-75.

543. Brook, M.G., et al., *Management of HIV-related lymphoma in HIV treatment centres in North Thames Region.* Int J STD AIDS, 2004. **15**(11): p. 765-6.

544. Cheung, M.C., et al., *Physician perceptions and preferences in the treatment of acquired immunodeficiency syndrome (AIDS)-related lymphoma.* Ann Hematol, 2007. **86**(9): p. 631-8.

545. Dunleavy, K. and W.H. Wilson, *Implications of the shifting pathobiology of AIDS-related lymphoma.* J Natl Cancer Inst, 2013. **105**(16): p. 1170-1.

546. Swan, J., et al., *Progress in cancer screening practices in the United States: results from the 2000 National Health Interview Survey.* Cancer, 2003. **97**(6): p. 1528-40.

547. Copenhagen HIV Programme *EuroSIDA: A multicentre study. Tracking HIV/AIDS Across Europe 2003*. 2003.

548. Kirk, O., et al., *Changes in use of antiretroviral therapy in regions of Europe over time. EuroSIDA Study Group.* Aids, 1998. **12**(15): p. 2031-9.

549. Podlekareva, D., et al., *The EuroSIDA study: Regional differences in the HIV-1 epidemic and treatment response to antiretroviral therapy among HIV-infected patients across Europe--a review of published results.* Cent Eur J Public Health, 2008. **16**(3): p. 99-105.

550. Kjaer, J. and B. Ledergerber, *HIV cohort collaborations: proposal for harmonization of data exchange.* Antivir Ther, 2004. **9**(4): p. 631-3.

551. Kowalska, J.D., et al., *The Coding Causes of Death in HIV (CoDe) Project: initial results and evaluation of methodology.* Epidemiology, 2011. **22**(4): p. 516-523.

552. Kowalska, J.D., et al., *A standardized algorithm for determining the underlying cause of death in HIV infection as AIDS or non-AIDS related: results from the EuroSIDA study.* HIV Clin Trials, 2011. **12**(2): p. 109-17.

553. Bannister, W.P., et al., *Transmitted drug resistant HIV-1 and association with virologic and CD4 cell count response to combination antiretroviral therapy in the EuroSIDA Study.* J Acquir Immune Defic Syndr, 2008. **48**(3): p. 324-33.

554. Peters, L., et al., *HIV and hepatitis C co-infection in Europe, Israel and Argentina: a EuroSIDA perspective.* BMC Infectious Diseases, 2014. **14**(Suppl 6): p. S13-S13.

555. Viard, J.P., et al., *Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study.* Aids, 2011. **25**(10): p. 1305-15.

556. Shepherd, L., et al., *Prognostic value of vitamin D level for all-cause mortality, and association with inflammatory markers, in HIV-infected persons.* J Infect Dis, 2014. **210**(2): p. 234-43.

557. Mocroft, A., et al., *Loss to follow-up in an international, multicentre observational study.* HIV medicine, 2008. **9**(5): p. 261-269.

558. Alabama, U.o.S. *Descriptive statistics*. 2014 [cited 2016; Available from: https://www.southalabama.edu/coe/bset/johnson/lectures/lec15.htm.

559. Pourhoseingholi, M.A., A.R. Baghestani, and M. Vahedi, *How to control confounding effects by statistical analysis.* Gastroenterology and Hepatology From Bed to Bench, 2012. **5**(2): p. 79-83.

560. Kirkwood, B. and J. Sterne, *Essential medical statistics.* Malden: Blackwell, 2003: p. 268-270.

561. Clayton, D., M. Hills, and A. Pickles, *Statistical models in epidemiology*. Vol. 161. 1993: IEA.

562. McCullagh, P. and J.A. Nelder, *Generalized linear models*. Vol. 37. 1989: CRC press.

563. Zhang, Z. and J. Sun, *Interval censoring.* Statistical methods in medical research, 2010. **19**(1): p. 53-70.

564. Collett, D., *Modelling survival data in medical research*. 2015: CRC press.

565. Wei, L.J., Lin, D. Y., and Weissfeld, L, *Regression Analysis of Multivariate Incomplete Failure Time Data by Modeling Marginal Distribution.* Journal of the American Statistical Association, 1989. **84**: p. 1065–1073.

566. Hanley, J.A., et al., *Statistical analysis of correlated data using generalized estimating equations: an orientation.* Am J Epidemiol, 2003. **157**(4): p. 364-75.

567. Brown, H. and R. Prescott, *Applied mixed models in medicine*. 2014: John Wiley & Sons.

568. Univeristy of Bristol Center for Multilevel Modelling. *Random slope models*. 2017 [cited 2017; Available from: http://www.bristol.ac.uk/cmm/learning/videos/random-slopes.html.

569. Jones, M.P., *Indicator and Stratification Methods for Missing Explanatory Variables in Multiple Linear Regression.* Journal of the American Statistical Association, 1996. **91**(433): p. 222-230.

570. Ziegler, J.L., et al., *Outbreak of Burkitt's-like lymphoma in homosexual men.* Lancet, 1982. **2**(8299): p. 631-3.

571. de Martel, C., et al., *Cancers attributable to infections among adults with HIV in the United States.* Aids, 2015. **29**(16): p. 2173-81.

572. Borges, A.H., et al., *Predicting risk of cancer during HIV infection: the role of inflammatory and coagulation biomarkers*, in *14th International Workshop on Co-morbidities and Adverse Drug Reactions in HIV*. 2012: Washington DC.

573. Crabb Breen, E., et al., *The Development of AIDS-Associated Burkitt's/Small Noncleaved Cell Lymphoma Is Preceded by Elevated Serum Levels of Interleukin 6.* Clinical Immunology, 1999. **92**(3): p. 293-299.

574. Breen, E.C., et al., *B-cell stimulatory cytokines and markers of immune activation are elevated several years prior to the diagnosis of systemic AIDS-associated non-Hodgkin B-cell lymphoma.* Cancer Epidemiol Biomarkers Prev, 2011. **20**(7): p. 1303-14.

575. Mahy, M., et al., *Increasing trends in HIV prevalence among people aged 50 years and older: evidence from estimates and survey data.* Aids, 2014. **28 Suppl 4**: p. S453-9.

576. Yin Z, B.A., Hughes G, Nardone A, Gill ON, Delpech VC & and contributors., *HIV in the United Kingdom 2014 Report: data to end 2013.* . 2014, Public Health England, London. .

577. Bruyand, M., et al., *Cancer risk and use of protease inhibitor or nonnucleoside reverse transcriptase inhibitor-based combination antiretroviral therapy: the D: A: D study.* J Acquir Immune Defic Syndr, 2015. **68**(5): p. 568-77.

578. Chao, C., et al., *Exposure to antiretroviral therapy and risk of cancer in HIV-infected persons.* AIDS, 2012.

579. Mbang, P.A., et al., *Association between Time on Protease Inhibitors and the Incidence of Squamous Cell Carcinoma of the Anus among U.S. Male Veterans.* PLOS ONE, 2015. **10**(12): p. e0142966.

580. Nunnari, G., J.A. Smith, and R. Daniel, *HIV-1 Tat and AIDS-associated cancer: targeting the cellular anti-cancer barrier?* Journal of Experimental & Clinical Cancer Research, 2008. **27**(1): p. 3.

581. Amini, S., K. Khalili, and B.E. Sawaya, *Effect of HIV-1 Vpr on cell cycle regulators.* DNA and cell biology, 2004. **23**(4): p. 249-260.

582. Zhou, F., et al., *HIV-1 Tat Promotes Kaposi's Sarcoma-Associated Herpesvirus (KSHV) vIL-6-Induced Angiogenesis and Tumorigenesis by Regulating PI3K/PTEN/AKT/GSK-3β Signaling Pathway.* PLOS ONE, 2013. **8**(1): p. e53145.

583. Vaccarella, S., et al., *Sexual Behavior, Condom Use, and Human Papillomavirus: Pooled Analysis of the IARC Human Papillomavirus Prevalence Surveys.* Cancer Epidemiology Biomarkers & Prevention, 2006. **15**(2): p. 326-333.

584. Robbins, H.A., et al., *Excess Cancers Among HIV-Infected People in the United States.* JNCI Journal of the National Cancer Institute, 2015. **107**(4): p. dju503.

585. Vesely, M.D., et al., *Natural innate and adaptive immunity to cancer.* Annu Rev Immunol, 2011. **29**: p. 235-71.

586. Clifford, G.M., et al., *Hodgkin lymphoma in the Swiss HIV Cohort Study.* Blood, 2009. **113**(23): p. 5737-42.

587. Shiels, M.S. and E.A. Engels, *Evolving epidemiology of HIV-associated malignancies.* Curr Opin HIV AIDS, 2017. **12**(1): p. 6-11.

588. De Vuyst, H., et al., *HIV, human papillomavirus, and cervical neoplasia and cancer in the era of highly active antiretroviral therapy.* Eur J Cancer Prev, 2008. **17**(6): p. 545-54.

589. Frisch, M., et al., *Human Papillomavirus-Associated Cancers in Patients With Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome.* Journal of the National Cancer Institute, 2000. **92**(18): p. 1500-1510.

590. Dal Maso, L., et al., *Risk of cancer in persons with AIDS in Italy, 1985-1998.* Br J Cancer, 2003. **89**(1): p. 94-100.

591. Grulich, A.E., et al., *Rates of non-AIDS-defining cancers in people with HIV infection before and after AIDS diagnosis.* Aids, 2002. **16**(8): p. 1155-61.

592. Kesselring, A., et al., *Immunodeficiency as a Risk Factor for Non–AIDS-Defining Malignancies in HIV-1–Infected Patients Receiving Combination Antiretroviral Therapy.* Clinical infectious diseases, 2011. **52**(12): p. 1458-1465.

593. Bedimo, R.J., et al., *Incidence of Non-AIDS-Defining Malignancies in HIV-Infected Vs. Non-Infected Patients in the HAART Era: Impact of Immunosuppression.* Journal of acquired immune deficiency syndromes (1999), 2009. **52**(2): p. 203.

594. Silverberg, M.J., et al., *Risk of cancers during interrupted antiretroviral therapy in the SMART study.* AIDS, 2007. **21**(14): p. 1957-63.

595. Lacerda, H.R. and D. Kitner, *Mortality of the elderly is still exceedingly high at diagnosis of AIDS despite favourable outcomes after highly active antiretroviral therapy in Recife, Brazil.* Int J STD AIDS, 2008. **19**(7): p. 450-4.

596. WHO. *International Statistical Classification of Diseases and Related Health Problems - 10th revision*. 2010 8 Jan 2014]; 10:[Available from: http://apps.who.int/classifications/icd10/browse/2010/en.

597. WHO, *WHO expert committee Physical status: The use and interpretation of anthropometry*, in *WHO technical report series*. 1995.

598. Hanley, J.A., *A heuristic approach to the formulas for population attributable fraction.* Journal of Epidemiology and Community Health, 2001. **55**(7): p. 508-14.

599. Rockhill, B., B. Newman, and C. Weinberg, *Use and misuse of population attributable fractions.* American Journal of Public Health, 1998. **88**(1): p. 15-19.

600. Miettine.Os, *Proportion of Disease Caused or Prevented by a Given Exposure, Trait or Intervention.* American Journal of Epidemiology, 1974. **99**(5): p. 325-332.

601. Burgi, A., et al., *Incidence and risk factors for the occurrence of non-AIDS-defining cancers among human immunodeficiency virus-infected individuals.* Cancer, 2005. **104**(7): p. 1505-11.

602. Petoumenos, K., et al., *Cancers in the TREAT Asia HIV Observational Database (TAHOD): a retrospective analysis of risk factors.* J Int AIDS Soc, 2010. **13**: p. 51.

603. Richardson, S., I. StÜCker, and D. HÉMon, *Comparison of Relative Risks Obtained in Ecological and Individual Studies: Some Methodological Considerations.* International Journal of Epidemiology, 1987. **16**(1): p. 111-120.

604. Diamond, C., et al., *Changes in acquired immunodeficiency syndrome-related non-Hodgkin lymphoma in the era of highly active antiretroviral therapy: incidence, presentation, treatment, and survival.* Cancer, 2005. **106**(1): p. 128-35.

605. Dolcetti, R., et al., *Pathogenetic and histogenetic features of HIV-associated Hodgkin's disease.* European Journal of Cancer, 2001. **37**(10): p. 1276-1287.

606. Engels, E.A., et al., *Immunologic and virologic predictors of AIDS-related non-hodgkin lymphoma in the highly active antiretroviral therapy era.* J Acquir Immune Defic Syndr, 2010. **54**(1): p. 78-84.

607. Ekstrom-Smedby, K., *Epidemiology and etiology of non-Hodgkin lymphoma--a review.* Acta Oncol, 2006. **45**(3): p. 258-71.

608. Epeldegui, M., E. Vendrame, and O. Martínez-Maza, *HIV-associated immune dysfunction and viral infection: role in the pathogenesis of AIDS-related lymphoma.* Immunologic research, 2010. **48**(1): p. 72-83.

609. Hleyhel, M., et al., *Risk of non-AIDS-defining cancers among HIV-1-infected individuals in France between 1997 and 2009: results from a French cohort.* AIDS, 2014. **28**(14): p. 2109-2118.

610. Gjærde, L.I., et al., *Trends in Incidences and Risk Factors for Hepatocellular Carcinoma and Other Liver Events in HIV and Hepatitis C Virus–coinfected Individuals From 2001 to 2014: A Multicohort Study.* Clinical Infectious Diseases, 2016. **63**(6): p. 821-829.

611. Popova, S., et al., *Comparing alcohol consumption in central and eastern Europe to other European countries.* Alcohol Alcohol, 2007. **42**(5): p. 465-73.

612. Wolfe, D., M.P. Carrieri, and D. Shepard, *Treatment and care for injecting drug users with HIV infection: a review of barriers and ways forward.* Lancet, 2010. **376**(9738): p. 355-66.

613. Strickler, H.D., et al., *Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women.* J Natl Cancer Inst, 2005. **97**(8): p. 577-86.

614. Rowhani-Rahbar, A., et al., *The impact of HIV status and type on the clearance of human papillomavirus infection among Senegalese women.* J Infect Dis, 2007. **196**(6): p. 887-94.

615. Ahdieh, L., et al., *Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women.* J Infect Dis, 2001. **184**(6): p. 682-90.

616. Borges, A.H., et al., *Immediate Antiretroviral Therapy Reduces Risk of Infection-Related Cancer During Early HIV Infection.* Clin Infect Dis, 2016. **63**(12): p. 1668-1676.

617. Engels, E.A., *Human immunodeficiency virus infection, aging, and cancer.* Journal of clinical epidemiology, 2001. **54 Suppl 1**: p. S29-34.

618. Howlader N, et al. *SEER Cancer Statistics Review, 1975-2011*. 2014 April 2014; Available from: http://seer.cancer.gov/csr/1975_2011/.

619. cancer research UK. *Cancer incidence by age*. 2016 [cited 2016 1 June 2016]; Available from: ttp://www.cancerresearchuk.org/health-professional/cancer-statistics/incidence/age#4ymkVy1LDaDvQizO.99.

620. U.S. Department of Health and Human Services, *The Health Consequences of Smoking: A Report of the Surgeon General.*, in *Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health*. 2004.

621. Helleberg, M., et al., *Risk of cancer among HIV-infected individuals compared to the background population: impact of smoking and HIV.* AIDS, 2014. **28**(10): p. 1499-1508.

622. IARC, *Tobacco smoke and involuntary smoking.* IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 2004. **83**: p. 1-1438.

623. Hessol, N.A., et al., *Lung cancer incidence and survival among HIV-infected and uninfected women and men.* Aids, 2015. **29**(10): p. 1183-93.

624. Engels, E.A., E.R. Cho, and S.H. Jee, *Hepatitis B virus infection and risk of non-Hodgkin lymphoma in South Korea: a cohort study.* Lancet Oncology, 2010. **11**(9): p. 827-34.

625. Chen, C.J., M.W. Yu, and Y.F. Liaw, *Epidemiological characteristics and risk factors of hepatocellular carcinoma.* J Gastroenterol Hepatol, 1997. **12**(9-10): p. S294-308.

626. Fwu, C.W., et al., *Mortality after chronic hepatitis B virus infection: a linkage study involving 2 million parous women from Taiwan.* Journal of Infectious Diseases, 2010. **201**(7): p. 1016-23.

627. Chen, G., et al., *Chronic hepatitis B virus infection and mortality from non-liver causes: results from the Haimen City cohort study.* International Journal of Epidemiology, 2005. **34**(1): p. 132-7.

628. Kiyosawa, K., et al., *Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus.* Hepatology, 1990. **12**(4 Pt 1): p. 671-5.

629. Tong, M.J., et al., *Clinical outcomes after transfusion-associated hepatitis C.* N Engl J Med, 1995. **332**(22): p. 1463-6.

630. Engels, E.A., et al., *Immunoblastic lymphoma in persons with AIDS-associated Kaposi's sarcoma: a role for Kaposi's sarcoma-associated herpesvirus.* Mod Pathol, 2003. **16**(5): p. 424-9.

631. Mariotto, A.B., et al., *Multiple cancer prevalence: a growing challenge in long-term survivorship.* Cancer Epidemiol Biomarkers Prev, 2007. **16**(3): p. 566-71.

632. Ibrahim, E.M., et al., *Risk of second breast cancer in female Hodgkin's lymphoma survivors: a meta-analysis.* BMC Cancer, 2012. **12**: p. 197.

633. Caini, S., et al., *The risk of developing a second primary cancer in melanoma patients: a comprehensive review of the literature and meta-analysis.* J Dermatol Sci, 2014. **75**(1): p. 3-9.

634. Salani, R., et al., *Posttreatment surveillance and diagnosis of recurrence in women with gynecologic malignancies: Society of Gynecologic Oncologists recommendations.* American Journal of Obstetrics and Gynecology, 2011. **6**(204): p. 466-478.

635. Khan, N.F., E. Watson, and P.W. Rose, *Primary care consultation behaviours of long-term, adult survivors of cancer in the UK.* The British Journal of General Practice, 2011. **61**(584): p. 197-199.

636. Shepherd, L., et al., *Infection-related and -unrelated malignancies, HIV and the aging population.* HIV Medicine, 2016. **17**(8): p. 590 - 600.

637. Petoumenos, K. and M.G. Law, *Smoking, alcohol and illicit drug use effects on survival in HIV-positive persons.* Curr Opin HIV AIDS, 2016. **11**(5): p. 514-520.

638. Clifford, G.M., et al., *Lung cancer in the Swiss HIV Cohort Study: role of smoking, immunodeficiency and pulmonary infection.* Br J Cancer, 2012. **106**(3): p. 447-52.

639. Hasse, B., et al., *Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study.* Clin Infect Dis, 2011. **53**(11): p. 1130-9.

640. Secretan, B., et al., *A review of human carcinogens—Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish.* The Lancet Oncology, 2009. **10**(11): p. 1033-1034.

641. WHO. *World Health Statistics data visualizations dashboard, Prevalence of tobacco smoking*. 2016 [cited 2017; Available from: http://apps.who.int/gho/data/node.sdg.3-a-viz?lang=en.

642. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2016.* CA: A Cancer Journal for Clinicians, 2016. **66**(1): p. 7-30.

643. IARC, *World cancer report*. 2014.

644. Parkin, D.M., L. Boyd, and L.C. Walker, *16. The fraction of cancer attributable to lifestyle and environmental factors in the UK in 2010.* Br J Cancer, 2011. **105**(S2): p. S77-S81.

645. Swerdlow, A.J., R. Peto, and S.D. Richard, *Epidemiology of cancer*. 'Oxford University Press': Oxford, UK.

646. Jha, P., *Avoidable global cancer deaths and total deaths from smoking.* Nat Rev Cancer, 2009. **9**(9): p. 655-664.

647. Doll, R., et al., *Mortality in relation to smoking: 50 years' observations on male British doctors.* BMJ : British Medical Journal, 2004. **328**(7455): p. 1519-1519.

648. Pirie, K., et al., *The 21st century hazards of smoking and benefits of stopping: a prospective study of one million women in the UK.* Lancet, 2013. **381**(9861): p. 133-141.

649. Banks, E., et al., *Tobacco smoking and all-cause mortality in a large Australian cohort study: findings from a mature epidemic with current low smoking prevalence.* BMC Medicine, 2015. **13**(1): p. 38.

650. Jha , P., et al., *21st-Century Hazards of Smoking and Benefits of Cessation in the United States.* New England Journal of Medicine, 2013. **368**(4): p. 341-350.

651. Doll, R., et al., *Mortality in relation to smoking: 40 years' observations on male British doctors.* Bmj, 1994. **309**(6959): p. 901-11.

652. The Medical Research, C., *Tobacco Smoking and Lung Cancer.* British Medical Journal, 1957. **1**(5034): p. 1523-1524.

653. Doll, R. and A.B. Hill, *Smoking and Carcinoma of the Lung.* British Medical Journal, 1950. **2**(4682): p. 739-748.

654. IARC, *Reversal of Risk After Quitting Smoking*, in *IARC Handbook of Cancer Prevention*. 2007: Lyon, France.

655. Khuder, S.A., *Effect of cigarette smoking on major histological types of lung cancer: a meta-analysis.* Lung Cancer, 2001. **31**(2-3): p. 139-48.

656. Pesch, B., et al., *Cigarette smoking and lung cancer—relative risk estimates for the major histological types from a pooled analysis of case–control studies.* International Journal of Cancer, 2012. **131**(5): p. 1210-1219.

657. Services, U.S.D.o.H.a.H., *Surgeon General's Report: The Health Consequences of Smoking—50 Years of Progress*. 2014.

658. Anthonisen, N.R., et al., *The effects of a smoking cessation intervention on 14.5-year mortality: a randomized clinical trial.* Ann Intern Med, 2005. **142**(4): p. 233-9.

659. Peto, R., et al., *Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies.* Bmj, 2000. **321**(7257): p. 323-329.

660. Khuder, S.A. and A.B. Mutgi, *Effect of smoking cessation on major histologic types of lung cancer.* Chest, 2001. **120**(5): p. 1577-83.

661. Fry, J.S., et al., *How rapidly does the excess risk of lung cancer decline following quitting smoking? A quantitative review using the negative exponential model.* Regulatory Toxicology and Pharmacology, 2013. **67**(1): p. 13-26.

662. Lee, P.N., B.A. Forey, and K.J. Coombs, *Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer.* BMC Cancer, 2012. **12**: p. 385.

663. Ordóñez-Mena, J.M., et al., *Quantification of the smoking-associated cancer risk with rate advancement periods: meta-analysis of individual participant data from cohorts of the CHANCES consortium.* BMC Medicine, 2016. **14**(1): p. 62.

664. Orellana-Barrios, M.A., et al., *Electronic Cigarettes—A Narrative Review for Clinicians.* The American Journal of Medicine, 2015. **128**(7): p. 674-681.

665. Bourke, L., et al., *E-cigarettes and Urologic Health: A Collaborative Review of Toxicology, Epidemiology, and Potential Risks.* Eur Urol, 2017. **71**(6): p. 915-923.

666. Farsalinos, K.E., et al., *Electronic cigarette use in the European Union: analysis of a representative sample of 27 460 Europeans from 28 countries.* Addiction, 2016. **111**(11): p. 2032-2040.

667. Bullen, C., et al., *Electronic cigarettes for smoking cessation: a randomised controlled trial.* Lancet, 2013. **382**(9905): p. 1629-37.

668. Hartmann-Boyce, J., et al., *Electronic cigarettes for smoking cessation.* Cochrane Database of Systematic Reviews, 2016(9).

669. Cumberbatch, M.G., et al., *The Role of Tobacco Smoke in Bladder and Kidney Carcinogenesis: A Comparison of Exposures and Meta-analysis of Incidence and Mortality Risks.* European Urology, 2016. **70**(3): p. 458-466.

670. Martínez-Sánchez, J.M., et al., *Perception of electronic cigarettes in the general population: does their usefulness outweigh their risks?* BMJ Open, 2015. **5**(11).

671. Hua, M. and P. Talbot, *Potential health effects of electronic cigarettes: A systematic review of case reports.* Preventive Medicine Reports, 2016. **4**: p. 169-178.

672. Cheng, T., *Chemical evaluation of electronic cigarettes.* Tobacco Control, 2014. **23**(suppl 2): p. ii11-ii17.

673. Silverman, D., et al., *Cancer epidemiology and prevention.* Bladder Cancer, 2006. **2**: p. 1156-79.

674. Pisinger, C. and M. Døssing, *A systematic review of health effects of electronic cigarettes.* Preventive Medicine, 2014. **69**: p. 248-260.

675. Boffetta, P., et al., *Occupational exposure to arsenic, cadmium, chromium, lead and nickel, and renal cell carcinoma: a case–control study from Central and Eastern Europe.* Occupational and Environmental Medicine, 2011. **68**(10): p. 723-728.

676. Williams, M., et al., *Metal and Silicate Particles Including Nanoparticles Are Present in Electronic Cigarette Cartomizer Fluid and Aerosol.* PLOS ONE, 2013. **8**(3): p. e57987.

677. Hess, C.A., et al., *E-cigarettes as a source of toxic and potentially carcinogenic metals.* Environ Res, 2017. **152**: p. 221-225.

678. Gritz, E.R., et al., *Smoking behavior in a low-income multiethnic HIV/AIDS population.* Nicotine Tob Res, 2004. **6**(1): p. 71-7.

679. Burkhalter, J.E., et al., *Tobacco use and readiness to quit smoking in low-income HIV-infected persons.* Nicotine Tob Res, 2005. **7**(4): p. 511-22.

680. Webb, M.S., et al., *Cigarette smoking among HIV+ men and women: examining health, substance use, and psychosocial correlates across the smoking spectrum.* J Behav Med, 2007. **30**(5): p. 371-83.

681. Marshall, M.M., et al., *Tobacco use and nicotine dependence among HIV-infected and uninfected injection drug users.* Addict Behav, 2011. **36**(1-2): p. 61-7.

682. Lifson, A.R., et al., *Smoking-related health risks among persons with HIV in the Strategies for Management of Antiretroviral Therapy clinical trial.* Am J Public Health, 2010. **100**(10): p. 1896-903.

683. Tainya C. Clarke, P.D., Brian W. Ward, Ph.D., Gulnur Freeman, M.P.A., and Jeannine S. Schiller, M.P.H. *Early Release of Selected Estimates Based on Data From the January–September 2015 National Health Interview Survey*. 2016 [cited 2017; Available from: https://www.cdc.gov/nchs/data/nhis/earlyrelease/earlyrelease201602.pdf.

684. Feldman, J.G., et al., *Association of cigarette smoking with HIV prognosis among women in the HAART era: a report from the women's interagency HIV study.* Am J Public Health, 2006. **96**(6): p. 1060-5.

685. Mdege, N.D., et al., *Tobacco use among people living with HIV: analysis of data from Demographic and Health Surveys from 28 low-income and middle-income countries.* The Lancet Global Health, 2017. **5**(6): p. e578-e592.

686. Shuter, J., S.L. Bernstein, and A.B. Moadel, *Cigarette smoking behaviors and beliefs in persons living with HIV/AIDS.* Am J Health Behav, 2012. **36**(1): p. 75-85.

687. Reynolds, N.R., *Cigarette smoking and hiv: more evidence for action.* AIDS education and prevention : official publication of the International Society for AIDS Education, 2009. **21**(3 Suppl): p. 106-121.

688. Crothers, K., et al., *Impact of cigarette smoking on mortality in HIV-positive and HIV-negative veterans.* AIDS Educ Prev, 2009. **21**(3 Suppl): p. 40-53.

689. Rahmanian, S., et al., *Cigarette smoking in the HIV-infected population.* Proc Am Thorac Soc, 2011. **8**(3): p. 313-9.

690. Crothers, K., et al., *The impact of cigarette smoking on mortality, quality of life, and comorbid illness among HIV-positive veterans.* J Gen Intern Med, 2005. **20**(12): p. 1142-5.

691. Helleberg, M., et al., *Mortality attributable to smoking among HIV-1-infected individuals: a nationwide, population-based cohort study.* Clin Infect Dis, 2013. **56**(5): p. 727-34.

692. Hirschtick, R.E., et al., *Bacterial pneumonia in persons infected with the human immunodeficiency virus. Pulmonary Complications of HIV Infection Study Group.* N Engl J Med, 1995. **333**(13): p. 845-51.

693. Gordin, F.M., et al., *Pneumonia in HIV-infected persons: increased risk with cigarette smoking and treatment interruption.* Am J Respir Crit Care Med, 2008. **178**(6): p. 630-6.

694. Kohli, R., et al., *Bacterial pneumonia, HIV therapy, and disease progression among HIV-infected women in the HIV epidemiologic research (HER) study.* Clin Infect Dis, 2006. **43**(1): p. 90-8.

695. Elssner, A., et al., *HIV-1 infection does not impair human alveolar macrophage phagocytic function unless combined with cigarette smoking.* Chest, 2004. **125**(3): p. 1071-6.

696. Wewers, M.D., et al., *Cigarette smoking in HIV infection induces a suppressive inflammatory environment in the lung.* Am J Respir Crit Care Med, 1998. **158**(5 Pt 1): p. 1543-9.

697. Benard, A., et al., *Bacterial pneumonia among HIV-infected patients: decreased risk after tobacco smoking cessation. ANRS CO3 Aquitaine Cohort, 2000-2007.* PLoS One, 2010. **5**(1): p. e8896.

698. Miguez-Burbano, M.J., et al., *Increased risk of Pneumocystis carinii and community-acquired pneumonia with tobacco use in HIV disease.* Int J Infect Dis, 2005. **9**(4): p. 208-17.

699. Christensen, P.J., et al., *Pneumocystis murina infection and cigarette smoke exposure interact to cause increased organism burden, development of airspace enlargement, and pulmonary inflammation in mice.* Infect Immun, 2008. **76**(8): p. 3481-90.

700. Slama, K., et al., *Tobacco and tuberculosis: a qualitative systematic review and meta-analysis.* Int J Tuberc Lung Dis, 2007. **11**(10): p. 1049-61.

701. Crothers, K., et al., *Increased COPD among HIV-positive compared to HIV-negative veterans.* Chest, 2006. **130**(5): p. 1326-33.

702. Lin, H.H., M. Ezzati, and M. Murray, *Tobacco smoke, indoor air pollution and tuberculosis: a systematic review and meta-analysis.* PLoS Med, 2007. **4**(1): p. e20.

703. Bates, M.N., et al., *Risk of tuberculosis from exposure to tobacco smoke: a systematic review and meta-analysis.* Arch Intern Med, 2007. **167**(4): p. 335-42.

704. van Zyl Smit, R.N., et al., *Global lung health: the colliding epidemics of tuberculosis, tobacco smoking, HIV and COPD.* Eur Respir J, 2010. **35**(1): p. 27-33.

705. Kirk, G.D., et al., *HIV infection is associated with an increased risk for lung cancer, independent of smoking.* Clinical Infectious Diseases, 2007. **45**(1): p. 103-10.

706. Sigel, K., et al., *HIV as an independent risk factor for incident lung cancer.* AIDS, 2012. **26**(8): p. 1017-25.

707. Sigel, K., A. Makinson, and J. Thaler, *Lung cancer in persons with HIV.* Curr Opin HIV AIDS, 2017. **12**(1): p. 31-38.

708. De, P., et al., *Systematic review and meta-analysis: influence of smoking cessation on incidence of pneumonia in HIV.* BMC Med, 2013. **11**(15): p. 1741-7015.

709. Marshall, M.M., M.C. McCormack, and G.D. Kirk, *Effect of Cigarette Smoking on HIV Acquisition, Progression, and Mortality.* AIDS education and prevention : official publication of the International Society for AIDS Education, 2009. **21**(3 Suppl): p. 28-39.

710. Friis-Moller, N., et al., *Class of antiretroviral drugs and the risk of myocardial infarction.* N Engl J Med, 2007. **356**(17): p. 1723-35.

711. Petoumenos, K., et al., *Rates of cardiovascular disease following smoking cessation in patients with HIV infection: results from the D:A:D study.* HIV Medicine, 2011. **12**(7): p. 412-421.

712. Aliyu, M.H., et al., *Cigarette smoking and fetal morbidity outcomes in a large cohort of HIV-infected mothers.* Nicotine Tob Res, 2013. **15**(1): p. 177-84.

713. Jeganathan, S., et al., *Predictors of oral health quality of life in HIV-1 infected patients attending routine care in Australia.* J Public Health Dent, 2011. **71**(3): p. 248-51.

714. Walker Harris, V. and T.T. Brown, *Bone loss in the HIV-infected patient: evidence, clinical implications, and treatment strategies.* J Infect Dis, 2012. **205 Suppl 3**: p. S391-8.

715. Borderi, M., et al., *Metabolic bone disease in HIV infection.* Aids, 2009. **23**(11): p. 1297-310.

716. Hansen, A.B., et al., *Incidence of low and high-energy fractures in persons with and without HIV infection: a Danish population-based cohort study.* Aids, 2012. **26**(3): p. 285-93.

717. Arcavi, L. and N.L. Benowitz, *Cigarette smoking and infection.* Arch Intern Med, 2004. **164**(20): p. 2206-16.

718. Crothers, K., et al., *HIV infection and risk for incident pulmonary diseases in the combination antiretroviral therapy era.* Am J Respir Crit Care Med, 2011. **183**(3): p. 388-95.

719. Gordon, S.B., et al., *HIV-1 infection is associated with altered innate pulmonary immunity.* J Infect Dis, 2005. **192**(8): p. 1412-6.

720. Appay, V., et al., *Accelerated immune senescence and HIV-1 infection.* Exp Gerontol, 2007. **42**(5): p. 432-7.

721. Appay, V. and A.D. Kelleher, *Immune activation and immune aging in HIV infection.* Curr Opin HIV AIDS, 2016. **11**(2): p. 242-9.

722. Winstone, T.A., et al., *Epidemic of lung cancer in patients with HIV infection.* Chest, 2013. **143**(2): p. 305-314.

723. Morlat, P., et al., *Causes of death among HIV-infected patients in France in 2010 (national survey): trends since 2000.* Aids, 2014. **28**(8): p. 1181-91.

724. Dal Maso, L., et al., *Pattern of cancer risk in persons with AIDS in Italy in the HAART era.* Br J Cancer, 2009. **100**(5): p. 840-7.

725. Engels, E.A., et al., *Elevated incidence of lung cancer among HIV-infected individuals.* J Clin Oncol, 2006. **24**(9): p. 1383-8.

726. Bower, M., et al., *HIV-related lung cancer in the era of highly active antiretroviral therapy.* Aids, 2003. **17**(3): p. 371-5.

727. Chaturvedi, A.K., et al., *Elevated risk of lung cancer among people with AIDS.* Aids, 2007. **21**(2): p. 207-13.

728. Shiels, M.S., et al., *Lung cancer incidence and mortality among HIV-infected and HIV-uninfected injection drug users.* J Acquir Immune Defic Syndr, 2010. **55**(4): p. 510-5.

729. Marcus, J.L., et al., *Immunodeficiency, AIDS-related pneumonia, and risk of lung cancer among HIV-infected individuals.* AIDS, 2017. **31**(7): p. 989-993.

730. Tirelli, U., et al., *Lung carcinoma in 36 patients with human immunodeficiency virus infection. The Italian Cooperative Group on AIDS and Tumors.* Cancer, 2000. **88**(3): p. 563-9.

731. Brock, M.V., et al., *Delayed diagnosis and elevated mortality in an urban population with HIV and lung cancer: implications for patient care.* J Acquir Immune Defic Syndr, 2006. **43**(1): p. 47-55.

732. D'Jaen, G.A., et al., *Human immunodeficiency virus-associated primary lung cancer in the era of highly active antiretroviral therapy: a multi-institutional collaboration.* Clin Lung Cancer, 2010. **11**(6): p. 396-404.

733. Suneja, G., et al., *Disparities in cancer treatment among patients infected with the human immunodeficiency virus.* Cancer, 2016. **122**(15): p. 2399-2407.

734. Suneja, G., et al., *Cancer treatment disparities in HIV-infected individuals in the United States.* J Clin Oncol, 2014. **32**(22): p. 2344-50.

735. Keith M. Sigel, K.C., Sheldon T. Brown, Cynthia L. Gibert, Matthew B. Goetz, Roger Bedimo, Roxanne Wadia, Maria Rodriguez-Barradas, Fatma Shebl, Lesley Park, *Treatment and Outcomes of Non-Small Cell Lung Cancer in Later Antiretroviral-Era HIV Infection*, in *CROI*. 2017: Seattle.

736. Doll, R., *An epidemiological perspective of the biology of cancer.* Cancer Res, 1978. **38**(11 Pt 1): p. 3573-83.

737. Smith, B.D., et al., *Future of Cancer Incidence in the United States: Burdens Upon an Aging, Changing Nation.* Journal of Clinical Oncology, 2009. **27**(17): p. 2758-2765.

738. Niaura, R., et al., *Human immunodeficiency virus infection, AIDS, and smoking cessation: the time is now.* Clin Infect Dis, 2000. **31**(3): p. 808-12.

739. Humfleet, G.L., et al., *Characteristics of HIV-positive cigarette smokers: a sample of smokers facing multiple challenges.* AIDS education and prevention, 2009. **21**(3 Suppl): p. 54-64.

740. Reynolds, N.R., *Cigarette smoking and HIV: more evidence for action.* AIDS Educ Prev, 2009. **21**(3 Suppl): p. 106-21.

741. Nahvi, S. and N.A. Cooperman, *Review: the need for smoking cessation among HIV-positive smokers.* AIDS Educ Prev, 2009. **21**(3 Suppl): p. 14-27.

742. Brath, H., et al., *Prevalence and Correlates of Smoking and Readiness to Quit Smoking in People Living with HIV in Austria and Germany.* PLOS ONE, 2016. **11**(2): p. e0150553.

743. Harris, J.K., *Connecting discovery and delivery: the need for more evidence on effective smoking cessation strategies for people living with HIV/AIDS.* Am J Public Health, 2010. **100**(7): p. 1245-9.

744. Tron, L., et al., *Tobacco smoking in HIV-infected versus general population in france: heterogeneity across the various groups of people living with HIV.* PLoS One, 2014. **9**(9): p. e107451.

745. Garfinkel, L. and S.D. Stellman, *Smoking and lung cancer in women: findings in a prospective study.* Cancer Res, 1988. **48**(23): p. 6951-5.

746. Guaraldi, G., et al., *Premature Age-Related Comorbidities Among HIV-Infected Persons Compared With the General Population.* Clinical Infectious Diseases, 2011. **53**(11): p. 1120-1126.

747. Sigel, K., *HIV infection is an independent risk factor for lung cancer*, in *17th CROI*. 2010.

748. Ng, M., et al., *Smoking prevalence and cigarette consumption in 187 countries, 1980-2012.* JAMA, 2014. **311**(2): p. 183-192.

749. Diaz, P.T., et al., *Increased susceptibility to pulmonary emphysema among HIV-seropositive smokers.* Ann Intern Med, 2000. **132**(5): p. 369-72.

750. Twigg, H.L., et al., *Lymphocytic alveolitis, bronchoalveolar lavage viral load, and outcome in human immunodeficiency virus infection.* Am J Respir Crit Care Med, 1999. **159**(5 Pt 1): p. 1439-44.

751. Morris, A.M., et al., *Permanent declines in pulmonary function following pneumonia in human immunodeficiency virus-infected persons. The Pulmonary Complications of HIV Infection Study Group.* Am J Respir Crit Care Med, 2000. **162**(2 Pt 1): p. 612-6.

752. Morris, A., et al., *Airway obstruction is increased in pneumocystis-colonized human immunodeficiency virus-infected outpatients.* J Clin Microbiol, 2009. **47**(11): p. 3773-6.

753. Turner, M.C., et al., *Chronic obstructive pulmonary disease is associated with lung cancer mortality in a prospective study of never smokers.* Am J Respir Crit Care Med, 2007. **176**(3): p. 285-90.

754. Shebl, F.M., et al., *Pulmonary infections and risk of lung cancer among persons with AIDS.* Journal of acquired immune deficiency syndromes (1999), 2010. **55**(3): p. 375-379.

755. Ballaz, S. and J.L. Mulshine, *The potential contributions of chronic inflammation to lung carcinogenesis.* Clin Lung Cancer, 2003. **5**(1): p. 46-62.

756. Agostini, C., et al., *Alveolar macrophages as a cell source of cytokine hyperproduction in HIV-related interstitial lung disease.* J Leukoc Biol, 1995. **58**(5): p. 495-500.

757. Pacht, E.R., et al., *Alveolar fluid glutathione decreases in asymptomatic HIV-seropositive subjects over time.* Chest, 1997. **112**(3): p. 785-8.

758. Giagulli, C., et al., *Opposite Effects of HIV-1 p17 Variants on PTEN Activation and Cell Growth in B Cells.* PLOS ONE, 2011. **6**(3): p. e17831.

759. Ramalingam, S., et al., *Lung cancer in young patients: analysis of a Surveillance, Epidemiology, and End Results database.* Journal of Clinical Oncology, 1998. **16**(2): p. 651-657.

760. Wistuba, I.I., C. Behrens, and A.F. Gazdar, *Pathogenesis of non-AIDS-defining cancers: a review.* AIDS Patient Care STDS, 1999. **13**(7): p. 415-26.

761. Picone, G. and F. Sloan. *Smoking cessation and lifestyle changes*. in *Forum for Health Economics & Policy*. 2003. De Gruyter.

762. Stein, C.J. and G.A. Colditz, *Modifiable risk factors for cancer.* Br J Cancer, 2004. **90**(2): p. 299-303.

763. Keith, A., et al., *Behavioral Interventions for Tobacco Use in HIV-Infected Smokers: A Meta-Analysis.* J Acquir Immune Defic Syndr, 2016. **72**(5): p. 527-33.

764. Shuter, J., et al., *Feasibility and preliminary efficacy of a web-based smoking cessation intervention for HIV-infected smokers: a randomized controlled trial.* J Acquir Immune Defic Syndr, 2014. **67**(1): p. 59-66.

765. Cropsey, K.L., et al., *An Algorithm Approach to Determining Smoking Cessation Treatment for Persons Living With HIV/AIDS: Results of a Pilot Trial.* J Acquir Immune Defic Syndr, 2015. **69**(3): p. 291-8.

766. Ferketich, A.K., et al., *Safety of varenicline among smokers enrolled in the lung HIV study.* Nicotine Tob Res, 2013. **15**(1): p. 247-54.

767. Lloyd-Richardson, E.E., et al., *Motivation and patch treatment for HIV+ smokers: a randomized controlled trial.* Addiction, 2009. **104**(11): p. 1891-900.

768. Keith Sigel, K.C., Kirsha Gordon, Sheldon Brown, David Rimland, Maria Rodriguez-Barradas, Cynthia Gibert, Matthew B. Goetz, Roger Bedimo, Robert Dubrow, *CD4 Measures as Predictors of Lung Cancer Risk and Prognosis in HIV Infection*, in *CROI conference*. 2015: Seattle.

769. Almodovar, S., *The Complexity of HIV Persistence and Pathogenesis in the Lung Under Antiretroviral Therapy: Challenges Beyond AIDS.* Viral Immunology, 2014. **27**(5): p. 186-199.

770. Cribbs, S.K., et al., *Healthy HIV-1-Infected Individuals on Highly Active Antiretroviral Therapy Harbor HIV-1 in Their Alveolar Macrophages.* AIDS Research and Human Retroviruses, 2015. **31**(1): p. 64-70.

771. Altavilla, G., et al., *Enhancement of Chemical Hepatocarcinogenesis by the HIV-1 tat Gene.* The American Journal of Pathology, 2000. **157**(4): p. 1081-1089.

772. Knight, K., S. Wade, and L. Balducci, *Prevalence and outcomes of anemia in cancer: a systematic review of the literature.* The American Journal of Medicine, 2004. **116**(7): p. 11-26.

773. Ludwig, H., et al., *The European Cancer Anaemia Survey (ECAS): A large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients.* European Journal of Cancer, 2004. **40**(15): p. 2293-2306.

774. Giovannucci, E., et al., *Diabetes and Cancer: A consensus report.* Diabetes Care, 2010. **33**(7): p. 1674-1685.

775. Burns, D.M. *Smoking cessation: recent indicators of what's working at a population level*. in *National Cancer Institute. Population Based Smoking Cessation: Proceedings of a Conference on What Works to Influence Cessation in the General Population. Smoking and Tobacco Control Monograph*. 2000.

776. Burns, D.M., et al., *Cigarette smoking behavior in the United States.* Changes in cigarette-related disease risks and their implication for prevention and control. Smoking and Tobacco Control Monograph, 1997. **8**: p. 13-42.

777. Boffetta, P., *I. Epidemiology of adult non-Hodgkin lymphoma.* Annals of Oncology, 2011. **22**(suppl_4): p. iv27-iv31.

778. Thomas, R.K., et al., *Part I: Hodgkin's lymphoma—molecular biology of Hodgkin and Reed-Sternberg cells.* The Lancet Oncology, 2004. **5**(1): p. 11-18.

779. Küppers, R. and M.-L. Hansmann, *The Hodgkin and Reed/Sternberg cell.* The International Journal of Biochemistry & Cell Biology, 2005. **37**(3): p. 511-517.

780. Stein, H. and M. Hummel, *Hodgkin's disease: biology and origin of Hodgkin and Reed-Sternberg cells.* Cancer Treatment Reviews, 1999. **25**(3): p. 161-168.

781. group, T.l.w. *Epic Study*. 2017 2017 [cited 2017 28 April 2017]; Available from: http://epic.iarc.fr/research/cancerworkinggroups/lymphoma.php.

782. Torre, L.A., et al., *Global cancer statistics, 2012.* CA: A Cancer Journal for Clinicians, 2015. **65**(2): p. 87-108.

783. SEER. *Cancer fact sheets*. 2017 [cited 2017 25 May 2017]; Available from: https://seer.cancer.gov/faststats/selections.php?#Output.

784. Cancer research UK. *Non-Hodgkin lymphoma incidence statistics*. 2017 [cited 2017; Available from: http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/non-hodgkin-lymphoma/incidence#ref-2.

785. Cancer research UK. *Hodgkin lymphoma incidence statistics*. 2017 [cited 2017; Available from: http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/hodgkin-lymphoma/incidence#heading-Two.

786. IARC. *EUCAN: Hodgkin lymphoma*. 2012 2012 [cited 2017 28 April 2017]; Available from: http://eco.iarc.fr/eucan/Cancer.aspx?Cancer=37.

787. Salati, M., et al., *Epidemiological Overview of Hodgkin Lymphoma across the Mediterranean Basin.* Mediterranean Journal of Hematology and Infectious Diseases, 2014. **6**(1): p. e2014048.

788. Grulich, A.E. and C.M. Vajdic, *The epidemiology of non-Hodgkin lymphoma.* Pathology, 2005. **37**(6): p. 409-19.

789. Cerhan, J.R., et al., *Medical history, lifestyle, family history, and occupational risk factors for diffuse large B-cell lymphoma: the InterLymph Non-Hodgkin Lymphoma Subtypes Project.* J Natl Cancer Inst Monogr, 2014. **2014**(48): p. 15-25.

790. Ekstrom-Smedby, K., et al., *Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph Consortium.* Blood, 2008. **111**(8): p. 4029-38.

791. Fallah, M., et al., *Autoimmune diseases associated with non-Hodgkin lymphoma: a nationwide cohort study.* Ann Oncol, 2014. **25**(10): p. 2025-30.

792. Baecklund, E., et al., *Lymphoma development in patients with autoimmune and inflammatory disorders--what are the driving forces?* Semin Cancer Biol, 2014. **24**: p. 61-70.

793. Altieri, A., J.L. Bermejo, and K. Hemminki, *Familial risk for non-Hodgkin lymphoma and other lymphoproliferative malignancies by histopathologic subtype: the Swedish Family-Cancer Database.* Blood, 2005. **106**(2): p. 668-72.

794. Chang, E.T., et al., *Family history of hematopoietic malignancy and risk of lymphoma.* J Natl Cancer Inst, 2005. **97**(19): p. 1466-74.

795. Brennan, M.F., et al., *Cancer: Principles and Practice of Oncology*. 2008, Lippincott, Williams & Wilkins, Philadelphia, Pa, USA.

796. Niederhuber, J., *Abeloff's Clinical Oncology. Philadelphia, Pa.: Churchill Livingstone Elsevier; 2014*. 2014.

797. Carbone, A., et al., *HIV-associated Hodgkin lymphoma.* Curr Opin HIV AIDS, 2009. **4**(1): p. 3-10.

798. Goldin, L.R., et al., *Familial aggregation of Hodgkin lymphoma and related tumors.* Cancer, 2004. **100**(9): p. 1902-8.

799. Besson, H., et al., *Tobacco smoking, alcohol drinking and Hodgkin's lymphoma: a European multi-centre case-control study (EPILYMPH).* Br J Cancer, 2006. **95**(3): p. 378-384.

800. Kamper-Jørgensen, M., et al., *Cigarette smoking and risk of Hodgkin lymphoma and its subtypes: a pooled analysis from the International Lymphoma Epidemiology Consortium (InterLymph).* Annals of Oncology, 2013.

801. van Leeuwen, F.E., et al., *Second cancer risk following Hodgkin's disease: a 20-year follow-up study.* J Clin Oncol, 1994. **12**(2): p. 312-25.

802. Gibson, T.M., et al., *Risk of non-Hodgkin lymphoma subtypes in HIV-infected people during the HAART era: a population-based study.* AIDS, 2014. **28**(15): p. 2313-8.

803. Van Leeuwen, M.T., Vajdic, C. M., Middleton, M. G., McDonald, A. M., Law, M., Kaldor, J. M., & Grulich, A. E. , *Continuing declines in some but not all HIV-associated cancers in Australia after widespread use of antiretroviral therapy.* AIDS, 2009. **23**(16): p. 2183–2190.

804. Bibas, M. and A. Antinori, *EBV and HIV-Related Lymphoma.* Mediterranean Journal of Hematology and Infectious Diseases, 2009. **1**(2): p. e2009032.

805. Glaser, S.L., et al., *Epstein-Barr virus-associated Hodgkin's disease: epidemiologic characteristics in international data.* Int J Cancer, 1997. **70**(4): p. 375-82.

806. Gasser, O., et al., *HIV Patients Developing Primary CNS Lymphoma Lack EBV-Specific CD4+ T Cell Function Irrespective of Absolute CD4+ T Cell Counts.* PLOS Medicine, 2007. **4**(3): p. e96.

807. Bohlius, J., et al., *Prognosis of HIV-associated non-Hodgkin lymphoma in patients starting combination antiretroviral therapy.* AIDS, 2009. **23**(15): p. 2029-37.

808. Kaech, S.M. and R. Ahmed, *CD8 T Cells Remember with a Little Help.* Science, 2003. **300**(5617): p. 263-265.

809. Piriou, E., et al., *Loss of EBNA1-specific memory CD4+ and CD8+ T cells in HIV-infected patients progressing to AIDS-related non-Hodgkin lymphoma.* Blood, 2005. **106**(9): p. 3166-74.

810. Herndier, B.G., et al., *High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of HIV-associated Hodgkin's disease.* American Journal of Pathology, 1993. **142**(4): p. 1073-9.

811. Grogg, K.L., R.F. Miller, and A. Dogan, *HIV infection and lymphoma.* Journal of Clinical Pathology, 2007. **60**(12): p. 1365-1372.

812. Tirelli, U., et al., *Hodgkin's disease and human immunodeficiency virus infection: clinicopathologic and virologic features of 114 patients from the Italian Cooperative Group on AIDS and Tumors.* Journal of Clinical Oncology, 1995. **13**(7): p. 1758-67.

813. Carbone, A., et al., *Human immunodeficiency virus-associated Hodgkin's disease derives from post-germinal center B cells.* Blood, 1999. **93**(7): p. 2319-26.

814. Siebert, J.D., et al., *Human immunodeficiency virus-associated Hodgkin's disease contains latent, not replicative, Epstein-Barr virus.* Human Pathology, 1995. **26**(11): p. 1191-5.

815. Polesel, J., et al., *Non-Hodgkin lymphoma incidence in the Swiss HIV Cohort Study before and after highly active antiretroviral therapy.* AIDS, 2008. **22**(2): p. 301-306.

816. Zoufaly, A., et al., *Cumulative HIV viremia during highly active antiretroviral therapy is a strong predictor of AIDS-related lymphoma.* J Infect Dis, 2009. **200**(1): p. 79-87.

817. Bhaskaran, K., et al., *Systemic non-Hodgkin lymphoma in individuals with known dates of HIV seroconversion: incidence and predictors.* Aids, 2004. **18**(4): p. 673-81.

818. Bonnet, F., et al., *Factors Associated with the Occurrence of AIDS-Related Non-Hodgkin Lymphoma in the Era of Highly Active Antiretroviral Therapy: Aquitaine Cohort, France.* Clinical Infectious Diseases, 2006. **42**(3): p. 411-417.

819. Achenbach, C.J., et al., *HIV viremia and incidence of non-Hodgkin lymphoma in patients successfully treated with antiretroviral therapy.* Clin Infect Dis, 2014. **58**(11): p. 1599-606.

820. Rubinstein, P.G., D.M. Aboulafia, and A. Zloza, *Malignancies in HIV/AIDS: from epidemiology to therapeutic challenges.* AIDS, 2014. **28**(4): p. 453-465 10.1097/QAD.0000000000000071.

821. Dolcetti, R., P. De Paoli, and D. Serraino, *Degree of immune suppression and risk of HIV-related Hodgkin lymphoma: time points matter.* Blood, 2009. **114**(11): p. 2354; author reply 2354-5.

822. Engels, E.A., et al., *Cancers associated with Kaposi's sarcoma (KS) in AIDS: a link between KS herpesvirus and immunoblastic lymphoma.* British Journal of Cancer, 2001. **85**(9): p. 1298-1303.

823. Deloose, S.T., et al., *High incidence of Kaposi sarcoma-associated herpesvirus infection in HIV-related solid immunoblastic/plasmablastic diffuse large B-cell lymphoma.* Leukemia, 2005. **19**(5): p. 851-5.

824. Sullivan, R.J., et al., *Epidemiology, pathophysiology and treatment of Kaposi sarcoma-associated herpesvirus disease: Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman disease.* Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 2008. **47**(9): p. 1209-1215.

825. Du, M.Q., C.M. Bacon, and P.G. Isaacson, *Kaposi sarcoma-associated herpesvirus/human herpesvirus 8 and lymphoproliferative disorders.* J Clin Pathol, 2007. **60**(12): p. 1350-7.

826. Oksenhendler, E., et al., *High incidence of Kaposi sarcoma-associated herpesvirus-related non-Hodgkin lymphoma in patients with HIV infection and multicentric Castleman disease.* Blood, 2002. **99**(7): p. 2331-6.

827. Stebbing, J., et al., *Antiretroviral Treatment Regimens and Immune Parameters in the Prevention of Systemic AIDS-Related Non-Hodgkin's Lymphoma.* Journal of Clinical Oncology, 2004. **22**(11): p. 2177-2183.

828. Kowalkowski, M.A., et al., *Longer duration of combination antiretroviral therapy reduces the risk of Hodgkin lymphoma: a cohort study of HIV-infected male veterans.* Cancer epidemiology, 2014. **38**(4): p. 386-392.

829. Lanoy, E., et al., *HIV-associated Hodgkin lymphoma during the first months on combination antiretroviral therapy.* Blood, 2011. **118**(1): p. 44-9.

830. Wang, Q., et al., *Chronic hepatitis B and C virus infection and risk for non-hodgkin lymphoma in hiv-infected patients: A cohort study.* Annals of Internal Medicine, 2017. **166**(1): p. 9-17.

831. Matsuo, K., et al., *Effect of hepatitis C virus infection on the risk of non-Hodgkin's lymphoma: a meta-analysis of epidemiological studies.* Cancer Sci, 2004. **95**(9): p. 745-52.

832. Dal Maso, L. and S. Franceschi, *Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(11): p. 2078-85.

833. Waters, L., et al., *Hepatitis C infection is not associated with systemic HIV-associated non-Hodgkin's lymphoma: a cohort study.* Int J Cancer, 2005. **116**(1): p. 161-3.

834. Franceschi, S., et al., *Hepatitis C virus and non-Hodgkin's lymphoma: findings from the Swiss HIV Cohort Study.* Br J Cancer, 2006. **95**(11): p. 1598-1602.

835. Chao, C., et al., *Survival of non-Hodgkin lymphoma patients with and without HIV infection in the era of combined antiretroviral therapy.* Aids, 2010. **24**(11): p. 1765-70.

836. Jacobson, C.A. and J.S. Abramson, *HIV-Associated Hodgkin's Lymphoma: Prognosis and Therapy in the Era of cART.* Advances in Hematology, 2012. **2012**: p. 8.

837. Cole, S.R., et al., *Copy-years viremia as a measure of cumulative human immunodeficiency virus viral burden.* Am J Epidemiol, 2010. **171**(2): p. 198-205.

838. Morton, L.M., et al., *Etiologic heterogeneity among non-Hodgkin lymphoma subtypes: the InterLymph Non-Hodgkin Lymphoma Subtypes Project.* J Natl Cancer Inst Monogr, 2014. **2014**(48): p. 130-44.

839. Hoffmann, C., et al., *Hodgkin lymphoma is as common as non-Hodgkin lymphoma in HIV-positive patients with sustained viral suppression and limited immune deficiency: a prospective cohort study.* HIV Med, 2015. **16**(4): p. 261-4.

840. Dolcetti, R., et al., *A lymphomagenic role for HIV beyond immune suppression?* Blood, 2016. **127**(11): p. 1403-1409.

841. Carbone, A., et al., *Epstein–Barr virus associated lymphomas in people with HIV.* Current Opinion in HIV and AIDS, 2017. **12**(1): p. 39-46.

842. Reekie, J., et al., *Fatal and nonfatal AIDS and non-AIDS events in HIV-1-positive individuals with high CD4 cell counts according to viral load strata.* AIDS, 2011. **25**(18): p. 2259-2268.

843. Pietersma, F., E. Piriou, and D. van Baarle, *Immune surveillance of EBV-infected B cells and the development of non-Hodgkin lymphomas in immunocompromised patients.* Leuk Lymphoma, 2008. **49**(6): p. 1028-41.

844. Arvey, A., et al., *The tumor virus landscape of AIDS-related lymphomas.* Blood, 2015. **125**(20): p. e14-22.

845. van Baarle, D., et al., *Dysfunctional Epstein-Barr virus (EBV)–specific CD8 T lymphocytes and increased EBV load in HIV-1 infected individuals progressing to AIDS-related non-Hodgkin lymphoma.* Blood, 2001. **98**(1): p. 146-155.

846. Landgren, O., et al., *Circulating Serum Free Light Chains As Predictive Markers of AIDS-Related Lymphoma.* Journal of Clinical Oncology, 2010. **28**(5): p. 773-779.

847. Bibas, M., et al., *Role of serum free light chains in predicting HIV-associated non-Hodgkin lymphoma and Hodgkin's lymphoma and its correlation with antiretroviral therapy.* Am J Hematol, 2012. **87**(8): p. 749-53.

848. Dolcetti, R., et al., *Role of HIV-1 matrix protein p17 variants in lymphoma pathogenesis.* Proceedings of the National Academy of Sciences of the United States of America, 2015. **112**(46): p. 14331-14336.

849. Vardhana, S. and A. Younes, *The immune microenvironment in Hodgkin lymphoma: T cells, B cells, and immune checkpoints.* Haematologica, 2016. **101**(7): p. 794-802.

850. Gloghini, A. and A. Carbone, *Why would the incidence of HIV-associated Hodgkin lymphoma increase in the setting of improved immunity?* Int J Cancer, 2007. **120**(12): p. 2753-4.

851. Aldinucci, D., et al., *The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape.* The Journal of Pathology, 2010. **221**(3): p. 248-263.

852. Sopori, M.L. and W. Kozak, *Immunomodulatory effects of cigarette smoke.* Journal of Neuroimmunology, 1998. **83**(1–2): p. 148-156.

853. Matthews, G.V., et al., *Changes in acquired immunodeficiency syndrome–related lymphoma since the introduction of highly active antiretroviral therapy.* Blood, 2000. **96**(8): p. 2730-2734.

854. Vajdic, C.M., et al., *Are antibody deficiency disorders associated with a narrower range of cancers than other forms of immunodeficiency?* Blood, 2010. **116**(8): p. 1228-34.

855. Jarrett, R.F., *Viruses and lymphoma/leukaemia.* The Journal of Pathology, 2006. **208**(2): p. 176-186.

856. Thompson, M.P. and R. Kurzrock, *Epstein-Barr Virus and Cancer.* Clinical Cancer Research, 2004. **10**(3): p. 803-821.

857. Moir, S. and A.S. Fauci, *B cells in HIV infection and disease.* Nature reviews. Immunology, 2009. **9**(4): p. 235-245.

858. De Milito, A., *B lymphocyte dysfunctions in HIV infection.* Curr HIV Res, 2004. **2**(1): p. 11-21.

859. Moir, S. and A.S. Fauci, *Pathogenic mechanisms of B-lymphocyte dysfunction in HIV disease.* The Journal of allergy and clinical immunology, 2008. **122**(1): p. 12-21.

860. Gloghini A, C.A., Pantanowitz L. *HIV-associated lymphomas*. Atlas Genet Cytogenet Oncol Haematol. in press 2016; Available from: http://AtlasGeneticsOncology.org/Anomalies/LymphomAssocHIVID1732.html.

861. Pantanowitz, L., A. Carbone, and R. Dolcetti, *Microenvironment and HIV-related lymphomagenesis.* Semin Cancer Biol, 2015. **34**: p. 52-7.

862. Schnittman, S.M., et al., *Direct polyclonal activation of human B lymphocytes by the acquired immune deficiency syndrome virus.* Science, 1986. **233**(4768): p. 1084-6.

863. De Paoli, P. and A. Carbone, *Microenvironmental abnormalities induced by viral cooperation: Impact on lymphomagenesis.* Seminars in Cancer Biology, 2015. **34**: p. 70-80.

864. Cesarman, E., *Gammaherpesviruses and lymphoproliferative disorders.* Annu Rev Pathol, 2014. **9**: p. 349-72.

865. Carbone, A., A. Gloghini, and G. Dotti, *EBV-Associated Lymphoproliferative Disorders: Classification and Treatment.* The Oncologist, 2008. **13**(5): p. 577-585.

866. Yao, Q.Y., A.B. Rickinson, and M.A. Epstein, *Oropharyngeal shedding of infectious Epstein-Barr virus in healthy virus-immune donors. A prospective study.* Chin Med J (Engl), 1985. **98**(3): p. 191-6.

867. Tierney, R.J., et al., *Epstein-Barr virus latency in blood mononuclear cells: analysis of viral gene transcription during primary infection and in the carrier state.* J Virol, 1994. **68**(11): p. 7374-85.

868. Callan, M.F., et al., *Large clonal expansions of CD8+ T cells in acute infectious mononucleosis.* Nat Med, 1996. **2**(8): p. 906-11.

869. Miyashita, E.M., et al., *Identification of the site of Epstein-Barr virus persistence in vivo as a resting B cell.* J Virol, 1997. **71**(7): p. 4882-91.

870. Cesarman, E., *Gammaherpesvirus and lymphoproliferative disorders in immunocompromised patients.* Cancer Letters, 2011. **305**(2): p. 163-174.

871. Bornkamm, G.W., *Epstein-Barr virus and its role in the pathogenesis of Burkitt's lymphoma: an unresolved issue.* Semin Cancer Biol, 2009. **19**(6): p. 351-65.

872. Price, A.M., et al., *Analysis of Epstein-Barr Virus-Regulated Host Gene Expression Changes through Primary B-Cell Outgrowth Reveals Delayed Kinetics of Latent Membrane Protein 1-Mediated NF-κB Activation.* Journal of Virology, 2012. **86**(20): p. 11096-11106.

873. Fangazio, M., L. Pasqualucci, and R. Dalla-Favera, *Chromosomal Translocations in B Cell Lymphomas*, in *Chromosomal Translocations and Genome Rearrangements in Cancer*. 2015, Springer. p. 157-188.

874. Cleary, M.L., et al., *Immunoglobulin gene rearrangement as a diagnostic criterion of B-cell lymphoma.* Proceedings of the National Academy of Sciences, 1984. **81**(2): p. 593-597.

875. Arnold, A., et al., *Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms.* New England Journal of Medicine, 1983. **309**(26): p. 1593-1599.

876. Gloghini, A., R. Dolcetti, and A. Carbone, *Lymphomas occurring specifically in HIV-infected patients: From pathogenesis to pathology.* Seminars in Cancer Biology, 2013. **23**(6, Part A): p. 457-467.

877. Swartz, M.A., *The physiology of the lymphatic system.* Advanced Drug Delivery Reviews, 2001. **50**(1–2): p. 3-20.

878. Baron, S., *Medical Microbiology*. 1996: University of Texas Medical Branch at Galveston.

879. Janeway, C.A., et al., *Immunobiology: the immune system in health and disease*. Vol. 1. 1997: Current Biology Singapore.

880. Kodituwakku, A.P., et al., *Isolation of antigen-specific B cells.* Immunol Cell Biol, 2003. **81**(3): p. 163-170.

881. Alberts, B., et al., *Molecular Biology of the Cell 4th Edition: International Student Edition*. 2002, Routledge.

882. Lane, H.C., et al., *Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome.* N Engl J Med, 1983. **309**(8): p. 453-8.

883. Martinez-Maza, O., et al., *Infection with the human immunodeficiency virus (HIV) is associated with an in vivo increase in B lymphocyte activation and immaturity.* J Immunol, 1987. **138**(11): p. 3720-4.

884. Bradwell, A.R., *Serum free light chain measurements move to center stage.* Clin Chem, 2005. **51**(5): p. 805-7.

885. Murray, P.R., K.S. Rosenthal, and M.A. Pfaller, *Medical microbiology*. 2015: Elsevier Health Sciences.

886. Jenner, E., *Serum free light chains in clinical laboratory diagnostics.* Clinica Chimica Acta, 2014. **427**: p. 15-20.

887. Hutchison, C.A. and O. Landgren, *Polyclonal immunoglobulin free light chains as a potential biomarker of immune stimulation and inflammation.* Clin Chem, 2011. **57**(10): p. 1387-9.

888. Solomon, A., *Light chains of human immunoglobulins.* Methods Enzymol, 1985. **116**: p. 101-21.

889. Ng, V.L., *B-Lymphocytes and Autoantibody Profiles in HIV Disease*, in *AIDS Allergy and Rheumatology*, N.E. Lane, Editor. 1997, Humana Press: Totowa, NJ. p. 11-28.

890. Grulich, A.E., et al., *B-cell stimulation and prolonged immune deficiency are risk factors for non-Hodgkin's lymphoma in people with AIDS.* AIDS, 2000. **14**(2): p. 133-40.

891. Breen, E.C., et al., *Elevated serum soluble CD30 precedes the development of AIDS-associated non-Hodgkin's B cell lymphoma.* Tumour Biol, 2006. **27**(4): p. 187-94.

892. Biggar, R.J., et al., *Immunoglobulin subclass levels in patients with non-Hodgkin lymphoma.* International Journal of Cancer, 2009. **124**(11): p. 2616-2620.

893. Ellison-Loschmann, L., et al., *Immunoglobulin E Levels and Risk of Lymphoma in a Case-Control Study in Spain.* Cancer Epidemiology Biomarkers & Prevention, 2007. **16**(7): p. 1492-1498.

894. Tittle, V., et al., *Serum-free light chains in HIV-associated lymphoma: no correlation with histology or prognosis.* AIDS, 2015. **29**(10): p. 1201-4.

895. Shiels, M.S., et al., *Free light chains and the risk of AIDS-defining opportunistic infections in HIV-infected individuals.* Clin Infect Dis, 2012. **55**(10): p. e103-8.

896. Zemlin, A.E., et al., *Serum free light chains in patients with HIV infection: their association with markers of disease severity and antiretroviral use.* J Clin Pathol, 2015. **68**(2): p. 148-53.

897. The Binding Site, *Freelite® Human Kappa Free kit for use on the SPAPLUS ® Insert Code: SIN231*. 2014.

898. The Binding Site, *Freelite® Human Lambda Free kit for use on the SPAPLUS® Insert Code: SIN232*. 2014.

899. The Binding Site, *Human IgG and IgG subclass liquid reagent kits for use on the SPAPLUS® Insert Code: SIN128.* 2014.

900. The Binding Site, *Human IgA liquid reagent kit for use on SPAPLUS ® Insert Code: SIN143.* 2014.

901. The Binding Site, *Human IgM kit for use on SPAPLUS® Insert Code: SIN144.* 2014.

902. The Binding Site, *Human IgD Kit for use on SPAPLUS® Insert Code: SIN169.* 2014.

903. Cockcroft, D.W. and M.H. Gault, *Prediction of creatinine clearance from serum creatinine.* Nephron, 1976. **16**(1): p. 31-41.

904. Mosteller, R.D., *Simplified calculation of body-surface area.* N Engl J Med, 1987. **317**(17): p. 1098.

905. Fawcett, T., *An introduction to ROC analysis.* Pattern Recognition Letters, 2006. **27**(8): p. 861-874.

906. Iio, A., et al., *The metabolism of IgE in patients with immunodeficiency states and neoplastic conditions.* J Clin Invest, 1977. **59**(5): p. 743-55.

907. Hansen, D.A., et al., *Identification of monoclonal immunoglobulins and quantitative immunoglobulin abnormalities in hairy cell leukemia and chronic lymphocytic leukemia.* Am J Clin Pathol, 1994. **102**(5): p. 580-5.

908. Rozman, C., E. Montserrat, and N. Vinolas, *Serum immunoglobulins in B-chronic lymphocytic leukemia. Natural history and prognostic significance.* Cancer, 1988. **61**(2): p. 279-83.

909. Bibas, M., *Polyclonal Serum Free Light Chains: A Biomarker of Disease Prognosis or of Immune Senescence?* Journal of Clinical Oncology, 2012. **30**(24): p. 3033-3034.

910. Baker, J.V., et al., *Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection.* J Acquir Immune Defic Syndr, 2011. **56**(1): p. 36-43.

911. Peters, L., et al., *HIV and hepatitis C co-infection in Europe, Israel and Argentina: a EuroSIDA perspective.* BMC Infect Dis, 2014. **14 Suppl 6**: p. S13.

912. Terrier, B., et al., *Serum-free light chain assessment in hepatitis C virus-related lymphoproliferative disorders.* Ann Rheum Dis, 2009. **68**(1): p. 89-93.

913. Basile, U., et al., *Assessment of free light chains in HCV-positive patients with mixed cryoglobulinaemia vasculitis undergoing rituximab treatment.* Liver Int, 2015. **35**(9): p. 2100-7.

914. Hutchison, C.A., K. Basnayake, and P. Cockwell, *Serum free light chain assessment in monoclonal gammopathy and kidney disease.* Nat Rev Nephrol, 2009. **5**(11): p. 621-628.

915. Mottet, N., et al., *EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent.* European Urology, 2017. **71**(4): p. 618-629.

916. Cancer research UK. *Prostate cancer risks and causes*. 2015 6 June 2014 [cited 2016 8 July 2016]; Available from: http://www.cancerresearchuk.org/about-cancer/type/prostate-cancer/about/prostate-cancer-risks-and-causes.

917. Lloyd, T., et al., *Lifetime risk of being diagnosed with, or dying from, prostate cancer by major ethnic group in England 2008–2010.* BMC Medicine, 2015. **13**(1): p. 171.

918. Bruner, D.W., et al., *Relative risk of prostate cancer for men with affected relatives: systematic review and meta-analysis.* Int J Cancer., 2003. **107**.

919. Steinberg, G.D., et al., *Family history and the risk of prostate cancer.* Prostate, 1990. **17**(4): p. 337-47.

920. Kote-Jarai, Z., et al., *BRCA2 is a moderate penetrance gene contributing to young-onset prostate cancer: implications for genetic testing in prostate cancer patients.* Br J Cancer, 2011. **105**(8): p. 1230-4.

921. Bancroft, E.K., et al., *Targeted prostate cancer screening in BRCA1 and BRCA2 mutation carriers: results from the initial screening round of the IMPACT study.* Eur Urol, 2014. **66**(3): p. 489-99.

922. Castro, E., et al., *Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer.* J Clin Oncol, 2013. **31**(14): p. 1748-57.

923. Morgentaler, A. and E.L. Rhoden, *Prevalence of prostate cancer among hypogonadal men with prostate-specific antigen levels of 4.0 ng/mL or less.* Urology, 2006. **68**(6): p. 1263-7.

924. Huggins, C. and C.V. Hodges, *Studies on prostatic cancer: I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. 1941.* J Urol, 2002. **168**(1): p. 9-12.

925. Roddam, A.W., et al., *Endogenous sex hormones and prostate cancer: A collaborative analysis of 18 prospective studies.* Journal of the National Cancer Institute, 2008. **100**(3): p. 170-183.

926. Monath, J.R., et al., *Physiologic variations of serum testosterone within the normal range do not affect serum prostate-specific antigen.* Urology, 1995. **46**(1): p. 58-61.

927. Debruyne, F.M.J., et al., *Testosterone treatment is not associated with increased risk of prostate cancer or worsening of lower urinary tract symptoms: prostate health outcomes in the Registry of Hypogonadism in Men.* BJU International, 2017. **119**(2): p. 216-224.

928. Klap, J., M. Schmid, and K.R. Loughlin, *The Relationship between Total Testosterone Levels and Prostate Cancer: A Review of the Continuing Controversy.* The Journal of Urology, 2015. **193**(2): p. 403-414.

929. Haider, A., et al., *Incidence of Prostate Cancer in Hypogonadal Men Receiving Testosterone Therapy: Observations from 5-Year Median Followup of 3 Registries.* The Journal of Urology, 2015. **193**(1): p. 80-86.

930. Dennis, L.K., C.F. Lynch, and J.C. Torner, *Epidemiologic association between prostatitis and prostate cancer.* Urology, 2002. **60**(1): p. 78-83.

931. Jiang, J., et al., *The Role of Prostatitis in Prostate Cancer: Meta-Analysis.* PLoS ONE, 2014. **8**(12): p. e85179.

932. Moreira, D.M., et al., *Baseline prostate inflammation is associated with a reduced risk of prostate cancer in men undergoing repeat prostate biopsy: Results from the REDUCE study.* Cancer, 2014. **120**(2): p. 190-196.

933. Dankner, R., et al., *Diabetes, prostate cancer screening and risk of low- and high-grade prostate cancer: an 11 year historical population follow-up study of more than 1 million men.* Diabetologia, 2016. **59**(8): p. 1683-1691.

934. Kasper, J.S. and E. Giovannucci, *A Meta-analysis of Diabetes Mellitus and the Risk of Prostate Cancer.* Cancer Epidemiology Biomarkers & Prevention, 2006. **15**(11): p. 2056-2062.

935. Catalona, W.J., et al., *Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men.* The Journal of Urology, 1994. **151**(5): p. 1283-90.

936. Catalona, W.J., D.S. Smith, and D.K. Ornstein, *Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination. Enhancement of specificity with free PSA measurements.* JAMA, 1997. **277**(18): p. 1452-5.

937. Public Health England, *Prostate cancer risk management programme (PCRMP): benefits and risks of PSA testing*. 2016.

938. Richie, J.P., et al., *Effect of patient age on early detection of prostate cancer with serum prostate-specific antigen and digital rectal examination.* Urology, 1993. **42**(4): p. 365-74.

939. Carter, H.B., et al., *Early detection of prostate cancer: AUA guideline.* J Urol, 2013. **190**(2): p. 419-426.

940. Humphrey, P.A., *Gleason grading and prognostic factors in carcinoma of the prostate.* Mod Pathol, 2004. **17**(3): p. 292-306.

941. Ilic, D., et al. *Screening for prostate cancer.* Cochrane Database of Systematic Reviews Issue 1. Art. No.: 2013; Available from: http://summaries.cochrane.org/CD004720/PROSTATE_screening-for-prostate-cancer#sthash.tUaQdGjw.dpuf.

942. Wolf, A.M., et al., *American Cancer Society guideline for the early detection of prostate cancer: update 2010.* CA Cancer J Clin, 2010. **60**(2): p. 70-98.

943. Practitioners, R.A.C.o.G., *Guidelines for preventive activities in general practice 8th edition*. 9.8 Prostate cancer. 2012, East Melbourne: Royal Australian College of General Practitioners.

944. Moyer, V.A., *Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement.* Ann Intern Med, 2012. **157**(2): p. 120-34.

945. Draisma, G., et al., *Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer.* J Natl Cancer Inst, 2003. **95**(12): p. 868-78.

946. Schröder, F.H., et al., *Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up.* The Lancet, 2014. **384**(9959): p. 2027-2035.

947. Andriole, G.L., et al., *Prostate Cancer Screening in the Randomized Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial: Mortality Results after 13 Years of Follow-up.* Journal of the National Cancer Institute, 2012. **104**(2): p. 125-132.

948. Brenner, H. and V. Arndt, *Long-Term Survival Rates of Patients With Prostate Cancer in the Prostate-Specific Antigen Screening Era: Population-Based Estimates for the Year 2000 by Period Analysis.* Journal of Clinical Oncology, 2005. **23**(3): p. 441-447.

949. Schröder , F.H., et al., *Screening and Prostate-Cancer Mortality in a Randomized European Study.* New England Journal of Medicine, 2009. **360**(13): p. 1320-1328.

950. de Koning, H.J., et al., *Prostate cancer mortality reduction by screening: power and time frame with complete enrollment in the European Randomised Screening for Prostate Cancer (ERSPC) trial.* Int J Cancer, 2002. **98**(2): p. 268-73.

951. Berry, S.J., et al., *The development of human benign prostatic hyperplasia with age.* J Urol, 1984. **132**(3): p. 474-9.

952. Holman, C.D., et al., *Mortality and prostate cancer risk in 19,598 men after surgery for benign prostatic hyperplasia.* BJU Int, 1999. **84**(1): p. 37-42.

953. Independent UK Panel on Breast Cancer Screening, *The benefits and harms of breast cancer screening: an independent review.* The Lancet, 2012. **380**(9855): p. 1778-1786.

954. Bibbins-Domingo, K., D.C. Grossman, and S.J. Curry, *The US preventive services task force 2017 draft recommendation statement on screening for prostate cancer: An invitation to review and comment.* JAMA, 2017. **317**(19): p. 1949-1950.

955. De Angelis, R., et al., *Cancer survival in Europe 1999–2007 by country and age: results of EUROCARE-5—a population-based study.* The Lancet Oncology, 2014. **15**(1): p. 23-34.

956. Finne, P., et al., *Lead-time in the European Randomised Study of Screening for Prostate Cancer.* Eur J Cancer, 2010. **46**(17): p. 3102-8.

957. Neppl-Huber, C., et al., *Changes in incidence, survival and mortality of prostate cancer in Europe and the United States in the PSA era: additional diagnoses and avoided deaths.* Annals of Oncology, 2012. **23**(5): p. 1325-1334.

958. Rimland, D. and J. Guest. *Increasing incidence of prostate cancer in the Atlanta VA cohort study (HAVACS)*. in *14th Conference on Retroviruses and Opportunistic Infections. Los Angeles, CA*. 2007.

959. Sigel, K., et al., *Cancer Screening in Patients Infected with HIV.* Current HIV/AIDS Reports, 2011. **8**(3): p. 142-152.

960. Ong, W.L., et al., *Clinicopathological characteristics and management of prostate cancer in the human immunodeficiency virus (HIV)-positive population: experience in an Australian major HIV centre.* BJU International, 2015. **116**: p. 5-10.

961. British HIV Association (BHIVA), *British HIV Association guidelines for HIV-associated malignancies 2014.* HIV medicine, 2014. **15**(S2): p. 1-92.

962. Thompson , I.M., et al., *Prevalence of Prostate Cancer among Men with a Prostate-Specific Antigen Level ≤4.0 ng per Milliliter.* New England Journal of Medicine, 2004. **350**(22): p. 2239-2246.

963. Hori, S., J.-S. Blanchet, and J. McLoughlin, *From prostate-specific antigen (PSA) to precursor PSA (proPSA) isoforms: a review of the emerging role of proPSAs in the detection and management of early prostate cancer.* BJU International, 2012: p. no-no.

964. Balk, S.P., Y.-J. Ko, and G.J. Bubley, *Biology of Prostate-Specific Antigen.* Journal of Clinical Oncology, 2003. **21**(2): p. 383-391.

965. Stephan, C., et al., *PSA and other tissue kallikreins for prostate cancer detection.* European Journal of Cancer, 2007. **43**(13): p. 1918-1926.

966. Lilja, H., D. Ulmert, and A.J. Vickers, *Prostate-specific antigen and prostate cancer: prediction, detection and monitoring.* Nat Rev Cancer, 2008. **8**(4): p. 268-278.

967. Catalona, W.J., et al., *Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening.* JAMA, 1995. **274**(15): p. 1214-20.

968. Partin, A.W., et al., *Analysis of percent free prostate-specific antigen (PSA) for prostate cancer detection: influence of total PSA, prostate volume, and age.* Urology, 1996. **48**(6A Suppl): p. 55-61.

969. Makarov, D.V., et al., *Biomarkers for prostate cancer.* Annu Rev Med, 2009. **60**: p. 139-51.

970. Catalona, W.J., et al., *Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial.* JAMA, 1998. **279**(19): p. 1542-7.

971. Sfoungaristos, S. and P. Perimenis, *PSA density is superior than PSA and Gleason score for adverse pathologic features prediction in patients with clinically localized prostate cancer.* Canadian Urological Association Journal, 2012. **6**(1): p. 46-50.

972. Doublet, J.D., et al., *The value of PSA density for the diagnosis of prostatic cancer and for the indication of radical prostatectomy.* Prog Urol, 1995. **5**(2): p. 211-9, 220; discussion 219-20.

973. Carter, H.B., et al., *Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease.* Jama, 1992. **267**(16): p. 2215-20.

974. Vickers, A.J., et al., *Systematic review of pretreatment prostate-specific antigen velocity and doubling time as predictors for prostate cancer.* J Clin Oncol, 2009. **27**(3): p. 398-403.

975. Arlen, P.M., et al., *Prostate-Specific Antigen Working Group's Guidelines on PSA Doubling Time.* The Journal of urology, 2008. **179**(6): p. 2181-2186.

976. Schmid, H.P., J.E. McNeal, and T.A. Stamey, *Observations on the doubling time of prostate cancer. The use of serial prostate-specific antigen in patients with untreated disease as a measure of increasing cancer volume.* Cancer, 1993. **71**(6): p. 2031-40.

977. Loeb, S. and W.J. Catalona, *The Prostate Health Index: a new test for the detection of prostate cancer.* Therapeutic Advances in Urology, 2014. **6**(2): p. 74-77.

978. Grulich, A.E. and J.M. Kaldor, *Trends in HIV incidence in homosexual men in developed countries.* Sex Health, 2008. **5**(2): p. 113-8.

979. Ohlsson, C., et al., *Genetic Determinants of Serum Testosterone Concentrations in Men.* PLOS Genetics, 2011. **7**(10): p. e1002313.

980. Crum, N.F., et al., *A review of hypogonadism and erectile dysfunction among HIV-infected men during the pre- and post-HAART eras: diagnosis, pathogenesis, and management.* AIDS Patient Care STDS, 2005. **19**(10): p. 655-71.

981. Silberstein, J., et al., *HIV and prostate cancer: a systematic review of the literature.* Prostate Cancer Prostatic Dis, 2009. **12**(1): p. 6-12.

982. Gould, D.C., M.R. Feneley, and R.S. Kirby, *Prostate-specific antigen testing in hypogonadism: implications for the safety of testosterone-replacement therapy.* BJU Int, 2006. **98**(1): p. 1-4.

983. Raynaud, J.P., et al., *Prostate-specific antigen (PSA) concentrations in hypogonadal men during 6 years of transdermal testosterone treatment.* BJU Int, 2013. **111**(6): p. 880-90.

984. Kaufman, J.M. and A. Vermeulen, *The decline of androgen levels in elderly men and its clinical and therapeutic implications.* Endocr Rev, 2005. **26**(6): p. 833-76.

985. Hammond, G.L., T.S. Wu, and M. Simard, *Evolving utility of sex hormone-binding globulin measurements in clinical medicine.* Curr Opin Endocrinol Diabetes Obes, 2012. **19**(3): p. 183-9.

986. World health organisation. *International Statistical Classification of Diseases and Related Health Problems -10th revision*. 2010 8 Jan 2014]; 10:[Available from: http://apps.who.int/classifications/icd10/browse/2010/en.

987. Legler, J.M., et al., *The Role of Prostate-Specific Antigen (PSA) Testing Patterns in the Recent Prostate Cancer Incidence Decline in the United States.* Cancer Causes & Control, 1998. **9**(5): p. 519-527.

988. Melia, J. and S. Moss, *Survey of the rate of PSA testing in general practice.* British journal of cancer, 2001. **85**(5): p. 656.

989. Drummond, F.J., et al., *Trends in prostate specific antigen testing in Ireland: lessons from a country without guidelines.* Irish Journal of Medical Science, 2010. **179**(1): p. 43-49.

990. Zhang, K., C.H. Bangma, and M.J. Roobol, *Prostate cancer screening in Europe and Asia.* Asian Journal of Urology, 2017. **4**(2): p. 86-95.

991. Zeliadt, S.B., et al., *Influence of Publication of US and European Prostate Cancer Screening Trials on PSA Testing Practices.* JNCI: Journal of the National Cancer Institute, 2011. **103**(6): p. 520-523.

992. Jemal, A., et al., *Prostate cancer incidence and psa testing patterns in relation to uspstf screening recommendations.* JAMA, 2015. **314**(19): p. 2054-2061.

993. Loeb, S., et al., *Overdiagnosis and Overtreatment of Prostate Cancer.* European urology, 2014. **65**(6): p. 1046-1055.

994. Gann, P.H., C.H. Hennekens, and M.J. Stampfer, *A prospective evaluation of plasma prostate-specific antigen for detection of prostatic cancer.* JAMA, 1995. **273**(4): p. 289-94.

995. Walker, J., et al., *Prevalence, associations, and adequacy of treatment of major depression in patients with cancer: a cross-sectional analysis of routinely collected clinical data.* The Lancet Psychiatry, 2014. **1**(5): p. 343-350.

996. Mitchell, A.J., et al., *Depression and anxiety in long-term cancer survivors compared with spouses and healthy controls: a systematic review and meta-analysis.* The Lancet Oncology, 2013. **14**(8): p. 721-732.

997. Crum, N.F., C.R. Spencer, and C.L. Amling, *Prostate carcinoma among men with human immunodeficiency virus infection.* Cancer, 2004. **101**(2): p. 294-299.

998. Preston, D.M., et al., *Prostate-specific antigen levels in young white and black men 20 to 45 years old1.* Urology, 2000. **56**(5): p. 812-816.

999. Vianna, L.E., Y. Lo, and R.S. Klein, *Serum prostate-specific antigen levels in older men with or at risk of HIV infection.* HIV medicine, 2006. **7**(7): p. 471-476.

1000. Bosch, J.L., et al., *Establishing normal reference ranges for PSA change with age in a population-based study: The Krimpen study.* Prostate, 2006. **66**(4): p. 335-43.

1001. Fang, J., et al., *Low levels of prostate-specific antigen predict long-term risk of prostate cancer: results from the Baltimore Longitudinal Study of Aging.* Urology, 2001. **58**(3): p. 411-6.

1002. Crum-Cianflone, N.F., et al., *Erectile dysfunction and hypogonadism among men with HIV.* AIDS Patient Care STDS, 2007. **21**(1): p. 9-19.

1003. Oesterling, J.E., et al., *Serum prostate-specific antigen in a community-based population of healthy men: Establishment of age-specific reference ranges.* JAMA, 1993. **270**(7): p. 860-864.

1004. Stamey, T.A., et al., *Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate.* N Engl J Med, 1987. **317**(15): p. 909-16.

1005. Richardson, T.D. and J.E. Oesterling, *Age-specific reference ranges for serum prostate-specific antigen.* Urologic Clinics of North America, 1997. **24**(2): p. 339-351.

1006. Tchetgen, M.B., et al., *Ejaculation increases the serum prostate-specific antigen concentration.* Urology, 1996. **47**(4): p. 511-6.

1007. Shepherd, L., et al., *Predictive value of prostate specific antigen in a European HIV-positive cohort: does one size fit all?* Antivir Ther, 2016. **21**(6): p. 529-534.

1008. Fossati, N., et al., *Clinical performance of serum isoform [-2]proPSA (p2PSA), and its derivatives %p2PSA and the Prostate Health Index, in men aged <60 years: results from a multicentric European study.* BJU International, 2015. **115**(6): p. 913-920.

1009. Guazzoni, G., et al., *Prostate-Specific Antigen (PSA) Isoform p2PSA Significantly Improves the Prediction of Prostate Cancer at Initial Extended Prostate Biopsies in Patients with Total PSA Between 2.0 and 10 ng/ml: Results of a Prospective Study in a Clinical Setting.* European Urology, 2011. **60**(2): p. 214-222.

1010. Haese, A., et al., *Clinical Utility of the PCA3 Urine Assay in European Men Scheduled for Repeat Biopsy.* European Urology, 2008. **54**(5): p. 1081-1088.

1011. D'Agostino, R.B., Jr. and R.B. D'Agostino, Sr., *Estimating treatment effects using observational data.* Jama, 2007. **297**(3): p. 314-6.

1012. Sedgwick, P., *Cohort studies: sources of bias.* BMJ, 2011. **343**.

1013. Sterne, J.A.C., et al., *Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls.* BMJ, 2009. **338**.

1014. Neri, A., et al., *Epstein-Barr virus infection precedes clonal expansion in Burkitt's and acquired immunodeficiency syndrome-associated lymphoma.* Blood, 1991. **77**(5): p. 1092-5.

1015. Bornkamm, G.W., *Epstein-Barr virus and the pathogenesis of Burkitt's lymphoma: More questions than answers.* International Journal of Cancer, 2009. **124**(8): p. 1745-1755.

1016. de-The, G., et al., *Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Ugandan prospective study.* Nature, 1978. **274**(5673): p. 756-61.

1017. Wareham, N.E., et al., *Epstein-Barr Virus (EBV) DNAemia and Post-Transplant Lymphoproliferative Disorders (PTLD) Among Transplant Recipients.* Open Forum Infectious Diseases, 2015. **2**(suppl_1): p. 1202-1202.