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**ANTIRETROVIRAL DRUG RESISTANCE AND TREATMENT OUTCOMES OF
HUMAN IMMUNODEFICIENCY VIRUS TYPE 1, IMPLICATIONS FOR LOW
AND MIDDLE INCOME COUNTRIES**

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Antiretroviral drug resistance and treatment outcomes of human immunodeficiency virus type 1, implications for low and middle income countries

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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1947-1999

SUMMARY

My thesis contains comprehensive studies of antiretroviral treatment (ART) naïve and experienced patients from the beginning of the Swedish HIV-1 epidemic. **Paper I** was the first study to describe the ART usage and occurrence acquired drug resistance mutations (DRM) at a population level in Sweden over time and we used data derived between 1997-2011. We showed that there was a general decrease of DRM to PIs and NRTIs, while an increase of DRM towards NNRTIs was found in patients from low-and middle-income countries (LMICs) with ART initiation after 2007. We therefore suggest that treatment regimens without NNRTI and their effectiveness tested for non-B-subtypes should be considered as first-line choice for patients from LMICs. In **Papers II, III and IV** I focused on the impact of different subtypes. At the start of the epidemic HIV-1B dominated in high-income countries and due to migration and traveling changes occurred in the global subtype distribution. Temporal trends of various subtypes in Sweden were unknown until analyzes presented in **Paper II** were performed. In that study we showed an increasing trend of HIV-1C and recombinant forms and a decline of HIV-1B in newly diagnosed patients. Our results suggest that this is partly due to spread of non-B subtypes among heterosexuals and MSM within the country. **Papers III, IV and V** analyzed treatment outcomes with special focus on subtype HIV-1C (**III** and **IV**) and consequences of reasons for treatment switch (**V**). In **Paper III** we demonstrated a significantly higher risk of viral failure for patients infected with HIV-1C using PI-based ART compared to HIV-1B. In **Paper IV** we analyzed drug resistance to second-generation NNRTI rilpivirine (RPV) among patients failing NNRTIs in Europe and in ART-naïve HIV-1C infected patients from Ethiopia and India. RPV-inhibition and binding affinity assays on HIV-1C reverse transcriptase was also performed. Our findings indicate that the use of RPV has limitations in HIV-1C dominated countries where laboratory monitoring is not standard of care. In **Paper V** we studied the effects of different reasons for therapy switch and found that higher viral load (VL) at switch from first-line ART had a negative effect on time to second line ART failure, nevertheless no effect of VL-level and DRM on the CD4⁺ T-cell gain, AIDS or death was found. In conclusion, the treatment outcome of HIV-1 infected patients in Sweden has shown remarkable improvements and is now very good. Prevalence of DRM is low and once viral failure with or without DRM occurs patients are managed effectively. Nonetheless the virus and the epidemic are highly heterogeneous and constantly changing, requiring close surveillance. Also our studies reveal new future challenges for the optimization of ART in patients infected in LMICs in terms of effectiveness on non-B subtypes.

LIST OF SCIENTIFIC PAPERS

- I. Bontell I, **Hägglom A**, Bratt G, Albert J, Sönnernborg A. Trends in antiretroviral therapy and prevalence of HIV drug resistance mutations in Sweden 1997-2011. *PLoS One*. 2013;8(3):e59337.
- II. Neogi U*, **Hägglom A***, Santacatterina M, Bratt G, Gisslén M, Albert J, Sönnernborg. ***Equal contribution.** Temporal trends in the Swedish HIV-1 epidemic: increase in non-B subtypes and recombinant forms over three decades. *PLoS One*. 2014 Jun 12; 9 (6): e99390.
- III. **Hägglom A**, Svedhem V, Singh K, Sönnernborg A, Neogi U. Increased risk of virological failure in HIV-1 subtype C infected patients on boosted protease inhibitors based regimen in a real-life cohort. *Manuscript submitted*.
- IV. Neogi U, **Hägglom A**, Singh K, Rogers LC, Rao SD, Amogne W, Schülter E, Zazzi M, Arnold E, Sarafianos SG, Sönnernborg A. Factors influencing the efficacy of rilpivirine in HIV-1 subtype C in low- and middle-income countries. *J Antimicrob Chemother*. 2015 Oct 30. pii: dkv359.
- V. **Hägglom A**, Santacatterina M, Neogi U, Sönnernborg A. Effect of therapy switch on time to second line antiretroviral treatment failure in HIV-infected patients. *Manuscript in preparation*.

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LIST OF ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ATV	Atazanavir
CRF	Circulating Recombinant Forms
d4T	Stavudine
ddI	Didanosine
DNA	Deoxyribonucleic acid
DRM	Drug resistance mutations
DRV	Darunavir
DTG	Dolutegravir
ECDC	European Center for Disease Prevention and Control
EFV	Efavirenz
ETR	Etravirine
EVG	Elvitegravir
fAPV	Fosamprenavir Calcium
FTC	Emtricitabine
GFATM	Global fund to fight AIDS, Tuberculosis and Malaria
GRT	Genotypic resistance test
HIV	Human immuno deficiency virus
HIV-1B	HIV-1 subtype B
HIV-1C	HIV-1 subtype C
IDV	Indinavir
IN	Integrase
INI	Integrase inhibitors
LMICs	Low-and middle-income countries
LPV/r	ritonavir-boosted Lopinavir
MSM	Men who have sex with men
MVC	Maraviroc
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors

LIST OF ABBREVIATIONS, CONT.

NVP	Nevirapine
PEPFAR	President's Emergency Plan for AIDS Relief
PI	Protease inhibitor
PR	Protease
PWID	People who inject drugs
RAL	Raltegravir
RNA	Ribonucleic acid
RPV	Rilpivirine
RT	Reverse transcriptase
RTV	Ritonavir
SIV	Simian immunodeficiency virus
SQV	Saquinavir mesylate
SSA	Sub-Saharan Africa
T-20	Enfuvirtide
TDF	Tenofovir disoproxil fumarate
TDR	Transmitted drug resistance
TPV	Tipranavir
UNAIDS	United Nations program on HIV-1/AIDS
URF	Unique recombinant forms
VF	Viral failure
VL	Viral load
WHO	World Health Organization
ZDV, AZT	Zidovudine, azidothymidine

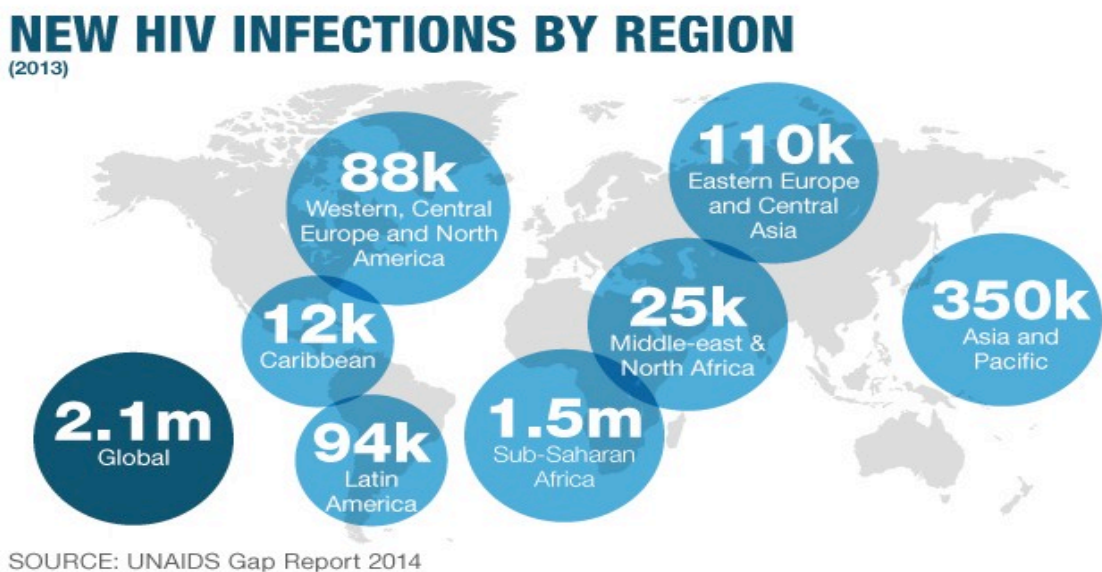
1 INTRODUCTION

1.1 THE GLOBAL HIV EPIDEMIC

Since the recognition of the human immune deficiency virus (HIV) in 1983 as the cause of the acquired immunodeficiency syndrome (AIDS), approximately 78 million individuals have become infected with HIV and around 39 million have died of AIDS [1].

The region of Sub-Saharan Africa (SSA) is the most affected (Figure 1). In 2014, almost 70% of the new HIV infections occurred in SSA. Furthermore the epidemic is still spreading rapidly in the WHO/Europe region. In 2014, the largest number ever (140,000) of newly diagnosed HIV cases was reported in the region [2]. Until today no cure for HIV-1 has been discovered. Antiretroviral therapy (ART) limits the transmission of HIV-1 [3] and reduces mortality [4, 5]. Though, patients in resource-poor settings still have increased mortality rates during the first months on ART due to late diagnosis with low CD4⁺ T-cell counts [5]. In 2014, 14.9 million HIV-1 infected patients received ART and 13.5 million were living in low-and middle-income countries (LMICs) [6]. Since the first effective combination therapy became available in 1996, new drugs with improved potency and tolerability have been developed [7]. Even if ART has changed the once deadly disease into a chronic but manageable condition, drug resistance remains a major concern in a subset of patients because of its association with poorer clinical prognosis [8, 9]. Also, it contributes to increased costs of HIV-1 care [10]. Currently over 100 drug resistance mutations (DRM), that reduce susceptibility to at least one drug have been identified in the HIV-1 genome [11]. However routine clinical testing for DRM is not feasible in LMICs where there are only a limited number of treatment options [12].

Figure 1. UNAIDS report, new infections by regions

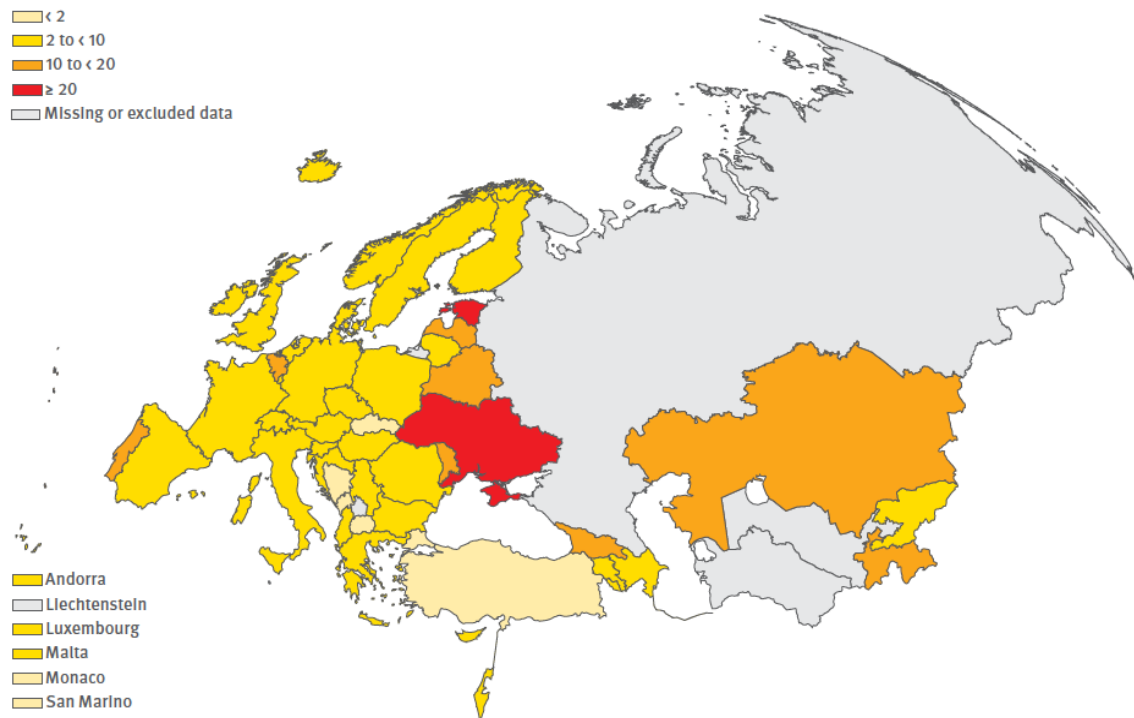


1.1.1 HIV-1 in Europe

Surveillance data from the European Center for Disease Prevention and Control (ECDC), states that despite efforts of disease prevention, the rate of new HIV-1 diagnoses has not declined considerably in the EU/EEA countries and the epidemic is increasing drastically in the WHO/Europe region (Figure 2) [2]. HIV-1 is most prevalent among the following key populations: men who have sex with men (MSM), migrants from high endemic countries (primarily SSA), and people who inject drugs (PWID). The proportion of late diagnoses is high in all countries that are reporting data on CD4⁺ T-cell count at diagnosis [13]. Patients from highly endemic countries and PWID are most often diagnosed later than other subgroups [14]. The most common subtype in European countries is HIV-1 subtype B (HIV-1B) [15-17]. However while HIV-1B remains the most common subtype among the native population, the subtype distribution among people born abroad corresponds to the country of birth. This implies that most patients that are immigrants acquired the infection in the originating country or became infected by compatriots while living in Europe [15]. Most likely the prevalence trends of non-B infection among newly diagnosed varies depending on the population risk group, migration and travel [15, 16]. Prevalence of transmitted drug resistance (TDR) to non-nucleoside reverse transcriptase inhibitors (NNRTI) is increasing, due to the low genetic barrier and frequent use of NNRTIs in first line ART regimens in Europe [18]. TDR varies depending on risk group and is presently most prevalent in MSM [19]. Unpublished data from Sweden has also shown that TDR is most prevalent within the MSM group and that the overall prevalence of TDR has increased in recent calendar years [21].

A brief report about trends of DRM in treatment-exposed patients in Western Europe revealed that trends of acquired drug resistance are declining. Factors associated with increased odds of detecting DRM were MSM, non-B subtypes, duration of ART exposure, second or higher lines of ART [20].

Figure 2 HIV-1 diagnoses per 100000 population, 2013 [13]



1.1.2 HIV-1 in Sweden

All known cases of HIV-1 must be reported according to the Swedish Infectious Diseases Act [22] and referred to one of the 30 specialist clinics in Sweden. Since 2009 all infectious disease clinics register real-time patient data in InfCare HIV, a decision support tool for daily clinical work and a national quality register [23], developed by Prof Anders Sönnernborg and Health Solutions. From the beginning of the epidemic until December 2014, a total of 10,014 HIV-1 cases and 2086 deaths had been reported in Sweden (Source: InfCare HIV-1 database [23]). A few cases have been diagnosed retrospectively from blood specimens collected as far back as 1979. Also one individual case originating from a highly endemic country and cared for at Roslagstulls Hospital, Stockholm, was retrospectively diagnosed in a sample drawn in 1976 (Sönnernborg, personal communication). Of all cases reported, 3264 (33%) were females and 6745 (67%) males (14 cases had missing information about gender). A majority 4408 (46%) had been infected through heterosexual contact, 3360 (35%) men through MSM contact, 982 (10%) cases were reported to be PWID. One hundred eighty-nine patients (1.95 %) supposedly received HIV-1 through mother to child transmission of whom 16 reported to be born in Sweden. Two hundred fifteen (2.22%) had been infected through blood contact or blood transfusion. Eight hundred sixty-one (8.6%) had other or unknown route of transmission.

The number of reported new diagnoses of HIV-1 has been at similar level for the past ten years in Sweden. Variations between calendar years are primarily due to differences in the number of reported cases among migrants and thereby depending on changes on migrant flows to Sweden from highly endemic countries [24, 25].

During 2014, 281 new cases of HIV-1 infection were reported and the majority, 167 (59%), were reported to have acquired HIV-1 in connection with a stay abroad or prior to immigration to Sweden, while 58 people (26%) had become HIV-1 infected in Sweden. Fifty-six individuals (20%) had not reported country of infection (Source: InfCare HIV-1 database [23]). Unpublished data suggests that the number of migrants infected after arrival to Sweden is likely to be high (Brännström *et al*, manuscript in preparation).

1.2 ANTIRETROVIRAL TREATMENT

Antiretroviral drugs can be classified by the phase of the HIV-1 lifecycle, which the drug inhibits. HIV-1 is an enveloped virus with two ribonucleic acid (RNA) strands in the core. The viral core also contains enzymes obligatory for the viral replication. These enzymes are: reverse transcriptase (RT), integrase (IN) and protease (PR). Presently there are four categories of antiretroviral drugs aimed at inhibiting these enzymes, and in addition combination preparations in single pill format containing two or more drugs have been developed [26]. The HIV-1 replication includes different steps; at first there is an interaction between the CD4-receptor (on the host cell) and the two glycoproteins, gp120 and gp41 in the envelope of the virus. Then the virus binds to the co-receptors CCR5 or the CXCR4, resulting in a change in the conformation allowing the cell membrane and the virus to fuse [26]. Entry or fusion inhibitors block the binding, fusion and/or entry of HIV-1 into the host cell [27].

There are two kinds of entry inhibitors: 1) CCR5 receptor antagonist blocks the viral attachment to the CD4⁺ T-cell by targeting the binding of the virus to the CCR5 -receptor on the surface of the CD4⁺ T-cell [28]; 2) Fusion inhibitors bind to gp41 and thereby block the conformational change of the virus that is needed to fuse with the host cell [26].

When HIV-1 has entered the host cell it uses the RT to reverse transcribe its RNA genome into deoxyribonucleic acid (DNA). There are two classes of RT inhibitors; nucleoside reverse transcriptase inhibitors (NRTI) and NNRTIs. The NRTI drug is competing with the naturally occurring substrates and integrates into the viral DNA strands as a false nucleotide. Thereby NRTIs inhibits the RT activity by acting as a chain terminator in the synthesis of proviral DNA

[29]. Currently there are 7 different NRTI drugs and five products containing two or more NRTIs.

The NNRTI drugs are not incorporated into the viral DNA like the NRTIs. Instead they block the RT by binding to distinct sites of the enzyme. By the NNRTI binding, RT changes the conformation and disrupts the catalytic site of the enzyme [30, 31]. There are four approved NNRTIs and two formulated single pill products containing NNRTI approved for use Sweden in the latest guidelines [32].

The enzyme integrase catalyzes the insertion of the viral DNA into the host cell genome [27]. Once the DNA of the virus is integrated in the host genome, it is replicated together with the genome of the host cell. There are three different integrase inhibitors (INI) approved for use in Sweden. The activity of the PR enzyme is essential to generate the components of the mature protein that is needed for HIV-1 to be infectious. The PR cleaves the large viral precursor polypeptides chain into efficient proteins in the final stage of the HIV-1 replication [26]. The six protease inhibitors (PI) presently registered inactivate the protease enzyme and thereby the newly synthesized virions become non-infectious [33].

1.2.1 Antiretroviral treatment in Sweden

Swedish HIV-1 treatment guidelines currently recommends 24 different antiretroviral drugs (Table 1) and several combination pills [32]. Therapy is considered for all patients regardless of CD4⁺ T-cell count. Delayed start of therapy can be considered only if the patient is not fully prepared to initiate treatment and has acceptable immune status (CD4⁺ T-cell count > 350 cells/ μ L) or if the patient has a stable CD4⁺ T-cell count of \geq 500 cells / μ L and non-detectable or very low viral levels. For previously untreated patients any of the combination regimens are recommended: **2 NRTIs + a ritonavir-boosted PI (PI/r), 2 NRTIs and 1 NNRTI, 2 NRTIs + one INI**. Since the guidelines are frequently updated, please see www.rav.nu where the latest guidelines are published [32].

Table 1. Antiretroviral drugs approved for use in Sweden and recommended in the 2014 treatment guidelines

Brand Name	Generic Name		Date of approval
Nucleoside Reverse Transcriptase Inhibitors			
Emtriva	Emtricitabine	FTC	24/10/03
Epivir	Lamivudine	3TC	15/11/01
Retrovir	Zidovudine, azidothymidine	ZDV, AZT	19/02/93
Videx	Enteric coated, didanosine	ddI	08/09/00
Viread	Tenofovir disoproxil fumarate	TDF	22/11/12
Zerit	Stavudine	d4T	08/05/96
Ziagen	Abacavir sulfate	ABC	08/07/99
Non-nucleoside Reverse Transcriptase Inhibitors			
Edurant	Rilpivirine	RPV	28/11/11
Intelence	Etravirine	ETR	06/03/13
Stocrin	Efavirenz	EFV	28/05/99
Viramune	Nevirapine	NVP	16/09/11
Protease Inhibitors			
Aptivus	Tipranavir	TPV	23/06/09
Crixivan	Indinavir	IDV	04/10/96
Invirase	Saquinavir mesylate	SQV	25/05/05
Kaletra	Lopinavir and ritonavir	LPV+RTV	27/06/06
Telzir	Fosamprenavir Calcium	fAPV	12/07/04
Norvir	Ritonavir	RTV	29/11/99
Prezista	Darunavir	DRV	24/10/12
Reyataz	Atazanavir sulfate	ATV	02/03/04
Fusion Inhibitors			
Fuzeon	Enfuvirtide	T-20	27/05/03
Entry Inhibitors - CCR5 co-receptor antagonist			
Celsentri	Maraviroc	MVC	18/09/07
Integrase inhibitors			
Isentress	Raltegravir	RAL	20/12/07
Tivicay	Dolutegravir	DTG	16/01/14
Vitekta	Elvitegravir	EVG	13/11/13

1.2.2 Monitoring of treatment effects

Plasma viral load (VL) and CD4⁺ T-cell count serve as prognostic markers of HIV-1 infection and are used in follow-up of both treated and untreated patients. The virological goal of the treatment is that HIV-1 RNA in plasma declines substantially after four weeks of treatment and to non-detectable virus levels within 3-6 months after treatment start [32]. In general a VL < 50 copies/ml and a CD4⁺ T-cell count preferably > 500 cells/ μ l are proposed as indicators of successful treatment. If treatment fails and VL levels rise to > 100 copies/ml a genotypic test should be considered to determine the presence of major DRM [34], and the level of clinically relevant resistance [35]. Despite suppressed VL some patients fail to recover the level of CD4⁺ T-cell count. CD4⁺ T-cell count serves as an important indicator of immune function and plasma VL predicts the rate of decrease of CD4⁺ T-cells in untreated patients [36]. A low CD4⁺ T-cell count at ART initiation and older age may affect immune recovery even if the patient has reached viral suppression, however the pathogenesis of immunologic failure is not fully understood [37]. Patients are followed-up continuously, untreated patients every 4-6 month and treated every 6-12 if the clinical status is stable [32]. Early linkage, retention, and an efficient continuum of care will lead both the better health for the individual and an improved public health situation since efficient treatment reduces community VL and the risk for onward transmission. Community VL is defined as an accumulated quantity of VL for a defined geographic location [38].

1.2.3 Switches of antiretroviral agents

Treatment switches occur for several reasons. Among patients with adequate treatment response the purposes of switch are e.g. to obtain lower costs, less toxic effects or due to manifest adverse effects. Although virological treatment failures are relatively uncommon, at least in Sweden and many western countries, they do occur and viral failure is still an important cause for switch. If patients have suspected side effects, the drugs could be exchanged to others within same class but with different patterns of adverse events. Patients with repeated measures of VL > 150-500 copies /mL are usually considered having treatment failure but patients with a suspected treatment failure are always individually assessed. Before any change of therapy is done, several factors are taken into account such as treatment history, any earlier genotypic resistance tests, adherence, the routines of medicine intake, possible drug interactions, diets and other potential causes of impaired absorption.

In **Paper V** we compare the second-line treatment response between patients switching therapy despite adequate first-line therapy response, patients with viral failure and acquired drug resistance, and patients with viral failure without detectable DRM.

1.2.4 Antiretroviral treatment strategies in low- and middle-income countries

General access to ART first increased in 2001 when the Doha declaration was adopted. Shortly thereafter two large funds investing in health programs were launched; US President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund to fight AIDS, Tuberculosis and Malaria (GFATM).

In 2003, the World Health Organization (WHO) initiated their 3 by 5 initiative aiming to provide 3 million HIV-1 infected patients with ART by 2005, and in 2007 the goal was met [39]. In 2014, WHO reported that there are 13.5 million HIV-1 infected receiving ART in LMICs [6]. Individualized patient management with all available ART, routine monitoring of biomarkers by high technological laboratories and specialized doctors are however scarce in LMICs [12, 40, 41]. Therefore WHO initiated a public health approach to increase access to ART for the HIV-1 infected patients [12]. Standardized first and second line ART are used: the first line choice is 2 NRTIs + 1 NNRTI and PIs are reserved for second line. The public health approach was first initiated in 2001 and at the first implementation of the approach VL monitoring was not required. In the 2013 WHO guidelines, VL was recommended to be measured for the first time, initially every 6-month and thereafter every 12-month. Current WHO guidelines recommend ART initiation among all HIV-1 infected adults, children and adolescents regardless of clinical status and CD4⁺ T- cell count [42]. In 2014, United Nations Program on HIV/AIDS (UNAIDS) launched the goal 90-90-90 by 2020, with the aim to diagnose 90% of the HIV-1 infected populations, have 90% of them on ART and have 90% of those treated virally suppressed [43]. According to a recent meta-analysis of virological outcomes of first-line ART in LMICs, VL suppression during the first 5 years of ART was high but rates declined thereafter due to loss to follow-up, deaths or interruption of ART. Furthermore uptake of second-line regimen was low in general [44].

The NNRTIs efavirenz and nevirapine are presently the cornerstones of ART in LMICs together with 2 NRTI, frequently TDF and 3TC. In high-income countries, however, a second generation NNRTI, rilpivirine, with an improved safety and tolerability profile as compared to efavirenz and nevirapine, has become a frequently used drug. However the feasibility of usage in LMICs is under debate [45].

In **Paper IV** we therefore examined both clinical and biochemical findings of this particular drug to evaluate the usefulness of RPV in LMICs where HIV-1C dominates the epidemic.

1.3 HIV-1 DRUG RESISTANCE

Drug resistance remains a problem even if new drugs are developed. The error prone RT [46], together with the high replication rate [47] of the virus promotes development of DRM if the replication is not inhibited completely with efficient ART [48].

The viral population within infected patients consists of a swarm of different genetic variants, so called viral quasispecies [49]. Within this heterogeneous virus pool, the quasispecies that are best adapted to the environment dominate. When the host environment changes for example during incomplete drug pressure, quasispecies with mutations that reduce susceptibility to ART will have an advantage and with time outgrow the wild type population [50-52]. However, usually the replicative capacity of the mutated quasispecies is not as effective as that of the wild type virus. Therefore the wild type variants predominate once drugs are no longer in the environment [53-55]. DRM with little impact on viral fitness can sustain for longer time and are more prone to transmission between individuals [56, 57].

How fast and easy DRM develop is depending on several factors of which the so-called genetic barrier of the drug is very important. All boosted PIs have a high genetic barrier and mutations are slow to develop despite ongoing replication during treatment failure. Also, this category of drugs requires several mutations to induce clinical relevant drug resistance [58, 59]. Some NRTIs (3TC, FTC) and NNRTIs (efavirenz, nevirapine) have a low genetic barrier and only a single mutation is required for emergence of drug resistance [60, 61], while DRM develop somewhat slower to other NRTI (AZT, ABC, ddI, TDF) or NNRTI (RPV, etravirine). For integrase inhibitors resistance develops quickly to the first generation INI (raltegravir, elvitegravir) but resistance develops very slowly, or not at all, towards the second generation INI dolutegravir. Cross-resistance can also occur within drug classes [62].

1.3.1 NRTI resistance

There are two main mechanisms for NRTI resistance; one is modification of the RT enzyme allowing it to discriminate between NRTIs and analog substrates, leading to a reduced incorporation of the NRTIs into the DNA chain. The other mechanism is eliminating the NRTI incorporated into the viral DNA [63, 64]. The point mutations, K65R, L74V, Q151M and M184V, are typical examples of discriminatory DRM [35]. The DRM leading to elimination of

NRTIs is known as thymidine analogue mutations (TAMs). Examples are M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E, and they include resistance to all NRTIs except lamivudine (3TC) [35].

There are also distinctive TAM pathways, which induce greater NRTI resistance. For example the appearance of M41L and L201W can predict later appearance of point mutations at codons 41, 67, 210, and 215. A second TAM pathway is derived from the K70R and K219Q/E/N that predicts development of mutations 67, 70, and 219 [35].

1.3.2 NNRTI resistance

NNRTIs bind to HIV-1 RT and NNRTI mutations are located in the enzyme pocket where NNRTIs bind and thereby reducing the affinity of the NNRTIs to the enzyme [65, 66]. If the HIV-1 replication is not completely suppressed or if the NNRTI is used as monotherapy, drug resistance emerges fast. The rapid emergence of DRM is due to selection of pre-existing mutant viruses within an individual [67-70]. Studies have shown that NVP used as single dose therapy in mother-to-child prevention in LMICs can select for NNRTI DRMs [69, 71]. Examples of NNRTI mutations are L100I, K101EP, K103NS, V106AM, E138AGKQ and Y181CIV [35]. Currently two second-generation NNRTIs also exist, rilpivirine (RPV) and etravirine (ETR). RPV has a reduced side-effect profile compared with the older NNRTIs [72]. Also, RPV and ETR have a higher genetic barrier compared to first generation NNRTIs [73]. Common DRM associated with RVP and ETR are: L100I, E138AGKQ, Y181CIV, Y188L, G190ASE and M230L [35].

1.3.3 Resistance to protease inhibitors

The HIV-1 enzyme PR is responsible for the maturation of the infectious HIV-1 particles, by cleavage of the two precursor proteins Gag and GagPol [74-76]. PIs prevent cleavage of the two proteins by binding to the active site of the PR. Thereby immature non-infectious virus particles are produced. Primary mutations are positioned at the substrate-binding cleft of the PR and interfere with the binding of the PI to the PR [34, 77-80].

Primary mutations alone may have little impact on resistance to PIs and they frequently cause decrease fitness of the virus. However when patients continue their therapy secondary mutations emerge that together with the primary mutations lead to high-level resistance and increased fitness [81-83]. Examples of PI mutations are: D30N, V32I, L33F, M46IL, I47AV [34].

1.3.4 Resistance to entry inhibitors

The resistance pathways differ markedly between entry inhibitors and other antiretroviral drugs. The fusion inhibitors block the gp41-mediated fusion of the HIV-1 and CD4⁺ T-cell membrane and the co-receptor inhibitors block the binding of the gp120 to the co-receptors CCR5 or CXCR4 [26, 27]. The key mechanism of resistance to fusion inhibitors are changes in a domain involving 10 amino acids between position 36 and 45 in the HR1 region of gp41 [84]. For the co-receptor inhibitors, a shift in co-receptor usage or multiple changes in different gp120 domains (V3, C2, C4 and V4) appear to be responsible for causing drug resistance [85].

1.3.5 Resistance to integrase inhibitors (INI)

For the first generation INI (raltegravir, elvitegravir), resistance develops rapidly at viral failure. Specific primary INI DRM develop which are followed by secondary mutations that further decrease the susceptibility and/or increase the viral fitness [86-89]. Examples of INI resistance mutations are T66AIK, E92Q, E138KA, and G140SA [35]. For the second generation INI, dolutegravir, resistance has been described *in vitro* but the barrier to resistance *in vivo* seems to be very high and very few patients have developed this mutation *in vivo* [90].

1.4 HIV-1 DRUG RESISTANCE IN SWEDEN

The first description ever of transmitted resistance to antiretroviral drugs was based on data from Sweden in which transmission of resistance to AZT was described [91]. After this novel publication articles have been published about transmitted and acquired drug resistance in Sweden [92, 93]. One study analyzed the trends of transmitted drug resistance between 2003 and 2010 and included 44% (N=1463) of all patients diagnosed in Sweden during this time period. A prevalence of 5.6% (95% CI: 4.5%–6.9%) was found without any significant time variations [94]. **Paper I** was the first description of acquired drug resistance and described the DRM patterns among Swedish HIV-1 treatment experienced patients between 1997–2011 [93].

1.5 HIV-1 DRUG RESISTANCE IN LOW AND MIDDLE INCOME COUNTRIES

The drawback of acquired and transmitted drug resistance was originally limited to resource-rich countries. Since scale up of ART, the problem has expanded to LMICs countries. In resource limited countries drug resistance measurement is not available for routine clinical practice. Instead sentinel sites have been used to monitor both acquired and transmitted drug resistance at population level [95]. A recent global meta-analysis among 50, 870 individuals from 111 countries, found that the odds of transmitted drug resistance in SSA were increasing due to the national scale-up of ARV with an increase of transmitted NNRTI resistance. NNRTI-associated TDR also have increased in Latin America/Caribbean, North America and upper-income Asian countries [96].

WHO studies of acquired HIV-1 drug resistance in LMICs from 2007-2010 showed an increase in prevalence from 4.8% (95% CI 3.8%–6.0%) in 2007 to 6.8% (95% CI 4.8%–9.0%) in 2010. Around 10-30% of the patients have viral failure per year. Because of the low genetic barrier around 70% of the patients with viral failure on NNRTI have NRTI/ NNRTI drug resistance mutations [97-99]. The lack of routine VL monitoring in clinical practice can result in long periods of virological failure before changing treatment [100]. Because of migration and travel, increase of both acquired and transmitted drug resistance in LMICs can potentially affect ART effectiveness globally.

1.6 HIV-1 SUBTYPES

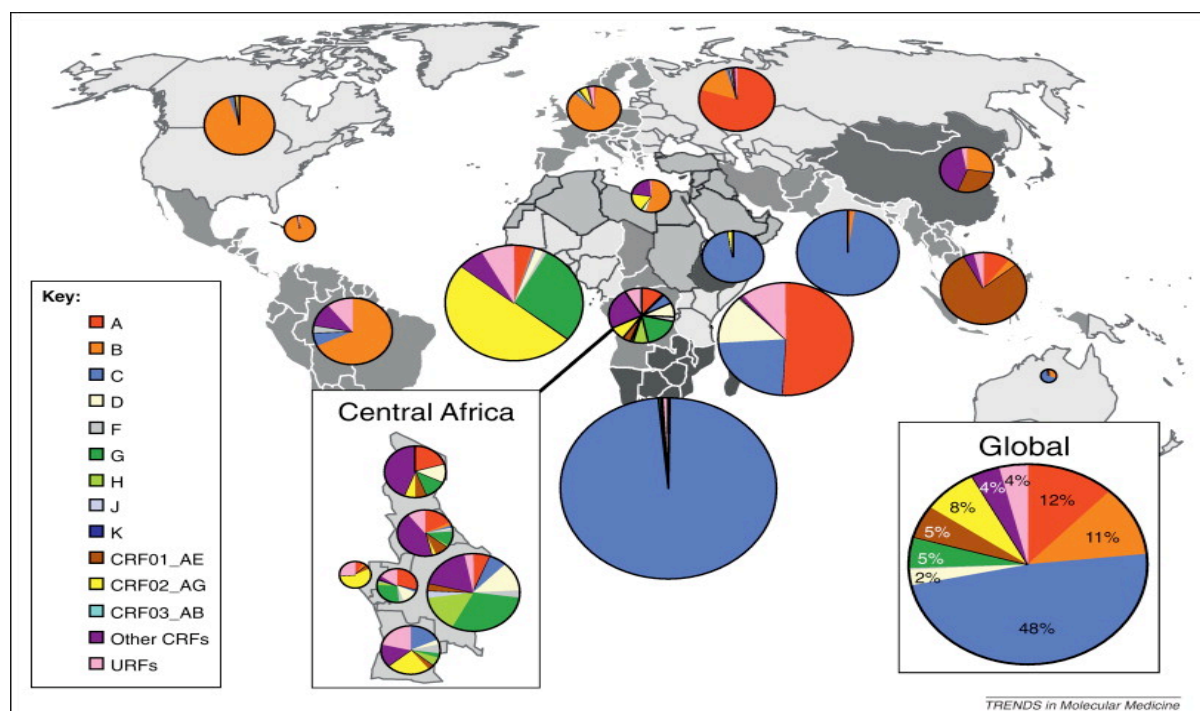
Several zoonotic transmissions from primates to humans have resulted in HIV-1 and HIV-2 and different HIV-1 lineages. HIV-1 is divided into four groups M, N, O, P and HIV-2 in groups A-H. The extreme variability of the virus does not only enhance the development of DRM but has also led to development of numerous HIV-1 subtypes and recombinants between them. HIV-1 group M (Major) is the main cause of the global pandemic [101] and has diversified into further genetic strains. At present HIV-1 group M is classified into nine subtypes (A, B, C, D, F, G, H, J, K) [102], and 70 circulating recombinant forms (CRF) (<http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html>). Three further groups of HIV-1 (O, N and P) exist, which is a result of several cross-species transmissions of simian immunodeficiency viruses (SIVs) [16]. Within the different subtypes there can be variations of up to 43% on the nucleotide level, depending on the regions examined and the subtypes. Full genome sequencing has also shown that combinations between subtypes are common. Recombinant virus between different HIV-1 M subtypes are called circulating recombinant

forms (CRF), if found in three or more individuals with different sources of the HIV-1M infection. If not found in three or more individuals they are named unique recombinant forms (URF). Subtypes originating from specific regions are distributed with distinct geographical patterns (Figure 3). HIV-1 diversity may have an impact on disease progression [103, 104], transmission [105], drug resistance development [106-108], diagnosis, VL monitoring [109], immune response and vaccine development [110-112]. In **Papers III and IV** we have analyzed in detail the effect of differences between HIV-1B and HIV-1C response to ART.

1.6.1 Global HIV-1 molecular epidemiology and subtype distribution

At the population level, mobility and migration to other geographical regions and transmission between risk groups result in a constant change and establishment of new sub-epidemics and recombinant forms of HIV-1. In European countries, HIV-1B is still the most common, however multiple strains co-circulate and non-B subtypes are no longer restricted to persons from endemic areas and certain risk groups [16, 113, 114]. See Figure 3 for the global distribution of HIV-1 subtypes. In **Paper II**, we analyze the molecular trends of subtype distribution in Sweden.

Figure 3. Regional distribution of HIV-1 subtypes and recombinants



Pie charts represent the geographical distribution of HIV-1 subtypes and recombinants between 2000–2003 and 2004–2007. Size of the pie chart represents the relative number of HIV-1 infected living in the region. The colors representing the different HIV-1 subtypes are indicated in the legend on the left-hand side of the figure. The subtype distributions found around the world and within central African countries are shown separately inside the figure. Source [102] with permission.

1.6.2 Impact of HIV-1 subtype M diversity on therapy effectiveness

Though non-B subtypes dominate the global HIV-1 epidemic, almost all studies on HIV-1 drug resistance, drug susceptibility and knowledge of changes in the protease and RT regions have been performed on HIV-1B from high income countries [115]. Previous studies have reported differences in the genetic barrier [116, 117], phenotypic characteristics [107] and different frequencies of typical DRM between HIV-1 subtypes [114]. Although cohort studies have shown similar outcomes between subtypes, these often compare treatment outcomes between HIV-1B versus non-B, grouping non-B together [103, 118, 119]. However one clinical trial has shown that HIV-1C was linked with increased risk of viral failure [120]. Pooling non-B subtypes as a unique genetic entity should be avoided since they are as distinct from each other as they are from HIV-1B [121]. Variations in the *pol*-gene between subtypes affect the phenotypic characteristics of the PR and RT enzymes, phenotypic susceptibility to antiretroviral drugs, and evolution of subtype-specific genotypic patterns of drug resistance [107, 122]. The viral protease is the target for protease inhibitors [123] and they are currently the second line therapy for LMICs where HIV-1C is predominant [102, 124]. Drug resistance differences among subtypes can probably be explained by the enzymatic efficiency of PR and RT [107]. DRM can cause increased or decreased catalytic activity, or affect inhibition or stability of the PR [125-129]. Both the high mutation rate within the HIV-1 PR and polymorphisms among different subtypes challenge the efficacy of PIs [107]. The protease-positions 20, 36, 63, 82 and 93 can affect PI treatment and there are several different studies that have reported higher frequencies of those polymorphisms among non-subtype B [130-132]. To analyze altered enzymatic characteristics on a molecular level of resistant phenotypes, crystal structures are needed [133]. The protease is a dimer and each monomer consists of 99 amino acids [134]. The flap is a flexible structure in the protease [135] where the protease inhibitors bind. Close to the flap is the hinge region, which is associated with movement and stability of the flap. The flap unit has to be flexible and undertake movements to let substrate that should be catalyzed (and the PI) to enter and bind. Therefore it is important for the flaps to be flexible, however the probable consequences of too much flexibility are reduced substrate flexibility and decreased binding of a protease inhibitor [136, 137]. To analyze altered enzymatic characteristics on a molecular level of resistant phenotypes crystal structures are needed [133]. The consensus sequences for different subtypes are found in the Los Alamos HIV-1 sequence database, <http://www.hiv.lanl.gov/>. The consensus HIV-1C PR has been compared to the consensus HIV-1B PR and polymorphisms that cause dynamic differences have been found [138]. The

consensus HIV-1C PR has increased flap flexibility and it is likely that this contributes to an increased level of drug resistance[139, 140]. As PIs are the second line choice in LMICs, there are an increasing number of patients with non-subtype B infection with access to PI drugs, more knowledge about drug resistance and treatment response is needed. There are several mutation lists for epidemiological surveillance of drug resistance however these lists are based on data available for subtype HIV1-B [141-144], algorithms to interpret DRM need to be updated constantly [115]. In **Paper III** we have performed molecular docking analyses to test the hypothesis that HIV-1C has a lower binding affinity to PIs compared to HIV-1B. Furthermore we have analyzed the clinical long-term impact of PI treatment response differences between the subtypes. In **Paper IV** we used patient-derived HIV-1C RT and performed inhibition and binding affinity assays with RPV.

2 AIMS AND OBJECTIVES

General aim

To describe any differences between categories of HIV-1 patients and viral subtypes with regard to disease progression, treatment outcome and drug resistance development, as well as analyze predictors of these outcomes, using the Swedish InfCare HIV-1 cohort.

Specific aims

1. *To describe the antiretroviral usage and drug resistance development of all treatment experienced patients in Sweden, 1997–2011:* The purpose was to assess the development leading up to the situation in 2011 and to identify possible challenges in the coming years.
2. *To describe the detailed changes and temporal trends of HIV-1 subtypes and recombinant forms in Sweden since the start of the epidemic:* Information suggested that the epidemic had shifted from a mono-phylogenetic HIV-1B epidemic to a more diverse pattern over time; therefore we aimed at describing this and to predict the pattern for the coming years.
3. *To identify any significant characteristics of HIV-1C with regard to response to protease inhibitor therapy:* We aimed at analyzing the virological and immunological responses to PIs in patients with HIV-1C and HIV-1B. Furthermore, we investigated structural differences relating to these differences and estimated binding energy between HIV-1C and HIV-1B PR.
4. *To identify any significant characteristics of HIV-1C with regard to response to the NNRTI rilpivirine:* We aimed at describing any subtype specific DRM patterns to RPV and to acquire a detailed understanding about the efficacy of RPV on patient-derived HIV-1C versus HIV-1B RT.
5. *To evaluate the consequences of first-line ART failure on the outcome of second-line ART over a period of 15 years, 1999-2014:* We aimed to compare the second-line treatment outcome between different reasons for first-line therapy switch. E.g first line-therapy switch with and without detectable viral load and first line failure with detectable DRM.

3 MATERIALS AND METHODS

Following is a short summary of materials and methods used in the articles/manuscripts constituting the Thesis. For details about study methods and statistical analyses for each study, please see the respective paper.

3.1 INFCARE HIV

In all papers, the database InfCare HIV was used for selection of patients. InfCare HIV is a national HIV database that includes >99.9% of all diagnosed HIV infected patients in Sweden. It has been used since 2003 at Karolinska University Hospital, Östra/Sahlgrenska Hospital, Gothenburg, and South Hospital, Stockholm. Since January 2009 all HIV clinics in Sweden are included. InfCare HIV is run by a steering committee (present chair: Prof Anders Sönnernborg) consisting of representatives from the larger HIV clinics, smaller clinics and from the Swedish Association of Infectious Disease Physicians. The health informatic company Health Solutions AB is responsible for the technical development.

Data since the beginning of the HIV-1 epidemic up to 2003/2009 have been entered retrospectively. Thereafter all data have been entered prospectively. InfCare HIV creates real-time reports at cohort level as well as at clinic and patient levels. Data are entered in the daily work with patients and the data quality is very high. Viral RNA levels, viral sequences, CD8⁺ and CD4⁺ T-cell counts (absolute and percentage) are transferred electronically directly from the laboratories. InfCare HIV contains four modules: decision support, quality assurance, consulting support and a research database. It is the national HIV quality assurance registry since 2004 (present chair: Dr Veronica Svedhem-Johansson) with the highest certification level no 1, confirming the high quality of the registry (<http://kvalitetsregister.se/hittaregister/registerarkiv/hiv.191.html>). The database is connected to an operational MsAccess and is updated every night. The data is anonymised and structured in a relational database that enables advanced analyses. All research is conducted in accordance to ethics committee guidelines and approval.

Data for all studies in the thesis was downloaded from InfCare HIV at different occasions and different inclusion criteria were used for each study depending on the study purpose.

3.2 VIRAL LOAD MONITORING

Different real time PCR HIV RNA quantification tests have been used for VL monitoring over time: In the year 1994 NASBA® (Organon Teknika) was used, then in year 1997 Roche® Amplicor HIV-1 Monitor Test and v 1,5 came in 1998. From 1999 COBAS® AmpliPrep sample preparation system was used, followed by COBAS® Amplicor HIV-1 monitor version 1.5 in year 2002 and Cobas TaqMan® HIV-1 v1.0 in year 2007, and since 2010 v2.0 (Roche Molecular Systems, Basel, Switzerland). Between 1996-1999 the lower detection level of VL was <500 copies/mL, between 1999-2010 the lower detection level was <50 copies/mL, and after 2010 the lower detection limit was <20 copies/ml.

3.3 CD4⁺ T-CELL COUNTS

CD4⁺ T-cell counts were measured using routine flow cytometry.

3.4 HIV DRUG RESISTANCE TESTING

Population based sequences of the *pol* gene including regions encoding RT, protease, and integrase, were generated with ViroSeq™ HIV-1 Genotyping System (Abbott, US) and entered into the InfCare HIV database. Major DRM were defined according to the IAS-USA list and the level of clinically relevant resistance for each drug was determined using the Stanford HIV db algorithm. At the time point of the study the current available IAS-USA list and Stanford HIVdb algorithm were used.

3.5 HIV-1 SUBTYPING

For HIV-1 subtyping, different methods were used:

- i) REGA subtyping tool version 3 (REGA v3), which uses an improved decision-tree algorithm and has greater sensitivity for identification of pure subtypes and recombinants [145]. The URFs were further characterized using bootscan analysis incorporated in the Rega v3 tool and validated in the SimPlot v3 software [146].
- ii) COMET (<http://comet.retrovirology.lu/>), which uses context based modelling for expeditious typing of HIV-1 viruses. Both the tools showed best performances for pure subtypes and CRFs [145].

- iii) Recombinant identification program version 3 (RIP 3.0) with 200 nt window size, to determine recombinations of shorter sequences (700 nt) [<http://www.hiv.lanl.gov/content/sequence/RIP/RIPexplain.html>].
- iv) HIV-1 BLAST, available in the HIV Los Alamos Database [http://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html] was used to identify the nearest HIV-1 sequences from different geographical proximity.

3.6 MOLECULAR MODELLING AND BINDING KINETICS

In **Paper III**, molecular modelling of PRs and docking of PIs was used. The details are described in the method section of the paper.

In **Paper IV**, rilpivirine inhibition and binding kinetics assay was performed [147], additionally molecular modelling was carried out as in **Paper III**.

4 RESULTS AND DISCUSSION

In this thesis the InfCare HIV database was downloaded at three different time points to provide the most updated versions. A database downloaded in January 2011 was used in **Paper I**, January 2012 was used in **Papers II** and **IV**, and January 2014 was used in **Papers III** and **V**. The comprehensive result was presented in each of the articles or manuscripts. Here I summarize the major findings.

4.1 Demographic changes in patients characteristics between 1997–2011

In **Paper I** we included all ART-experienced patients (N=6537, 75% of all patients). Several demographic changes have occurred over time: the proportions of MSM and PWID have decreased, while the proportions of women and heterosexually infected patients have increased. The proportion of patients born and infected abroad has also increased, with large patient groups coming from SSA and South-East Asia. The increase of patients born abroad has resulted in a raised number of non-B subtypes, especially subtype C and CRF01_AE.

4.2 Trends in antiretroviral therapy usage in Sweden 1997–2011

Treatment options and clinical guidelines have improved over time and we summarized the major changes in **Paper I**. Between 1987 and 1996 most patients were given monotherapy or dual therapy, and in the following five-year period a greater diversity was seen; a total of 110 different combinations were prescribed as first-line therapy in the period 1997–2001; most common were 2 NRTIs (3TC+ZDV or d4T) plus an unboosted PI (IDV or NFV), but ZDV monotherapy was still initiated in 5.9% of all patients. During the next period, 2002–2006, the NRTI backbone 3TC+ZDV was increasing to 66.2%, and was combined with boosted LPV or EFV or NVP. In the last five-year period (2007–2011), the most common NRTI backbones were FTC+TDF (51.1%) and 3TC+ABC (30.7%), combined with EFV or LPV/r or ATV/r (Table 2).

Table 2 The 10 most common first line ART regimens in Sweden during different periods of time

	Group I: 1987-1996, N=1741	Group II: 1997-2001, N=1181	Group III: 2002-2006, N=1434	Group IV: 2007-2011, N=2181
1	ZDV (67.4%)	3TC, ZDV, IDV (17.9%)	3TC, ZDV, LPV/r (29.1%)	FTC, TDF, EFV (26.9%)
2	ZDV, ddI (8.8%)	3TC, ZDV, NFV (10.8%)	3TC, ZDV, EFV (14.6%)	3TC, ABC, EFV (11.8%)
3	3TC, ZDV, IDV (7.8%)	3TC, d4T, NFV (8.5%)	3TC, ZDV, NFV (5.8%)	3TC, ZDV, LPV/r (9.6%)
4	3TC, ZDV (6.9%)	3TC, ZDV (7.8%)	3TC, ZDV, NVP (4.0%)	FTC, TDF, ATV/r (9.1%)
5	ddI (3.1%)	3TC, ZDV, EFV (6.3%)	3TC, ABC, ZDV (3.2%)	3TC, ABC, LPV/r (8.3%)
6	ZDV, ddI (0.7%)	ZDV (5.9%)	3TC, TDF, EFV (3.0%)	FTC, TDF, LPV/r (7.8%)
7	ABC (0.7%)	3TC, d4T, IDV(4.5%)	FTC, TDF, EFV (2.8%)	3TC, ABC, ATV/r (7.3%)
8	3TC, ZDV, RTV (0.5%)	3TC, ZDV, LPV/r (2.8%)	3TC, TDF, ATV/r (2.7%)	FTC, TDF, DRV/r (4.0%)
9	3TC (0.5%)	3TC, ZDV, IDV/r (2.5%)	3TC, d4T, NVP (2.6%)	3TC, ZDV, EFV (1.7%)
10	3TC, d4T (0.4%)	3TC, ABC, ZDV (1.9%)	3TC, ABC, LPV/r (2.1%)	3TC, ZDV, NVP (1.6%)

Describes the percentages (%) of all first line ART prescribed during the periods: 1987-1996, 1997-2001, 2002-2006, and 2007-2011.

4.3 Prevalence of major drug resistance mutations over time

The number of patients on ART has increased from N= 1546 in 1997 to N=5272 in 2011. Even though a genotypic resistance test (GRT) is recommended in the clinical guidelines the number of GRTs at treatment failure has not increased over time. When the DRM results were categorized by year of first ART initiation it was found that NNRTI DRM have increased among patients initiating their first ART between the years 2007–2011. The increasing number of patients can partly explain this. Nevertheless this continuous development of DRMs among patients starting ART since 2007 shows that NNRTI differs from the other drug classes used until 2011, since the reverse declining pattern of DRM was seen for the other drug classes. Full-class resistance, defined as intermediate or high-level resistance to all available drugs in a class, was initially high, over 15% of GRTs until 2001, but declined in 2002 and has leveled off to around 1.0–1.5% of patients with GRTs between 2009 and 2011 (Table 3).

Table 3. Total numbers of patients on ART, with GRT and major DRMs per year 1997-2011

Year	Patients on ART	Patients with GRT	≥1 major DRM to any class	NRTI DRM	PI DRM	NNRTI DRM	2 class DRM	3 class DRM	FI or II DRM
1997	1546	219	150 (9.70%)	145 (9.38%)	38 (2.46%)	11 (0.71%)	41 (2.65%)	4 (0.26%)	
1998	1714	202	137 (7.99%)	130 (7.58%)	48 (2.80%)	14 (0.82%)	48 (2.80%)	8 (0.47%)	
1999	1835	181	97 (5.29%)	86 (4.69%)	44 (2.40%)	9 (0.49%)	39 (2.13%)	6 (0.33%)	
2000	1990	206	121 (6.08%)	109 (5.48%)	54 (2.71%)	30 (1.51%)	62 (3.12%)	14 (0.70%)	
2001	2103	229	140 (6.66%)	129 (6.13%)	77 (3.66%)	49 (2.33%)	87 (4.14%)	31 (1.47%)	
2002	2197	195	126 (5.74%)	114 (5.19%)	66 (3.00%)	42 (1.91%)	73 (3.32%)	23 (1.05%)	
2003	2388	220	147 (6.16%)	134 (5.61%)	55 (2.30%)	61 (2.55%)	84 (3.52%)	19 (0.80%)	
2004	2643	154	105 (3.97%)	90 (3.41%)	42 (1.59%)	58 (2.19%)	66 (2.50%)	20 (0.76%)	1 (0.038%)
2005	2951	173	98 (3.32%)	78 (2.64%)	41 (1.39%)	44 (1.49%)	49 (1.66%)	18 (0.61%)	2 (0.068%)
2006	3257	142	84 (2.58%)	67 (2.06%)	30 (0.92%)	38 (1.17%)	39 (1.20%)	13 (0.40%)	0 (0.000%)
2007	3638	155	65 (1.79%)	57 (1.57%)	27 (0.74%)	22 (0.60%)	35 (0.96%)	7 (0.19%)	0 (0.000%)
2008	4077	156	62 (1.52%)	53 (1.30%)	22 (0.54%)	26 (0.64%)	32 (0.78%)	7 (0.17%)	1 (0.025%)
2009	4526	154	71 (1.57%)	54 (1.19%)	18 (0.40%)	27 (0.60%)	25 (0.55%)	4 (0.09%)	2 (0.044%)
2010	4956	193	78 (1.57%)	60 (1.21%)	17 (0.34%)	38 (0.77%)	31 (0.63%)	6 (0.12%)	2 (0.040%)
2011	5272	216	85 (1.61%)	56 (1.06%)	18 (0.34%)	45 (0.85%)	33 (0.63%)	3 (0.06%)	4 (0.076%)

The prevalence of patients with different types of DRMs is in relation to the all the patients on treatment that year.

The **highest prevalence** for each type of DRM is marked in **regular bold script**, while the *lowest prevalence is in bold italics*.

4.4 Highly diverse HIV-1 epidemic in Sweden

In **Paper II**, we showed that all eleven pure subtypes and sub-subtypes (A1/A2, B, C, D, F1/F2, G, H, J and K) within HIV-1 group M (3107/3967; 78%), 17 CRFs (757/3967; 19%), and 32 URFs (103/3967; 3%) were present in Swedish HIV-1 epidemic. Among the subtypes, HIV-1B dominated (47%) followed by HIV-1C (18%) and CRF01_AE (12%). HIV-1B dominated in MSM (91%) and PWID (66%). Among the PWID, 27% were infected with CRF01_AE. A diverse pattern was seen in heterosexually infected patients among whom HIV-1C dominated (31%). Among the cities Stockholm (Huddinge, South Hospital, Solna) represented the maximum diversity.

4.5 Significant increase in HIV-1 non-B subtypes in Sweden over three decades

There was a significant increase of newly diagnosed HIV-1C, recombinants, and other pure subtypes over time compared to HIV-1B (set as reference) ($p < 0.01$), both among migrants and patients infected in Sweden (Figure 2 in **Paper II**). The multinomial regression model was adjusted for year of diagnosis tested for non-linearity however it was not significant. By extending the graph, the model predicted an excess of recombinant forms by 2015. As there was a significant increase of non-B subtypes in Sweden over time, we decided to analyze in **Paper III** and **Paper IV** whether there was any difference in therapy response among the two predominant HIV-1 subtypes, i.e. HIV-1B and HIV-1C, with regard to one of the two most preferred NNRTIs, rilpivirine, and to protease inhibitors.

4.6 HIV-1C infected patients and patients initiated with PI/r based regimen have higher risk of viral failure

In **Paper III**, we looked into the use of PI/r based regimens in HIV-1C infected patients and factors affecting the efficacy. Both primary (if a regimen failed to suppress the VL within nine months of ART initiation i.e. VL > 500 copies/mL between 1996-1998 or VL > 50 copies/mL between 1999-2015) and secondary viral failure (VF) (if one VL was > 500 copies/mL or two consecutive VL were > 50 copies/mL after nine months on ART) were increased in the HIV-1C patients ($p = 0.04$; $p < 0.001$, respectively) compared to HIV-1B. Pre-therapy higher VL, HIV-1C infection (Odds ratio, OR: 1.66; $p = 0.03$) and PI/r-based regimen (OR: 1.63; $p = 0.001$) showed increased risk of primary VF without having any difference in odds in missing doses of therapy (adherence). HIV-1C individuals, who were given second line PI/r therapy, had a significantly higher hazard ratio (HR) compared to HIV-1B subjects (adjusted HR 1.92, $p = 0.002$) for time-to-secondary VF. Further, molecular modeling and induced-fit-docking suggested lower affinity for PIs to HIV-1C protease than for HIV-1B.

Our findings suggest an increased risk of VF in HIV-1C patients on PI/r-based regimens, despite specialist physician management and modern laboratory monitoring, which may limit the utmost efficacy of PI/r in LMICs as standardized second-line drugs.

4.7 Rilpivirine may have limitations in HIV-1C -dominated epidemics in LMICs

In **Paper IV**, we looked into the potential use of RPV; a second generation NNRTI, in HIV-1C infected patients and factors affecting its efficacy. In this study, we pooled data from the Swedish InfCare cohort together with data from a large European network (EuResist) as well as from two cohorts from the LMICs India and Ethiopia. We also used biochemical data and molecular modeling to find out the optimal efficacy of RPV in HIV-1C.

Our clinical and biochemical findings indicate that the usefulness of RPV has limitations in HIV-1C-dominated epidemics in LMICs. Primary RPV resistance was rare, but the proportion of patients with >100,000 HIV-1 RNA copies/mL pre-ART was high in patients from India and Ethiopia, limiting the usefulness of RPV as a first-line drug in LMICs. *In vitro* inhibition assays showed ~2-fold higher RPV IC₅₀ for HIV-1C RT than HIV-1B RT. Pre-steady-state determination of RPV-binding affinities revealed 3.7-fold lower RPV binding to HIV-1C than HIV-1B RT. Structural analysis indicated that naturally occurring polymorphisms close to the NNRTI-binding pocket might reduce RPV binding, leading to lower susceptibility of HIV-1C to RPV. However, in patients failing first-line NNRTI treatments, cross-resistance patterns suggested that 73% of the patients could benefit from switching to RPV-based therapy.

4.8 Effect of therapy switch and viral load level on second line ART outcomes

In **Paper V**, we found that the frequencies of DRM and the level of VL were low at first line treatment failure, and that treatment modifications were commonly carried out due to other reasons. Out of the 869 patients included in our study, 495 (57.0%) switched to second-line ART without a VF, 250 (28.8%) switched with a VF without DRM, and 124 (14.2%) switched with a VF and PI, NNRTI and/or NRTI DRM. Patients switching from first-line to second-line ART with virologic failure but without any DRM or with virologic failure and DRM, showed a significantly negative difference in time to second-line VF in all studied survival percentiles compared with patients switching without failure.

For example, the first 50% (median) of virologic failures occurred within 4.53 years of second-line ART among patients who switched without failure (Reference group) and within 3.43 years (1.1 year before) among patients who switched with a DRM. We also analyzed the effect of VL level at first line treatment failure on time to second line treatment-failure. The patients initiating second-line ART with VL 201-500, 501-1,000, 1,001-10,000, 10,001-100,000 and >100,000 copies/mL, respectively, showed a significantly negative difference in time to second line VF in the 30th, 40th and median survival percentile compared with patients who initiated

second-line therapy with a VL between 0 and 200 copies/mL. However an important finding in this study was that once viral failure occurs and DRM are detected, treatment failure is managed in an efficient way in modern clinical practice because type of switch and VL at second-line ART initiation did not show any significant effect on median CD4⁺ T-cell counts at 12 and 24 months, time to AIDS or death. This result has also implications for several LMICs that do not use VL monitoring because it shows the importance of VL monitoring in countries where its use is not part of standard-of-care. If VL monitoring implemented, the treatment switch can efficiently being monitored, which will increase the health status of HIV-1 infected patients in LMICs.

5 CONCLUSIONS

This thesis provides an overview of the HIV-1 epidemic and antiretroviral treatment outcomes in Sweden, including the changes of ART usage, DRM patterns and subtype distribution over time. It reveals how changes in Sweden are influenced by travel and migration and the country is thereby a part of the global epidemic. Although our studies are performed mostly on data from Sweden, our results also have implications for low and middle-income countries and several conclusions can be drawn:

1. NNRTI resistance increased after 2007 and was primarily found among patients infected in LMICs who had initiated ART in recent years. This increase may partly be a result of undetected transmitted drug resistant virus or unknown ART exposure prior to arrival in Sweden **(I)**.
2. There is a trend of increasing numbers of newly diagnosed patients carrying HIV-1C and recombinant forms, compared to HIV-1B due to migration to Sweden and to a spread among heterosexually infected patients and MSM within the country. The epidemic in Sweden was at the time of the study one of the most diverse epidemics outside west-central Africa **(II)**.
3. Treatment outcome of HIV-1C is not equal to HIV-1B. Despite the well-developed standardized clinical care given in Sweden with modern laboratory monitoring and focus on adherence support, HIV-1C infected patients on protease inhibitors have a higher risk of viral failure. A potential contributing cause to the increased risk of viral failure among patients infected with HIV-1C can be the naturally occurring polymorphisms in the HIV-1C protease that may affect the binding of PIs **(III)**.
4. There are indications of limited utility of the NNRTI rilpivirine in countries where GRT and VL monitoring is not available, if the country has a HIV-1C-dominated epidemic. Rilpivirine is currently not approved as a first-line drug but can be beneficial for patients failing first-line therapy if genotypic resistance testing is performed and VL level assessed **(IV)**.
5. Toxicity and/or convenience are the far most common reasons to switch from first-line ART. When switching between first and second line ART, there is an increased risk of viral failure if the patient has a high VL. However, there is no significant effect on CD4 T-cell count, AIDS, and death **(V)**.

6 RECOMMENDATIONS FOR FUTURE STUDIES

My research has raised many questions in need of further investigations:

1. Because of the diversity of the Swedish HIV-epidemic, continued molecular surveillance should be pursued to understand the nature of transmission in the country, related to local spread as well to migration.
2. Papers III and IV indicated a subtype specific difference in therapy response. Non-B subtypes, more specifically HIV-1C, showed a decreased treatment response against PI/r that are frequently used in Sweden, despite lack of detectable DRM. It is therefore important to understand and identify any unrevealed resistance mechanisms of non-B subtypes against PI.
3. We also recognize the significance to perform treatment outcome studies of non-B subtypes, especially HIV-1C, that are responsible for the vast majority of the global HIV-burden. Further research should consider subtype specific long-term treatment responses to different antiretroviral drugs in real-life cohorts.
4. Finally given the present limited hope for HIV-vaccine for the near future, the new drug development should focus on non-B subtypes also that is responsible for 88% of the global infections.

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