# Use and safety on antiretrovirals in pregnancy: filling the gap between regulatory recommendation and clinical practice

A thesis presented for the degree of Doctor of Philosophy

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# Declaration

I, Virginia Rasi, 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

Antiretroviral therapy (ART) contributed enormously to the reduction of HIV vertical transmission rate whilst treating maternal disease. Nowadays the proportion of women living with HIV (WLWH) knowing their HIV status and conceiving whilst being on effective combination of antiretroviral agents (ARVs) has stabilized at high level. However, early treatment initiation means prolonged infants *in utero* exposure to ARVs and consequent potential toxic effect to the developing embryo (i.e. congenital anomalies) and adverse pregnancy outcomes (including stillbirths and preterm delivery). Aim of this thesis was to evaluate safe and effective use of ARVs in pregnant WLWH, to evaluate pregnancy outcomes - with a main focus on detection of congenital anomalies (CAs), and to assess whether early exposure to combinations of ARV was associated with increased risk for CAs. Furthermore, a gap-analysis evaluating real-world use of ARVs and regulatory recommendation for the safe and effective use of ARV agents was conducted.

I used data from the National Surveillance of HIV in Pregnancy and Childhood (NSHPC), an ongoing surveillance study of all pregnancies in WLWH and their infants across the UK/Ireland. The analysis included data on pregnancies reported to the NSHPC from 2008 to 2018. For the gap-analysis, I have assessed publicly available data from the European Medicines Agency through their website as source for regulatory recommendations, while the NSHPC was the data source for the real-world use of ARV agents.

This thesis identified three main findings: an increased earlier use in pregnancy and from before conception of combinations of ARV (34.5%, 412/1,194 of pregnancies in 2008 started ART prior conception vs 80.6%, 478/593 in 2018) and a wider range of available ARV combinations for pregnant WLWH in the UK between 2008-2018. A gap between real-world use of ART and regulatory/clinical recommendations for pregnant WLWH, with regulatory recommendation “catching up” with real-world use of ARVs and only in recent years timely amended their guidelines whenever a safety signal from the real-world evidence has been detected. No evidence of increased risk of CAs in infants with *in utero* exposure to ART with an overall CA prevalence of 2.03% (95%CI 1.77-2.31, 227 CA/111,197 liveborn infants) nor of any particular patterns of CAs affecting the same organs/systems by the rule of three.

# Impact statement

Currently there is still only one antiretroviral agent for HIV treatment with a regulatory recommendation for use in pregnancy despite the growing number of effective antiretroviral agents. Although these are not recommended in pregnancy by regulators, they are widely used in clinical practice, as real-world data demonstrate. A major cause of this disconnect is the gender bias in HIV clinical studies, with relatively few women included, plus systematic exclusion of pregnant women. Therefore, there is a general paucity of safety and efficacy data in pregnancy for new drugs.

Data on antiretroviral use in pregnancy has mostly arisen from the post-marketing phase through registries, prospective cohorts, surveillance studies, etc. This generates an important time-lag between market-authorisation of a new antiretroviral agent being granted and the first data on safety and effectiveness in pregnancy becoming available. This time-lag is prolonged when safety data for rare events such as congenital anomalies need to be generated. Through a gap-analysis comparing real-world use of antiretrovirals in the UK and regulatory recommendations, I identified two issues: no antiretroviral up to 2018 has had clear recommendation for use in pregnancy despite accumulating data, and the most restrictive recommendations are either due to data unavailability or the consequence of efficacy/safety risk only identified years after market-authorisation. Therefore, my study evidenced a second time-lag between availability of data and recommendation updates, which prevents women benefiting from new drugs and calls for action to exploit and incorporate new findings into regulatory recommendations systematically and promptly.

When I started my PhD, I decided to include Dolutegravir (DTG) as a case-study being a recently authorised agent with limited pregnancy data from clinical trials and initial (limited) reassuring post-marketing safety data. In 2018 a safety signal from a post-marketing surveillance study suggested increased risk for neural tube defects with periconception DTG exposure, prompting changes in both regulatory and clinical recommendations. I evaluated DTG use in pregnancy in the UK through the National Surveillance of HIV in Pregnancy and Childhood and in Europe through the European Pregnancy and Paediatric HIV Cohort Collaboration and found an increased use of DTG over time (prior to the signal) but no increased risk for NTDs or other congenital abnormalities. My findings have already been shared with the EMA, WHO and other groups, informing updated guidelines, policies and regulatory decisions.

Furthermore, I have impacted on the ongoing surveillance of HIV in pregnancy in the UK by introducing changes for data collection and management on congenital abnormalities.

The new approach channels data to avoid inaccurate reporting, improving overall data quality. This is important particularly given the large number of different regimens being used in pregnancy, mostly started from before conception. Information on use and safety of real world antiretroviral use is important to share with various stakeholders (e.g. Public Health England, the British HIV Association, patient groups) and for future research addressing drug safety. The findings presented in this thesis impact regulatory guidelines and clinical practice, and public health policies toward an increased focus on pregnant women living with HIV.

# Acknowledgments

This thesis is dedicated to all women, pregnant and nonpregnant, HIV infected and uninfected, patients, healthcare professionals, professors, mothers and single ladies, constantly inspiring, motivating and guiding my journey to grow as a woman and a better healthcare professional.

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# Acronyms and abbreviations

AIDS Acquired immune deficiency syndrome
aOR Adjusted odds ratio
ART Antiretroviral therapy
B-R Benefit-Risk assessment
CA congenital anomalies
CDC Centres for Diseases Control and Prevention
CI Confidence interval
EDD Estimated delivery date
EMA European Medicines Agency
EPPICC European Pregnancy Paediatric HIV And Cohort Collaboration
FDA Food and Drug Administration
HIC High-income country
HIV-DR HIV drug resistance
INSTI Integrase strand transfer inhibitor
LMIC Low- and middle-income countries
MAH marketing authorisation holder
MSM Men who have sex with men
NNRTI Non-nucleoside reverse transcriptase inhibitor
NTDs neural tube defects
OR Odds ratio
PrEP Pre-exposure prophylaxis
PI Protease inhibitor
PK-PD pharmacokinetic-pharmacodynamic studies
RCT randomised clinical trial
RR relative risk
SGA small for gestational age
SSA Sub-Saharan Africa
VL Viral load
WHO World Health Organisation
APR Antiretroviral Pregnancy Registry
ARVs antiretroviral agents/ antiretrovirals
BHIVA British HIV Association
cART Combined antiretroviral therapy
CD4 CD4-presenting T-cell
CS Caesarean section
DF Degrees of freedom
EUROCAT European Surveillance of Congenital Anomalies and Twins
EPAR European public assessment report
FDC fixed dose combination
GW gestation week
HIV Human Immunodeficiency virus
IDU Injecting drug use
IQR Interquartile range
LBW low birth weight
MHRD Maximum recommended Human Dose
NOEL no observed adverse effect level
NRTI Nucleos(t)ide reverse transcriptase inhibitors
NSHPC National Surveillance of HIV in Pregnancy and Childhood
PHE Public Health England
PLWH People living with HIV
PTD premature delivery
RMP Risk Minimization Plan
SB stillbirth
SmPC Summary of Product Characteristics
UNAIDS Joint United Nations Programme on HIV/AIDS
VT vertical transmission
WLWH women living with HIV

# 1 Introduction : HIV

## 1.1 Epidemiology of HIV

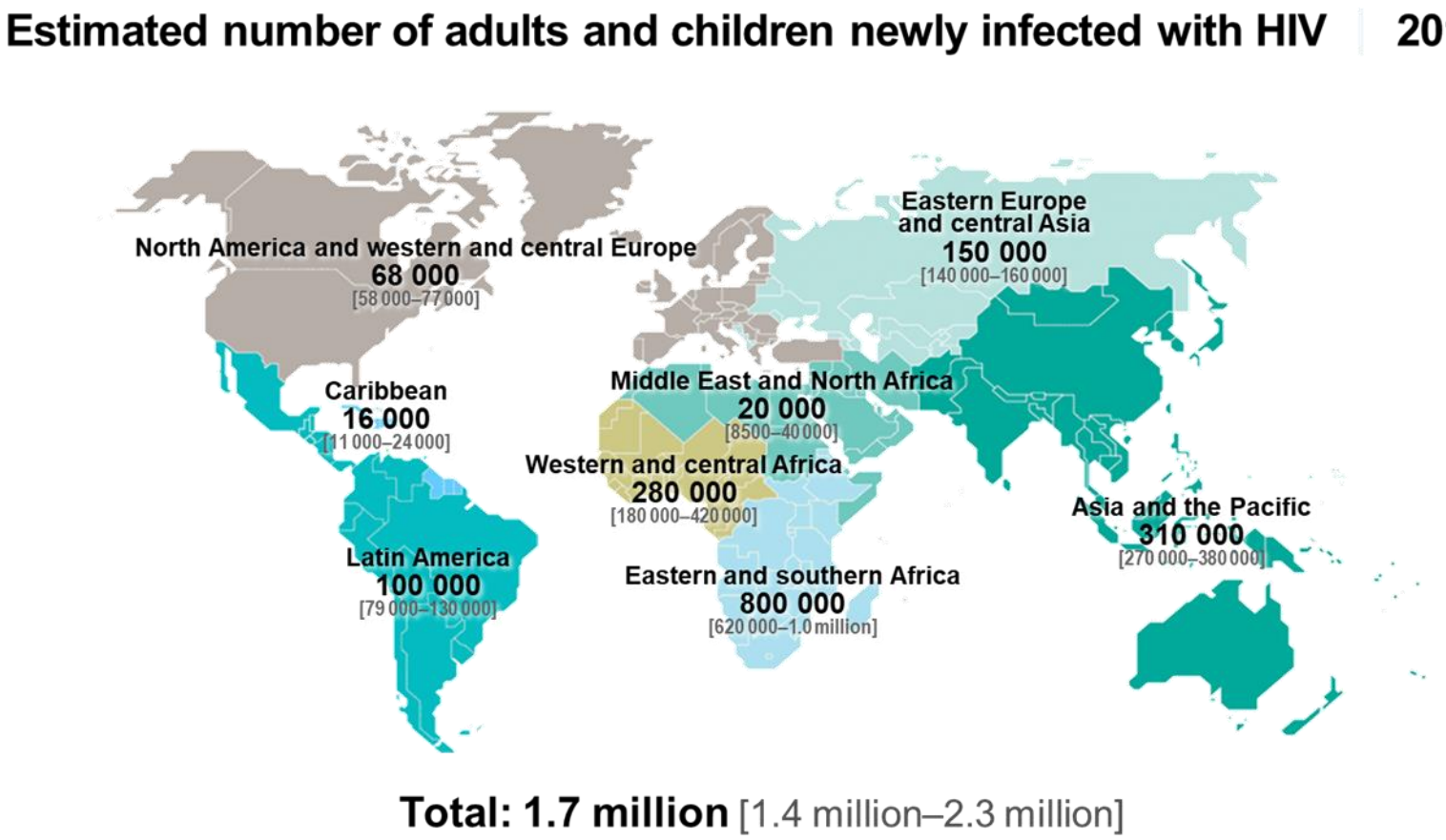
### 1.1.1 Global HIV epidemiology

In 2018 an estimated 37.9 million people worldwide were living with Human Immunodeficiency Virus (HIV), with a global prevalence among adults of 0.8%. Of the people living with HIV (PLWH), 36.2 million were adults and 1.7 million were children under the age of 15 according to the latest Joint United Nations Programme on HIV/AIDS (UNAIDS 2019a, UNAIDS 2019b).

Globally, around 1.7 million new HIV infections had been reported by the end of 2018. Since the beginning of the epidemic, there has been a significant decline in the number of new infections, but the pace at which reduction of new HIV infections occurs varies by age group, gender and region, with some countries achieving greater reductions and others experiencing rises in both new infections and Acquired Immune deficiency syndrome (AIDS)-related deaths. Since 2010 the number of new HIV infections has declined globally by an estimated 16%, from 2.1 million to 1.7 million in 2018; the number of deaths from AIDS-related illness has also decreased from 1.2 million people in 2010 to 770,000 people reported in 2018 (UNAIDS 2019a, UNAIDS 2019b).

Linked to these declines, the number of people accessing antiretroviral therapy (ART) has increased from 7.7 million (7.7/31.7, 24%) in 2010 to 23.3 million (23.3/37.9, 62%) in 2018, an increase of almost 40% in ART usage (UNAIDS 2019a, UNAIDS 2019b). According to the latest UNAIDS reports, as of the end of June 2019, the number has further increased with about 24.5 million PLWH (64.6%) now accessing ART (UNAIDS 2019a).

HIV testing has similarly increased over time, with latest numbers reporting 79% of PLWH globally knowing their HIV status, but with still one in five (the remaining 21%) not being aware of their infectious status (UNAIDS 2019a). Worldwide HIV distribution is reported in Figure 1.1 and Table 1.1.




**Source: UNAIDS, July 2019 Core epidemiology slides, from: https://www.unaids.org/en**

**Figure 1.1 HIV distribution worldwide by the end of 2018, by region**

## Table 1.1 HIV distribution worldwide by the end of 2018, by region

| Region | N of people living with HIV | N of new infections | N of AIDS-related deaths | Treatment coverage |
|---|---|---|---|---|
| **Global, total** | **37.9 million (100%)** | **1.7 million** | **770,000** | **23.3 million (62%)** |
| Asia and the Pacific | 5.9 million (16%) | 310,000 | 200,000 | 3.1 million (54%) |
| The Caribbean | 340,000 (<1%) | 16,000 | 6,700 | 186,000 (55%) |
| East and Southern Africa | 20.6 million (54%) | 800,000 | 310,000 | 13.7 million (67%) |
| Eastern Europe and Central Asia | 1.7 million (4%) | 150,000 | 38,000 | 648,000 (38%) |
| Latin America | 1.9 million (5%) | 100,000 | 35,000 | 1.2 million (62%) |
| Middle East and North Africa | 240,000 (<1%) | 20,000 | 8,400 | 78,800 (32%) |
| West and Central Africa | 5.0 million (13%) | 280,000 | 160,000 | 2.5 million (51%) |
| Western and Central Europe and North America | 2.2 million (6%) | 68,000 | 13,000 | 1.7 million (79%) |

Sources: UNAIDS. Adapted from AIDSinfo website (UNAIDS 2019a)

Most HIV-positive people live in low and middle-income countries (LMICs) with Sub-Saharan Africa (SSA) being the hardest hit region worldwide, followed by Asia and the Pacific (UNAIDS 2019a). As shown in Table 1-1, 25.6 million HIV-positive people live in SSA, with 20.6 million in East Southern Africa, accounting for more than half (54%) of all PLWH, followed by Asia and the Pacific, accounting for 16% of all the PLWH worldwide (UNAIDS 2019a).

Notably, reports from Eastern Europe and Central Asia have shown an accelerating trend in the HIV epidemic, now suffering the greatest growth worldwide, with an annual number of new HIV cases 29% higher than in 2010 (from 120,000 new HIV cases in 2010 to 150,000 in 2018) (UNAIDS 2019a).

Over the years, key populations at higher risk of infection have been identified and are mostly represented by people who inject drugs , gay men and men who have sex with men (MSM), transgender people, sex workers and prisoners.

Worldwide most of the new infections (54%) are among these key populations and their sexual partners. In particular, r MSM account for 17% of new infections, people who inject drugs  for 12%, sex workers for 6% and transgender women 1%. The sexual partners of these groups account for an additional 18% of new HIV infections (UNAIDS 2019a).

Further, when evaluating the relative risk of HIV acquisition, by population groups and compared with the general population, data suggest that the risk of acquiring HIV among MSM is 22 times higher than among all adult men. Similarly, the risk for people who inject drugs is 22 times higher than for people who do not inject drugs; 21 times higher for sex workers than adults aged 15 to 49 years and 12 times higher for transgender woman than adults aged 15 to 49 years (UNAIDS 2019a).

### 1.1.2 HIV epidemiology in the UK

According to the latest report from Public Health England (PHE) in 2018 an estimated 101,600 people were living with HIV, with 4,453 new diagnoses, of which 3,266 were in males and 1,185 in females. Of the estimated 101,600 individuals living with HIV, 97% were receiving ART and overall 87% of PLWH in the UK have an undetectable viral load (PHE 2019). Furthermore, annual numbers of new infections among key populations, have more than halved from a peak of 2,700 cases in 2012 to 1,200 cases in 2017 (PHE 2018). Similarly, there has been a decline in new HIV diagnoses acquired through heterosexual sex irrespective of ethnicity and gender. For example, new HIV diagnoses in both black African and black Caribbean heterosexuals have been decreasing steadily over the past 10 years (black African from 2,424 in 2008 to

to 542 in 2017 and black Caribbean from 231 to 52). Over the years, the steepest decline in new HIV diagnoses have been observed among gay and bisexual men (30% decline, from 2,709 in 2009 to 1,908 in 2018), white (46% decline, from 2,353 in 2015 to 1,276 in 2018), born in the UK (46% decline from 1,627 in 2015 to 873 in 2018), and residing in London (63%, from 1,135 in 2009 to 416 in 2018) (PHE 2019).

London accounts for the largest proportion of HIV diagnoses in the UK (34%, 1,504/4,453 in 2018), followed by the Midlands and East of England region (23%, 1,004/4,453); nationally, most people newly diagnosed are aged 25 to 49 years (67%, 3,000/4,453). The number of late diagnoses, (defined as having a CD4 counts of less than 350 cell/$m^3$ within 91 days of HIV diagnosis) has decreased from 3,895 in 2008 to 1,879 in 2017 and was higher among heterosexual men and women (59%, 307/523 and 50%, 312/624, respectively) and lowest among gay and bisexual men (33%, 524/1,571) (PHE 2019).

### 1.1.3 HIV in women

Women account for half (18.8 million, 50%) of all adults living with HIV worldwide and for 43% (740,000/1.7 million, 43.5%) of all the new HIV infections globally in 2018 (UNAIDS 2019a) (Table 1.2).

Young women are disproportionately affected by the HIV infection with several identified contributing factors to their increased susceptibility to acquire the virus, including gender inequalities, gender-based violence and differential access to healthcare services. For example, in 2018, worldwide, numbers of new HIV infections among women aged 15 to 24 years were 60% higher than among young men of the same age (310,000 new cases in young women vs 200,000 in young men) (UNAIDS 2019a).

Gender inequality is a key player in women's increased susceptibility to HIV infection. Many countries, for example, require parental and spousal consent in order to access sexual and reproductive services, with studies showing this to be associated with lower service uptake (The Lancet 2019). Women who do not conform to such norms can face discrimination and stigma related to their sexual and reproductive health. Gender minorities are also at greater risk of discrimination and stigma, with data showing transgender women being 49 times more likely to acquire HIV than the general population (Baral et al. 2013). They will also be more likely to engage in street-based sex, be homeless, use substances, have partners who are at higher risk for

HIV and lack access to healthcare services, including gender-affirming care (Bradford et al. 2013, Poteat et al. 2014). Gender-based violence remains a major public health issue with much of this violence meted out by intimate partners and a global estimation of nearly 30% of women experiencing physical and/or sexual violence by their intimate partners at least once in their lifetime (WHO 2013a). Evidence from regions where HIV prevalence is high, such as SSA suggests that intimate partner violence increases susceptibility to HIV and is also associated with both lower access to treatment and treatment adherence and consequent inability to reach viral load suppression (Li et al. 2014, Hatcher et al. 2015).

Sex between young women and older men has been shown to increase the risk of HIV infection, for example by almost 7-fold for women five to seven years younger than their male partners in KwaZulu Natal, South Africa (de Oliveira et al. 2017). Data from the same study have also shown that men who infect young women are more likely to have acquired HIV from women aged 25-40 years and either unaware of their infection status and/or had detectable VL (de Oliveira et al. 2017).

This sex-networking is thought to be the main cause of the SSA high incidence of HIV in young women, where in 2018 they accounted for 26% of all new HIV infections (210,000/800,000) with around 6,000 new cases every week (UNAIDS 2019a, UNAIDS 2019c). Furthermore, in East and Southern Africa, women are thought to acquire HIV five to seven years earlier than men, with Cameroon, Cote d'Ivoire and Guinea being the most striking example where female adolescents (15-19) are five times more likely to acquire the virus than boys of the same age (Dellar et al. 2015, UNAIDS 2016).

Sex workers and women engaging in transactional sexual activity remain an important key population, with sex workers having been reported at the highest risk of HIV acquisition in nearly every setting studied (Baral et al. 2012). This population is at higher risk of overlapping HIV-risk factors, such as substance use, use of illicit injecting drugs, sexual abuse and assault and inability to negotiate safe sex (Baral et al. 2012).

Finally, evidence suggests women to be biologically more susceptible to HIV infection; this is where factors causing inflammation in the female genital tract, whether due to alterations in the thickness or in the integrity of the mucosal layer or due to alterations of normal acid-vaginal microbiome (e.g. due to sexually transmitted infections or micro-abrasions due to sexual activity) or due to hormonal alteration (e.g. oestrogen and progesterone induced changes in vaginal microbiota) have been demonstrated to increase the risk for a women to acquire HIV (Roberts et al. 2012, Adimora et al. 2013, Hapgood et al. 2018).

**HIV epidemiology in the UK**

According to latest PHE reports, in the UK in 2017 an estimated 28,669 women were living with HIV, making up one-third (31%, 28,669/93,385) of people living with diagnosed HIV infection(PHE 2019). There were 1,106 women (aged ≥15 years) newly diagnosed in the UK in 2017 accounting for around a quarter (1,106/4,334) of all the new adult HV infections, with a 13% decline compared to the previous year (1,265 diagnoses in 2016). In 2017, 94% of the newly diagnosed women, with data on HIV acquisition route (794/848) acquired the virus through heterosexual sex, with a steady decline over the past decade (66%, from 2,328 in 2008 to 794 in 2017).

Similar to global trends, despite the overall decline in HIV new infections among women, the pace at which this decline occurs in women is still 15 to 25% slower than in heterosexual men over the past decade (~2,500 new HIV infection in women vs ~1,500 in men in 2008 and ~1,100 new HIV infection in women vs ~770 in men in 2017)(PHE 2019).

Late diagnosis has declined (740 in 2013 to 560 in 2017), but the proportion of late HIV diagnosis remains high among women diagnosed in England, at 50% in 2017, with estimates suggesting that a woman with a late diagnosis has been living with an undiagnosed infection for around 3 to 5 years (PHE 2019).

## Table 1.2 HIV distribution in women worldwide by the end of 2018, by region

| Region | N of women living with HIV | | N of new HIV infections | | N of AIDS-related deaths | | Treatment coverage |
|---|---|---|---|---|---|---|---|
| | Women (15+) | Young women (15-24) | Women (15+) | Young women (15-24) | Women (15+) | Young women (15-24) | Women (15+) |
| **Global, total** | **18.8 million** | **2.2 million** | **740,000** | **310,000** | **270,000** | **25,000** | **12,7 million** |
| Asia and the Pacific | 2.1 million | 160,000 | 95,000 | 31,000 | 60,000 | 1,900 | 1.3 million |
| The Caribbean | 160,000 | 17,000 | 6,200 | 2,400 | 2,200 | <200 | 97,800 |
| East and Southern Africa | 12 million | 1.6 million | 420,000 | 210,000 | 130,000 | 16,000 | 8.6 million |
| Eastern Europe and Central Asia | 580,000 | 22,000 | 47,000 | 5,900 | 8,800 | <200 | 264,000 |
| Latin America | 580,000 | 40,000 | 28,000 | 68,00 | 11,000 | <500 | 260,000 |
| Middle East and North Africa | 85,000 | 8,700 | 6,200 | 1,700 | 2,500 | <200 | 30,100 |
| West and Central Africa | 2.8 million | 400,000 | 130,000 | 58,000 | 55,000 | 5,900 | 1.7 million |
| Western and Central Europe and North America | 490,000 | 13,000 | 13,000 | 2,400 | 2,400 | <100 | ---- |

Sources: UNAIDS. Adapted from AIDSinfo website (UNAIDS 2019a)

## 1.2 HIV natural history and treatment overview

### 1.2.1 The virus, its life cycle and natural history

*HIV subtypes*

HIV is a retrovirus of the subfamily of lentiviruses, first identified in the early 1980s. There are currently two known HIV subtypes, HIV-1 and HIV-2 (Gallo et al. 1983, Gallo et al. 1984). These exhibit diversity in their genetic profile – with identified distinct subtypes or *clades*, different virulent potency and geographical prevalence (Peeters et al. 2013). HIV-1 is the more virulent, infectious and widespread worldwide, while HIV-2 (Lemey et al. 2003) is characterized by a longer asymptomatic phase, slower CD4+ T cell depletion and mostly confined to West Africa (Peeters et al. 2013).

*HIV life cycle*

HIV primary receptor is CD4, which is present on the surface of CD4+ T cell lymphocytes of various immune cells such as monocytes/macrophages lineage; the high-affinity interaction with the CD4+ molecules on the T-cell surface, allows the viral envelope to fuse with the CD4 cell membrane (Holmes 2001, McCune 2001, Goodsell 2015) (Figure 1.2). Once the fusion is completed HIV is absorbed into the human cell and with a set of enzymes, such as reverse transcriptase converts its genetic material – HIV RNA into HIV DNA. HIV then enters the CD4 nucleus, combines with the cell's genetic material and through the action of the viral enzyme integrase inserts its viral DNA into the DNA of the host cell (Holmes 2001). Inside the nucleus the virus begins to use CD4 cell machinery to create long chains of HIV proteins –the building blocks for more HIV. New HIV RNA and HIV proteins –now made by the host cell– move towards the surface of the cell and assemble into immature HIV (Sundquist et al. 2012). Immature HIV is then pushed out of the host cell in the so called budding phase and once outside the human cell, a protease breaks up the long protein chains converting immature virus into the mature and infectious form (Sundquist et al. 2012).

*Stages of HIV infection*

HIV disease and its progression are usually caused by high concentration of active replicative virus and consequent destruction of CD4 cells. Viral replicative activity also defined as viral load (VL) is a numerical expression of the quantity of virus (HIV RNA) is a given volume (serum or plasma). VL testing measures the number of HIV particles, i.e. how many HIV RNA copies are present in a millimetre of serum or plasma and is usually expressed as copies/mL. Through VL testing is possible monitor individuals' response to ART (Dasgupta et al. 2014). Therefore, VL along with CD4

cell counts represent the best laboratory markers to manage HIV/AIDS patients, predict disease progression and treatment success.

The “acute phase” or primary infection is characterized by active engagement of the immune system and active and massive virus replication inside CD4 cells.

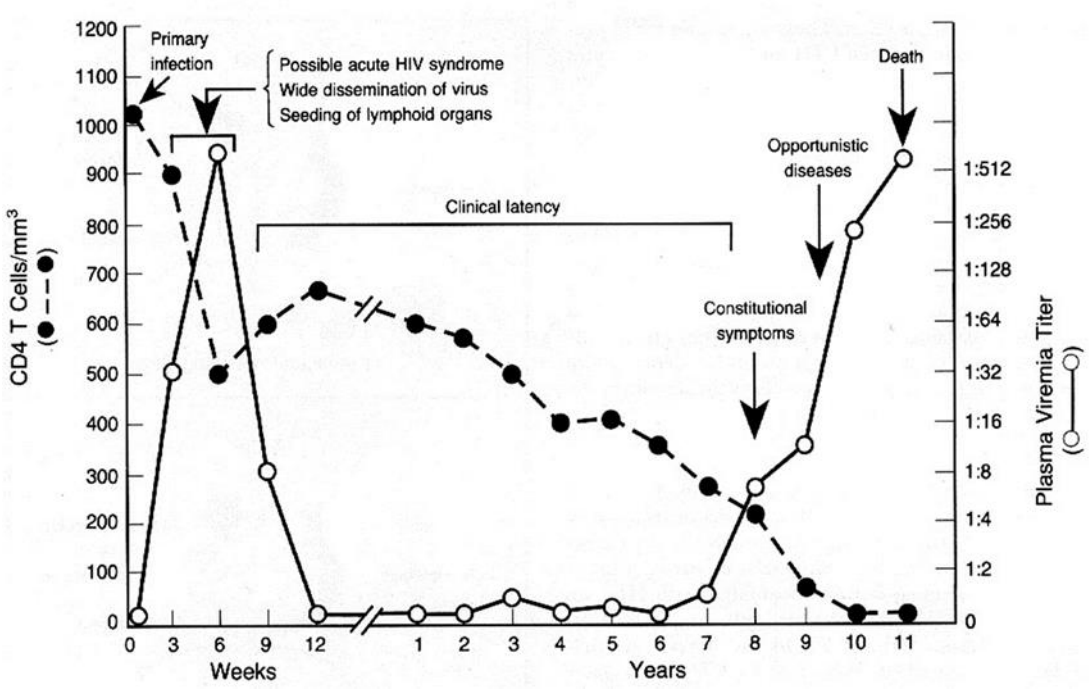
Regardless of the portal of HIV entry, the major anatomic sites for the establishment and propagation of HIV are lymphoid tissues (Kasper 2015). The virus then disseminates through the blood to the whole body while permanently destroying a very large proportion of CD4 cells, mostly originating from the stomach and gut-associated lymphoid tissue (Veazey et al. 1998, McCune 2001). During this phase, the viral replication is very high, usually millions of copies of HIV RNA per millilitre of plasma increasing greatly the likelihood of HIV transmission (Mindel et al. 2001, Kasper 2015). The immune system then recognises the virus and develops an immune response by production of antibodies - a phase known as s*eroconversion* (Gaines et al. 1987, Ariyoshi et al. 1992). In the acute phase, some infected individuals are asymptomatic, whilst others can either manifest flu-like symptoms including fever, fatigue and rash or in less common cases, develop very severe symptoms requiring hospitalization. Regardless of the situation this phase is followed, after approximately 6 weeks, by a relative CD4 cell recovery even in the absence of medicines and a concomitant progressive decline in viral replication (Fauci 2003).

Once the infection has been established the virus succeeds in escaping complete immune-mediated clearance and the “chronic phase” of infection starts (Kasper 2015). Chronic infection is characterized by continuous viral replication, but with varying degrees and a relatively slow pace and slow progression of HIV-related symptoms that can last for years. Considering the natural evolution of the disease, if HIV is untreated or if treated but with no adequate control of VL, signs and symptoms of AIDS and/or non-AIDS associated events such as opportunistic infections and cancer will manifest (Pantaleo et al. 1993, El-Atrouni et al. 2006). AIDS is the final stage, the most severe, characterized by high VL, high risk of transmission and in absence of treatment by very poor survival chance (Pantaleo et al. 1993, Mocroft et al. 1998) (Figure 1.2).

*The clinical stages*

Historically, organizations such as the WHO (WHO 2007) and the Centres for Disease Control and Prevention (CDC) (CDC 1992) developed case-definitions to standardize description of HIV disease and its progression to AIDS. The CDC classification relies mostly on laboratory criteria, such as multi-test algorithm or a positive HIV virologic

test/CD4 cell counts, along with the identification and monitoring of conditions associated with those with HIV. The WHO provides a more widely set of criteria, taking into consideration differences by countries and resource-settings, using both immunologic (CD4 cell counts) and clinical criteria dividing disease progression in four stages (clinical stages I to IV). However, since evidence showing early treatment initiation reduces the risk of HIV- related illness and AIDS-deaths, international guidelines, including WHO started to recommend ART for all patients regardless of their CD4 counts and/or clinical manifestation and these clinical classifications have become less relevant (Insight Start Study Group et al. 2015, WHO 2015a).

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## Figure 1.2 Typical course of HIV infection

**Comment**: The dashed line indicates CD4 T cells counts while the continuous line the HIV RNA load. After primary infection within the first 6 weeks (x-axis weeks), there is a rapid viral replication (up to $10^6$ copies/mL plasma of HIV RNA) and a concomitant drop of CD4 T cell counts (around 500 cells/$mm^3$). Then by week 12 there is a relative CD4 T cells recovery and a period of clinical latency of approximately 7-9 years. When CD4 T cell counts then starts to fall and viral replication spikes, firstly opportunistic infections and symptomatic AIDS will manifest and then death will occur.

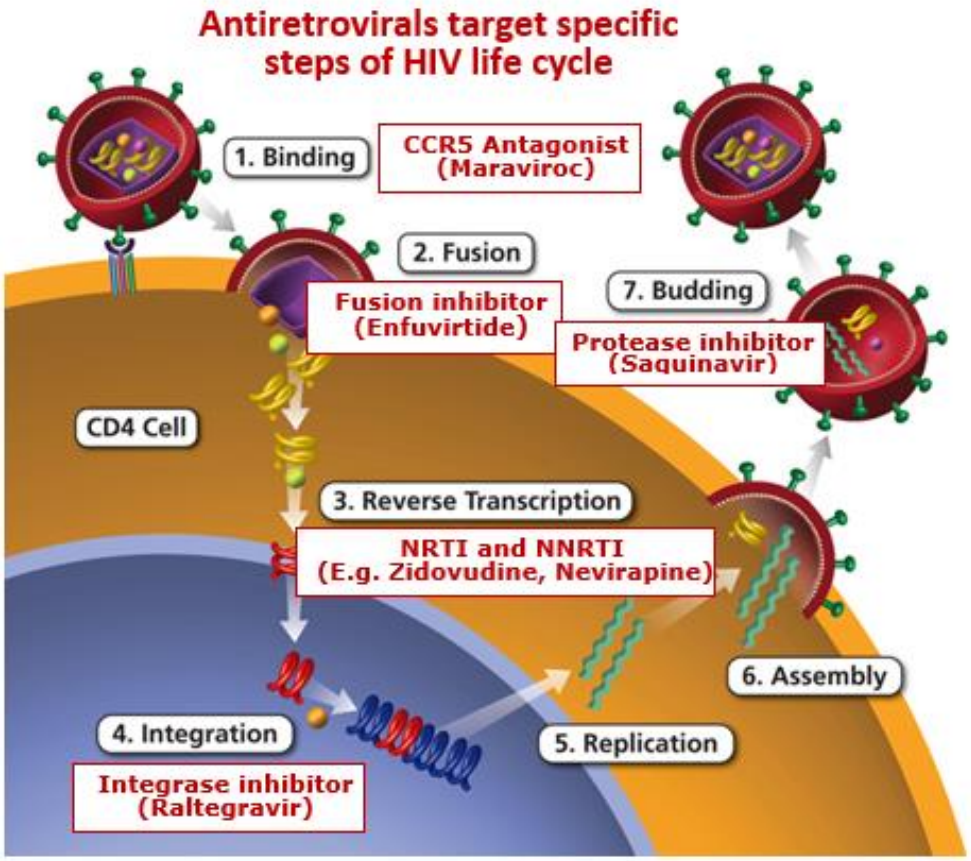
### 1.2.2 Brief history of HIV treatment

Over the last 30 years, antiretrovirals (ARVs) have been developed to target different steps of the HIV life-cycle with the goal of reaching undetectable levels of viral replication, i.e. when VL has fallen below the detection limit of laboratory assay (e.g. <50 copies/mL).

There are currently five main classes of ARVs, targeting different stages of the HIV life-cycle (Figure 1.3, Table 1.3 & 1.4). The first class of ARVs to be marketed were nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs) in the late 1980s, followed by protease inhibitors (PIs) from 1995 and non-nucleoside reverse transcriptase inhibitors (NNRTIs) a year later. In 2003, the entry or fusion inhibitors were added, followed by the integrase strand transfer inhibitors (INSTIs) in 2008. A timeline of HIV/AIDS main events, breakthroughs and HIV organisations' campaigns is reported in Figure 1.4.

**Table 1.3 Antiretroviral agents, by class and mechanism of action**

| Class drug | Activity/ targets | Examples |
|---|---|---|
| **Nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs)** | Prevent transcription by inhibiting the reverse transcriptase enzyme, and cause DNA chain termination by incorporation into new HIV RNA | Zidovudine, Lamivudine, Abacavir, Tenofovir-DF |
| **Non-nucleoside reverse transcriptase inhibitors (NNRTIs)** | Directly inhibit transcription by binding reverse transcriptase enzyme and causing disruption of its activity | Efavirenz, Nevirapine, Rilpivirine |
| **Protease inhibitors (PIs)** | Prevent assembly of proteins and consequent production of new viral particles by inhibiting the protease enzyme | Atazanavir, Darunavir, Lopinavir |
| **Integrase strand transfer inhibitors (INSTIs)** | Prevent integration of viral DNA into host DNA by inhibiting the integrase enzyme | Raltegravir, Elvitegravir, Dolutegravir |
| **Entry or Fusion inhibitor** | Disrupt early stages (i.e. binding, fusion or entry) of HIV interaction with the host by binding to HIV's targets (e.g. chemokine receptors). | Maraviroc, Enfuvirtide |




Adapted from AIDS*info,* available at https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/1596/life-cycle

## Figure 1.3 Antiretroviral agents and their targets in the HIV life-cycle

**Comment**: In black the seven steps of the HIV life-cycle are illustrated. In red the antiretroviral drugs' mechanisms of action, either inhibit key viral enzymes or antagonize essential steps of the HIV life-cycle.

## Table 1.4 Antiretroviral agent, by year of marketing authorisation in Europe and the U.S.

| NRTI | Year of approval | | NNRTI | Year of approval | | PI | Year of approval | | EI/FI | Year of approval | | INSTI | Year of approval | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EMA | FDA | | EMA | FDA | | EMA | FDA | | EMA | FDA | | EMA | FDA |
| **Zidovudine (ZDV)** | _ | 1987 | **Nevirapine (NVP)** | 1998 | 1996 | **Saquinavir (SQV)** | 1996 | 1995 | **Enfuvirtide (ENF)** | 2003 | 2003 | **Raltegravir (RAL)** | 2008 | 2007 |
| **Didanosine (ddl)** | _ | 1991 | **Efavirenz (EFV)** | 1999 | 1998 | **Ritonavir (rit/r)** | 1996 | 1996 | **Maraviroc (MCV)** | 2007 | 2007 | **Dolutegravir (DTG)** | 2014 | 2013 |
| **Zalcitabine (ddC)** | _ | 1992 | **Etravirine (ETR)** | 2008 | 2008 | **Indinavir (IDV)** | 1996 | 1996 | | | | **Elvitegravir (EVG)** | 2014 | 2013 |
| **Stavudine (D4T)** | 1996 | 1994 | **Delavirine (DLV)** | 2011 | 2001 | **Nelfinavir (NFV)** | 1998 | 1997 | | | | **Bictegravir (BIC)** | 2018 | 2018 |
| **Lamivudine (3TC)** | 1996 | 1995 | **Rilpivirine (RPV)** | 2011 | 2011 | **Amprenavir (APV)** | 2000 | 1999 | | | | | | |
| **Abacavir (ABC)** | 1998 | 1999 | | | | **Lopinavir/r (LPV/r)** | 2001 | 2000 | | | | | | |
| **Tenofovir-DF (TDF)** | 2002 | 2001 | | | | **Atazanavir (ATV)** | 2004 | 2003 | | | | | | |
| **Emtricitabine (FTC)** | 2003 | 2002 | | | | **Fosamprenavir (FPV)** | 2004 | 2004 | | | | | | |
| **Tenofovir-AF (TAF)** | 2017 | 2016 | | | | **Tipranavir (TPV)** | 2005 | 2005 | | | | | | |
| | | | | | | **Darunavir (DRV)** | 2007 | 2006 | | | | | | |

NRTIs, Nucleoside reverse transcriptase inhibitors; NNRTIs, Non-nucleoside reverse transcriptase inhibitors; PI, Protease inhibitors; INSTIs, Integrase strand transfer inhibitors; EI/FI, Entry inhibitor/Fusion inhibitor; ST/FDC, single tables/fixed-dose combination

The first efficacious treatment for HIV was Zidovudine (ZDV) monotherapy approved by the US regulatory agency Food and Drug Administration (FDA) in 1987. ZDV is a NRTI, first studied in clinical trials in the late 1980s and shown to increase survival while reducing opportunistic HIV-related infections (Fischl et al. 1987). After ZDV, several other NRTIs such as Didanosine (ddI), Stavudine (d4T) and Zalcitabine were developed; however treatment with these drugs was quickly limited by the virus's rapid development of resistance and drugs toxicities (Simpson et al. 1995, Vella et al. 2012) (for resistance issues in HIV, see later in this section).

The main breakthrough in HIV treatment came with the development of combination ART (cART) also known (historically) as highly active antiretroviral therapy (HAART) in 1996, following the licensing of two new classes of ARVs, the first PI Saquinavir (SQV) and then the first NNRTI Nevirapine (NVP) (Broder 2010, Palmisano et al. 2011, Vella et al. 2012).

The ACTG 320 trial a randomised clinical trial (RCT) in which people with HIV-1 infection were stratified according to CD4 cell counts (50 or fewer vs. 51 to 200 cells/$mm^3$) and received either ZDV (or d4T) and Lamivudine (3TC) or the same regimen with a PI (Indinavir) showed that combining ARV agents from different classes was more effective than ZDV or any other NRTI monotherapy in preventing disease progression (6% progressed to AIDS or death in cART vs 11% in dual-combination; estimated hazard ratio (HR) 0.50; 95% CI 0.33-0.76; $p$=0.001 ) and in suppression of VL (VL decreased by log 2.18, 1.55, and 0.90 in the cART, ZDV+ddI, and ZDV+NVP groups, respectively ($p$<0.05) (Hammer et al. 1996, Hogg et al. 1998).

Following cART development, ART regimens generally consisted of a "backbone 2+1", where the "2" are usually two NRTIs and the "+1" or third agent is another ARV from a different drug class, either a NNRTI or a PI. The "backbone 2+1" since 1997 has been adopted as the standard of care in high-income-settings with substantial declines in AIDS-related morbidity and mortality (Mocroft et al. 1998, Palella et al. 1998). Palella et al reported the mortality rate to have declined in a cohort study in the United States from 29.4 per 100 person-years in 1995 to 8.8 per 100 person-years by the second quarter of 1997 and a similar decline occurred for the incidence of serious opportunistic infections which fell from approximately 50 per 100 person-years in 1995 to 13.3 per 100 person-years by the beginning of 1997 (Palella et al. 1998).

Similar results were also reported from a European study where the mortality rate declined from 23.3 deaths per 100 person-years in 1995 to 4.1 per 100 person-years of follow up (Mocroft et al. 1998). Both studies showed that much of the reduction in AIDS-related mortality and morbidity were attributable to treatment changes.

Another important step came in 2003 with the development of fixed-dose combination (FDC) therapies (Vella et al. 2012). This had a significant impact in scaling-up cART, particularly in resource-limited settings, allowing reduction in the number of pills from 15-20 per day to as few as two (usually one pill, twice a day). FDC also allowed more affordable cost of cART and consequent increased access to treatments. The reduction of daily drug consumption and number of pills along with the cost reduction contributed to both the improvement of long-term adherence to treatment and the reduction of AIDS-related deaths (Vella et al. 2012). FDC also played a role in the development of generics and their impact on treatment accessibility, particularly for LMIC. Before generics were introduced to the market, limited numbers of newly HIV diagnosed people living in the regions with highest HIV prevalence had access to cART, while with their introduction costs of ARVs were significantly reduced (Vella et al. 2012, Danzon et al. 2015).

Therefore, FDC and generics fostered the reduction of AIDS-related deaths, particularly in LMIC, where rates were still considerably higher than in HIC. Indeed, since adaption of the "backbone 2+1" in HIC, AIDS-related death had started to reduce already from the late 1990s, from 55,000 deaths in 1996 to 32,000 in 1997 and 19,000 in 2008 (UNAIDS 2019a). Whereas in LMIC, particularly in SSA, it was not before 2008 that the number of AIDS-related deaths started to decline, from 1.2 million in 2004 to 950,000 in 2008 (Mocroft et al. 1998, Palella et al. 1998, UNAIDS 2019a).

Following introduction of cART, several clinical trials and observational studies confirmed triple therapy's ability to reduce AIDS-related mortality and morbidity (Detels et al. 1998, Mocroft et al. 1998, Palella et al. 2003). Studies have also shown the significant beneficial effects of early initiation of cART, meaning before symptoms appear and before their CD4 cell counts fall under 350cells/$mm^3$ (Detels et al. 1998, Hogg et al. 1998, Palella et al. 2003).

For example, a prospective observational US-based study, collecting data on HIV positive patients between 1994 and 2002, demonstrated how individuals with CD4 cell counts between 200-350 cells/$mm^3$ and 351-500 cells/$mm^3$ who started ART were more likely to have undetectable VL (p=0.03 and 0.04, respectively) and have reduced mortality rates that those who delayed ART initiation after CD4 cell counts has dropped <350cells/$mm^3$ (rate ratio0.27; 95%CI 0.14-0.55, $p$< 0.001) (Palella et al. 2003).

This was then confirmed and further explored, almost 10 years later, by the Strategic Timing of Antiretroviral Therapy (START) study, which evaluated the benefits and risks of immediate initiation of cART in asymptomatic PLWH with CD4 cell counts

over 500 cells/mm$^3$ compared with deferral of treatment to when CD4 cell counts had fallen under 350 cells/mm$^3$ (Insight Start Study Group et al. 2015). The START study proved the beneficial effect of immediate cART for both serious AIDS-related and non-AIDS-related events (1.8%; 0.60 events per 100 person-years in the immediate-initiation group vs 4.1%; 1.38 events per 100 person-years in the deferred-initiation group) (Insight Start Study Group et al. 2015).

Similarly, results from the Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa (TEMPRANO) on a total of 2,056 patients, of whom 41% had a baseline CD4 cell count of ≥500 cells/mm$^3$, reported the risk of death or severe HIV-related illness to be lower with early ART initiation than with deferred (adjusted HR 0.56; 95%CI 0.41-0.76 and adjusted HR among patients with a baseline CD4 cell count over 500 cells/mm$^3$ of 0.56; 95%CI, 0.33-0.94) (Temprano et al. 2015).

Introduction of cART, FDC and generics allowed a progressive and global cART coverage that expanded from thousands of people in 2000 to 12.9 million by 2013, reducing the number of annual AIDS-related deaths from 2.4 million in 2005 to 1.5 million in 2013 and to 770,000 in 2018 (UNAIDS 2016, UNAIDS 2019a). Additionally, ART coverage contributed to reducing the number of new HIV infections from the peak of 2.9 million in 1997 to the current 1.7 million (UNAIDS 2016, UNAIDS 2019a).

Access to ART and progressive global availability also translated into increased life expectancy and decreased years of life lost (Antiretroviral Therapy Cohort 2017). Analysis from the Antiretroviral Therapy Cohort Collaboration showed how 20-year-old patients from Europe and North America who started ART with three or more ARVs between 1999 and 2010 had an increased life expectancy by about 9 years in women and 10 in men (Antiretroviral Therapy Cohort 2017).

ART scale-up also contributed to prevention of onward transmission as reported in 2010 by the Partners in Prevention prospective cohort study; this study demonstrated how cART initiation by the infected partner within a serodiscordant heterosexual couple resulted in a lower transmission rate than where the infected partner did not take cART (0.37 transmission rate per 100 person-years vs 2.24, respectively) (Donnell et al. 2010) .

A further breakthrough came in 2011 when the HPTN 052 trial, a multicentre RCT randomizing participants to early ART with CD4 350-550 cells/mm$^3$ versus delayed ART until CD4 dropped to ≤350 cells/mm$^3$, showed a significant reduction in HIV heterosexual transmission by 96% in those infected and treated with ART, an important first step towards the idea of treatment as prevention (Cohen et al. 2011).

Therefore, early initiation of ART not only reduces rates of AIDS-related mortality and morbidity and positively impacts life expectancy, but also reduces HIV transmission in couples who immediately after positive HIV diagnosis start ART, by rapid achievement and sustainment of viral suppression (Cohen et al. 2011, Grinsztejn et al. 2014).

In 2011 another important concept came through: for an individual to fully benefit from cART, they need to know their infection status, be engaged in HIV care and receive and adhere to effective cART (Gardner et al. 2011). This is now known as the care continuum or HIV treatment cascade and identifies sequential stages that PLWH should go through between HIV diagnosis and effective and sustained viral suppression, including linkage to care, retention in care and receipt of ART (Gardner et al. 2011). Since the care continuum model came out, its adaptation at Federal, State and local levels have been used or adapted to identify gaps in HIV care and to support strategies to improve engagement in care and outcomes for PLWH and has formed the basis of the UNAIDS 90-90-90 targets.

This initiative aims for 90% of the PLWH to be diagnosed, for 90% of those diagnosed to access ART and for 90% of those accessing treatment to effectively suppress VL by 2020 (UNAIDS 2014) (Figure 1.4).

Furthermore, the 2012 FDA's approval of the first pre-exposure prophylaxis (PrEP) treatment, a regimen of TDF/FTC for HIV-negative individuals at risk of HIV acquisition, was another important step towards the control of the HIV pandemic (Calabrese et al. 2016, McCormack et al. 2016). PrEP is intended for any individual at risk of HIV acquisition due to risky behaviour (e.g. sexual and/or use of injection drugs), including MSM and transgender women (Grant et al. 2010), heterosexual couples (Baeten et al. 2012) and heterosexual men and women (Thigpen et al. 2012).

Increased access to ART and consequent increased ARV coverage along with evidence of beneficial early initiation have also contributed to shifting the concept of treatment eligibility. In the late 1990s, cART was generally deferred among asymptomatic patients with CD4 cell counts >200 cells/mm$^3$ mostly due to the side effects and toxicities of the first generation ARVs (IAPAC 2002, Yeni et al. 2002). Between 2006 and 2009 the threshold to start ART was raised to 350 cells/mm$^3$ worldwide and between 2009 and 2013, most guidelines further set the threshold to 500 cells/mm$^3$ following evidence from the HPTN 052 trial, with special priority given to individuals with severe or advanced HIV disease (WHO clinical stages 3 or 4) and those with CD4 counts ≤350 cells/mm$^3$ (Cohen et al. 2011, WHO 2013b, Eholie et al. 2016).

Following the two RCTs START and TEMPRANO, in 2015 the WHO's guidelines changed, recommending all PLWH to start treatment as soon as diagnosis is made, regardless of CD4 cell counts and individual's clinical status (i.e. symptomatic/ asymptomatic), known as the "test and treat" approach (Insight Start Study Group et al. 2015, Temprano et al. 2015, WHO 2015a).

**Where are we now**?

Over the past decade, global ART uptake for all people with HIV has increased from 7.7 million (24%) in 2010 to 23.3 million (62%) in 2018 and to 24.5 million (65%) by the end of June 2019, an increase of almost 41% (UNAIDS 2019a). Global scale up of ART also increased the number of averted AIDS-related deaths from 940,000 in 2010 to 1.3 million in 2018, preventing 360,000 AIDS-related deaths. Improvements in the treatment cascade have also been apparent over time, with a global achievement by the end of 2018 of 79% of PLWH knowing their status, 78% of those being on ART and 86% of those on effective viral suppression (UNAIDS 2019a) (Figure 1.4).

Resistance, adherence, safety and efficacy are some of the major stimuli in the development of new drugs, and the ARV drug pipeline is strong. Newly approved drugs are usually annually reported alongside any changes in recommendations, doses and formulations, such as the ongoing development of long-acting injectable formulations (i-base 2019, Vitoria et al. 2019).


**Figure 1.4 Timeline of HIV/AIDS related events**

**Resistance**

One of the major limitations for the full success of ART, both at an individual and ART programme level is the development of antiretroviral-drug resistance. This is especially a concern for those countries with restricted drug options, limited adherence, support and lab monitoring.

To understand development of HIV drug resistance (HIV-DR) it is important to keep in mind two concepts: high rate of HIV infection and high rate of viral mutation. The first relates to the very high rate at which new host cells need to be infected in order for the virus to maintain a steady infectious state, particularly given the short half-life of infected cells (i.e. one to two days). The second regards the (high) tendency of reverse transcriptase to make errors while transcribing from viral RNA to DNA, introducing an average of one mutation for each viral genome transcribed. These mutations can confer a selective advantage for the virus to withstand ARVs' effect (Clavel et al. 2004) .

With the introduction of cART and proof of its superiority compared to monotherapy in both reducing VL and preventing disease progression, it was also demonstrated that cART resulted in reduced chances for the virus to develop resistance against multiple agents (Hammer et al. 1996, Hogg et al. 1998). Indeed to do so, resistance to all the drugs in a regimen, i.e. multiple mechanism each generating multiple and different mutations was necessary (Hammer et al. 1996, Montaner et al. 1998, Clavel et al. 2004).

However, viral resistance to cART is possible whenever the levels of drugs are not sufficient enough to block viral replication but sufficient enough to (positively) select variants of virus able to escape the drugs' effects, causing gradual emergence of resistance to all the drugs in a regimen (Clavel et al. 2004).

This is defined as induced resistance or acquired HIV-DR, which is most often the consequence (and not the cause) of initial treatment failure and once established, generates a vicious circle of progressive higher treatment failure and higher levels of resistance. Thus, acquired HIV-DR is mostly caused by interruption of treatment and/or suboptimal adherence (Clavel et al. 2004, WHO 2019a).

Another type of HIV-DR, transmitted HIV-DR or primary resistance occurs with primary infection with a "drug-resistant" virus, i.e. resistant to a single or multiple ARVs. This is mostly the consequence of transmission from patients whose resistance developed while on treatment or due to some naturally resistant HIV strains (Clavel et al. 2004, WHO 2019a).

Cross-resistance is defined as the resistance to an ARV to which the virus has not been previously exposed but as the consequence of a mutation selected by using other(s) ARVs, for example, within the same drug class. This is particularly important when switching from ARVs of the same class in order to select efficacious alternative regimens and is usually, whenever possible, tested prior to treatment changes (Clavel et al. 2004).

### 1.2.3 HIV transmission

HIV is primarily transmitted by sexual contact across mucosal surfaces with an infected person. Additionally, it is passed by percutaneous inoculation through re-use of infected needles (e.g. through injecting drug use or unsafe medical procedures), blood transfusion and other contaminated blood products and by vertical transmission (see next section) (Kasper 2015).

The risk of transmitting HIV through unprotected sex with an infected partner depends on a variety of factors such as type of sexual practice, stage of infection, presence of co-infections and infected partner plasma VL (Quinn et al. 2000, Castilla 2005). For example, a campaign launched in 2016 supports the statement of “Undetectable = Untransmittable” or U=U, which means that people living with HIV under effective ART and on effective VL suppression ( i.e. undetectable VL, <50 copies/mL) for at least 6 months, do not infect their partners. This is now based on more than 10 years of accumulating scientific evidence and has been endorsed by more than 350 HIV organisations worldwide including the International AIDS Society (IAS), UNAIDS and the British HIV Association (BHIVA).

Proof of the concept was already published in 2008 with the Swiss Statement reviewing more than 25 studies and finding an estimated risk of transmission of less than 1 in 100,000 (0.001%) for those couples whose HIV positive partner was on suppressive cART (Vernazza et al. 2008).

Further evidence came in 2011 with results from the HPTN 052 study on more than 1,700 heterosexual couples, showing significant advantage of early cART initiation in reducing rates of sexual transmission and clinical events, with 39 HIV transmissions, 28 virologically linked to the infected partner and only one occurring in the early-initiation group, with an incidence rate of 0.1 per 100 person-years in the early treatment group (95% CI 0.0, 0.4) and 1.7 per 100 person-years in the delayed-treatment group (95% CI 1.1, 2.5), with an HR of 0.04 (95% CI, 0.01,0.27; $p$<0.001) (Cohen et al. 2011).

These findings were then confirmed after four more years of follow-up showing how early ART initiation was associated with a 93% lower risk of linked partner infection than in those delaying ART (HR, 0.07; 95% CI, 0.02, 0.22) (Cohen et al. 2016).

Following this study, others reported zero transmissions for serodiscordant couples who had sex without condoms when VL was undetectable (i.e. VL<200 copies/ml), including the PARTNER study on heterosexual couples (95%CI 0.0-0.30 per 100 person-years) (Rodger et al. 2016); the Opposites Attract study on gay male couples in Australia, Thailand and Brazil (upper CI limit of 1·59 per 100 couple-years of follow-up for transmission rate) (Bavinton et al. 2018); and the PARTNER2 study, an extension of the PARTNER study in gay male couples (upper 95% CI 0·23 per 100 couple-years of follow-up) (Rodger et al. 2019).

In UK and Ireland, over 95% of PLWH have acquired the virus through sex without a condom, and according to recent reports, mostly at risk and primarily affected remain gay, bisexual and men who have sex with men. New HIV diagnoses, acquired by heterosexual sex have decreased in both Black African and Black Caribbean heterosexuals, from 2,424 in 2008 to 542 in 2017 and from 231 to 52, respectively (PHE 2018).

Injecting drug use (IDU) accounts for a high proportion of infections in settings with limited or insufficient access to harm reduction services and high prevalence of IDU, including Eastern Europe and Central Asia, North Africa, the Middle East, and many parts of Asia (Stone 2016). Eastern Europe and Central Asia had a significant increase of new HIV infections over time with over 57% rise between 2010 and 2015 (United Nation 2016, Larney et al. 2017). Eastern and South-Eastern Europe, and particularly Russia and Ukraine, have the highest prevalence of IDU in the world at 1.27% versus the global rate of 0.25%, among people aged 15-64 years (Bailey et al. 2017), which was originally a driving force in the HIV epidemic in this region. In the UK, HIV diagnoses among people who inject drugs has a steady low rate, accounting for around 4% of all cases ever diagnosed in the UK, since the beginning of the epidemic (PHE 2018).

## 1.3 HIV in pregnancy: epidemiology and vertical transmission

### 1.3.1 Epidemiology of HIV in pregnancy

According to data reported from the UNAIDS (UNAIDS 2017), around 1.4 million HIV positive women become pregnant every year and about 1 million receive a combination of ARV agents to prevent vertical transmission (VT) and for treatment to prevent disease progression (Siemieniuk et al. 2017). Latest data on pregnant and breastfeeding women report an 82% coverage with ARVs to prevent VT.

In the UK and Ireland, according to the National Surveillance of HIV in Pregnancy and Childhood (NSHPC) there are around 35,000 women living with HIV (WLHIV), with 1,200 becoming pregnant every year (Peters 2018). In the UK and Ireland between 2015 and 2016 a record low VT rate of 0.28% (95% CI 0.08%, 0.715%) was reported, with only four transmissions among the 1,438 infants born to diagnosed HIV women (Figure 1.5) (Peters 2018). This is the lowest rate of VT across the European region, followed by Denmark with 0.5% and France with 0.7 % (von Linstow et al. 2010, Aebi-Popp et al. 2013, Mandelbrot et al. 2015).

Success in reducing VT requires prompt identification of pregnant women with HIV infection, and the very low VT rates in Europe have been facilitated by high uptake of antenatal HIV screening (e.g. currently estimated at 99% of all pregnant women in the UK). Awareness of HIV status enables prompt treatment and appropriate obstetric management. Another key factor behind the VT declines in the UK and other European countries is the increasing proportion of pregnant women who conceived whilst on ART; in the UK, this has increased from 19% (512/2,717) for the period of 2000-2003 to 65% (2,193/3,366) for 2013-2015 (Sconza 2017), and since 2015, 80% of births are to women who were taking ART at conception (NSHPC 2019). This trend, alongside earlier treatment starts among women diagnosed in pregnancy, means that a very large proportion of women have now reached the goal of effective suppressive VL at the time of delivery, estimated to be at 93% in 2015-16 in the UK (Peters 2018).

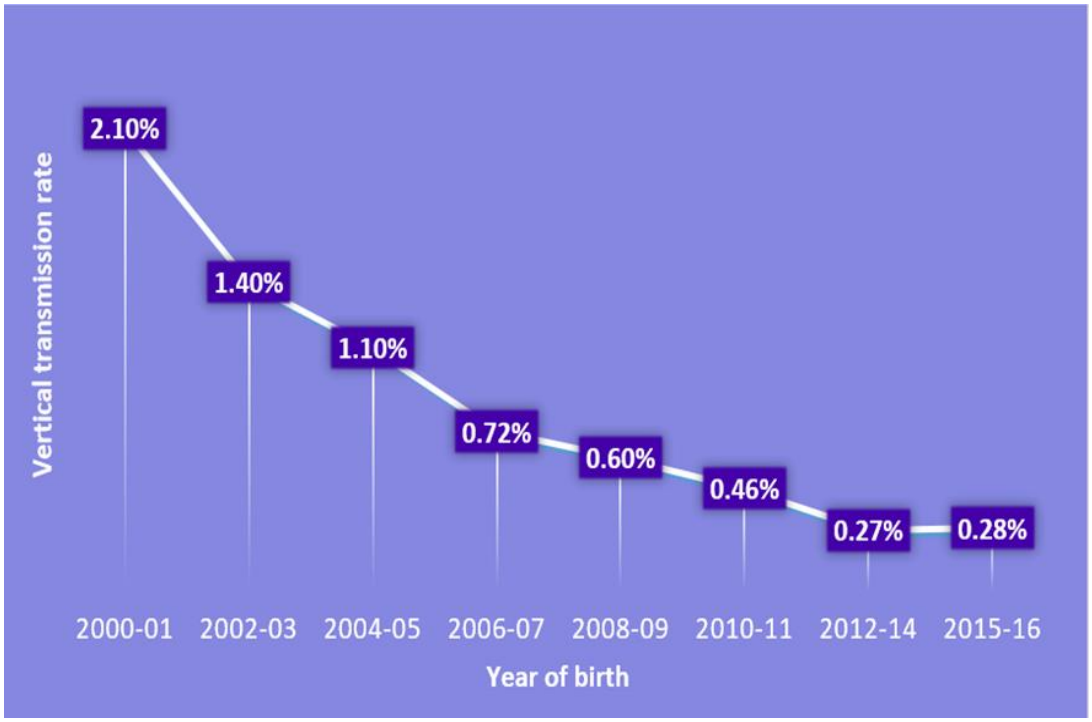



**Figure 1.5 Trends of VT among HIV diagnosed women, UK and Ireland, 2000-16**

Source: data from the NSHPC

### 1.3.2 Vertical transmission: mechanism and timing

VT can take place during pregnancy, intrapartum and postnatally through breastfeeding. VT remains an important means of HIV transmission (the most important route of acquisition for children) and without any intervention, it is estimated that approximately 15 to 45% children will acquire the virus from their mothers (Newell et al. 1993, Stevens et al. 2014, UNAIDS 2017); the VT rate in untreated breastfeeding women in LMICs was estimated at 25 to 45%, being somewhat lower in untreated non-breastfeeding women in resource-rich settings, at 15 to 30% (De Cock et al. 2000).

During pregnancy, or *in utero* transmission mainly depends on maternal VL plasma levels and fetal exposure to cell-free-HIV in the amniotic fluid. HIV infects certain cells within the placenta and it is whether or not the placenta is infected that determines whether or not the embryo becomes eventually infected, most likely explaining the low rates of *in utero* HIV transmission (Miller et al. 1998, Miller 2018). *In utero* transmission can also depend on other placenta-related risk factors such as chorioamnionitis – an infection of the fetal membranes (amnion and chorion) or can occur in more advanced stages of infection when VL is high and CD4 cell counts is low (Newell et al. 1993, Thorne et al. 2003a).

*Intra-partum* transmission can be the result of micro-transfusions between mother and fetal blood during uterine contractions or follow ascending infection from the vagina and cervix to the amniotic fluid because of premature or prolonged membranes rupture. Data on premature rupture of membranes (PROM) are conflicting and differ between pre-cART and cART era. Pre-cART studies mostly suggested an increasing risk of VT in relation to duration of PROM (Landesman et al. 1996, International Perinatal 2001), while results from cART-era showed the risk to be significantly related to maternal VL at delivery, rather than duration of PROM (Cotter et al. 2012, Mark et al. 2012). Furthermore, results from a large UK-based study looking at 2,116 pregnancies from 2007-12 and defining undetectable VL as less than 50 copies/mL, showed no difference in VT rates in relation to time of rupture of membranes (Peters et al. 2016). However, direct exposure to mother's blood and genital secretions during labour and through birth canal passage is believed to be the main mechanism (Thorne et al. 2003a, Kourtis et al. 2006). In non-breastfeeding populations most transmission takes place around the time of delivery.

Finally, *postnatal* transmission can occur through breast milk at any point during lactation and the cumulative probability of HIV acquisition increases with the duration of breastfeeding; studies report an overall double risk of VT if breastfeeding is

prolonged and the risk to last as long as breastfeeding continues (Breastfeeding HIV International Transmission Study Group et al. 2004, Horvath et al. 2009). This route was first highlighted by cases of infection in breastfed infants of women who had acquired the virus postnatally through transfusion or heterosexual routes (Hira et al. 1990). Evidence of reduced transmission through formula feeding came only in 2000, when Nduati and colleagues performed a clinical trial showing a 37% rate of VT in infants randomised to breastfeeding versus 20% in those randomised to formula feeding at 24 months in an African population not receiving ART (Nduati et al. 2000).

Timing of HIV transmission in non-breastfeeding settings prior to availability of cART, was defined as *in utero* infection when a virological test in an exposed infant within 72 hours from birth was positive and as *peripartum* if a virological test were negative within the first 72 hours after birth but then positive after 4-6 weeks of age (Mofenson 1997).

Maternal risk factors for VT include plasma VL, CD4 cell count and the stage of the infection (Nduati et al. 2000, Castilla et al. 2005). Maternal VL is considered the best individual predictor of VT risk with the risk increasing linearly with the level of maternal plasma viraemia (Newell et al. 1996, Cooper et al. 2002, Castilla et al. 2005, Palmisano et al. 2011). Proof that high VL values significantly increase the risk of transmission from mother to infant came from a large US-based prospective cohort study evaluating 800 mother-infant pairs (Garcia et al. 1999). The study reported no transmissions among 57 women with <1000 copies/mL, a VT rate of 16.6% among women with VL between 1000-10,000 copies/mL (32/193), 21.3% for those with 10,001-50,000 copies/mL (39 of 183), 30.9% for those with 50,001-100,000 copies/mL (17/54), and 40.6% among women with >100,000 copies/mL (26/64), $p<0.001$. The highest rate of transmission occurred among women with VL >100,000 copies/mL who did not receive ZDV (63.3%, 19/30 women) (Garcia et al. 1999). However, this and other studies could only prove and predict the risk of HIV transmission in relation to maternal viremia but not the VL threshold below which VT never occurs, which is why U=U seems not to be applicable to VT (The European Collaborative Study 1999, Ioannidis et al. 2001, Waitt et al. 2018).

### 1.3.3 Prevention of vertical transmission

*Overview*

Interventions to reduce VT mostly target the three phases at which VT can occur, namely during pregnancy, at the time of delivery and during breastfeeding. Prevention of VT consists of a range of different interventions including prevention of HIV infection among women of reproductive age (i.e. educational material and contraception); prevention of unplanned pregnancies among WLWH (i.e. family planning and reproductive health services) and prevention of transmission by providing lifelong ART (WHO and IATT 2007). Knowledge of HIV status and consequent timely engagement with antenatal care is an important part of the preventive measures and therefore antenatal screening programmes and HIV testing have become an important tool to prevent VT.

*Mode of delivery*

Elective caesarean section (CS) is another preventive measure, which was the recommended mode of delivery for pregnant WLHW in the pre-cART era in resource-rich settings, reflecting both observational and clinical trial evidence of reduced risk of VT compared with natural vaginal delivery. The European Collaborative Study, a prospective cohort study on 373 mothers-infants pairs was the first to show association of lower risk of VT in women delivering by elective CS, with 5% (4/83) of the infants delivered by elective CS infected vs 20% (52/264) of those delivered vaginally or by emergency CS ($p<0.001$) (The European Collaborative Study 1999).

Additional evidence supporting the protective role of elective CS came from an international RCT (European Mode of Delivery 1999) enrolling 436 women, who were then randomised to vaginal natural delivery or elective CS; results reported three infected (1.8%, 3/170) infants born to women in the CS group compared with 21 (10.5%, 21/200) born to women in the vaginal delivery group ($p<0.001$), with elective CS lowering the risk of VT by 80% (multivariate OR 0.2, 95% CI 0.1-0.6) (European Mode of Delivery 1999).

Further, a trans-Atlantic meta-analysis on 15 prospective cohort studies included more than 8,533 mother-infant pairs and through fitting logistic regression models including mode of delivery and adjusting for ART use, advanced maternal disease and low birth weight, found elective CS to be strongly associated with lower risk of VT (OR 0.43, 95%CI 0.33-0.56) (International Perinatal et al. 1999). This association remained regardless of ART administration (transmission rate in the elective CS without ART group was 10.4% (95%CI 7.8-12.9%) vs 19.0% (95%CI 17.9-20.0%) in

the group with other modes of delivery and without ART (International Perinatal et al. 1999).

However, most of these studies were conducted in the pre-cART-era with limited data on maternal VL, while results from studies in the cART-era and from resource-rich settings have showed no evidence of additional protection from elective CS against VT in women with effective suppression of VL prior to delivery (Townsend et al. 2008a, Briand et al. 2013, Townsend et al. 2014, Kennedy et al. 2017). Furthermore, two UK-based analyses reported no difference in VT rates among women on cART when comparing elective CS vs planned natural vaginal delivery (Townsend 2008, 2014). The first analysis based on deliveries between 2000 and 2006, reported no statistically significant difference between elective CS (0.7%, 17/2286) and planned vaginal delivery (0.7%, 4/559; AOR=1.24, 95% CI: 0.34–4.52, *p*=0.746, adjusted for sex and VL) (Townsend et al. 2008a). The second analysis reported an overall transmission rate in women with undetectable VL (<50 copies/mL) of 0.09% with no significant difference between elective CS and planned natural vaginal delivery (0.11% vs 0.15%, *p*=0.53), however with limitations due to lack of statistical power (Townsend et al. 2014).

*Antiretroviral drugs to prevent VT*

Up to the late 1990s standard of care in western Europe and USA were to follow the Pediatric AIDS Clinical Trials Group 076 (PACTG076) protocol regimen. This consisted of an oral fixed dose of ZDV during pregnancy, followed by an intravenous infusion in labour and then administration of ZDV to the neonate for his/her first 6 weeks of life (Connor et al. 1994). The PACTG 076 trial results were published in 1994. This US-France based double-blind, placebo-controlled RCT assessed the safety and efficacy of ZDV for the prevention of VT. Pregnant women HIV diagnosed were enrolled between 14 to 34 weeks of gestation with CD4 cell counts >200 cell/mm$^3$ with no indication for ART. Primary analysis of efficacy evaluated 409 mother-infant pairs and estimated the proportion of infants infected to be 8.3% (95% CI 18.4-32.5%) in the ZDV group and 25.5% (95% CI 18.4-32.5%) in the placebo group. The estimated absolute difference between the two groups who were infected was 17.2% (95%m CI 8.9-25.5%), corresponding to a 67.5% (95% CI 40.7-82.1%) relative reduction in risk of VT (Connor et al. 1994). The combination of ZDV and elective CS was shown to reduce VT rates further, e.g. to 2% (95 % CI 0.1- 4.0%) in the international meta-analysis (International Perinatal et al. 1999) and to less than 1% regardless of maternal VL levels in the European Mode of Delivery trial (European Mode of Delivery 1999). The efficacy of ZDV to reduce VT was subsequently demonstrated in several other large RCTs investigating short course regimens.

For example, one RCT in Thailand showed a 50% reduction in VT when ZDV was started at week 36 without a neonatal component in non-breastfeeding populations (9.4%, 95% CI 5.2-13.5% in the ZDV arm vs 18.4%, 95% CI 13.2-24.2% in placebo arm, p=0.006) and falling to 30% with a similar regimen in breast-fed babies (Shaffer et al. 1999). These approaches were mostly adopted in LMICs, where the PACTG076 protocol could not be used mostly due to its complexity and cost (Shaffer et al. 1999, Lallemant et al. 2000).

Following the widespread use of cART in resource-rich settings, VT rates decreased even further, given ART's ability to decrease VL to undetectable plasma levels. Although not many clinical trials of cART for prevention of VT have been conducted, studies both in Europe and USA have demonstrated ARVs to be highly effective in preventing VT (Calmy et al. 2007). The Women and Infant Transmission Study in the United States, for example, reported VT at 20% (95% CI 16.1%-23.9%) for women not receiving antenatal ART compared to 10.4% (95% CI 8.2%-12.6%) for those on ZDV monotherapy and 1.2% (95% CI 0-2.5%) for those on cART in an analysis of more than 1,500 women (Cooper et al. 2002).

Meanwhile, in Europe following widespread use of antenatal cART, rates of VT decreased from 5.1% (95% CI 3.02%-7.87%) in 1997-98 to 0.99% (95% CI 0.32%-2.30%) in 2001-2003 (European Collaborative 2005). This reduction was significantly associated with maternal cART use and with time of cART initiation, with transmission occurring in 11.5% (18/157) of the untreated mothers compared with 1.20% (11/918) of those receiving cART ($p$<0.001). Further reductions in VT risk were seen in women initiating cART before pregnancy (1/397, 0.25%) compared with those starting cART during pregnancy (10/521, 1.92%, p=0.02)(European Collaborative 2005).

Both longer duration and earlier initiation of cART and their association with reduced rates of VT were also reported from two European studies. Data from both the NSHPC and the ANRS French Perinatal Cohort reported a significantly increased risk of VT associated with late start of cART in pregnancy compared with earlier start (Warszawski et al. 2008, Townsend et al. 2014). Both studies reported a VT probability declining rapidly during the first 9 weeks after starting cART, reaching about 1% at 9 weeks duration, then declining more slowly, levelling off at around 0.5% after 13 weeks (Warszawski et al. 2008, Townsend et al. 2014). In more recent years, the Promoting Maternal and Infant Survival Everywhere (PROMISE) trial demonstrated the superiority of maternal cART initiation in pregnancy over ZDV-monotherapy in immunocompetent women (high CD4 count) (Fowler et al. 2016). This RCT compared efficacy and safety of various ART regimens for prevention of VT by enrolling pregnant women with CD4 cell counts ≥350 cells/mm$^3$ (or country specific

threshold for treatment) and then randomly allocated women to three groups: ZDV alone (ZDV plus single-dose of NVP at onset of labour, followed by postnatal "tail" of TDF/FTC for 6-14 days); ZDV-based cART (ZDV/3TC +LPV/r during third trimester); TDF-based cART (TDF/FTC+LPV/r during third trimester). The rate of transmission was significantly lower with ART-based combinations than with ZDV alone (0.5% in the combined ART groups vs.1.8%) (Fowler et al. 2016).

**Policies to prevent VT, with a focus on UK**

Effective prevention of VT also comprises the development of clinical guidelines for the management of pregnant WLWH and their infants. Across Europe, countries have developed national guidelines, usually tailored to the country's specific needs and capacities (i.e. human and economic). Widespread use of cART, knowledge about its beneficial effect on viral suppression and consequent significant reduction in VT rate with undetectable VL, contributed to changes in recommendation over the past decades.

A 2012 study surveyed 25 European countries (chosen to represent all Europe) about temporal and geographical patterns of antenatal ART and mode of delivery and identified international variability in national VT prevention guidelines (Aebi-Popp et al. 2013). For example, in the early 2000s in the UK, 'opt out' antenatal HIV testing was introduced to increase ascertainment of HIV in pregnancy, with all women booking for antenatal care receiving HIV screening unless opting out. This replaced the opt-in system, which resulted in missed opportunities for identifying pregnant women with HIV (Tookey et al. 1998); women who declined the first time should be offered the test again with more time for discussion. Women at high risk of infection, such as those with an HIV-positive partner are offered repeated testing later in pregnancy (Bull et al. 2015).

Regarding mode of delivery, to date elective CS is recommended for those with a VL> 400 copies/mL at 36 weeks, while with a VL between 50-399 copies/mL, CS can still be considered, when accounting for the actual VL trajectory alongside the treatment. Nowadays, whenever VL is <50 copies/ml a planned vaginal delivery should recommended in the absence of obstetric contraindications (BHIVA 2019a).

Concerning breastfeeding, the recommended approach in the UK is abstinence, regardless of VL levels; this is in line with other HIC guidelines, recommending exclusive formula feeding regardless of maternal VL or therapy, since even with sustained undetectable VL, the risk of VT is low but not zero (Waitt et al. 2018, Prendergast et al. 2019). However, the current BHIVA guidelines support women choosing to breastfeed if they are virologically suppressed and have good adherence, recommending a monthly review of VL during breastfeeding and two months after stopping breastfeeding, for both mother and infant (BHIVA 2019a).

## 1.4 Pregnancy: general treatment consideration, including toxicity

*Overview*

There are some specific issues around the provision of any kind of medication in pregnancy that require careful consideration. A key issue relates to the natural changes that a pregnant body undergoes over gestation, particularly the metabolic processes, the immunological alterations and cardiovascular changes enabling the development of the placenta. An important prerequisite for successful maintenance of pregnancy, for example, include changes in the maternal immune system response to prevent foetus rejection. To do so, changes in the T-cell helper Th1 and Th2 populations, important in the immune response modulation, need to occur. Specifically, a shift in the cellular immune response ratio Th1/Th2 to Th2>Th1, meaning a down-regulation of Th1 and up-regulation of Th2 response is needed; if this shift fails it can compromise normal pregnancy development (Wegmann et al. 1993, Sykes et al. 2012).

During pregnancy, the majority of organs and systems are affected by both anatomical and physiological changes, in particular, changes in fat distribution, delayed gastric emptying, prolongation of gastrointestinal transit time, reorganisation of water-compartments and consequent haemodilution that can alter drug pharmacokinetics and pharmacodynamics and drug bioavailability (Mirochnick 2000, Pacheco 2013). These changes can affect drug absorption, distribution, metabolism, excretion and placental transfer (Loebstein et al. 1997, Pacheco 2013, Costantine 2014). For further information on the general concept of pharmacokinetics and pharmacodynamics, see chapter 2 section 2.3.

### 1.4.1 Pharmacokinetics in pregnancy

Some of the pregnancy-induced pharmacokinetic changes in medications are the result of expression of specific metabolising enzymes, their transporters and transcriptional regulators (Costantine 2014). For example, as a result of the reduction of plasma albumin and alpha-1-acid glycoprotein concentrations a decrease in plasma protein binding can occur; decreased protein-binding means that higher concentrations of free drug are available, favouring more distribution to tissues and consequently reduced drug plasma concentration (Feghali et al. 2015).

Increase of cardiac output, ventilation rates and blood flow to organs such as the liver and kidneys can increase the clearance and distribution of drugs (usually as a direct

result of expanded blood volume). Changes in liver enzyme activity have also been observed in pregnant women and can result in changes in drug metabolism and drug-drug interaction (Feghali et al. 2015, Illamola et al. 2018). For example, studies have shown how the superfamily of enzymes cytochrome P450 (CYPP450) are involved in the first phase of hepatic drug metabolism, where activities of some of these enzymes (i.e. CYP3A4) may be increased during pregnancy leading to increased metabolism of drugs such as nifedipine (a calcium channel blocker drug to treat high blood pressure) or indinavir (Feghali et al. 2015, Illamola et al. 2018).

Further, gestational-specific changes can alter bioavailability and volume distribution of drugs, mostly as the result of the expanded uterine perfusion and development of the feto-placental compartment. These can act as additional compartments, leading to increased drug accumulation and apparent increase in volume of distribution (Feghali et al. 2015). Placental permissiveness to medicines and mechanisms by which substances can cross the placenta can also influence the extent to which medicines might potentially affect a normal embryo-fetal development (Illamola et al. 2018).

Therefore, because of these pharmacologic alterations, whenever medicines are administered in pregnancy, potential adjustments in dose, timing and duration of treatment should be considered and are important concepts to understand why some drugs, including ARVs, might not be as effective as expected in pregnancy (Loebstein et al. 1997, Pacheco 2013, Costantine 2014, Illamola et al. 2018).

### 1.4.2 Teratogenicity and congenital anomalies

*Teratogenicity*

Teratogenicity has been defined as a toxicity caused "*by any substance, agent or process that can interfere with normal prenatal development, causing the formation of developmental abnormalities of the embryo or foetus* (DIA) (Tassinari 2015). In the early 19$^{th}$ century, the uterus was believed to be an impenetrable barrier protecting the developing embryo from any external threat, hence teratogenic effects were thought to be attributable to genetic variations (Wilson 1977). This theory changed when in the early 1940s an Australian ophthalmologist, Norman Gregg, established a connection between maternal rubella infection in pregnancy and the triad of cataracts, heart malformations and deafness (Gregg 1991). Furthermore, following several animal studies detecting other teratogens, such as the chemotherapy agents' nitrogen mustards, the idea of an environmental aetiology for teratogenicity took hold.

Environmental agents such as drugs, viruses and physical/chemical elements, upon contact with the developing embryo can cause functional or morphological changes. These were addressed in the late 1980s by teratologist Thomas Shepard and criteria for the definition of human teratogens were established on the basis of three pillars: the exposed parent-child pair, the chemical and biological effects of the agent and the identification of specific exposure syndromes (Shepard 1982). These are now known as Shepard's seven criteria to establish human teratogenicity (Shepard 1994) (Table 1.5).

However, it was with the thalidomide catastrophe that a further step was taken – both in understanding that the placenta was not an impenetrable barrier and that teratogenesis did indeed have an environmental component to its aetiology (Fraser 1988). Placenta studies have since progressed and many important discoveries on placental mechanisms and function have been made. For example, alterations in the physiological placentation are now a recognised factor for the development of preeclampsia – a pregnancy-induced high blood pressure condition (Brosens et al. 1970, Fisher 2015). Nowadays the multifactorial aetiology of birth defects is widely accepted including both environmental and genetic elements (Miller 2018).

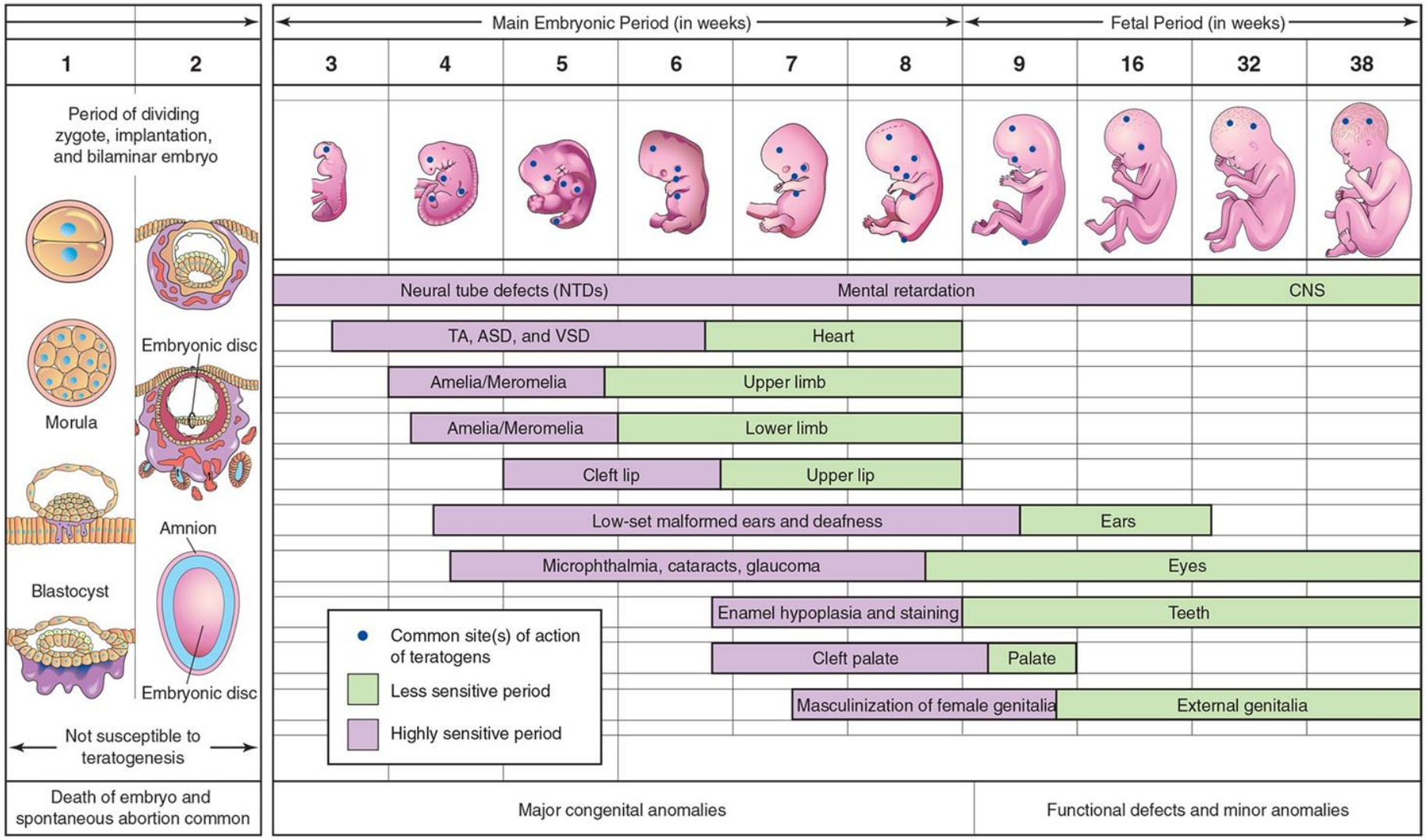
Teratogenicity is strictly related to *time of exposure,* time at which a teratogen can exert its action on embryological development (Moore et al. 2015, Bleyl et al. 2017). A normal human pregnancy usually lasts 40 to 42 weeks divided into three trimesters, each characterized by fundamental embryological growth steps, with some more critical than others (Figure 1.6) (Scheuerle et al. 2016).

## Table 1.5 Shepard's criteria for proof of teratogenicity in humans

| | Amalgamation of criteria for proof of human teratogenicity[1] |
|---|---|
| **1** | Proven exposure to agent at critical time(s) in prenatal development (prescriptions, physicians' records, dates) |
| **2** | Consistent findings by two or more epidemiologic studies of high quality:<br>**a)** control of confounding factors,<br>**b)** sufficient numbers,<br>**c)** exclusion of positive and negative bias factors,<br>**d)** prospective studies, if possible, and<br>**e)** relative risk of six or more (?) |
| **3** | Careful delineation of the clinical cases. A specific defect or syndrome, if present, is very helpful |
| **4** | Rare environmental exposure associated with rare defect. Probably three or more cases (e.g. oral anticoagulants and nasal hypoplasia) |
| **5** | Teratogenicity in experimental animals important but not essential |
| **6** | The association should make biological sense |
| **7** | Proof in an experimental system that the agent acts in an unaltered state. Important information for prevention |

[1]Items 1-3 or 1,3 and 4 are essential criteria. Items 5-7 are helpful but not essential

Adapted from the original article (Shepard 1994) and (Shepard 1986, 1988, 1992)




Reproduced with permission from Moore, T.V.N et al., Before we were born: essentials of embryology and birth defects, 2020 (Moore et al. 2020)

**Figure 1.6 Teratogens and timing of their effect on embryo-fetal development**

These critical phases consequently have a greater susceptibility to genetic mistakes and potential toxic effects of exogenous substances such as medicines. During the pre-implantation period, teratogenic effects might result in an “all or nothing” effect because at this stage zygotes and blastocysts (the future embryo) contain omnipotent stem cells without any differentiation, hence the exposure is either sufficient to kill the embryo or the embryo survives, implants and proceeds to normal structural development (Finnell 1999). Disruption of the very early developmental stages in the majority of cases thus results in miscarriage often so early that a woman might not even be aware of her status. Disruption within early stages of morphogenesis and organogenesis – the *embryonic period*, often results in major structural anomalies, given that most structural growth happens in the first 8 weeks. Disruption during the *fetal period*, where the majority of fetal growth occurs, usually results in anomalies of organ differentiation, growth and function, such as ovarian cysts and cataracts (Scheuerle et al. 2016)

Maternal diabetes and maternal alcohol consumption can better explain this concept (Bleyl et al. 2017). If diabetes arises early in pregnancy – known as gestational diabetes – it is associated with a wide range of structural birth defects, mostly affecting a normal development of neural-tube, heart (mostly septal) and renal structures, while late-onset diabetes usually result in macrosomia and neonatal hyperglycaemia. Similarly, maternal alcohol consumption causes distinctive structural patterns of brain and facial appearance, and growth alterations when exposure occurs within the first trimester, whereas it is mostly associated with impaired cognition and behavioural disorders when consumption occurs late in pregnancy (Bleyl et al. 2017).

Temporal association between exposure to a medication and presence of a specific defect depends on whether the exposure to the given medication preceded or coincided with the development of the specific organ/body part (and thus the defect in question) (Table 1.5, Figure 1.6). For example, if a medicine is started in second/ third trimesters (e.g. 20 gestational weeks) and a typical first trimester (forming) defect (e.g. neural tube defect) is detected, then the medicine cannot be said to have contributed to the defect, because the exposure occurred after the closure of the neural tube (typically by 28 days from conception) (Scheuerle et al. 2016). This is an important concept when balancing the benefit-risk ratio of medicines use in pregnancy.

*Congenital anomalies*

Congenital anomalies (CAs) or birth defects encompass a wide range of developmental disorders affecting the embryo and the foetus, defined by the WHO as "any structural or functional anomalies that occur during intrauterine life and can be identified prenatally, at birth or later in life"(WHO 2016a).

CAs are usually defined as *structural* and *functional*, where s*tructural* occur as alterations of critical development points of embryogenesis, especially during the first trimester (from conception to 8-12 weeks) and *functional* as consequence of underlying genetic defects or chromosomal abnormalities, carried by one or both parents or due to *de novo* mutations (Wellesley et al. 2005).

CAs can be also defined as isolated; part of a syndrome (i.e. when occurring with other CAs and with distinctive signs and symptoms); as a sequence (i.e. when a single known developmental defect causes a cascade of *subsequent* other CAs); or as an association, when two or more anomalies not pathogenetically related occur more frequently than expected by chance (Hersh et al. 2002, Jones et al. 2013).

Generally, the aetiology of CAs is thought to be multifactorial and mostly a consequence of complex interactions between genetic and environmental factors and in most cases (30 to 45%) of unknown origin (Czeizel 2005, Kumar 2008, Dolk et al. 2010, Sarkar et al. 2013). Genetic factors are estimated to account for about 10 to 30% of CAs and include chromosomal aberrations like Down's syndrome and Mendelian single-gene defects. Environmental factors (including maternal exposures to hazards) account for 5 to 10% and multifactorial inheritance for about 20 to 30%. Multifactorial inheritance is defined as the combination of genetic and environmental factors, where a combination of genetic profiles of both parents, embryo and environmental factors during preconception and early gestation produce the condition/malformation (Kumar 2008, Feldkamp et al. 2017).

For some CAs, progress in understanding the genetic contribution has been made, for example the role of microdeletions (i.e. deletion 22q11) in the development of certain heart defects and cleft palate or how novel single gene mutations (i.e. CHD7 mutations) contribute to the development of the CHARGE syndrome (acronym for Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and development and Ear abnormalities and deafness) (Amati et al. 1995, Takahashi et al. 1995, Vissers et al. 2004).

Similarly, for common isolated CAs such as hypospadias, environmental factors acting as triggers on polygenic liability (predisposition) is a recognised aetiology (Czeizel 2005). Recently, the Utah Birth Defect Network, a population-based case

cohort study collected data on 5,504 infants with major CAs (overall prevalence 2.03%, 5,504/27,0878 total births, livebirths and stillbirths) from 2005-2009 and reported an unknown aetiology for the majority (80%) of cases (n=4390) with only a fifth having known aetiology (Feldkamp et al. 2017). Of the 1,114 cases with known aetiology, 90% were of chromosomal or genetic origin (namely the three common trisomies [21, 18, 13], Turner syndrome, structural chromosomal abnormalities, and single gene disorders) with 4.1% due to teratogens (mostly poorly controlled pregestational diabetes) and 1.4% due to twinning (conjoined or a-cardiac) (Feldkamp et al. 2017).

Environmental factors affecting pregnant women include chemical pollutants, dietary imbalance, ionizing radiation, exposure to teratogenic medications, and socioeconomic and demographic factors (Rasmussen et al. 2009). In addition, infections such as rubella, cytomegalovirus and zika virus; smoking and alcohol consumption; maternal pre-existing conditions (e.g. diabetes and hypertension) and pregnancy-associated conditions (e.g. gestational diabetes, preeclampsia) are all considered contributing factors in the development of CAs (Lechat et al. 1993, Reefhuis et al. 2004, Rasmussen et al. 2009).

According to latest WHO estimates, 303,000 newborns die annually within the first four weeks of birth because of CAs and worldwide every year 3-6% infants are born with CAs (Dolk et al. 2010, WHO 2016a). Prevalence differs between HICs and LMICs, with more than 90% of CAs occurring in LMICs, presumably due to most deliveries occurring there, and lack of screening and in utero detection (Sitkin et al. 2015, Lanzoni et al. 2017).

In Europe, approximately one in 40 pregnancies (2.5%) of the annual 5.2 million births have a CA: 80% in liveborn infants (2.5% died in the first week of life), 2.0% were stillbirths or fetal deaths from 20 week gestation and 17.6% of all cases were terminations of pregnancy for fetal anomaly (TOPFA) (Dolk et al. 2010). Antenatal screening and consequent early detection of birth defects are increasing thanks to screening programmes and improvements in technologies, allowing women to have diagnosis at early stages. For example, in 2017, the timing of diagnosis of CA in the UK was known for most of the affected infants (91.4%) and identification of defects occurred antenatally for 62.2% of these (NCARDRS 2017).

Congenital Heart Defects (CHD) are the most common birth defect, for example, in the UK they affect up to eight in every 1,000 infants and are a leading cause of CA-related deaths (Mendis et al. 2011). CHD include a range of structural defects of the heart and great vessels. Signs and symptoms vary based on the severity and the type of defect. The most common are septal defects(aperture between the wall normally

separating right and left heart), coarctation of aorta (narrowing of the major artery), and pulmonary valve stenosis (narrow opening of pulmonary valve). Some identified maternal risk factors include diabetes, increased body mass index, smoking and alcohol consumption and assisted reproductive technologies (Stothard et al. 2009, Liu et al. 2013).

According to the European Surveillance of Congenital Anomalies and Twins (EUROCAT), chromosomal anomalies contribute to 28% of stillbirths/fetal deaths from 20 GW and 48% of TOPFA, with a prevalence of 3.6 per 1,000 births (Dolk et al. 2010). Down's syndrome or trisomy 21 is the most common chromosomal anomaly accounting for 8% of all cases of CAs in Europe and with a worldwide prevalence of 10 per 10,000 livebirths (Weijerman et al. 2010). In UK the prevalence per 10,000 total births for Down's Syndrome was 28.3 (95% CI: 27.6-29.1) in the latest national report (NCARDRS 2017). Chromosomal disorders, and particularly Down's syndrome, are significantly related to advanced maternal age, with 90% of errors occurring during maternal oogenesis (Allen et al. 2009, Stothard et al. 2009).

From a public health perspective, CAs are often described as major and minor, where major anomalies identify those with a significant impact on life expectancy, physical and/or social capacities and usually require surgical intervention (Rasmussen et al. 2014). Examples of major CAs are cleft lip, gastroschisis, spina bifida, and some CHD, such as atrial and ventricular septal defects. Minor CAs are those of limited or no impact on health or on short- or long-term function and usually involve non-vital organs. Examples include single transverse palmar crease and fifth finger clinodactyly (Czeizel 2005, Rasmussen et al. 2014). Minor CAs are helpful for the diagnosis of syndromes of known aetiology, such as chromosome abnormalities or single gene disorders; for example, bilateral single transverse palmar creases are seen in more than 30% of Down's syndrome diagnoses (Rasmussen et al. 2014).

In the past CAs were classified as *lethal*, *severe* and *mild. Lethal* anomalies are those incompatible with life, usually ending in stillbirths, infant death or in termination of pregnancies (i.e. anencephaly). *Severe* are those requiring medical attention in order to avoid severe disability or death (i.e. some heart conditions such as Tetralogy of Fallot). *Mild* defects require medical intervention but their impact on life expectancy is minimal if the defect is corrected (e.g. undescended testis) (Czeizel et al. 1993, Czeizel 2005).

Most of the current classification system consider *lethal* and *severe* defects as *major* CAs and *mild* defects as *minor* CAs; others such as the EUROCAT systems- a European network of population-based registries for epidemiological surveillance of CAs do not consider most of the mild CAs to be classifiable as minor CAs (i.e. not

considered defect at all), unless they have a medical or cosmetic consequence (e.g. preauricular tags, low-set ears or sacral dimple are not considered minor CAs) (Czeizel 2005, EUROCAT 2014).

Prevention of CAs is possible through primary, secondary and tertiary prevention measures. Primary prevention consists of avoidance of the cause, for example by vaccinating for rubella. Secondary prevention comprises early detection and treatment; for example, undescended testis can be corrected with medications administered right after birth. Tertiary prevention involves early surgical intervention resulting in complete recovery, for example, from certain types of congenital cardiovascular malformations (Czeizel 2005).

## 1.5 HIV in pregnancy: adverse pregnancy outcomes

### 1.5.1 Adverse pregnancy outcome

*Overview*

Adverse pregnancy outcomes refer to a variety of unfavourable pregnancy events, with multifactorial aetiology and classification. In this review, adverse pregnancy outcomes describe preterm delivery; embryo/fetal growth alterations resulting in small for gestational age and low birth weight (LBW) infants; miscarriages and stillbirths. Definitions of such outcomes are reported in Table 1.6.

**Table 1.6 definitions of adverse perinatal outcomes**

| Outcome | Definition |
|---|---|
| **Preterm delivery (PTD)[1]** | Infants born alive before 37 completed GW (less than 259 days). There are sub-categories, based on gestational age: e*xtremely preterm*: born at less than 28GW; *very preterm*: 28-32GW; *moderate to late preterm*: 32-36 completed GW. |
| **Low birth weight (LBW)[1]** | Infants with a weight at birth of less than 2,500g, (up to (and including 2,499g) regardless of gestational age. Very-LBW: weight <1,500g; Extremely LBW: weight <1,000g |
| **Small for gestational age (SGA)[2]** | Infants born with a birth weight less than the 10$^{th}$ centile for gestational age |
| **Stillbirth (SB)[3]** | Infant born with no sign of life at or after 28 GW |
| **Miscarriage[4]** | Involuntary, spontaneous loss of pregnancy before 20-24 completed GW (depending on the country and classification criteria) |

GW: gestational week; 1(WHO 2010a); 2(de Onis et al. 1996); 3(WHO 2015b) 4(ACOG et al. 2018)

Table 1.7 reports the most common identified risk factors for adverse perinatal outcomes in the general population. For example, maternal age at both extremes (i.e. young and advanced age) has been associated with several of the presented adverse perinatal outcomes. Particularly, younger age and adolescent pregnancies have been associated with both LBW and PTD, while advanced age is associated with both SB and miscarriage (Goldenberg et al. 2008, Gibbs et al. 2012). A recent systematic review from a Norwegian study evaluated around 420,000 pregnancies and reported the risk of miscarriage according to maternal age, with this lowest in women aged 25-29 (10%), with the absolute lowest risk at age 27(9.5%), then rising almost linearly

after age 30, reaching 54% in women aged ≥45 years (Magnus et al. 2019). Similarly, advanced maternal age has been associated with the risk of stillbirth in a systematic review and meta-analysis on over 180,000 stillbirths, showing increased risk (OR 1.75, 95%CI 1.62-1.89) for women aged ≥35 compared with women aged <35, with a population attributable risk of 4.7% (Lean et al. 2017).

Miscarriages are estimated to affect one in four pregnancies and even though exact causes are not always identifiable, about half are associated with chromosomal aberration – either extra or missing chromosomes, and mostly due to random errors rather than inherited chromosomal abnormalities (Kroon et al. 2011). Low progesterone levels have also been associated with early pregnancy loss. Recently, data from two studies found evidence of medical benefits from progesterone administration in those women presenting with early pregnancy bleeding to prevent miscarriage (Coomarasamy et al. 2020). Both studies were large multicentre placebo-controlled trials, the PROgesterone in recurrent MIScarriage trial evaluating women with unexplained recurrent miscarriages and the PRISM trial (Progesterone In Spontaneous Miscarriage) evaluating women with early pregnancy bleeding. Results suggested supplementation of 400mg of progesterone twice-daily was useful to reduce the rate of miscarriage in subsequent pregnancies, with live birth rate of 72% (98/137) in the progesterone group vs 57% (85/148) in the placebo (95%CI 1.08–1.51; *p*=0.004) and estimated to prevent 8,450 miscarriages (Coomarasamy et al. 2020).

Outcomes such as miscarriage and stillbirths carry some intrinsic difficulties in their detection and monitoring. For example, estimates of miscarriage incidence might be limited by under-ascertainment of early and very early pregnancy losses, when a woman might not be yet engaged in prenatal care or might not be symptomatic or might misinterpret her symptoms for those of a late menstrual period (Lechat et al. 1993, Li et al. 2018). Challenges in the measurement of stillbirth include non-uniformity of classification systems – i.e. differences in definition of stillbirth and differences in classification systems for stillbirth causes, with more than 30 active classification systems making detection and collection and comparable national estimates quite a challenge (International Stillbirth Alliance et al. 2017, Alliance for Maternal Newborn 2018). Miscarriage and stillbirth might also occur because of CAs especially in early deaths. However, for early stillbirths, when a malformation might not be an obvious external one, a pathological examination to confirm the cause might be necessary, and consequently detection of such a malformation might depend on both the availability of a specialized foetal pathologist and the possibility to perform autopsies (Lechat et al. 1993)

## Table 1.7 Identified risk factors for perinatal adverse outcomes

| Outcome | Most frequently recognised risk factors | | | | | | |
|---|---|---|---|---|---|---|---|
| | Maternal age | Socioeconomic & nutrition status | Tobacco smoking | Alcohol & illicit drugs use | Diabetes | Anomalies in normal placentation | Others |
| **PTD** | ✓ | ✓ | ✓ | | ✓ | ✓ | Blood pressure, ART |
| **LBW** | ✓ | | ✓ | ✓ | | ✓ | Anemia, IUGR |
| **SGA** | | ✓ | ✓ | | | ✓ | Nulliparous |
| **SB** | ✓ | ✓ | ✓ | ✓ | ✓ | | Previous SB, PTD or SGA |
| **Miscarriage** | ✓ | | ✓ | ✓ | ✓ | | Low Progesterone, Thyroid disorders |

BMI: body max index; ART: Assisted Reproductive Therapies; IUGR: Intrauterine Growth Restriction.

### 1.5.2 Untreated HIV and adverse pregnancy outcomes

In pre-ART era, most adverse pregnancy outcomes in women living with HIV were the result of advanced maternal immunosuppression, high VL and disease progression / presence of opportunistic infections (Wedi et al. 2016, Zash et al. 2016a). Consequently, there was a significantly higher rate of adverse pregnancy outcomes particularly in LMIC (Wedi et al. 2016). Evidence of this has been reported from a systematic review and meta-analysis on studies published from 1980-2014, investigating the association between maternal HIV infection and eleven perinatal outcomes, namely PTD and very-PTD; LBW, very-LBW; term-LBW and preterm-LBW SGA and very-SGA; miscarriage, stillbirth and neonatal death (Wedi et al. 2016). The review included prospective and retrospective cohort studies and case-control studies and compared HIV-positive women naïve to ART and HIV-negative controls, including around 53,600 women from 35 studies (20 prospective and 12 retrospective cohort studies and 3 case-controls). The meta-analysis showed HIV infection to be strongly associated with increased risk of PTD in 14 prospective and eight retrospective cohort studies which included 34,337 pregnant women and showed a relative risk (RR) of 1.50 (95% CI 1.24-1.82) and a RR of 1.82 (95% CI 1.41-2.34), respectively (Wedi et al. 2016). In this meta-analysis, maternal HIV was also associated with LBW in 36,312 women from 16 prospective (RR 1.62 95% CI 1.41-1.86) and nine retrospective cohort studies (RR 1.93, 95%CI 1.48-2.52), showing a significant association (Wedi et al. 2016).

A further four prospective and three retrospective cohort studies reported on SGA risk, based on data from 14,315 women, with maternal HIV reported to be associated with increased SGA risk (RR 1.31 95%CI 1.14-1.51 and RR 2.08, 95%CI 1.26-3.46, respectively) (Wedi et al. 2016). Two studies in particular, both from SSA, reported a significant association between maternal HIV infection and stillbirth, with a RR of 1.67 (95%CI 1.05-2.66) (Bulterys et al. 1994, Ladner et al. 1998). The strongest evidence of such outcomes was identified in SSA with a RR of PTD 2.23, (95%CI 1.57-3.16) vs 1.00, (95%CI 0.56-1.81) in Europe from retrospective cohort studies (Wedi et al. 2016).

Furthermore, three prospective studies showed an increased risk of pregnancy adverse outcomes in relation to HIV progression, maternal immune status and adverse outcomes (Ryder et al. 1989, Temmerman et al. 1994, Coley et al. 2001). Ryder et al reported data from two hospitals in in Kinshasa, Zaire from 8,108-screened women and their infants. The study reported infants born to seropositive mothers to be more frequently premature, with LBW and higher neonatal mortality rates than those born to seronegative mothers (PTD:18% of women with AIDS symptoms vs 3%

seronegative, $p$<0.001; LBW: 33% of women with AIDS symptoms vs 10% seronegative, $p$<0.001; neonatal deaths: 6.2% vs. 1.2%, $p$<0.0001) (Ryder et al. 1989).

Temmerman et al, collected data from 1989 to 1991 in Kenya on 813 pregnant women (406 HIV-seropositive and 407 seronegative) enrolled before 28 weeks' gestation and followed up until 6 weeks after delivery. Results showed a strong association between maternal HIV infection and birthweight (with a median birthweight of 2913g for seropositive vs 3072g for seronegative mothers, $p$=0.0003), and with PTD (21.1% in seropositive vs 9.4% in seronegative, $p$<0.0001). Further, the study reported association between maternal immunological status and risk of PTD, which was higher for those women with CD4 cell percentages lower than 30% (26.3% vs 10.1%, $p$<0.001) (Temmerman et al. 1994).

In a prospective cohort study from Tanzania including 1,580 pregnant women (1,078 living with HIV vs 502 uninfected) enrolled between 12-27 weeks gestation, a significant higher risk of LBW and PTD was reported for pregnant women living with HIV and at WHO stage 2 or higher with a RR 2.29 (95%CI 1.34-3.92, $p$=0.03) for LBW and a RR of 1.93 (95%CI 1.35-2.77, $p$=0.0003) for PTD (Coley et al. 2001).

### 1.5.3 ART and adverse pregnancy outcomes

Pregnant WLWH are considered a special population, as they need to adhere fully to combined treatment for the entire duration of their pregnancies (and life-long) and many conceive whilst on treatment. This means both mother and their infants will be exposed to any potential drug's effect (beneficial or detrimental) throughout the entire pregnancy and fetal growth (Mitchell 2012).

As said in section 1.3.3 an increasing proportion of women now conceive while on treatment, with UNAIDS reporting an increase from an estimated 37% in 2009 to 77% in 2014 in 21 of the 22 countries most hit by HIV in 2009 (accounting for 90% of the global number of pregnant women living with HIV) (UNAIDS 2014, UNAIDS 2015, Zash et al. 2016a). In Italy, the number of women conceiving on ART regimen increased from 45% in 2010 to 58% in 2016 (Chiappini et al. 2018) and the UK this proportion rose from 60% in 20012-14 to 75% in 2015-16 according to the NSHPC, the national active surveillance in the UK (Peters 2018).

Similarly, in the US, the percentage of women conceiving on ART increased from 42% in 2012 to 54% in 2015 and in the same years, Botswana reported an increase from 27% to 50%,respectively (Zash et al. 2016a). This global shift mostly reflects the fact

that the majority of the 22 countries where 90% of the newly HIV diagnosed pregnant women live have adopted WHO's Option B+ (WHO 2015a).

As previously mentioned, exposure to medicines and time of exposure poses a risk for teratogenic and fetal developmental/growth toxicities. Following ART scale-up and longer durations on treatment, concerns as to whether this changing exposure to ART could increase the risk for toxic effects, particularly exposure during organogenesis, started to rise. Safe use of ART and limitations on data availability for pregnant women are addressed in chapter 2.

The first indications of adverse pregnancy outcomes being associated with use of ART in pregnancy were published in the late 1990s / early 2000s when observational studies in Europe noted high rates of PTD in women on cART (Lorenzi et al. 1998, European Collaborative et al. 2000). Accumulating evidence concerning ART exposure and increased risk of adverse perinatal outcomes subsequently suggested increased risk of certain adverse perinatal outcomes for those women initiating ART prior to conception (Chen et al. 2012, Zash et al. 2016a).

A systematic review and meta-analysis including 19,189 mother-infant pairs investigating safety and adverse perinatal outcomes related to ART showed a significant increased risk of adverse pregnancy outcomes for those women initiating ART prior to conception (Uthman et al. 2017). The review found women starting ART before conception to be significantly more likely to deliver preterm (pooled RR 1.20, 95%CI 1.01-1.44) or very-PTD (1.53, 95%CI 1.22-1.92) or to have LBW infants (1.30, 95%CI 1.04-1.62) than those who started ART in pregnancy. Higher rates of PTD were seen in LMIC (pooled RR 1.41, 95%CI 1.22−1.63) than those in HIC, but association was not significant (RR 0.89, 95%CI 0.54−1.47) (Uthman et al. 2017). Nevertheless, differences in background rates for such outcomes (greater in LMIC than in HIC), confounding factors such as lifestyle (i.e. substance, alcohol, tobacco use) and comorbidities should be considered when interpreting results (Hoffman et al. 2019).

*Preterm delivery*

Early European studies suggested association between maternal use of cART prior to conception and increased risk of PTD. Data from a pooled analysis of the European Collaborative Study and Swiss Mother and Child HIV cohort study of a total of 3,920 mother-infants pairs showed women on cART prior to pregnancy to be twice as likely to deliver preterm than those starting later in pregnancy (OR 2.17, 95%CI 1.03-4.58) (European Collaborative et al. 2000).

An updated analysis from the European Collaborative Study on 4,372 livebirths reported an association between cART and PTD of a similar magnitude (aOR 2.05, 95%CI 1.43- 2.95) (Thorne et al. 2004). In 2007 a meta-analysis of US and European data reported a similar association with a two-fold higher risk of PTD (OR 1.71, 95%CI 1.09-2.67) for those women starting ART before pregnancy or during first trimester compared with those starting ART later in pregnancy (Kourtis et al. 2007).

Following these studies, several others reported similar findings, and a growing literature reported increased risk of PTD with exposure to certain ART regimens when started prior to conception/first trimester, such as those containing boosted-PI. This included studies from SSA. The Mma Bana study, an RCT running between 2006 and 2008 in southern Botswana, randomised pregnant women with CD4 cell counts ≥200 cell/$mm^3$ to receive ABC/ZDV/3TC or LPV/r/ZDV/3TC, while women with CD4 cells count <200cell/$mm^3$ or with AIDS-related illness received the standard of care for Botswana (i.e. NVP/ZDV/3TC) (Shapiro et al. 2010). Women in the randomized groups started ART between 26-34 weeks' gestation, while the observational groups started between 18-24 weeks' gestation. The study reported an increased risk of PTD in the LPV/r arm (23%) compared to the arm receiving ABC/ZDV/3TC (15%, 95%CI for percentage-point difference, <1 to 16) (Shapiro et al. 2010).

A retrospective secondary analysis of the Mma Bana study data was performed among the 560 mother-infant pairs in the randomised treatment arms; higher rates of PTD were found among the 267 women in the PI group than in the 263 women in the NRTI group (21.4% vs 11.8%, $p$ =0.003), with PI-based cART being the most significant risk factor for PTD (OR 2.03, 95%CI 1.26–3.27, $p$ = 0.004) (Powis et al. 2011).

A US-based study also found an increased risk of PTD in women who initiated PI-based regimens prior to conception or during first trimester than those starting later in pregnancy (aOR 1.55, 95% CI 1.16–2.07, $p$= 0.003) (Watts et al. 2013). Meanwhile in Europe, a study from the French Perinatal Cohort study reported similar findings, with PTD more frequent in women taking Ritonavir-boosted PIs prior to conception compared with those starting ART later in pregnancy (aOR 1.31, 95%CI 1,11-1.55)

(Sibiude et al. 2012). UK data from the NSHPC evaluated 4,184 women on PIs-based ART regimens versus 1,889 on NNRTI-based regimens (mostly EFV and NVP) and showed an overall, increased risk of PTD in women conceiving on ART (10.4%, 629/6,073) and a particularly increased risk of PTD for women conceiving on LPV/r with low CD4 cells counts ≤ 350 cells/μL (aOR 1.99, 95%CI 1.02-3.85 vs NNRTI-based regimen) after adjusting for other factors associated with PTD (Favarato et al. 2018).

This is consistent with results from the PROMISE study, a RCT conducted in India and SSA evaluating both effectiveness and safe use of different ARV combinations initiated in pregnancy, which reported a statistically significant higher risk of PTD in the LPV/r+2 NRTIs arm compared with the monotherapy with ZDV + single dose of NVP arm (20.5% vs. 13.1%, $p<0.001$) (Fowler et al. 2016); TDF-based ART was associated with higher rates of very-PTD (<34 weeks) than ZDV-based ART (6.0% vs. 2.6%, $p=0.04$)(Fowler et al. 2016).

Two large studies from Tanzania and from Botswana reported greater risk associated with pre-conception initiation, with respect to NNRTI-based regimens (Chen et al. 2012, Li et al. 2016). Li et al reported data from a prospective observational study conducted at 10 HIV centres in Dar es Salaam, Tanzania and found, after adjusting for maternal CD4 cell counts and other potential covariates (e.g. age and hypertension), an increased risk of PTD for those women starting NVP or EFV-based ART regimens before pregnancy (univariate RR 1.40, 95%CI 1.23-1.59) compared to those starting ART during pregnancy (RR 0.95, 95%CI 0.80-1.12) (Li et al. 2016). Chen et al reported from a prospective observational study in Botswana, higher odds for PTD among women starting a NVP-based regimen prior to conception compared with those receiving ZDV-monotherapy (aOR 1.2, 95%CI 1.1-1.4) (Chen et al. 2012). Another and more recent multicentre retrospective study on 1,663 pregnancies from Ethiopia found a greater risk for PTD in those pregnancies exposed to NVP-based regimens when compared to EFV-based ART (aOR 1.44, 95%CI 1.06-1.96) (Ejigu et al. 2019).

*Low birth weight, small for gestational age*

Data on the associations between ART and birth weight are conflicting for both LBW and SGA. Results from Uthman and colleagues' systematic review found women who started ART before conception to be 30% more likely to give birth to infants of LBW than those starting ART during pregnancy (pooled RR 1·30, 95%CI 1·04−1·62), while frequency for SGA did not differ significantly between women starting ART prior vs during pregnancy (overall RR 1.13 95%CI 0.94-1.35) (Uthman et al. 2017). Chen et al. reported a significant risk of SGA for women starting ART before pregnancy (aOR, 1.8, 95%CI 1.6- 2.1) in their Botswana study, particularly for those starting combined treatments vs ZDV-monotherapy (aOR 1.5; 95%CI, 1.2-1.9)(Chen et al. 2012), while data from the Ethiopian study of Ejigu et al found no evidence of an association between ART initiation prior to versus during pregnancy and increased risk of SGA (aOR 1.00, 95%CI 0.76-1.32 vs aOR 1)(Ejigu et al. 2019). In addition, results from a South African retrospective cohort study on 2,500 singleton livebirths evaluating pre-

and post-conception exposure to TDF+3TC/FTC+EFV versus other ART regimens (i.e. NVP-based and other 3-drug EFV-based regimens) and risk of SGA, found no significant differences in adjusted analyses (Chetty et al. 2018).

In the PROMISE trial, an increased risk of LBW (<2500g) was reported for those women randomised to antenatal ZDV-based ART initiation versus those randomised to ZDV monotherapy (23.0% vs. 12.0%, *p*<0.001), with LBW also more frequent with TDF-based ART than with ZDV alone (16.9% vs. 8.9%, *p*=0.004) (Fowler et al. 2016).

*Miscarriage and stillbirth*

Studies on exposure to ART and adverse pregnancy outcomes such as miscarriage and stillbirth are scarce. The "HAART standard" a randomised study (component of the PROMISE trial) evaluated rates of miscarriage and SB in non-breastfeeding women with CD4 cell counts ≥400 cells/μL who started ART during pregnancy were randomized after delivery to either continue or discontinue ART (Hoffman et al. 2019). The study suggests that women randomized to continue ART who subsequently conceived were more likely to have miscarriage or stillbirth compared with women randomized to stop ART with 23.6% (33/140) in the continue-ART arm and 11.9% (15/126) in the discontinue-ART arm (RR 2.0, 95%CI 1.1–3.5, *p*=0.02); however in the as-treated analysis (i.e. categorizing women by their ART status at conception), the RR was reduced and no longer statistically significant (RR 1.4, 95%CI 0.8–2.4) (Hoffman et al. 2019).

Findings both from LMIC and HIC suggest that exposure to ART, particularly at conception, might be associated with adverse pregnancy outcomes such as miscarriage and stillbirth. For example, Chen et al reported an increased risk of stillbirth for women on ART at conception in Botswana (aOR 1.5, 95%CI 1.2-1.8), with 6.3% of women conceiving on NVP-based regimen having a stillbirth vs 4.7% of those starting ART later in pregnancy and 1.7% of those starting ZDV-monotherapy in pregnancy (Chen et al. 2012). Data on 47,000 births from a birth outcomes surveillance study in Botswana more recently assessed specific ART regimen started prior to conception and adverse perinatal outcomes.

The overall rate of stillbirth among births exposed to ZDV-3TC-NVP was 6.1% (83 of 1365); after adjusting for maternal age, gravida and educational attainment, the study reported ZDV-3TC-NVP exposure from conception to be associated with greater risk for stillbirth (aRR, 2.31; 95% CI, 1.64-3.26) compared with exposure to TDF-FTC-EFV (Zash et al. 2017). Additionally, recent analysis from the NSHPC (UK and Ireland) after adjusting for maternal origin, found a greater stillbirth rate in WLHIV than the

general population (8.6 vs 5.2 per 1000, between 2007-15), but did not find any association between exposure to ART from time of conception and increased risk of stillbirth (Favarato et al. 2019). This and other studies suggest that maternal immune status, ethnicity (Asian) and other pregnancy complications (i.e. pre-eclampsia and diabetes) are important contributing factors to the risk of stillbirth in WLWH, many of which apply to uninfected women too (Chi et al. 2007, Chen et al. 2012, Aminu et al. 2014).

*Congenital anomalies*

Historically, the Antiretroviral Pregnancy Registry (APR), an international prospective pregnancy exposure registry, reported a slight increase in the overall CA rate compared to population-based comparators only for exposure to Didanosine in first trimester 4.7% (20/427; 95%CI 2.9-7.1%) and second/third trimester 4.3% (20/464; 95%CI 2.7- 6.6%), but with no specific patterns of anomalies. Similar results of a modest, but statistically significant increase, were seen in the overall rates of CAs with Nelfinavir both with first trimester exposure 3.9% (47/1212; 95%CI 2.9-5.1%) and second/third trimester 3.1% (86/2733; 95%CI 2.5-3.9%)(APR 2019).

Over the years, several specific CAs have been associated with first trimester exposure to certain ARVs. For example, data from the ANRS French Perinatal Cohort study including 13,124 livebirths between 1994-2010 reported a significant association between first trimester exposure to ZDV-based regimens and CHD 2.3% (74/3267) compared to 1.1% (23/2,152) among infant not exposed to ZDV in the first trimester (aOR 2.2; 95%CI 1.3–3.7 $p$=0.003) (Sibiude et al. 2014).

Following this study, the French group conducted a second analysis re-evaluating all children with a diagnosis of CHD and confirmed their preliminary conclusion of an increased risk of CHDs with exposure to ZDV compared with no-exposure to ZDV in first trimester (1.5% vs 0.7%; aOR 2.2; 95%CI 1.3–3.7; $p$< 0.001). This association was particularly significant for ventricular septal defects (VSD) (1.1% vs 0.6%; $p$ = 0.001) and other CHDs (0.31% vs 0.11%; $p$ = 0.02) (Sibiude et al. 2015).

Nevertheless, data from the APR, after evaluating around 13,000 pregnancies exposed to ZDV-containing regimens in any trimester, found no significant difference in the risk for CHD, particularly for VSD when compared to non-ZDV-containing regimens; with a prevalence of VSD of 0.24% (95%CI 0.16- 0.34) in the ZDV-exposed group and of 0.21% (95%CI 0.07- 0.49) in the non-ZDV-exposed group with a relative risk when comparing the two of 1.13 (95%CI 0.44-2.90) (Vannappagari et al. 2016a).

In the US, the Surveillance Monitoring for ART Toxicities (SMARTT) study analysed data from HIV-positive women and their infants, and found a relative increase of CA, with a prevalence of 6.7% (175/2,580, 95%CI 5.8-7.82%) and higher odds of CA with first trimester exposure to ATV (26/222, 11.7%) vs no ATV in first trimester (145/2,295, 6.2% aOR 1.95, 95%CI 1.24-3.05) and particularly for musculoskeletal (CA rate of 5% in infants exposed to ATV in first trimester vs 2% for those non-exposed, aOR 2.57, 95%CI 1.30-5.08) and skin anomalies (CA rate of 1.4% vs 0.3%, aOR 6.01, 95%CI 1.43-25.3) (Williams et al. 2015).

*Efavirenz and congenital anomalies*

First concerns for a potential fetal teratogenicity with exposure to EFV arose from preclinical findings. A trial on 40 cynomolgus monkeys, 20 exposed to 600mg EFV/die (dose resulting in plasma concentrations comparable to systemic human therapeutic exposure) and 20 controls, found three of the 20 (15%) of the EFV-exposed monkeys to have significant anomalies including neural tube defects (NTDs) (one anencephaly with unilateral anophthalmia, one microphthalmia and one cleft palate) (Nightingale 1998).

Neural tube defects (NTDs) are a CA affecting the brain, the spine or spinal cord resulting in total or partial failure of the neural tube to close, a process usually completed within the first month of pregnancy (i.e. within the first 28 days from the day of conception) (Copp et al. 2013, Wilde et al. 2014, Zaganjor et al. 2016). Several risk factor have now been identified such as folate deficiency, obesity, uncontrolled diabetes and periconception use of several medication such as carbamazepine and valproic acid (Copp et al. 2013, Wilde et al. 2014, Atta et al. 2016, Greene et al. 2017). NTDs are rare defects affecting approximately 1 in 1,000 pregnancies in Europe, the majority are identified prenatally and approximately 70% of the detected pregnancies are terminated (EUROCAT 2014). NTDs manifest with a wide range of clinal severity; the three most common lesions are anencephaly (total failure of cranial neural tube closure, thus no formation of the skull vault), encephalocele (a persistent opening in the skull that allows the meninges to herniate creating an extra-cranial mass) (Rowland et al. 2006), and spina bifida (or myelomeningocele/open spina bifida; the most common type resulting from a failure of the closure of the neural tube along the body axis (Copp et al. 2010, Copp et al. 2013, Wilde et al. 2014)). Anencephaly is always lethal before or at birth, encephalocele can be lethal depending on the extent of the damage on the brain tissue caused by herniation and spina bifida is generally compatible with postnatal life but again can leave severe disabilities in the survivors (Copp et al. 2010, Copp et al. 2013, Wilde et al. 2014).

In light of the seriousness of these events, the initial report of identified NTDs led the FDA to classify EFV as a Class C drug (those for which “risk cannot be ruled out”). Several retrospective cases of central nervous system defects (three meningomyelocele and one Dandy-Walker syndrome) were reported in infants whose mothers were taking EFV from time of conception (De Santis et al. 2002, Fundaro et al. 2002, Saitoh et al. 2005). Fundaro et al. reported the first case of a NTD in a human fetus exposed from first month of pregnancy to maternal EFV. The women received daily folic acid supplementation from periconception time and delivered at 38 GW a neonate with a myelomeningocele (Fundaro et al. 2002). De Santis et al presented a case series of three women whose pregnancies had been exposed to EFV from conception, with one resulting in a myelomeningocele with cerebral ventriculomegaly (De Santis et al. 2002). Saitoh et al reported the third case of myelomeningocele in an infant whose mother was exposed to EFV during the first 16 weeks of pregnancy (Saitoh et al. 2005). Following these retrospective case reports, FDA reclassified EFV in 2005 as a Class D drug (those with “evidence of human fetal risk”) and recommended not to use it during the first trimester of pregnancy (Lewis-Hall 2005).

Additional data from a prospective, observational study the IMPAACT study, collecting data from 2002 and 2007 on 1,112 infants reported 61 CAs (61/1112, prevalence 5.49/100 livebirths, 95%CI 4.22–6.99) and found an increased risk of CAs with first trimester exposure to EFV (6/47, 12.8% CAs) vs no first exposure to EFV (41/47, 87.2%; OR 2.84, 95%CI 1.13-7.16) (Knapp et al. 2012). The ANRS French Perinatal Cohort Study collected data on 13,124 live births between 1994 and 2010, reporting a significant association between EFV and neurological defects using the Metropolitan Atlanta Congenital Defects Program classification (aOR 3.0, 95% CI 1.1-8.5), $p=0.04$, absolute risk +0.7%, 95%CI +0.07% +1.3%). However, the association was not significant using EUROCAT classification (aOR 2.1, 95% CI 0.7-5.9, $p=0.16$) (Sibiude et al. 2014).

Prospective reports from the APR and observational studies such as the NSHPC did not report evidence of an increased overall incidence of CA for those women exposed to EFV from time of conception, even though numbers were too small to detect increased risk of rare event such as NTDs (Townsend et al. 2007, APR 2009). It was only after years of data gathering that enough evidence accumulated to rule out safe and effective use of EFV in pregnancy with no increased risk for nervous system defects (Ford et al. 2014, Martinez de Tejada et al. 2019). Investigations to evaluate EFV were also sustained by the WHO, given their intention to recommend EFV-based regimen as preferred first-line treatment for adults and adolescents, including women of childbearing age and pregnant women; these included a systematic review and

meta-analysis, one in 2010 evaluating rates of CAs among liveborn infants whose mothers were exposed from preconception/first trimester to EFV-based regimens and those exposed to non-EFV-based regimens and found a non-significant relative risk (RR 0.87, 95%CI 0.61-1.24%, *p*=0.45) with one NTD (meningomyelocele) with first trimester exposure to EFV among all the 1,256 livebirths, giving a prevalence of 0.08% (95%CI 0.002-0.44%) (Ford et al. 2010). Ford et al then conducted an updated review including CAs reported up to 2014 and including 2,026 live births whose mother where either on EFV or non-EFV-based regimens during the first trimester. The study identified 41 CAs, giving a pooled proportion of 1.63% (95%CI 0.78-2.48), with only one NTD (incidence of 0.05%) and no differences in the overall risk of CAs between the two groups (RR 0.78, 95%CI 0.56-1.08) (Ford et al. 2014).

Additionally, data from a pooled analysis of observational data from 13 European and Thai studies evaluated the association between EFV exposure from time of conception / first trimester and the presence of CAs. The study included nearly 25,000 livebirths from 21,000 women and reported 412 infants with at least one CA, with a prevalence of 1.65% (95%CI 1.50 1.82) and a total of 453 CAs according to the EUROCAT classification criteria. There was a non-significant association between exposure from conception/first trimester to EFV- and non-EFV-based regimen (aOR 0.61, 95%CI 0.36-1.03, *p*= 0.067); and no NTDs among the 21 CAs in the 19 infants exposed to EFV (Martinez de Tejada et al. 2019).

In the United States, the Surveillance Monitoring for ART Toxicities (SMARTT) study evaluated more than 3,000 HIV-exposed but uninfected infants enrolled between 2007 and 2017 with respect to microcephaly (Williams et al. 2020). The study found exposure to EFV to be associated with a 2-fold increased risk of microcephaly by Nellhaus thresholds for children aged up to 3 years (9.9%, 14/141 exposed to EFV and had microcephaly vs 5.0%, 142/2,82 not exposed to EFV, aRR 2.02, 95%CI 1.16–3. 51, *p*=0.013). Similar results were also found using the SMARTT criteria for children aged over 3 years (aRR 2.56, 95%CI 1.22–5.37) and using the WHO standards for defining head circumference Z scores (6.5%, 8/124 of children exposed to EFV vs 2.0%, 44/2235 of children unexposed to EFV, aRR 3.69, 95%CI 1.77–7.70). This association was more pronounced when EFV was combined with ZDV/3TC vs TDF/FTC (aRRs of 4.38 and1.86 for microcephaly by Nellhaus criteria and 7.20 and 2.06 by SMARTT criteria, respectively) and persisted, although was less pronounced for first trimester EFV exposure (10/114, aRR 1.75, 95%CI 0.92–3.34) (Williams et al. 2020).

# 2 Introduction : EMA

## 2.1 History of regulators

Evidence of medicine use and its regulation dates far back. In the 120 BC, Mithridates VI formulated a "universal antidote" against poisoning held as a panacea. This was the most common remedy used for centuries without any quality control or evidence of any efficacy; it was the English *Apothecaries Wares, Drug and Stuffs Act* in 1540 that subjected, for the first time, this panacea and other medicines to supervision (Griffin 2004).

Furthermore, the first pharmacopoeias with both descriptive reference and effective regulatory value dates back to 1240 with the Salerno Medical Edict emitted by Fredrick II of Sicily, ordering remedies to always be prepared in the same way, the very first step towards standardisation. However, it was only in the 19$^{th}$ century that remedies turned into medicines, fostered by the steady progress of life science (Rago et al. 2008).

History of regulation instead has had unfortunate events as major drivers. The first catastrophe occurred in the US in 1937 when over 100 people died having been poisoned by exposure to sulphanilamide elixir (Akst 2013). This was the first stimulus for the development of *The Federal Food, Drug and Cosmetic Act* and for the obligation of a premarket notification for any new drug (Ballentine 1981).

In 1957 two events changed the concept of regulation in Europe. First, with the Treaty of Rome and the creation of the European Economic Community, the foundation of today's European Union (EU) were set. Second, and probably by far the biggest catalyst for modern medicine regulation, the marketing of Contergan. Contergan was intended to treat insomnia, anxiety and nausea and as an alternative to barbiturates, with fewer side effects. It also became widely used in the whole of Europe, between 1958 and 1960, to treat pregnancy-related nausea. We now best known Contergan for its active substance: thalidomide (Griffin 2004, Rago et al. 2008). The thalidomide disaster affected more than 10,000 infants born with phocomelia and other deformities and brought to the attention of European legislations the lack of institutions both to safeguard public health and to harmonize medicines' regulation (Rago et al. 2008).

Revision of the entire regulatory system resulted in the establishment in the early 1960s in the UK of a Committee on the Safety of Drugs, followed by a voluntary reporting system of adverse drug reactions – still known as the Yellow Card Scheme.

At the same time in the US, the FDA imposed for the first time the New Drug Applications, calling for any new drug to be proven safe as well as effective prior to marketing (Rago et al. 2008). In Europe, following the first pharmaceutical directive (65/65/EEC) in 1965 the EU law required a marketing authorisation for all medicines prior to being put on the EU market with aligned guidelines throughout the member states (Council 1965).

In 1995, the European Medicine Evaluation Agency was established driven by the need to generate a “common market” for medicines, renamed European Medicine Agency (EMA) as of 2009. Before the 1995 establishment inspection and monitoring of medicines was regulated at national or regional level in a heterogeneous and fragmented way. For example, in the UK different bodies were regulating different aspects of medicines at a national level (i.e. National Institute for Biological Standards and Controls, Medical Devices Agency and Office for National Statistic) until 2003 when the Medicines and Healthcare products Regulatory Agency (MHRA) was established also to liaise uniquely with the EMA (EMA 2019a).

## 2.2 European Medicines Agency

The EMA is an EU Agency set up to harmonize the evaluation of medicinal products in their *safety, quality and efficacy,* three fundamental requisites on which medicines' authorisation is based.

The EMA is governed by an independent management board composed by 36 members acting in the public interest and dealing with budgetary and approval of the EMA's annual work. The Agency is led by an executive director who is the legal representative and whose work is supported by the EMA's staff for all day-to-day operations. The EMA's main tasks are to evaluate benefit-risk ratio (B-R assessment) of new medicines applying for market authorisation in Europe and to monitor safety and efficacy of authorised medicines throughout their entire lifecycle (i.e. from molecule's discovery to post-authorisation phase) (Figure 2.1) (EMA 2019a).

The full process of evaluation for a new market authorisation is quite complex involving seven scientific committees and several expert groups before reaching the final opinion and recommendation (described fully in section 2.4). Therefore, the EMA provides different tools to foster patients' timely access to therapeutics innovations guiding the applicants/developers (e.g. pharmaceutic companies) through the process of robust evidence generation.

One of the main tools provided by the EMA is the provision of scientific advice (EMA 2019b); this consists of early engagement with developers in reviewing the study design and the methodology, given that the main scope is to ensure developers are performing the correct tests and studies. Scientific advice can be requested at any stage of the medicine development and has been shown to be most useful in situations such as the development of innovative medicines, where limited or no relevant EU guidelines are available; or when developers have limited knowledge about medicine's regulation procedures, such as small or medium sized enterprises or academic groups. Furthermore, scientific advice aims to facilitate proactive pharmacovigilance planning and integrate advice on safety, quality and efficacy (EMA 2019b).

The Agency then assures a continuous monitoring of medicines during their entire lifecycle, and particularly after the market authorisation is obtained (Figure 2.1). This is to ensure that a medicine's benefits always outweigh its risks and to monitor any other adverse reactions or rare events due to exposure to the medicine not previously seen. For this task, the EMA makes use of pharmacovigilance tools, risk minimisation plans and B-R assessments, discussed later in this chapter (section 2.4).

The complexities of rules and methodologies underpinning the regulatory process have been set with the aim of enabling a consistent and robust assessment of quality, safety and efficacy of medicines. However, regulatory bodies have frequently been subject to criticism (Eichler et al. 2008, Joshi et al. 2018). Developers typically favour a flexible environment, claiming that a rigid and complex legal-regulatory framework plays against the patients' need for early access to innovative medicines. Consumers and watchdogs have complained about the lack of transparency in the authorisation process and they also argue that evidence is largely generated by the developers, (i.e. pharmaceutical companies), questioning the independence of the evidence generated (Eichler et al. 2008, Gotzsche et al. 2011, Joshi et al. 2018).

The EMA instead claims to have a complete chain of quality and trust assurance measures, including the methodological guidelines and good practices (GLP - GCP) as well as international standards (ICH) when companies develop their studies (ICH 2017, EMA 2019c, EMA 2019d, EMA 2019f). These are inspected by regulatory authorities both on a routine basis and ad hoc when there are suspicions of improprieties. Extensive explanation of the full process, including transparency and ethical policies is publicly available in EMA website (EMA) (EMA 2019). Additionally, over the past 20 years enormous efforts to improve EMA's transparency and its engagement with the public and healthcare professionals have been made. For example, in 2000, the Committee for Orphan Medical Products was established and since then representatives of both patients and healthcare professionals' sit as full members on almost all the EMA's scientific committees, contributing to the important task of B-R assessment of medicines.

In 2012, another step forward was taken with the establishment of the Pharmacovigilance and Risk Assessment Committee, an essential body to monitor safety of medicines (Laroche et al. 2016). Once again, regulation progressed due to another safety crisis, the Benfluorex disaster, a drug approved as hypolipidemic and hypoglycemic found to cause severe heart valve disease (Frachon et al. 2010, Tribouilloy et al. 2010, Weill et al. 2010, Prescrire 2014).

Further, in 2015, a new policy on publishing clinical data marked a true milestone in the matter of transparency, allowing for academia and industry to assess and re-assess clinical data and for the EMA to build public trust in its decision-making processes (Bonini et al. 2014). This was the result of two landmark policies, one set in 2010 regarding the accessibility to documents related to products for human use and one, in 2014, on publication of clinical data of medicinal products intended for human use (EMA 2010, Bonini et al. 2014, EMA 2014c).

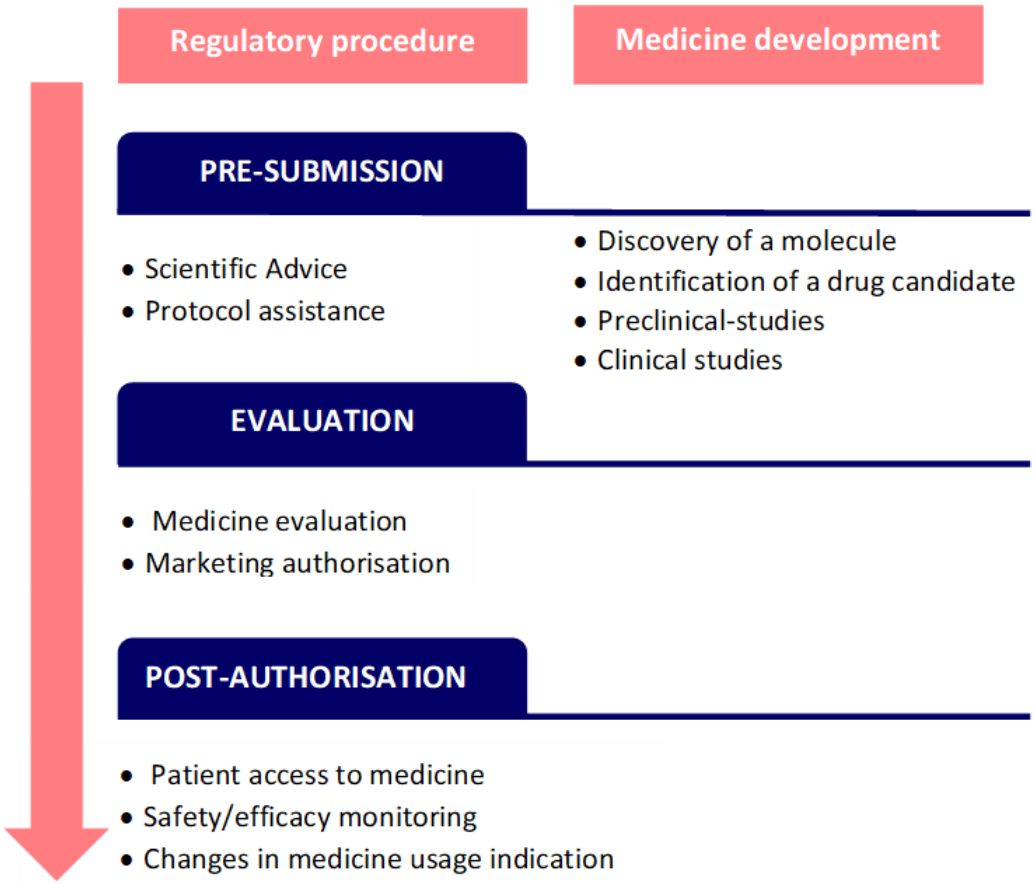



**Figure 2.1 Medicine's life cycle**

## 2.3 How does a medicine obtain a market authorisation?

### 2.3.1 General considerations

Every year pharmaceutical companies, academics, governmental research organizations, or a combination of these, investigate tens of thousands of molecules with a potential to become medicines (Taylor 2015). The development goes through many steps and phases. The discovery phase typically consists of the selection of a clinical condition, the identification of a target within the human body (e.g. a cell, an enzyme, a gene or molecular pathway) and the research to identify substances with the potential to interact with the identified target (Taylor 2015).

The transition between the discoveries of promising substances to preclinical studies is a continuum, where results from pharmacology and toxicology testing help select a candidate medicine. The medicine's developer generally conducts preclinical and clinical studies, so regulators do not play a direct role in the authorisation of preclinical or clinical studies, which is the responsibility of the national competent authorities in Europe. However, regulators (both in the EU and US) ensure that the medicine's developer complies with EU and international standards and follows *Good Clinical Practice (GCP)*, a set of rules on study design and results reporting to ensure ethical and scientific fairness for the patients enrolled in clinical studies (EMA 2019c).

Similarly, regulators assess the medicine's benefits and risks from a scientific point of view, generating recommendations for the medicine's use. In Europe, the EMA does not have authority on the actual marketing and access for patients across the different EU countries (EMA 2019a).

Figure 2.2 illustrates the necessary steps to obtain a marketing authorisation, while detailed information on each step is provided in subsections 2.3.2 and 2.3. 3.

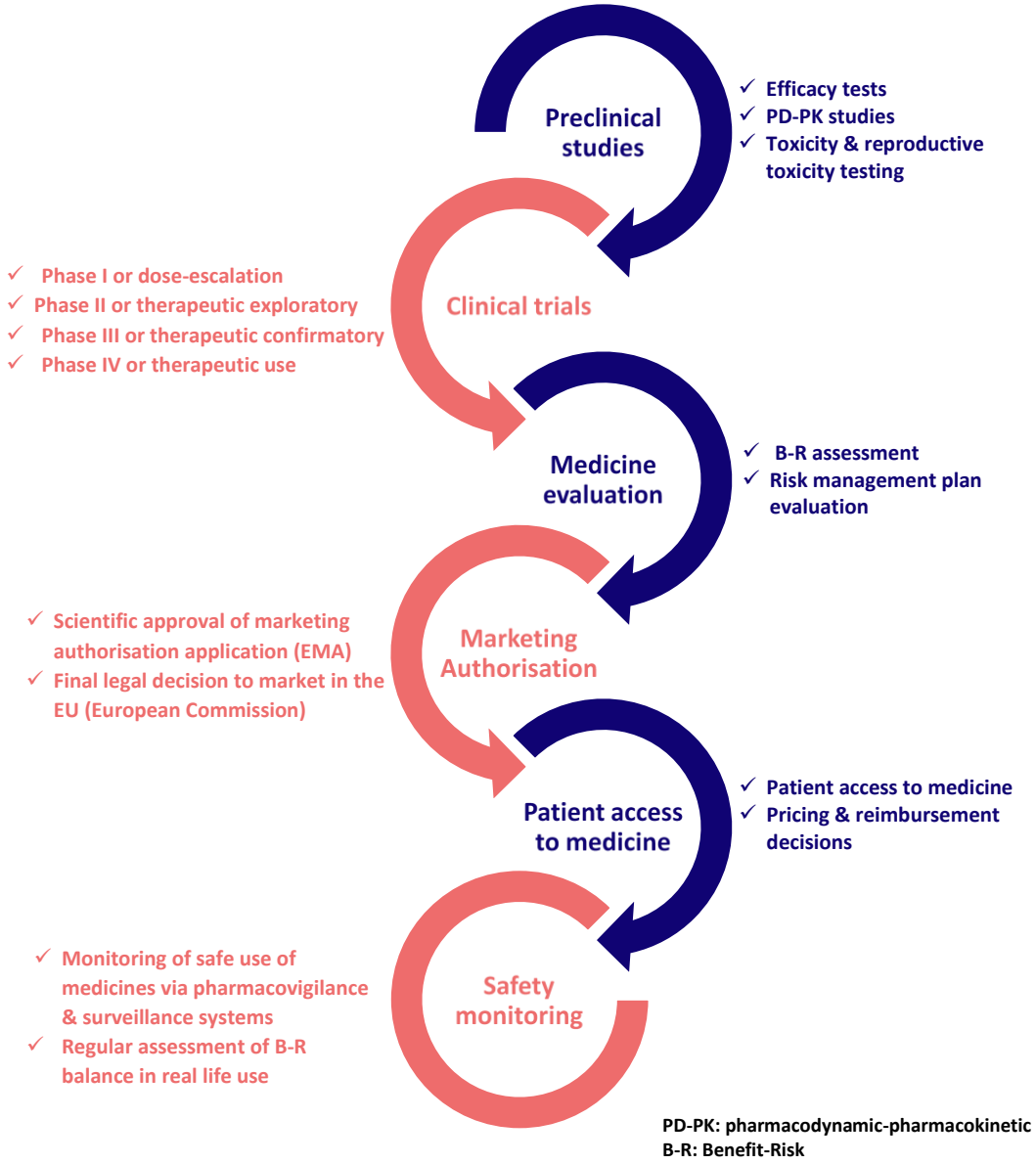



**Figure 2.2 Steps to obtain a medicine's marketing authorisation in Europe**

## 2.3.2 Preclinical and clinical studies

*Preclinical studies*

Preclinical studies usually precede human testing of medicines, mainly using animal models. Animal models have been used for decades to predict treatment outcome in-patient (efficacy) and to identify potential adverse events (safety) contributing to B-R assessment (Polson et al. 2012). They usually include a variety of different types of research such as pharmacodynamics, pharmacokinetics and toxicology testing and allow for the calibration of the safe-dose for the first-in-man-study (Polson et al. 2012).

Pharmacodynamics (PD) assesses how a drug affects an organism, while pharmacokinetics (PK) assesses how the organism affects the drug (Benet et al. 1995). PD measures the medicine's molecular, biochemical and physiologic effects, taking into consideration that for a medicine to produce an effect, it needs to interact at a molecular level, for example through receptor binding or chemical interactions with a target (Marino et al. 2020). Whereas PK assesses the interaction between an organism and a chemical substance and determines the onset, duration and intensity of a medicine's effect. Throughout the ADME scheme, an acronym for "absorption, distribution, metabolism and excretion" it is possible to study most of these complicated interactions (Benet et al. 1995). ADME describes how the organism alters a medicine in its level (concentration) and kinetics, influencing its pharmacological activity, i.e. the potential for beneficial or adverse effects. More in depth, an organism interferes with the absorption of a medicine – the process of entering the blood circulation; alters a medicine's distribution – meaning the medicine dispersion into the body's tissues and fluids; changes irreversibly by biotransformation or metabolism from the original substance into metabolites; and finally interferes with medicines' excretion – meaning the elimination of the medicine from the body (Benet et al. 1995).

PD-PK studies address the dose-response relationship between a drug and the human body. Both can be affected by patient- or disease-related factors or by the medicine's chemical properties (Blot et al. 2014). For example, PD can be altered by genetic mutations, body nutritional conditions (i.e. malnutrition), aging process, drug-drug interactions or presence of co-morbidities (Mangoni et al. 2004, Smith et al. 2012). All these conditions can alter the bioavailability of binding proteins (the transporters of medicines inside the body) or decrease /increase receptor responsiveness or compete for receptor binding sites with other drugs (Kristensen 1976).

Similarly, PK is influenced by the renal and/or hepatic function or can be altered by

gastrointestinal diseases or from drug-drug interactions and competition for receptors, but also from sex, age and genetic makeup(Kristensen 1976, Smith et al. 2012). Equally, fundamental to fully understand medicine-body interactions is the knowledge of the medicine's half-life, the time required for a substance to change from one concentration to another; the characteristics of the biological membranes that a medicine will cross (e.g. placenta and blood brain barrier) and how the medicine can cross them (i.e. mediated free diffusion or through carriers) (Benet et al. 1995).

Toxicology testing is another important component of preclinical studies, comprising *in vitro* and *in vivo* analyses (Steinmetz et al. 2009). The vast majority of toxicity testing is carried out in the context of regulatory requirements, which usually ask for laboratory testing on at least two animal species, one rodent (rats or mice) and one non-rodent (e.g. rabbits) and are now an integral component of medicine development. Toxicity testing usually comprises combined studies to assess the severity and the different types of toxicity due to exposure to a candidate medicine (Steinmetz et al. 2009, ICH 2017).

Examination of adverse effects that may occur on the first ever exposure to a substance is defined as *single-dose exposure* and is assessed through *acute toxicity studies,* which also establish the maximum non-lethal dose of medicine administration (Steinmetz et al. 2009). Acute toxicity studies can also be used to determine the Maximum Tolerated Dose (MTD), defined as the highest dosage of a medicine that does not cause unacceptable side effects (Chevret 2014). MTD is particularly useful for cancer and HIV medicine development, given the relatively high doses used in these fields to reach the greatest possible beneficial effects, in both animal models and in Phase I clinical trials (Chevret 2014).

Repeated-dose toxicity studies establish toxicities that may develop later due to the continuous exposure to the substance. These studies also identify the most affected organ(s) and determine the dose at which the required therapeutic effects will occur. They are also used to assess the NOAEL- no observed adverse effect level- in other words, the highest dose without significant adverse effects (see later in this section) (Steinmetz et al. 2009).

Reproductive toxicity studies are an important branch of toxicity studies, aiming to reveal any relevant effect on mammalian reproduction due to exposure to the medicine under development (EMA 2009) . Generally, reproductive toxicity studies utilize rats as the predominant rodent species and a second mammalian species for the evaluation of embryo-fetal toxicity, most commonly rabbits. Rabbits are particularly used in Embryo-Fetal Development studies because they have shown to

better identify human teratogens that might have not been detected previously by the rodent species. Rats and rabbits are commonly used given the extensive background knowledge on these species, the easy availability and for comparability and practicality reasons. The number of animals included varies by study, but for the detection of common adverse events such as major malformations, abortions, or total litter loss, 16 to 20 litters for rodents are the protocol (EMA 2009). Usually, reproductive toxicity studies follow acute- and repeated-dose toxicity studies of at least one-month duration, allowing for dose-identification and establishment of the Maximum Tolerated Dose (EMA 2009).

Reproductive toxicity studies, ideally, should include mature adult animals and cover exposure through all the stages of development, from conception to sexual maturity. Moreover, to allow evaluation of short and long-term effects, one complete lifecycle should be included, meaning from the conception of one generation (F0) to the conception of the following generation (F1) (known as two-generation study) (ICH 2017).

According to the International Conference of Harmonisation (ICH) S5(R3), assessment of reproductive function can be broken down in six stages (ICH 2017). Stage A includes tests from premating to conception and should utilize both adult males and females and aims to evaluate any toxic effect or disturbances from treatment prior to mating through mating and implantation. The histopathology of the reproductive organs from repeated-dose toxicity usually represent a good starting point to detect major effects on male and female fertility (ICH 2017). Stage B looks only at adult females from conception to implantation performing the same tests as before. Stage C looks at embryonic development and major organ formation, from implantation to closure of the hard palate (considered an important marker for physiological passage from embryonic to fetal period). This is the stage where Embryo-Fetal Development studies are performed to detect any effect on the embryo-fetal development due to medicine exposure during organogenesis. Stage D considers fetal and organ development and growth from closure of the hard palate to the end of the pregnancy. Stage E looks at the neonate adaptation to extrauterine life, from birth to weaning, while Stage F observes post-weaning development and attainment of full sexual function. From stages C to F, studies aim to detect any adverse effects following maternal exposure to the medicine from implantation to weaning, including development of the offspring and are defined as pre- and post-natal developmental toxicity studies (ICH 2017).

A final consideration about toxicity testing, also valid for reproductive toxicity testing, concerns their applicability to assess the NOAEL. NOAEL determine the level in which

a medicine is effective without producing adverse effects and allows for conversion to the Human Equivalent Dose and ultimately for the Maximum Recommended Human Dose (MHRD). This is an important parameter used in risk assessment and risk management to establish safe doses in humans. Therefore, by using NOAEL index, is possible from animal models to predict a medicine's potential for adverse effects in humans. For example, a NOAEL of <10-fold the human exposure at the MHRD observed in an animal model increases concern for reproductive or developmental toxicity in humans. Whereas a NOAEL of >10-fold the human exposure at the MHRD, reduces such concerns and if NOAEL is >25-fold the human exposure at the MRHD, concerns are considered to be minimal for the clinical use of the medicine. These units are often reported in the leaflet for healthcare professionals with the release of the medicine (ICH 2017).

Other tests include chronic-toxicity studies evaluating less-specific effects and targeted organs or systems and doses at which these effects are developed; genotoxicity testing to assess the potential of a substance to interact with genetic material and carcinogenicity testing to evaluate if the exposure to a certain substance can induce cancers (ICH 2017).

*Clinical trials*

Clinical trials (CT) consist of experimental and observational studies conducted in humans. According to the WHO definition, a CT for registrational purposes "is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions (i.e. drugs, cells, other biological products, etc.) to evaluate the effects on health outcomes". In their purest form, CT are designed to observe human subjects under "experimental" controlled conditions (Umscheid et al. 2011). CT aim to answer two main questions: if the new medicine works and, if so, if it is safe for patients to take it. Thus, through CT, data on safety and efficacy are generated. CT have some fundamental characteristics, such as being prospective; requiring the appropriate selection of a study population in both size and characteristics to best represent the target population; being voluntary regarding enrolment; requiring informed consent to participate; and being supervised by an ethical committee to safeguard patients' rights (Umscheid et al. 2011).

Historically, ethical considerations in human research were not addressed until the mid-20$^{th}$ century. First with the Nuremberg Code of 1949, in response to the criminal medical experimentation by the Nazis, ten basic principles for human research were articulated (Nuremberg 1949). This was then extended globally, in the 1964, as the Declaration of Helsinki, a cornerstone of human research ethics (World Medical 2013)

Further, the first informed consent adhering to principles regarding respect for patients' rights and acting in their best interests were formulated following the 1979 Belmont Report in responses to the Tuskegee syphilis experiment (Brandt 1978, Department of Health et al. 2014). Nowadays all clinical trials for registration of medicinal products must be subject to the GCP guidelines (Verma 2013) including the ICH and the WHO-Good Clinical Practice (EMA 1997, FDA 1997). These are the standard guidelines for the EU, Japan and the US, to facilitate mutual acceptance of CT data, to achieve greater global harmonization and to assure protection and preservation of human rights (Verma 2013).

Clinical trials consist of four phases, the first three precede marketing authorisation and are concentrated in the assessment of safety, while the fourth starts when the medicine is firstly prescribed and as long as the medicine continues to be used (Duffy 2006, Umscheid et al. 2011, Taylor 2015). Usually, a candidate medicine takes six to ten years to complete the first three phases of clinical trials.

Phase I or first-in-man-study (or "dose-escalation study") aims to confirm the preclinical findings by replicating the same analysis in human subjects and begins testing safety of the candidate medicine (Umscheid et al. 2011). In Phase I trials, a small number of healthy human volunteers (10-15 individuals) are enrolled and receive incremental doses of the medicine, without a control group, without randomisation, but under careful monitoring (Nuremberg 1949, Duffy 2006, Umscheid et al. 2011).

Phase II (or "therapeutic explanatory study") studies enrol a larger number of subjects (100-300) with the disease of interest (Umscheid et al. 2011). Phase II tests for safety, PD-PK and evaluates efficacy against the target disease. However, numbers are still too small to power phase II of a full efficacy-assessment but are enough to ascertain excessive toxicity (Duffy 2006, Umscheid et al. 2011). In addition, phase II sets the scene for several questions that phase III will address, such as optimal doses, dose frequencies, administration routes and endpoints.

Phase III (or "therapeutic confirmatory or pivotal trial") enrols a large number of participants (300-3000) to demonstrate the medicine's safety, to confirm its efficacy and monitor any side effects. Typically, two types of phase III studies are conducted: comparative efficacy and equivalency trials. The former aims to prove superiority of the new medicine by comparison to an established therapy or to placebo and for this reason are planned and performed as superiority trials (or placebo-controlled trials). Equivalency trials are intended to assess a new medicine's equivalent effects or non-inferiority to an established therapy, known as positive or non-inferiority trials (Christensen 2007, Umscheid et al. 2011).

In phase III trials, efficacy is demonstrated and lower-frequency side effects can be detected due to the higher statistical power and bigger sample size, e.g. any effect occurring in less than 1 in 100 people (Eypasch et al. 1995, Taylor 2015). This is also possible, because phase III uses two comparison groups and allocates randomly among the groups for comparison of treatment efficacy (Umscheid et al. 2011). However, the statistical power of phase III trials is not strong enough to detect rare events (1 in 1,000 people), highlighting the importance of phase IV for their identification. Typically, two pivotal phase-III are required for a market authorisation approval (Taylor 2015).

Phase IV (or “therapeutic use or post-marketing study”) starts after the candidate medicine has obtained market authorisation approval (Umscheid et al. 2011). Phase IV studies aim to detect less-common adverse drug reactions, rare and late events due to exposure to the medicine while also confirming the medicine’s efficacy in the long-term and real-world settings. Phase IV relies on surveillance systems from different stakeholders, such on-going surveillance studies or voluntary reporting systems of adverse drug reactions or from regulatory bodies such as pharmacovigilance and post-authorisation studies (Umscheid et al. 2011).

### 2.3.3 Steps towards marketing approval from a regulatory point of view

Once the medicine's developer has completed preclinical and clinical studies to assess safety and efficacy, it is ready to apply for a marketing authorisation. To do so, all data generated must be submitted to the EMA. The EMA meets with the developer, defined now as the marketing authorisation applicant (MAA) about 6 -7 months prior to the submission with representatives of various areas (e.g. paediatric and risk management experts) to validate the data submitted and check compliance with the relevant legal and regulatory requirements (Figure 2.2). Some of the mandatory information to be included in the application are preclinical data, benefits and side effects observed in patients during CT; posology (dosage) and route of administration; targeted groups of patients; the medicine's mechanism of action and results from PD-PK studies (EMA 2019d, EMA 2019e, EMA 2019f).

During the evaluation and assessment of a candidate medicine, the Committee for Medicinal Products for Human Use will establish, in collaboration with other committees and scientific bodies, if the benefits outweigh the risks (B-R assessment) as this is the prerequisite for a medicine to obtain marketing authorisation.

However, at time of first authorisation the complete safety profile of the medicine will not be available, so the other important evaluation is the way the risks will be minimised, managed, and monitored. Any information relative to potential or identified safety concerns and how the risks will be managed and monitored in phase IV should be included in a document called Risk Management Plan (RMP). The assessment step can take up to 210 'active' days, and it is interrupted by one or two "clock-stops": time for the MAA to address any questions raised by the Committee for Medicinal Products for Human Use. Overall, assessment of a new medicine takes up to a whole year.

Once a decision is made, whether positive or negative, a comprehensive document called European Public Assessment Report (EPAR) becomes publicly available on the EMA webpages. This is a very technical document with all the timings of the Committee for Medicinal Products for Human Use assessment and the reasons for approving (or refusing) the authorisation; with this step, the new medicine is ready to be marketed in Europe.

## 2.4 Benefit-risk assessment and pharmacovigilance

### 2.4.1 General consideration

Benefit-risk (B-R) assessment ensures that the benefits of a medicine always outweigh potential risks and aims to minimize any (potential or identified) risks. Therefore, B-R assessment is a continual process occurring from the pre- to post-marketing authorisation phase. Pre-marketing B-R assessment mostly relies on preclinical safety assessments (e.g. animal toxicology studies), clinical pharmacology and clinical trials; post-marketing B-R assessment makes use of non-experimental data such as case repots or case series, databases of spontaneous reporting of adverse effects, disease-based or drug-based registries, electronic medical records and administrative claims databases (Dal Pan et al. 2012).

However, the extent of information on the B-R collected in pre-marketing phase is a function of the number of patients enrolled, the duration of treatment and the specific safety evaluations performed. Therefore, full assessment of safety and efficacy of medicines can be limited by small sample sizes or short durations and/or be impacted by the homogeneity of the selected patient population (i.e. not being representative of the target population) (Dal Pan et al. 2012). Additionally, even when efficacy is evaluated, multiple sources of uncertainty arise, such as systematic random and gross experimental errors (e.g. non-validated surrogate endpoints) and bias that may be detected by regulators at time of medicinal approval (Pignatti et al. 2002). Further, most registrational studies are designed (and powered) to assess efficacy, not real-life effectiveness or safety, and yet more than half of potential new medicines that reach phases II and III of human trials fail because they cannot demonstrate efficacy (Eichler et al. 2008, Kimmelman et al. 2017). For example, a report evaluated reasons for phase II-failure of candidate's medicine and found 51% (44 out of the 87 reported reasons) to be because of insufficient efficacy (Arrowsmith 2011).

Assessment of B-R in the early phases of CT (i.e. phase I and II) greatly relies on the evidence gathered from preclinical studies. There are growing concerns whether data from animal models are a reliable and applicable source for safety and efficacy in humans. Over the last decade data from cross-sectional and protocol studies have raised some concerns on study design features such as lack of blocking, blinding or and reports from systematic reviews found general low rates of reporting of measures to reduce bias (Sena et al. 2010, Landis et al. 2012, Vogt et al. 2016) such as the number of animals needed (van der Worp et al. 2010, Wieschowski et al. 2018). Other limitations of pre-clinical studies include lack of power in most animal models to detect rare adverse events or toxicities despite higher dosage levels or longer duration of

drug administration and the fact that they generally include young, healthy animals with no other treatment concurrently (i.e. different to the likely scenario for human use) (Polson et al. 2012). For example, a recent systematic review evaluated 708 preclinical efficacy studies contained in 109 investigator brochures submitted for ethical review for phases I and II trials. The study found no reference to a published, peer-reviewed report for 89% of these studies, suggesting a lack of critical and independent evaluation and also found that 44% of the studies did not pre-specify endpoints or study design features such as blinded outcome assessment (Wieschowski et al. 2018).

Similarly, there is a growing literature reporting flaws in the design, conduct and analysis of some CTs, leading to bias and over- or under-estimation of efficacy of the medicine, leading on the one hand to the potential implementation of an ineffective and/or harmful intervention or, alternatively, to the non-implementation of an effective intervention (Page et al. 2016, Naci et al. 2019). For example, a large meta-epidemiological study of over 1,900 RCTs reported how the lack of blinding was associated with an average of 13% exaggeration of treatment effects (ratio of OR 0.87, 95%CI 0.79-0.96) among trials that reported subjectively associated outcomes; and a further analysis on over 2,000 RCTs in 228 meta-analyses evaluated treatment effect and found estimates to be exaggerated in trials with high risk-of-bias judgments (vs low) for allocation concealment (ratio of ORs 0.92, 95% CrI 0.86- 0.98) and blinding (ratio of ORs 0.87, 95%CrI 0.80-0.93) (Savovic et al. 2012, Savovic et al. 2018).

Consequently, at the time of a medicine's approval, knowledge about its B-R is still limited and often availability of data of specific populations (i.e. children, elderly and pregnant women) or for specific toxicities (i.e. teratogenicity) or for rare adverse events are scarce (Eichler et al. 2008, Dal Pan et al. 2012, Naci et al. 2019). Therefore, data are mostly generated from real-world use of medicine where it is generally used on a larger and more heterogeneous population, including a broad range of co-morbidities and co-medications, also with more severe underlying diseases (Dal Pan et al. 2012).

### 2.4.2 Pharmacovigilance and Risk Management Plan

Pharmacovigilance is defined by the WHO as “the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem” (WHO 2010). In Europe, the concept of pharmacovigilance is embedded in the B-R assessment along with that of risk management and was established in legislation by the Article 1 (28b) of Directive 2001/83 EG, defining risk management as “a set of pharmacovigilance activities and interventions designed to identify, characterize, prevent or minimize risk relating to a medicinal product, including the assessment of the effectiveness of those interventions”(European Parliament 2001).

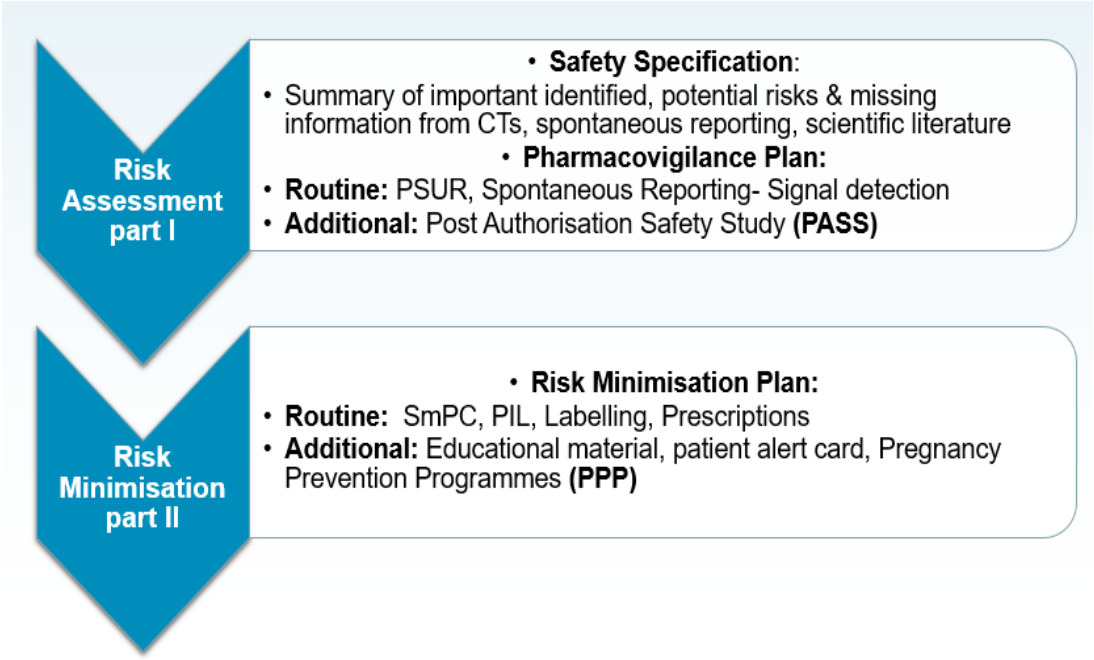
Over the past decades, EMA has proactively engaged in improving B-R assessment, through the establishment of RMPs and pharmacovigilance activities to better monitor and manage safety and effectiveness of medicinal products (EMA 2014). RMPs were introduced in Europe in 2005 as EU-RMP and are now part of the application process for authorisation, as already stated. EU-RMP can be, and are, usually requested by regulators if significant changes in the medicine’s indication are made or if the medicine contains a new active substance with limited safety data or if they are new products of a class for which a potential or a serious safety risk have been previously identified (Zomerdijk 2015).

EU-RMP consists of two parts, the first comprising the *safety specification* and *pharmacovigilance plan.* The former summarizes safety concerns of a medicine at a particular time-point in its lifecycle, potentially affecting its B-R, i.e. “important potential” or “important identified” risks, as well as areas where key information is missing. How these should be further investigated is covered by the *pharmacovigilance plan,* which includes routine and additional pharmacovigilance activities. Routine-pharmacovigilance activities are conducted for each medicine to detect safety signals including the reporting of suspected adverse drug reactions to regulatory authorities or submission of Periodic Safety Update Reports and other activities required by EU legislation. Meanwhile, additional pharmacovigilance activities address significant important or potential risks or significant missing information; these include monitoring of ongoing studies or registries (see section 2.5), and post-authorisation safety studies, which are carried out after marketing approval to obtain further information on safety and/or to measure the effectiveness of risk management measures (Zomerdijk 2015). The second part of an EU-RMP assesses whether each safety concern requires risk minimisation activities beyond the pharmacovigilance plan and whether routine or additional activities as part of RMPs are necessary to address the safety concern (Figure 2.3).

Risk minimisation measures (RMMs) are another tool to optimise the balance between the B-R and to minimize the risks of a medicine's use in clinical practice. RMMs either prevent or reduce the occurrence or the severity of adverse drug reactions by conditioning and restricting use (EMA 2014, EMA 2019g).

In Europe, there are two types of RMMs routine and additional. The former is required for all medicinal products and includes, for example, the patient information leaflet and the Summary of Product Characteristic (SmPC), which is the leaflet attached to a medicine providing all the relevant information on how to use it. The latter applies only to those medicines carrying serious and specific risks, required only when critical safety issues have not been sufficiently addressed by routine-RMMs (Figure 2.3). Examples of additional-RMMs include educational materials for health care professionals and/or patients, patient alert cards and prevention programmes, such as the Pregnancies Prevention Programmes (PPP).

PPP are for medicinal products with an identified human teratogenic risk requiring contraindication in pregnant women and those of childbearing age. The plans might include requirements for pregnancy test before starting treatment and repeated monthly during treatment, or compliance with effective contraceptive measures. Some PPP also have indications for lactation given the potential breastmilk exposure (of the medicinal product or its metabolites). Some PPP includes recommendations relating to the male partner, considering the potential teratogenic effects of the medicinal product (or its metabolites) on the semen and the possibility of pre-genetic, genetic and epigenetic transmission of anomalies to the foetus. For example, for known high-risk teratogens such as thalidomide, isotretinoin and vitamin A derivatives, PPP contraindicate medicines administration in pregnancy, requiring a pregnancy test before, during and after their use and the utilization of effective contraception methods for women of childbearing age (EMA 2014, Zomerdijk 2015, EMA 2019g).




PSUR: Periodic Safety Update Report; SmPC: Summary of Product characteristic; PIL: Patient Information Leaflet
Adapted from (EMA 2009, Zomerdijk 2015)

## Figure 2.3 Structure of EU risk management plan

### 2.4.3 Benefit-risk assessment in pregnancy

B-R assessment of medicines intended for pregnant and childbearing aged women carries additional levels of complexity. The first level of complexity is the “double risk and double benefit”: the maternal and the fetal. Administration of medicine in pregnancy requires, alongside the “standard” assessment as to whether a medicine’s benefit outweigh its risk (for the mother), the additional consideration as to whether the medicine could potentially detrimentally impact the physiological development of the fetus (Mitchell 2012). Hence the introduction of the concept of “innocent bystander” status of the fetus, who may be indirectly exposed to benefit of the maternal health improvement but is also exposed to unknown potential embryofetal and teratogenic risks (Mitchell 2012, Saint-Raymond et al. 2016).

Medicine’s embryofetal toxicity and teratogenicity has always been a major concern, for both clinicians and regulators, requiring different B-R assessments for different scenarios. For example, some pregnant women might require medicine to treat conditions exclusively related to pregnancy (i.e. eclampsia or gestational diabetes), some might require treatment for a condition arising during pregnancy (e.g. urinary tract infections or influenza) and some might require treatment for pre-existing chronic diseases where medicines cannot be discontinued or when availability of alternative treatments may be limited. Pre-existing condition such as asthma, diabetes mellitus, hypertension, epilepsy and depression all require continuation of treatments during pregnancy. Hyperglycaemia, for example, *per se* is a human teratogen, hence the control of glucose levels in pregnancy is critical and so it is the administration of anti-diabetic medicines (Goldman et al. 1985, Zabihi et al. 2010). Similarly, untreated hypertensive disorders can have severe consequences for both mother and foetus (ACOG 2013, Bernardes et al. 2019). Therefore, consideration of the risks of untreated maternal conditions needs to be balanced with the risks (or benefits) of maternal exposure to medicine with potential toxicity for the fetus.

Another level of complexity in the B-R assessment is the timing of exposure. Increasing number of women are now conceiving on medication, either because of their chronic conditions or because they are not aware of their status (about half of pregnancies are unplanned), exposing the fetus to potentially toxic effects in the most vulnerable phase, the organogenesis . A US-based study evaluated the number of medicines taken per pregnancy from the late 1970 to the early 2000 and reported a 68% increase (from 2.5 in 1976-1978 to 4.2 in 2006-08, range 0-28), with the average number of medicine taken during the first trimester increasing by 62% (from 1.6 to 2.6, range 0-25), with 82% of women in the most recent years using at least one medicine in first trimester (Mitchell et al. 2011).

B-R assessment is further complicated by the limited data availability on safety and efficacy from human pregnancies and the equally limited knowledge on embryofetal toxicity and teratogenicity. Data from the pre-marketing phase generally come from animal models and hardly ever from human pregnancies and if so, from number too small to detect teratogens. For example, a review on 172 medicine approved by the US FDA between 2000 and 2010 showed 98% of them not to have sufficient information to detect teratogenic risk in humans and for 73% no available data about their use in pregnancy (Adam et al. 2011). Similarly, in Europe a study evaluating 534 SmPCs found that 67% (361/534) of the medicines had no reported clinical experience in pregnancy and 95% (505/534) had a reported restriction for use in pregnancy; of those reporting a restriction, 90% (453/505) did not provide information on the medicine's ability to cross the placenta. Additionally, for those with a specific recommendation, 57% (299/525) were ambiguous and either lacking data on preclinical studies (9%, 28/299) or in pregnant women (21%, 64/299) (Arguello et al. 2015).

Therefore, assessment of embryofetal toxicity and teratogenic risks presents major limitations and the extrapolation of data from animal model cannot provide accurate prediction in humans (Riley et al. 2017). Findings in animal studies are a particular concern with regard to their applicability for human pregnancy and two good examples of these limitations are thalidomide and corticosteroids (Liggins et al. 1972, Bonanno et al. 2007). The former is nowadays a very well-known human teratogen but with no evaluation of teratogenicity from animal studies, while corticosteroids have shown toxic effects in animals but not in humans where it is still often used to treat preterm infants to allow lung development (Liggins et al. 1972, Bonanno et al. 2007).

Further, historically, pregnant women were excluded from CTs either a priori because of being pregnant or removed once becoming pregnant (Saint-Raymond et al. 2016). Awareness of potential teratogenic effect of prescription drugs arose first with the discovery that diethylstilbestrol- a substance given to pregnant women to reduce the chance of miscarriage- was the cause of a very rare vaginal carcinoma in the daughters exposed *in utero,* followed by the thalidomide disaster. This led to a series of recommendations to exclude pregnant women from CTs, such as the 1977 FDA's Guidelines advising for exclusion of pregnant women from phase I and II trials (FDA 1977). However, a first "wave" of CT reform started after about 20 years of evidence indicating underreporting of women in biomedical research (Lyerly et al. 2008). This led to the establishment in the US of the Women's Health Initiative at the National Institutes of Health (NIH) and the approval of the NIH-Revitalization Act of 1993, declaring inclusion of representative sample of subpopulations unless their exclusion

was justified “on a basis other than cost” (FDA 2011). One year later, the Institute of Medicine also stated that pregnant women are “presumed to be eligible for participation in CT” (Mastroianni et al. 1994). Furthermore, in 1997 the FDA established the Pregnancy Category Labelling System (FDA 1997) and the Second Wave Initiative was launched about 10 years after, “a broad, multipronged campaign to promote ethically responsible research with pregnant women” (Lyerly et al. 2009). More recently, the FDA replaced its pregnancy categories by the Pregnancy and Lactation Rule (PLLR, Final Rule), with the aim to further incentivise the clinical research for pregnant women (ACOG 2014).

Nevertheless, despite this and other efforts, pregnant women are still underrepresented and this conservative “policy of exclusion” from CTs is still widely in place (Lyerly et al. 2008, Roes et al. 2018). For example, a recent systematic review evaluating some key pregnancy outcomes (e.g. fetal losses, CAs, birth weight) in CTs on treatment for HIV infection, diabetes and hypertension between 1997 and 2017 found on the one hand, an overall increase in the number of studies including women (from a total of n=26 in 1997-02 to a total of n=44 in 2013-17) but on the other hand, a frequent underreporting of such outcomes. This highlights an important missed opportunity to collect information about potential consequence of fetal exposure to drugs. For example, the number of fetal losses were not reported in 76% (42/55) of CTs on diabetes, 81% (48/59) in CTs on HIV treatment and 72% (13/18) in CTs on hypertension; the number of CAs were not reported in 64%(35/55), 56% (33/59) and 94% (17/19) of the CTs on HIV, diabetes and hypertension, respectively (Aurich et al. 2020).

Furthermore, the recent outbreaks of Ebola and Zika virus offer a pertinent example of the complexity, and yet the necessity, of inclusion of pregnant and childbearing age women in registrational trials. Zika and Ebola viruses have shown to have some common characteristics: both have disproportionately affected pregnant women and their infants; both can directly affect the fetus by placental transfer; both can be (potentially) prevented by maternal immunization (Faucette et al. 2015, Rasmussen et al. 2016). To date, pregnant women infected by the Ebola virus have only survived after miscarriages, induced abortions or stillbirths, with an aggregate maternal mortality rate of 86% (WHO 2016 , Bebell et al. 2017, Gomes et al. 2017) and a perinatal mortality of 100% and with data from the WHO reporting no recorded case of infants, born to infected mothers, surviving more than a few days (Mupapa et al. 1999, Jamieson et al. 2014, WHO et al. 2014).

Zika virus can cause a pattern of CAs, known as congenital Zika syndrome (CZS), encompassing a spectrum of abnormalities such as microcephaly, craniofacial

disproportion, ocular manifestations and late-onset manifestation of developmental issues (Schwartz 2017, Zou et al. 2017).

The exclusion of pregnant women from nearly all vaccine trials meant that for the latest Ebola outbreaks, of the more than 25,000 available doses of experimental vaccine none were given to pregnant women (Haddad et al. 2018). The WHO Ethics Review Committee reviewed 14 protocols for international trials, including studies on brincidofovir (Dunning et al. 2016), favipiravir (Sissoko et al. 2016), convalescent plasma (van Griensven et al. 2016) and several phases of two vaccines, the rVSVΔG/ZEBOV-GP (Huttner et al. 2015) and the ChAd3-EBO-Z (De Santis et al. 2016) and found all protocols excluded pregnant women (Alirol et al. 2017). Some trials such as the brincidofovir trial based their exclusion on embryotoxicity findings in preclinical studies and others, such as the favipiravir trial, because of the failure to obtain insurance coverage, despite the awareness of the disease resulting in 100% human fetal loss (Dunning et al. 2016, Sissoko et al. 2016). In some other cases such as the rVSVΔG/ZEBOV-GP vaccine trials, 42 pregnant women were denied participation, even after an interim analysis proved the vaccine to be safe and effective in adults (Henao-Restrepo et al. 2017). Similarly, no pregnant women were included in the Zika virus vaccine trials, although prospective cohort studies included pregnant women to better understand the natural history of *in utero* Zika transmission (Ethics Working Group on ZIKV Research and Pregnancy 2017).

Maternal exposure to the investigational treatment (Ebola vaccine) would have offered a higher chance of maternal survival without adding or increasing risk to the fetus, given the current 100% fetal/neonatal mortality observed in absence of interventions (Bebell et al. 2017, Gomes et al. 2017). Similarly, vaccine against Zika virus could protect the fetus from maternal infection and prevent or minimize the risk for CZS, given that is estimated 1 out of 7 infants born to Zika infected mothers will present with CAs (Rice et al. 2018).

Furthermore, exclusion of pregnant women from CT precludes the possibility to detect pregnancy-induced PK changes, the body's response and ultimately the medicine's efficacy (described in chapter1, section 1.4.1) (Illamola et al. 2018, Roes et al. 2018). Medicines might require dosage adjustments or changes in the mode or frequency of administration, particularly for chronic conditions such as asthma and diabetes where good control of the disease enhances the likelihood of healthy infants; or for conditions such as HIV where sub-optimal treatment may result in increased risk of both disease progression and VT (EMA 2019g). Yet, PK studies including pregnant women have remained constant over time, representing only 1.3% of the total registered trials from

the 1960's to 2013 (Illamola et al. 2018). Furthermore, in the recent systematic review by Aurich et al., of the 132 CTs retrieved on diabetes, hypertension and HIV, overall only 33% (43/132) were PK studies, specifically 6% (3/55) on diabetes, 11% (2/18) on hypertension, and 64% (38/59) on HIV (Aurich et al. 2020).

### 2.4.4 Benefit-risk assessment in pregnant women living with HIV

The B-R assessment for pregnant WLWH carries some specific considerations. cART requires lifelong use and consequently, administration of cART should not be stopped after initiation, even during pregnancy; aside from treating maternal disease, cART also prevents VT and B-R assessment must thus consider this too (see chapter 1, section 1.3.3). In addition, B-R assessment is further complicated by cART being a combination of at least three drugs (often in a fixed dose), thus isolating and assessing toxicity of individual ARV drugs is complex (Zash 2018).

Although women globally account for half of the population living with HIV, they still are underrepresented in registrational trials (Berlin et al. 2009, Curno et al. 2016). According to a recent systematic review on 387 articles, women accounted for an average of 19% of the participants in cART studies, with female participation ranging from 0 to 94.5% (Curno et al. 2016). Furthermore, a recent study showed how registrational CT for new ARVs are not representative of the global HIV pandemic (Pepperrell et al. 2020). The study compared demographic characteristics of people living with HIV worldwide with those of people recruited in 20 phase III studies for marketing approval of DTG, Bictegravir, Tenofovir Alafenamide and Doravirine. The authors reported that although 42% of the global population PLWH are black and only 3% are white women, only 20% of participants enrolled in the CT were women, of these only 7% of the participant were black women,13% were white women, while the rest were man. Hence half the participants in the total were white men. The study also highlighted discrepancies in study settings, showing how even though 60% of PLWH live in LMICs, 76% studies assessing DTG were in HIC (which represent only 5% of the global HIV burden); overall white men were overrecruited by 44% compared with their global burden disease and black females were underrepresented by 35% (Pepperrell et al. 2020). So far, two major CTs have been conducted where only women were enrolled, the Women AntiretroViral Efficacy and Safety study (WAVES) and Antiretroviral Therapy Naïve Women (ARIA) trial.

WAVE is an international randomized controlled, double-blind phase 3 study and was the first trial on ARVs enrolling only women to assess safety and efficacy (Squires et al. 2016). ARIA is a randomized, open-label, multicentre, controlled, phase 3 study enrolling women aged over 18, but still excluding pregnant women (Orrell et al. 2017).

A recent study to evaluate inclusion of pregnant women in CTs for HIV-related research between 2001 and 2015, reviewed 63 trials and found that of the 14 ARVs approved by the FDA, only half (n=7) had been studied in pregnant women and those 7 ARVs were included only in 4 (6%) out of the 63 trials (Wickremsinhe et al. 2019).

Furthermore, most of the CTs in pregnancy are usually conducted on medicine already marketed (i.e. in phase IV) having been approved based on efficacy and safety data obtained from non-pregnant women and mostly from male subjects. For example, from the Wickremsinhe et al. study it was also reported that out of the 45 trials assessing HIV treatments 43 were investigating ARVs already approved by the FDA for the general population, with reports of an average delay of 4.4 years between the medicine's approval and the first pregnancy study (Wickremsinhe et al. 2019). Similarly, Colbers and colleagues found an average of 6-year lag between FDA approval of ARVs and first time publication of PK data in pregnancy (Figure 2.4) (Colbers et al. 2019).

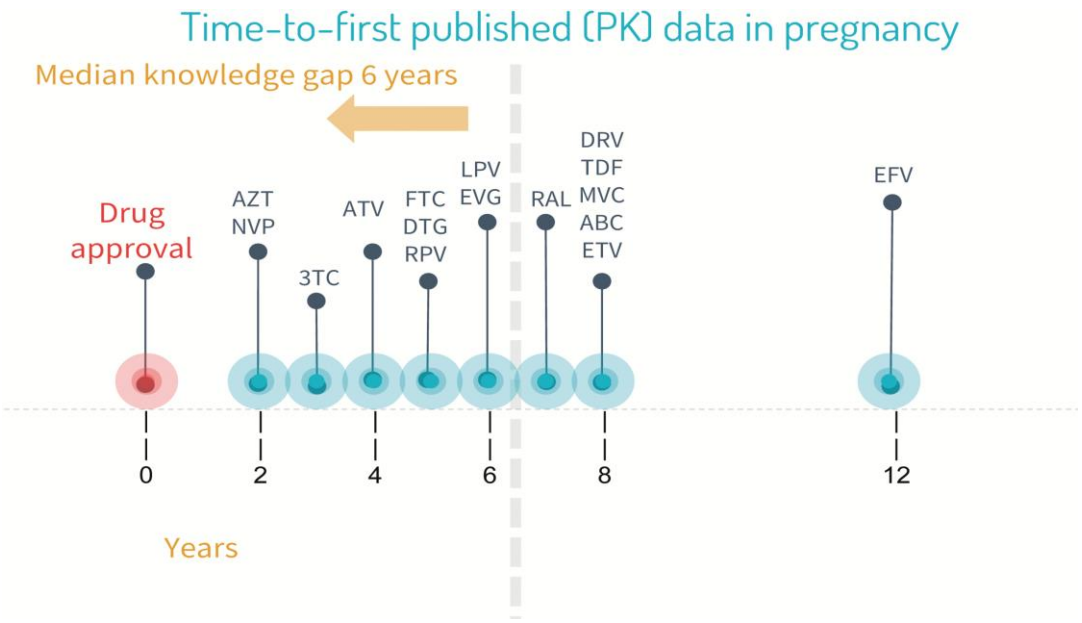



Figure reproduced under Creative Commons Licence, Colbers et al 2019, Clin Infect Dis

**Figure 2.4 Years between FDA approval and publication of PK data in pregnancy for different ARVs**

These findings highlight the strong reliance on post-marketing study for safety and efficacy data in pregnant women. Inclusion of pregnant women in phase I is needed to establish safe and effective dosage in second/third trimester, when pregnancy-induced changes might alter the medicine's profile, requiring dose changes (Colbers et al. 2019, Mofenson et al. 2019a).

Nowadays, the majority of PK data on ARVs in pregnant and lactating women are supplied by two large ongoing networks, the International Maternal Pediatrics Adolescent AIDS Clinical Trial Network (IMPAACT) and the Pharmacokinetics of Newly developed Antiretroviral Agents in HIV-Infected Pregnant Women (PANNA) studies (PANNA: ClinicalTrials.gov ID:NCT00825929, IMPAACT: ClinicalTrials.gov ID:NCT00042289). These utilise an opportunistic approach to allow for prompt enrolment of women whenever a new ARV is approved in order to collect and evaluate data on dosage, PK and safety during the second/third trimester and postpartum period (Mofenson et al. 2019a).

Results from a PANNA study showed a significant reduction to FTC exposure in the third trimester compared to the post-partum period (Colbers et al. 2013). Similarly, a study evaluating 228 women exposed to 3TC reported a 22% increased clearance in pregnant vs non-pregnant women (Benaboud et al. 2012). Another PANNA study, evaluating TDF found a 30% clearance increase during the last trimester compared to the postpartum period (Colbers et al. 2013). These alterations are most likely the consequence of NRTIs renal excretion and pregnancy-induced changes, particularly in the third trimester, with renal blood flow increases of up to 25-50% and a glomerular filtration up to 50%, subjecting NRTIs to increased renal elimination (Gilbert et al. 2015).

NNRTIs undergo CYP450-mediated metabolism; studies have found reduced concentrations of EFV and NVP in pregnancy, most likely as a result of progesterone-induced accelerated hepatic metabolism of CYP450 (Lamorde et al. 2010, Benaboud et al. 2011). Meanwhile, an increased plasma concentration of Etravirine (ETR) during pregnancy, with a decreased clearance of 52% in the third trimester vs postpartum attributed to the decreased expression of CYP2C19 induced by pregnancy (Best et al. 2015, Mulligan et al. 2016, Ramgopal et al. 2016). A more recent study also found a reduction up to 50% of RPV exposure in pregnancy, particularly in third trimester compared with the postpartum period (Colbers et al. 2017).

PIs also undergo CYP450 metabolism and several studies have demonstrated a reduced plasma exposure of PIs, particularly in late stages of pregnancy compared to postpartum (Gilbert et al. 2015). Additionally, PI activity requires – in all populations,

including pregnant women – administration with a pharmacokinetic enhancer or booster such as cobicistat or ritonavir to ensure therapeutic dose levels and prolonged plasma concentrations. Recent studies evaluated pregnancy and postpartum PK of such boosters and found a significant reduction in pregnancy exposure to cobicistat when given as a booster to ATV- and DRV-regimens compared to postpartum (Momper et al. 2018, Crauwels et al. 2019, Momper et al. 2019). These PK reductions are most likely the result of physiological pregnancy-related changes. Similarly, exposure to DRV boosted by cobicistat in its standard formulation of 800mg/150mg once daily showed a total exposure to DRV reduced by 50% (area under the curve, AUC) and 89% reduction of DRV minimum concentration ($C_{min}$) (Crauwels et al. 2019). Crauwels and colleagues also evaluated DRV boosted by ritonavir in its standard formulation of 800mg/100mg once daily and found 35% lower AUC and 50% lower $C_{min}$ in the third trimester vs postpartum period (Crauwels et al. 2016). These changes have shown to have an impact on drug exposure in pregnancy and an increased risk for maternal viral rebound and potential VT, leading to pregnant-specific dosing recommendations (Colbers et al. 2019).

INSTI are the most recent drug class, of which EVG is to date the only INSTI requiring cobicistat as booster; consequently, EVG is the only one with an altered PK in pregnancy (Best et al. 2017). Results from the IMPAACT P1026 study found that a EVG/cobicistat combination had a reduced viral suppression power, with only 75% of women with effective VL suppression by the time of delivery (Best et al. 2017). Raltegravir and Dolutegravir characteristics will be addressed separately in chapter 6.

Additionally, exclusion of pregnant women from CTs precludes gathering data about ARVs placental transfer. Placental transfer is an important determinant of fetal exposure to medicine and a surrogate marker to assess both the potential risk for physiological embryo-fetal development should the drug have toxic effects and the potential pre-exposure prophylactic effect with respect to VT (Benaboud et al. 2012, Stek et al. 2012, Colbers et al. 2013). However, data availability is overall limited and generated mainly from preclinical animal models or from cord blood sampling at the time of delivery (Colbers et al. 2019). Studies have shown NRTIs such as FTC, 3TC and TDF to have good placental transfer capacity; NNRTIs are equally able to cross the placenta, with NVP having the highest transfer probability due to its low protein binding power; however, PIs have proven to have poor placental transfer (Benaboud et al. 2011, Eley et al. 2013).

## 2.5 Post-authorisation phase

### 2.5.1 General consideration

The post-authorisation or post-marketing phase is generally where data on drug-related adverse events and rare toxicities, not identified in the pre-approval studies, are detected. This phase is also when most data on safety and effectiveness of a medicine's use in pregnant women are collected. The vast majority of drug-induced teratogenic effects, for example, are discovered in post-authorisation phase, by spontaneous adverse reaction reporting, follow-up cohort studies (e.g. pregnancy registries) and case-control surveillance studies (Dal Pan et al. 2012, Saint-Raymond et al. 2016). Limitations of pre-marketing studies may also be detected in the post-marketing phase; a US report estimated that 20% of marketed medicines acquired a warning during the post-marketing period (black box in the US and inverted black triangle in Europe) and 4% were withdrawn from the market for safety reasons (Lasser et al. 2002).

From a regulatory point of view, the post-authorisation phase makes use of PASS to obtain further information on a medicine's safety and Post-Authorisation Efficacy Study (PAES) to measure the effectiveness. These studies make use of pharmacovigilance activities including the implementation of additional-PhV plans when needed (Figure 2.2). Furthermore, since 2012 MAH must publish in their RMPs any post-authorisation experience, any evaluation of medicine's use in pregnancy and any proposal for identified risk management in pregnancy. For example, MAH must publish warnings and/or restrictions for a medicine's use or requirement to comply with specific PPP (e.g. delaying pregnancy or delaying treatment or using alternative medication whenever is possible) (EMA 2019g). However, whether a medicine will be used or not in real-life will remain a final B-R assessment of the healthcare professional and the patient, who, in the end, may have to take a decision with limited pregnancy-specific information (EMA 2019e).

### 2.5.2 Pregnancy registries and case-control surveillance studies

Follow-up or pregnancy registries and case-control surveillance studies are the most common sources to identify teratogens in post-marketing phase. Pregnancy registries are observational studies where an *a priori* hypothesis is usually made, data are collected longitudinally from early pregnancy to infancy and outcomes are compared with an internal or external control group (Lechat et al. 1993, APR 2017). Set up a registry starts with the prospective identification of pregnant women exposed to a given medicine, followed by their enrolment in the registry with data collection on pregnancy outcomes (and sometimes infant outcomes) through follow-up. Registries may be developed by the pharmaceutical company, public health bodies, regulators or by research groups (e.g. academia). They rely on voluntary reporting, where women and/or their health care providers submit data to the registry. The biggest advantage of such studies is the prospective nature, i.e. the enrolment of a woman exposed to drug of interest from early stages of pregnancy before the outcome is known. Consequently, they are designed to assess the safe use of medicine in human pregnancy, to monitor exposure and to identify risk contributing factors (e.g. dose, timing of exposure) and to detect any major developmental effects. Hence typically they have a primary objective such as detection of CAs and secondary objectives such as fetal growth abnormalities, spontaneous abortion, stillbirth and preterm birth (Dal Pan et al. 2012, EMA 2019g).

Pregnancy registries are usually powered enough to detect high risk teratogens, but their relatively small sample sizes are usually insufficient to detect rare CAs (e.g. those with background rates ranging from 1 in 1,000 to 1 in 10,000). Similarly, detection of moderate risk teratogens might be limited or biased by confounding factors such as concomitant use of other medicines or maternal lifestyle (e.g. smoking, alcohol); such information may not be collected by some registries. Registries are further limited by (self-)reporting bias, loss to follow-up and difficulties in finding a comparison group of “unexposed” women (Dal Pan et al. 2012).The Antiretroviral Pregnancy Registry (APR) (APR 2017) is an example of a pregnancy exposure registry. The APR is an ongoing international registry, established in the late 1980s with the aim to evaluate first trimester and later prenatal exposures to ARVs and providing early detection of any major teratogenic effects caused by exposure to any of the ARVs listed in the registry. Although, given they are post-marketing studies, there is a lag between time of ARVs’ approval and collection of enough data on first trimester exposure to detect potential increase of CAs, particularly for rare CAs. For example, it is estimated that 200 first trimester exposure is needed to rule out a two-

fold increase in overall CAs and 2,000 exposures to rule out a three-fold increase for rare events such as neural tube defects (considering background rates vary across countries between 0.12-0.06%) (Watts 2007, APR 2017, Mofenson et al. 2019a).

Case-control surveillance studies are another type of epidemiological study used to detect CAs. In this type of studies, the outcome is known (e.g. pregnancy outcome,

presence of CAs) and the data on maternal exposure to a given medicine are retrospectively reported (e.g. by interviewing the mothers). Case-control surveillance studies are characterised by higher statistical power than cohort studies, enabling detection of moderate-risk teratogen even among drugs used less frequently. Additionally, the advantage of having a control group foreseen by the study design allows for data collection on potential confounders (e.g. smoking, alcohol, assumption of others medicine) (Dal Pan et al. 2012).

Furthermore, establishment of prospective cohort studies has recently been proved as a promising source for data collection on potential exposures to teratogens through maternal medicine intake. These studies are particularly useful for detection of adverse effects on newly authorised medicine, by enrolment of women of childbearing age, who are then followed up, if becoming pregnant, and linked to the infant's birth outcome (Mofenson et al. 2019a).

### 2.5.3 Spontaneous reporting systems

Identification of a new safety issue often begins with clinical observations made by healthcare professionals during clinical practice or by patients. When a new side effect (symptoms or sign) not previously described is identified it is important to establish a temporal plausibility and to perform a differential diagnosis (Dal Pan et al. 2012).

A spontaneous reporting system (of adverse reactions or events) is another primary source of data in the post-authorisation phase to capture exposure to medicines with potential toxicity in pregnancy. The core of this system is the voluntary-base of reporting, either directly to an established national or regional centres or to the pharmaceutical company (which then must report to regulators). For example adverse drugs reactions can be directly reported to PhV databases such as the FDA Adverse Event Reporting System (FAERS) or the EMA EudraVigilance for medicines authorised in the EEA or the MHRA in the UK or the WHO VigiAccess (Dal Pan et al. 2012, Mofenson et al. 2019a).

However, such systems have several limitations such as under-reporting (given the voluntary base); reliance on the quality of the individual reporting; difficulty in ascertaining causality between the exposure and the adverse event; impossibility to measure prevalence due to lack of a denominator; risk of case duplicates (i.e. same case reported twice under different descriptions) and biased reporting of only adverse outcomes (Dal Pan et al. 2012, Hill et al. 2019).

# 3 Aim and Methods

## 3.1 Rationale

The arsenal of ARVs is constantly growing and, despite available treatments still being effective for a large part of the infected population, increasing drug resistance, toxicity concerns and sub-populations not benefitting from standard regimens have resulted in the demand for safer and more effective new therapeutic options. Even if an increasing number of new ARVs are now available, there are still some limitations for their use in specific populations such as women of childbearing age, pregnant and breastfeeding women.

As previously mentioned, the majority of women living with HIV are of childbearing age. Studies in the UK from the late 1990s / early 2000s reported an increased rate of pregnancies, mostly reflecting an increasing proportion of women accessing and remaining engaged with clinical care, along with improvements in HIV treatment and management (French et al. 2012, Huntington et al. 2013). Huntington et al. for example, reported an overall increase in the number of pregnancies (which rose from 156 in 2000/2001 to 450 in 2008/2009) and an increase in repeat pregnancies, i.e. subsequent pregnancies from women who had already had at least one reported pregnancy. This reflects both an increasing likelihood for diagnosed women to have a further pregnancy and a desire for women to create a family (Huntington et al. 2013). This increasing number of pregnancies also mirrors the widespread use of effective cART, which meant both a drastic reduction in VT rates and that women could live longer and healthier lives, most likely playing a key role in decision-making around childbearing (French et al. 2012, Huntington et al. 2013).

Nowadays the proportion of WLWH knowing their HIV status and conceiving whilst on effective cART regimens has stabilized at a high level, while the number of pregnancies per year in the UK has slightly declined, possibly reflecting completion of families and changes in the characteristics and clinical features of pregnant and childbearing aged women. For example, in resource-rich settings such as the UK, maternal age has increased, and studies have shown that older women tend to conceive whilst already on cART and are more likely to effectively suppress VL by delivery, possibly reflecting better treatment adherence and early engagement with antenatal care.

Early engagement with antenatal care might also reflect the fact that older women, particularly those over 40 years old, are more likely to experience fertility issues and/or adverse pregnancy outcomes such as obstetric complications (i.e. pre-eclampsia and gestational diabetes) and pregnancy complications (i.e. preterm delivery and stillbirths) (Huntington et al. 2013, Townsend et al. 2017).

These new trends of early cART initiation and hence prolonged infant *in utero* exposure to cART have led to concerns for potential toxic effects on the developing embryo. As outlined in Chapter 2, evidence generation on safety and efficacy for women of childbearing age, including those pregnant and breastfeeding, has proved to be particularly difficult (data collection from clinical experience is limited due to the frequent exclusion of women from registrational trials). Consequently, knowledge about potential embryo-fetal toxicity and teratogenicity caused by *in utero* exposure to ARVs, particularly for newly authorised ARVs and new ARV classes such as INSTIs is also limited.

This means that at the time of approval of a new ARV a fully satisfactory set of recommendations for its use in pregnancy cannot be provided. Therefore, regulatory and clinical guidelines often need to reconcile with real-world findings where combinations of ARVs are widely used by pregnant women throughout pregnancy and from which a growing evidence-base of pregnancy adverse outcomes have been reported, as mentioned in Chapter 1 section 1.5.3.

The above provides the rationale to describe and address the data gap in the use of ARVs in pregnancy by bridging data from regulators that form the basis of regulatory recommendations for use of ARVs (i.e. from preclinical studies and CTs) with extensive information from clinical experience on a population-level. This thesis analyses patterns of ARV use occurring between 2008 and 2018, focusing on the newly authorised class of INSTIs and the potential risk for CAs.

## 3.2 Aim and objectives

Aim:

To evaluate how antiretroviral agents are used in pregnant women living with HIV in the UK for treatment and prevention of VT, to explore safety and effectiveness aspects of their use and to assess consistency between regulatory recommendations and real-world evidence

Objectives:

1. To investigate the real-world use of ARVs in the UK between 2008 and 2018 by describing the patterns of ARV use in pregnant women living with HIV, including timing of earliest cART initiation for the considered pregnancy, type of regimen and temporal trends

2. To conduct a gap analysis of the safe and effective use of ARVs in pregnancy, using pre- and post-authorisation data obtained through publicly available datasets and regulatory data from the EMA

3. To examine the overall pregnancy outcomes in women with diagnosed HIV living in the UK in 2008-2018 and to conduct a detailed investigation of congenital anomalies in this population, specifically:

    a. evaluating potential temporal associations between periconception exposure to ARVs and detection of congenital anomalies
    b. describing types of congenital anomalies by organ/system
    c. exploring patterns of congenital anomalies by exposure to combinations of ARVs (rule of three)

4. As a case-study, to test the utility of data from multiple sources including a population-based surveillance study (NSHPC), a European cohort collaboration and the EMA to evaluate the Dolutegravir safety signal (i.e. increased risk of NTDs) and assess the potential for a drug class-safety signal.

## 3.3 Data sources:

I have used different data sources for this thesis, namely the National Surveillance of HIV in Pregnancy and Childhood (NSHPC) and the European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC) for analysis of individual patient data and the European Medicines Agency to collect publicly available safety and efficacy data on authorised medicines.

### 3.3.1 The National Surveillance of HIV in Pregnancy and Childhood

In the UK and Ireland, women living with HIV and their infants have been monitored since the late 1980s through a national study based at the UCL Great Ormond Street Institute of Child Health, London. The NSHPC was established in 1986, first known as the National Surveillance of Paediatric AIDS, then extended to include infection and perinatal exposure to HIV and renamed in 1989 as the National Study of HIV in Pregnancy and Childhood. As of 2019 the NSHPC has been known as the National *Surveillance* of HIV in Pregnancy and Childhood. Additionally, as of 2019, Irish data are not collected anymore.

The NSHPC is a population-based ongoing surveillance study collecting national data in the UK on all known cases of antenatal and perinatal exposure to HIV and paediatric HIV infection. Its main objectives are to monitor patterns of HIV infections and diagnosis in pregnant women and their infants; to evaluate changes in obstetric and therapeutic management of pregnant WLWH; to capture pregnancy and birth outcomes; to audit new perinatal HIV transmissions to better understand timing and circumstances of infant acquisition of infection; and to follow-up uninfected children born to WLWH and exposed to ART.

The NSHPC collects pseudonymized data on all women diagnosed with HIV prior to or during their current pregnancy and all infants exposed *in utero* to HIV (i.e. data are collected for the whole population and not as a sample). The study makes use of two parallel mechanisms for confidential and voluntary reporting schemes: a pregnancy and a paediatric scheme.

*Pregnancy scheme*

The pregnancy scheme was established under the auspices of the Royal College of Obstetricians and Gynaecologist (RCOG) in 1989 and collects data on all HIV positive

pregnant women through an electronic quarterly active reporting system that for the past couple of years has completely replaced the original paper-based system.

Every three months a named representative, defined as “responder”, from every maternity unit providing care to HIV positive women in the country, receives and completes an electronic notification card via a secure web-based reporting website (NSHPC Online), including null returns (i.e. no pregnancies occurred for the quarter). The responder is ideally a person with knowledge about all HIV positive pregnancies in the unit and able to access the required information, hence is usually the antenatal screening coordinator, but may sometimes be a specialist midwife, a consultant obstetrician or a health adviser. Through the notification card the number of pregnancies to HIV positive women seen in the previous three months are collected.

The NSHPC asks for data on any pregnancy outcome including terminations and miscarriages. For each case reported on the notification card, a pregnancy notification form (see appendix 9.1.1) is generated on the NSHPC Online to collect preliminary details about the women and her current pregnancy. For pregnancies expected to continue to term a pregnancy outcome form (see appendix 9.1.2) is generated closer to the Estimated Date of Delivery (EDD) to collect additional details on the pregnancy outcome.

These forms allow collection of maternal demographic and specific HIV-related characteristics and clinical management of pregnancy, labour and delivery (described in *Data items* and Table 3.1).

*Paediatric scheme*

The paediatric form was started in 1986 by the Royal College of Paediatrics and Children Health (RCPCH) via the British Paediatric Surveillance Unit (BPSU) through a monthly reporting scheme that is used for a number of different conditions. Through the notification card the number of all cases of HIV-exposed infants and HIV-infected children seen in the previous month were collected. The notification cards were sent to all consultant paediatricians in the country registered with the RCPCH (appendix 9.1.3), who then directly sent back the notification card to the NSHPC electronically or via the BPSU (appendix 9.1.4). However, as of January 2019 most paediatric respondents report directly online to the NSHPC Online, with very few received indirectly by the BPSU. Case identifiers (date of birth and NHS number) are collected via a paediatric notification form used to collect demographic data, birth details, and initial HIV test results. All notified children are then followed up, through a follow-up form, generated 18 months after the child’s data of birth to collect information on the

infant's infection status and other tests results, including final 18-month antibody test. Infants found to be infected are followed up annually until they are transferred to adult health care services or known to be lost-to-follow-up.

*NSHPC data items and data management*

All data are collected using standardized online data collection forms. These are periodically reviewed with new variables added if appropriate. No names or addresses are collected. Women are also assigned a unique NSHPC study number, which is retained for subsequent pregnancy reports so that new pregnancy report can be linked to previous for the same women.

Demographic information such as women's date of birth, ethnicity group, region of birth and parity are collected. Additionally, information on the probable source of HIV infection, mode of acquisition along with timing of diagnosis and whether the woman was diagnosed prior to or during the current pregnancy are also reported. Furthermore, information of cART, including whether the women was already on a treatment prior to conception or if started during the current pregnancy are collected (for more details see appendix 9.1).

The pregnancy form includes obstetric information such as date of delivery, pregnancy outcome, gestational age at delivery, planned and actual mode of delivery, CD4 cells count and VL closest to delivery as well as any switches for one ARVs to another are also recorded.

The paediatric form includes the infant's demographics, mode of delivery, *in utero* exposure to maternal cART, infant's birth-weight, presence of perinatal infections and congenital anomalies, post-partum prophylaxis, initial infection status and clinical details, and whether the infant was breastfed. Data from the pregnancy and the paediatric reports are linked to create a mother-infant pair and a substantial data linkage within the NSHPC database is carried out quarterly to link subsequent pregnancies in the same woman and to identify second-generation pregnancies (i.e. pregnancies in women who were reported to the NSHPC as children with vertical HIV). Consistency and validity checks are carried out every three months to produce a complete updated dataset for analysis. The NSHPC database as a unique form of one row per pregnancy, a unique identified for each pregnancy and a unique identifier for each women (i.e. the NSHPC unique study number mentioned above), so that pregnancy from the same women can be identified.

*Datasets used in this thesis*

For my thesis, I was provided with pre-specified datasets including only those variables needed for my analyses by the NSHPC Surveillance Manager (Table 3.1). These were transferred via a secure document gateway (i.e. a restricted shared drive) as an excel document where information has been divided into three separate excel sheets: one with women's demographic information, their pregnancies and infants data; one with cART combinations, starting and ending dates for each ARVs and any switches for each pregnancy; and one with data on VL and CD4 cell count values and dates per woman. Therefore, I received a restricted database according to the eligibility criteria I deemed relevant for the thesis objectives. Consequently, data were manually cleaned (i.e. inclusion/exclusion criteria were checked) and through the unique study identifiers each pregnancy was matched with the relevant and complete information (i.e. cART exposure/timing, VL levels and CD4 cell count, pregnancy outcomes, etc). To ensure the quality of the data prior to analyses presented in this thesis additional checks on the variables of interest were carried out particularly in respect to earliest exposure to combination of ARVs and congenital anomalies classification (explained in detail later in this chapter).

*Information governance*

The NSHPC carries out surveillance activities for Public Health England under Regulation 3 of the Health Service (Control of Patient Information), hence is included among those studies that by the Department of Health support "treatment or prevention" of sexually transmitted disease and therefore do not require individual patient consent for data collection and retention (Directions 2000 et al. 2000, NSHPC 2020). However, given the sensitive nature of those data, they are securely stored (historically, in locked cupboard at the ICH while the electronic data are stored as password protected files or databases on ISO27001-compliant secure drivers where access is limited to members of the NSHPC team.

## Table 3.1 Variable obtained from the NSHPC

| | |
|---|---|
| **Socio-demographic** | Maternal DOB (dd/mm/yyyy) |
| | Maternal ethnic origin |
| | Maternal country of birth (or region of birth) |
| **HIV-clinical** | Maternal HIV route of acquisition |
| | HIV Diagnosis in relation to the pregnancy (before/during) |
| **Pregnancy and delivery history** | Parity (previous livebirths, stillbirths, miscarriages/termination of pregnancies) |
| | Date of delivery/ child date of birth |
| | Gestational age (in weeks) |
| | Pregnancy outcome (livebirths, stillbirths, miscarriages or termination of pregnancy) |
| | Planned and actual mode of delivery |
| | VL near delivery (& dates) |
| | CD4 count during pregnancy & near delivery (& dates) |
| **ART-related** | cART combination used prior/during pregnancy (yes/no) |
| | Individual ARVs used in pregnancy (start/stop & switches dates) |
| **Infant history** | Sex |
| | Birth weight (kg) |
| | Twins (yes/no) |
| | Congenital anomalies (yes/no & details[1]) |
| | HIV infection status |
| | Mortality (as neonatal death and infant death)[2] |

DOB: date of birth; EDD: estimated date of delivery
[1]Details on CAs such as the organ and system affected were obtained from both the obstetric and the paediatric forms
[2]Neoantal death defined as a child that died within 28 days of birth and infant death defined as a child that died after 28 days of birth)

## European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC)

The European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC) is an international network of cohort and surveillance studies, mostly from European countries but also including cohorts outside of Europe (e.g. Thailand) set up in 2010 and conducting epidemiological research on pregnant women living with HIV, their infants and infants exposed *in utero* to HIV. EPPICC collects individual patient data and performs individual patient data meta-analyses, pharmacovigilance projects and other observational studies. Cohorts in EPPICC include an overall 30,000 HIV positive pregnant women and over 6,000 newly HIV diagnosed children (according to the last data merger in 2017). EPPICC is coordinated by the Penta Child Health (a global independent scientific network dedicated to paediatric research) and is coordinated at UCL jointly by the MRC CT Unit and the Great Ormond Street Institute of Child Health. EPPICC Pregnancy conducts research on pregnant women living with HIV with the aim of answering questions requiring large number of patients and in which the participating studies follow pregnant women and their infants to monitor their health. One of the main drivers in the establishment of EPPICC Pregnancy was the lack of CT data and the small size of individual cohort studies precluding meaningful analyses. EPPICC studies involve analysis of previously collected observational data, hence no study-specific interventions beyond those received in routine care are carried out. Examples of EPPICC pregnancy projects to date include the assessment of migrant women and whether they face inequalities in prevention of VT within the European Union (Favarato et al. 2018); evaluation of safe use of neonatal prophylaxis for high risk infants (Chiappini et al. 2013) and assessment of the risk of congenital anomalies in pregnancies exposed to EFV-containing regimen (Martinez de Tejada et al. 2019).

*Data merger and general methods*

The studies participating in EPPICC periodically provide pseudonymized individual patient data (i.e. no names, initials, hospital numbers, national ID or addresses of patients are collected) prepared according to a detailed standard operating procedure (SOP) and data specification which is based on a modified HIV Cohorts Data Exchange Protocol (HICDEP) (www.hicdep.org). HICDEP is based on a relational structure, with data presented in a series of tables, together with look-up tables for the codes to be used. Each table in the SOP has one or more identification numbers (ID) numbers (i.e. Patient, PREG_ID, FETAl_ID and BABY_ID) to allow match-up of data for the same woman, pregnancy and infants across tables. These are unique ID

assigned by the participating studies and are linkable to names and other identifiers only by the staff from each of the participating studies who have access to identifiers according to local approvals (e.g. clinical stuff and those entering the data). Table 3.2 provides the variables collected.

Each participating cohort/study is expected to be responsible for gathering, computerizing and submitting its own data for the EPPICC data merger. Pregnancy data are submitted to the data coordinating centre at UCL Great Ormond Street Institute of Child Health, where a comprehensive set of data quality checks are conducted, including validation checks, cardinality between tables and consistency and logic checks. Then individual cohort/studies are merged and analyses on the pooled dataset are conducted with oversight of each of the analyses provided by a Project Team.

*DOLOMITE-EPPICC*

In 2017 the DOLOMITE study was set up to investigate Dolutegravir (DTG) use and safety in pregnant women and *in utero* exposed infants in Europe and Canada, conducted within the NEAT-ID network and the EPPICC.

DOLOMITE-EPPICC conducts pooled analyses of observational prospectively collected pseudonymised individual patient data on pregnancies exposed to DTG from participating studies within the EPPICC framework. The aim of the study is to assess pregnancy and neonatal outcomes following DTG exposure during pregnancy in real-world settings.

The analyses I have conducted in chapter 6 in regard to DTG use in pregnancy in the NSHPC represent the UK data included in DOLOMITE-EPPICC and is explained in the relevant section of chapter 6.

## Table 3.2 Variables collected by EPPICC[1]

| | |
|---|---|
| **Maternal socio-demographic data** | Birth date (yyyy-mm-dd)<br>Ethnicity group<br>Origin (country of birth)<br>Date of HIV diagnosis (yyyy-mm-dd)<br>Co-infections (e.g. HCV, HBV, syphilis)<br>Mode of HIV infection[4] |
| | Date of last menstrual period (yyyy-mm-dd)<br>EDD (yyyy-mm-dd)<br>Parity<br>Pregnancy outcomes[7], N of fetus & date (yyyy-mm-dd) |
| **cART data** | ARVs contained in the regimen<br>Start/end date (yyyy-mm-dd)<br>Start time (i.e. from conception/during pregnancy)<br>Intrapartum use of ZDV<br>WHO stage 3 or 4 conditions |
| **Laboratory (CD4 & VL)** | Date of measurement (yyyy-mm-dd)<br>Values |
| **Newborns** | Date of delivery (yyyy-mm-dd)<br>Mode of delivery<br>Gender (sex of the baby)<br>Weight/height (at birth)<br>Neonatal death (date & cause of death)<br>Congenital anomalies<br>Baby lab, cART & other infections |

[1]For further details on this variables see appendix 9.2

### Data sets for the thesis

*Individual patient data (real world data)*

I have used several extracts of the NSHPC dataset in this thesis, each will be described in the relevant chapters and they are briefly summarized in Table 3.3.

## Table 3.3 Summary of the datasets used in this thesis

| Chapter | Type of analysis | Dataset |
|---|---|---|
| Chapter 4 | Snapshot analysis: to examine pattern and real-world use of ARVs | NSHPC data on antenatal use of ARVs in all singleton pregnancies with EDD from 2005 to 2016 |
| | Descriptive analysis: to examine trend and changes in pattern of ARVs use over time | NSHPC data on antenatal use of ARVs in all singleton pregnancies with DOB from 2008 to 2018 |
| | Descriptive and statistical analysis: to examine safe real-world use & pregnancy outcomes exposed to RPV and COBI | NSHPC data on antenatal exposure to RPV and COBI in all pregnancies with EDD from 2013 to 2017 |
| Chapter 5 | Descriptive and statistical analyses: to describe prevalence and explore patterns of CAs; to evaluate risk of CAs due to timing (i.e. preconception vs pregnancy) and ARVs exposure | NSHPC data on all singleton pregnancies with DOB from 2008 to 2018 delivering liveborn infants exposed to combination of ARVs and presenting with CAs |
| | Descriptive analysis: to examine pregnancy adverse outcomes (i.e. miscarriage, stillbirth, ToP) | NSHPC data on antenatal use of ARVs in all singleton pregnancies with DOB from 2008 to 2018 |
| Chapter 6 | Descriptive and statistical analysis: to examine RAL and EVG earliest exposure (i.e. at conception vs in pregnancy) and pregnancy outcomes | NSHPC data on antenatal use of RAL and EVG in all singleton and multiple pregnancies with EDD between 2008 and 2018 |
| | Descriptive and statistical analysis: to examine safe real-world use & pregnancy outcomes exposed to DTG | NSHPC data on antenatal exposure to DTG in all pregnancies with EDD from 2013 to 2017 |
| | Descriptive and statistical analysis: to examine pregnancy outcomes exposed to DTG | EPPICC and NSHPC data on antenatal use of DTG in all singleton pregnancies with DOB from 2008 to 2018 |

ARVs: antiretrovirals; RPV: rilpivirine, COBI: Cobicistat, DTG: Dolutegravir, EVG: Elvitegravir, RAL: Raltegravir; CAs: congenital anomalies; DOB: date of birth; EDD: estimated date of delivery; ToP: termination of Pregnancy.

### 3.3.2 European Medicines Agency

Through the European Medicines Agency (EMA) website, I have collected all publicly available data for each of the ARVs of interest, accessing and exanimating the European Public Assessment Report (EPAR), a document published once a medicine receives a positive decision from the European Commission. The information contained in the EPAR is updated throughout the medicine's lifecycle and any changes to the initial "terms and conditions" of use is included. Since 2005, this dossier includes the EU-Risk Management Plans (RMPs) now mandatory for all applicants and used to strengthen the B-R assessment of a medicinal products (as described in chapter 2). I have extracted additional information from the Periodic Safety Update Reports (PSUR) and from the relevant sections of the Summary of Product Characteristics (SmPC), (Figure 3.1).

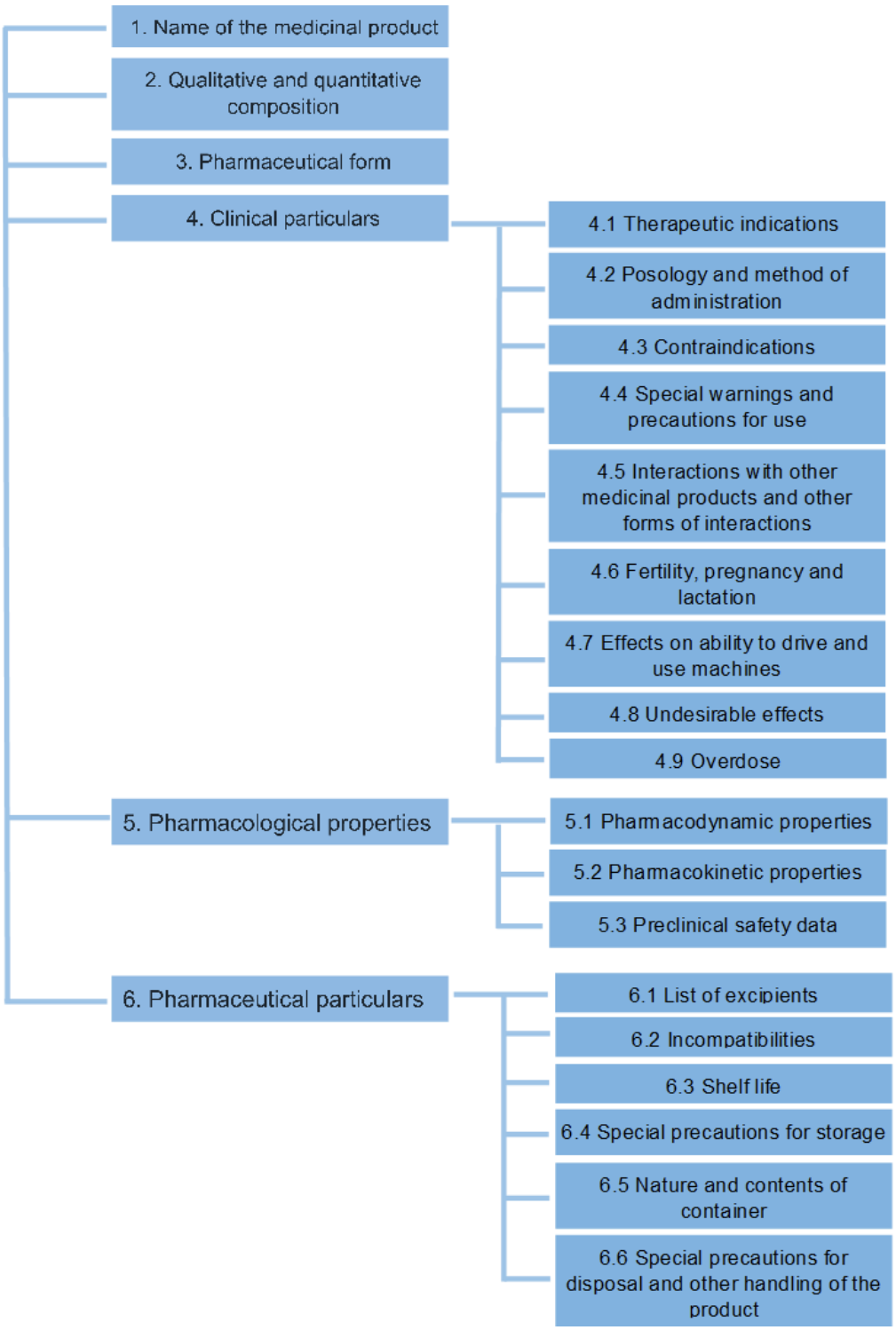
The SmPC is a legal document required as part of a medicine's marketing authorisation procedure and is the basis of information for healthcare professionals on how to prescribe the medicine safely and effectively. The SmPC is divided into sections, each addressing a specific important aspect of the medicine. For example, it contains the therapeutic indication(s), the target disease and population, the recommended dosage and method of administration, warnings and precautions or contraindication for its use for special population, etc. Data contained in the SmPC are updated throughout the life-cycle of the medicine, whenever new data are available.

*Data extraction*

Table 3.4 illustrates how I organized the data after their extraction from the relevant sections of the SmPCs and EPAR documents for each ARVs included in the analyses. In the first column are listed the ARV as single agents or ARVs in a fixed-dose combination, followed by the trade or commercial name and then by the year of authorisation (licencing year). The following six columns refer to specific sections of the SmPCs and I have populated them with all the relevant information for my thesis, i.e. anything that addressed pregnant, breastfeeding women and those of childbearing age. For example, in section 4.2 "*Posology and methods of administration*" information about drug-drug interaction such as with contraceptive hormonal pills might be listed. Similarly, in section 4.3 "*Contraindication*" specific recommendation to avoid the use of certain ARVs in pregnancy might be reported. In section 4.4 "*Special warnings and precautions*", more specific recommendations for pregnant women and those of childbearing age to comply with specific contraceptive

measure prior to starting the treatment or to avoid the treatment whilst being pregnant might be listed. Section 4.6 "*Fertility, pregnancy and lactation*" contains specific recommendations for women of childbearing age and those pregnant and/or breastfeeding and often cross-references with section 4.3, 4.4 and 5.3. Section 4.6 is also where all the relevant clinical safety data are reported, including sample size and consequently the level of strength of the recommendations (e.g. "large" (>3,000 or >1,000) or "limited" (<300) amount of data from human/clinical studies). Data on preclinical findings are also briefly reported here to sustain/justify the recommendation and then cross-referenced with section 5.3 "*Preclinical safety data*" where all data from animal model are reported. These section contains data from reproductive toxicities studies, fertility and genotoxicity (as mentioned in Chapter 2 section 2.3.2). Section 5.1 "*Pharmacodynamic properties*" and section 5.2 "*Pharmacokinetic properties*" are sections where information from PK-PD studies specifically addressing the PK-changes in pregnant women might be found. Specific data extraction, collection from the relevant sections of the SmPCs and EPAR documents that pertain to a single analysis have been described in detail in the relevant chapter, i.e. Chapter 4.

The final six columns of the table have been populated with information on whether the ARV had trans-placental transfer abilities (usually such information is reported for each of the ARVs contained in a fixed-dose combination) and whether this was observed in animal models or reported from CT studies or real-world data; whether the SmPCs reported recommendations for adjustments/changes in the ARV dosages tailored for pregnant women; whether clinical and PK data on pregnant women were available. Lastly, for all the ARVs, data on their half-life were retrieved, where half-life ($t$ ½) was defined as the time required by a substance to lose half of its pharmacological activity; a pharmacokinetic parameter used to evaluate how much time a substance requires to pass through the kidneys and the liver and be extracted from the body.




Information is presented according to a predefined structure; certain information that is appropriate for different sections are cross-referenced to avoid repetitive information

**Figure 3.1 Structure of the Summary of Product Characteristics (SmPC)**

**Table 3.4 Sample of the summary on safety data extracted from EMA-sources**

| | |
|---|---|
| **ARV(s)** | As single agents or in fixed-dose combination |
| **Trade name** | European commercial name |
| **Licencing year** | Year of European authorisation |
| **Sec. 4.2 "Posology and methods of administration"** | e.g. warnings of drug-drug interactions or reduced drugs concentration due to pregnancy |
| **Sec. 4.3 "Contraindication"** | e.g. compliance with contraceptive methods/avoidance in pregnancy |
| **Sec. 4.4 "Special warnings and precautions for use"** | e.g. compliance with contraceptive methods/avoidance in pregnancy & warnings of drug-drug interactions or reduced drugs concentration due to pregnancy |
| **Sec. 4.6 "Fertility, pregnancy and lactation"** | Data from CT, recommendation for drug's use for WCBA & in preg, B-F, cross-reference with sec 5.3 |
| **Sec 5.1 "Pharmacodynamic properties"**<br>**Sec. 5.2 "Pharmacokinetic properties"** | Any data from PK-PD studies on pregnant women |
| **Sec. 5.3 "Preclinical safety data"** | Any data on preclinical findings from animal models for each agent contained in a fixed-dose combination |
| **Placenta crossing** | Y/N; animal model/CTs/RW-data |
| **Dose changes** | Y/N |
| **CT data on pregnant women** | Y/N |
| **PK-PD data on pregnant women** | Y/N |
| **Half-life (t ½)** | For each ARV as single agents or in a fixed-dose combination |

CT: clinical trials; WCBA: women of childbearing age; preg: pregnant; B-F: breast-feeding; PK-PD: pharmacokinetic-pharmacodynamic; RW-data: real world data; Y/N: yes/no

## 3.4 Definitions and data analyses

Definitions used throughout the thesis are outlined here, while specific definitions appropriate for each analysis are specified in the relevant chapters.

*Maternal characteristics*

**Maternal age**: defined as maternal age at delivery is derived using the women's 'date of birth' and the child's 'date of birth' for live- and stillbirths, while the variable 'end of pregnancy' was used for the other outcomes.

**Parity**: defined as the number of previous pregnancies regardless of the outcomes, hence also including termination of pregnancies and miscarriages.

**Ethnic group**: for most of the analyses, ethnicity was classified as white, black African, black other, other and missing.

**Region of birth**: for most of the analyses these groups were used: Sub Saharan Africa, UK/Ireland, Europe, elsewhere, not known.

**HIV acquisition route**: defined as the maternal HIV acquisition route. Different categorical variables were created, namely heterosexual (through heterosexual intercourse), injecting drug use (IDU), vertical (defined as HIV acquisition during pregnancy, at time of labour and delivery or through breastfeeding), other (e.g. transfusion recipient or contact with infected blood) and not known.

**Timing of diagnosis**: defined as diagnosis of HIV made "before" or "during" the current pregnancy.

**First, second and third trimesters**: first trimester (T1) is defined as 1-12 completed gestational weeks (GW), second trimester (T2) as 13-26 GW and third trimester (T3) as more than 27 GW, respectively. These cut-off values have been also used to define the time of cART initiation, e.g. a cART regimen started at 12 GW, is a treatment started in T1, a cART regimen started at 13 GW is a treatment started from T2, including T3, while a cART regimen started "at conception" is defined as a treatment started prior to conceiving (see later in this section for more details).

**VL at delivery**: defined as VL within 30 days of delivery, i.e. ≤30 days before or ≤30 days after delivery. While baseline VL is defined as VL measure at diagnosis, or first result reported in pregnancy.

Undetectable VL is defined as ≤400 copies/μL following NICE guidelines, and ≤50 copies/μL following BHIVA guidelines; detectable VL is defined as >400 copies/μL.

**Mode of delivery and pregnancy outcomes**: classified as vaginal, elective CS (before the onset of labour or membrane's rupture) or emergency CS (after membrane's rupture or onset of labour). Pregnancy outcomes have been classified in livebirths, stillbirths (death occurring ≥24 GW), miscarriages (fetal deaths occurring <24 GW) and termination of pregnancy (ToP).

*Infant characteristics*

Infant deaths: classified as neonatal deaths where an infant died within the first 28 days of life and child deaths where a child died after 28 days of life.

*Treatment characteristics*

Antiretroviral agents (ARVs): refers to the single agents of an ART regimen, also defined as ARV combination.

Antiretroviral therapy (ART): refers to the combination of ARVs also referred to as cART.

Timing of initiation is expressed in slightly different ways for different analyses with details provided in the relevant results chapters (see above for definitions of trimesters).

Periconception exposure is defined as maternal exposure to any ARV initiated before conception and includes also T1.

An ARV was considered as used in pregnancy if there was any use in the pregnancy regardless of the duration.

### 3.4.1 European Surveillance of Congenital anomalies (EUROCAT)

The EUROCAT was founded in 1979 as the European Concerted Action on Congenital Anomalies and Twins a population-based network of congenital anomaly registries with the aim to conduct epidemiologic surveillance of CAs in Europe (Lechat et al. 1993, Boyd et al. 2011). EUROCAT collects data on major structural CAs detected pre- or post-natally (defined in chapter 1 section 1.4.2 “Teratogenicity and congenital anomalies”, (Rasmussen et al. 2014), while minor anomalies are excluded from data collection unless associated with major CAs or genetic syndromes (Lechat et al. 1993, Boyd et al. 2011). Nowadays, EUROCAT Guide 1.4 (EUROCAT 2016) tables and the classification criteria for major CAs by organ/system has become a widely used reference, also enabling comparison and pooled data analysis across European countries. For example, the EUROCAT organ/system classification was used to categorize the CAs collected in a recent pooled analysis on individual pregnancy data within EPPICC on seven observational studies on HIV positive pregnant women, across 13 European countries and Thailand (Martinez de Tejada et al. 2019). Furthermore, the value of combining data was reported by Morris et al., when they evaluated microcephaly prevalence in Europe, following reports of Zika virus and the risk for microcephaly using 24 EUROCAT registries covering approximately 570, 000 annual births in 15 countries, between 2003 and 2012 (Morris et al. 2016). Boyle et al. also avail EUROCAT when reporting an increased risk of Down syndrome for singleton pregnancies vs multiple, after collection on over 14 million births from 1990 and 2009, which is a relevant information for genetic counselling and prenatal screening (Boyle et al. 2014).

Therefore, I chose this classification to re-code all the CAs reported to the NSHPC (data collected from 2008 to 2018) and used these in Chapter 5 and 6 with two main rationales: the potential to compare my findings with those from other European studies and to resolve the issue of the many different systems/ classifications criteria leading to different definitions. Furthermore, I adapted the EUROCAT “prevalence data table” to my findings (Table 3.5), while the following sections from the “EUROCAT Guide 1.4” were used to re-classify all the CAs reported to the NSHPC: Sec. 3.2 “Minor anomalies for exclusion” (active since 2005); Sec. 3.3 “EUROCAT subgroups of CAs (v2014; implemented in EDMP December 2014, used for website prevalence tables from December 2014)”; Sec. 3.5 “Detailed CAs coding guidelines” and Sec. 3.6 “EUROCAT description of CAs subgroups” (EUROCAT 2016).

## Table 3.5 Example of the EUROCAT Prevalence Data Table

| Anomaly group | Tot Cases | LB | FD | TOPFA |
|---|---|---|---|---|
| **All Anomalies** | | | | |
| **Nervous system** | | | | |
| Neural Tube Defects | | | | |
| Anencephalus and similar | | | | |
| Encephalocele | | | | |
| Spina Bifida | | | | |
| Hydrocephalus | | | | |
| Severe microcephaly | | | | |
| Arhinencephaly/holoprosencephaly | | | | |
| **Eye** | | | | |
| Anophthalmos/micropthalmos | | | | |
| Anophthalmos | | | | |
| Congenital cataract | | | | |
| Congenital glaucoma | | | | |
| **Ear, face and neck** | | | | |
| Anotia | | | | |
| **Congenital heart defects** | | | | |
| Severe CHD Â§ | | | | |
| Common arterial truncus | | | | |
| Double outlet right ventricle | | | | |
| Transposition of great vessels | | | | |
| Single ventricle | | | | |
| Ventricular septal defect (VSD) | | | | |
| Atrial septal defect (ASD) | | | | |
| Atrioventricular septal defect (AVSD) | | | | |
| Tetralogy of Fallot | | | | |
| Tricuspid atresia and stenosis | | | | |
| Ebstein's anomaly | | | | |
| Pulmonary valve stenosis | | | | |
| Pulmonary valve atresia | | | | |
| Aortic valve atresia/stenosis Â§ | | | | |
| Mitral valve anomalies | | | | |
| Hypoplastic left heart | | | | |
| Hypoplastic right heart Â§ | | | | |
| Coarctation of aorta | | | | |
| Aortic atresia/interrupted aortic arch | | | | |
| Total anomalous pulmonary venous return | | | | |
| PDA as only CHD in term infants (>=37 weeks) | | | | |
| **Respiratory** | | | | |
| Choanal atresia | | | | |
| Cystic adenomatous malf of lung Â§ | | | | |
| **Oro-facial clefts** | | | | |
| Cleft lip with or without palate | | | | |
| Cleft palate | | | | |
| **Digestive system** | | | | |
| Oesophageal atresia with/without tracheo-oesophageal fistula | | | | |
| Duodenal atresia or stenosis | | | | |
| Atresia or stenosis of other parts of small intestine | | | | |

| | | | | |
|---|---|---|---|---|
| Ano-rectal atresia and stenosis | | | | |
| Hirschsprung's disease | | | | |
| Atresia of bile ducts | | | | |
| Annular pancreas | | | | |
| Diaphragmatic hernia | | | | |
| **Abdominal wall defects** | | | | |
| Gastroschisis | | | | |
| Omphalocele | | | | |
| **Urinary** | | | | |
| Bilateral renal agenesis including Potter syndrome | | | | |
| Multicystic renal dysplasia | | | | |
| Congenital hydronephrosis | | | | |
| Bladder exstrophy and/or epispadias | | | | |
| Posterior urethral valve and/or prune belly | | | | |
| **Genital** | | | | |
| Hypospadias | | | | |
| Indeterminate sex | | | | |
| **Limb** | | | | |
| Limb reduction defects | | | | |
| Club foot - talipes equinovarus | | | | |
| Hip dislocation and/or dysplasia | | | | |
| Polydactyly | | | | |
| Syndactyly | | | | |
| **Other anomalies/syndromes** | | | | |
| Skeletal dysplasias Â§ | | | | |
| Craniosynostosis | | | | |
| Congenital constriction bands/amniotic band | | | | |
| Situs inversus | | | | |
| Conjoined twins | | | | |
| Congenital skin disorders | | | | |
| VATER/VACTERL | | | | |
| Vascular disruption anomalies Â§ | | | | |
| Lateral anomalies Â§ | | | | |
| Teratogenic syndromes with malformations Â§ | | | | |
| Fetal alcohol syndrome | | | | |
| Valproate syndrome Â§ | | | | |
| Maternal infections resulting in malformations | | | | |
| Genetic syndromes + microdeletions | | | | |
| **Chromosomal** | | | | |
| Down Syndrome | | | | |
| Patau syndrome/trisomy 13 | | | | |
| Edward syndrome/trisomy 18 | | | | |
| Turner syndrome | | | | |
| Klinefelter syndrome | | | | |

Prevalence is given in per 10,000 births; Â§: incomplete or missing specification of IDC 10 codes LB: livebirths; FD: fetal deaths (≥20 GW); TOPFA: termination of pregnancy for fetal anomaly

Source: adapted from EUROCAT Website Database: https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence_en

## 3.5 Missing data

There was some missing information identified as part of the process of data cleaning from the NSHPC. As previously explained, different datasets have been used in this thesis, with each of the analyses requiring different level of details from the obtained data. Therefore, data cleaning and the way in which I have dealt with missing data differed for each analysis and is explained in the relevant chapters.

A key outcome variable with substantial missing data was VL at delivery. For example, this was missing for 36.3% (4,394/12,099) of deliveries occurring during 2008-2018 (based on data presented in Chapter 4). Previous NSHPC analyses (French 2014) have assumed that, given the high rates of women receiving cART, whenever a pregnancy had a missing value for VL at delivery, but the last available VL at any time during pregnancy was undetectable, then delivery VL was imputed as undetectable. For this thesis, I decided to be more restrictive and pregnancies without VL measures at delivery were considered to have missing data. However, in light of the large proportion of pregnancies excluded, a comparison of maternal and infant characteristics (i.e. exposure to cART, time of first exposure to ARVs, presence/or not of CAs and median maternal age at delivery) between those with and without VL at delivery was carried out, showing some significant differences between the two groups as reported in Table 3.6.

**Table 3.6 Maternal/infant characteristics for women with or without reported VL values at end of pregnancy, NSHPC 2008-2018**

| Maternal/infant characteristics | Women with reported VL | Women without reported VL | *p*-values |
|---|---|---|---|
| **Tot, *n* (%)** | **7,705** | **4,394** | |
| **Timing of first exposure to ART** | | | |
| Periconception | 4,677 (60.7%) | 3,010 (68.5%) | <0.001 |
| Pregnancy | 3,028 (39.3%) | 1,384 (31.5%) | |
| **cART** | | | |
| Yes | 4,396 ( 57.1%) | 2,853 (64.9%) | <0.001 |
| No | 3,309 (42.9%) | 1,541 (35.1%) | |
| **Congenital Anomalies** | | | |
| Yes | 162 (2.1%) | 105 (2.4%) | 0.303 |
| No | 7,543 (97.9%) | 4,289 (97.6%) | |
| **Maternal age at conception** | | | |
| Median (years) | 33.9 | 34.3 | 0.016 |

## 3.6 Statistical analysis

Data extraction from the NSHPC database was performed using MS Access 2016. Data were compiled using and analysed using R version 4.02 (R Core Team 2020). In this section I provide a general overview whilst specific statistical methods, and definitions of populations and inclusion/exclusion criteria will be described in detail in the methods sections of relevant chapters.

### 3.6.1 Descriptive analyses and test significance

Proportion were calculated among cases with known information on the variable of interest and were compared using the $\chi^2$ or Fisher's exact test (if there were less than five observations on any cell); trends in proportions were assessed using the $\chi^2$ test-for-trend (Kirkwood et al. 2003) and Pearson's' $\chi^2$ test with Yates' continuity correction was used whenever the total sample size was less than 40, or the expected number were small or if there was any expected less than five (when Yates' is used).

For categorical variables, the total number of studied subjects and measure of frequencies (i.e. percentage, %) are reported, while for continuous variables, the median and interquartile range (IQR) were used for skewed data. Binomial 95% CI were calculated with the exact method (Agresti 2013).

### 3.6.2 Construction of multivariable models

Multivariable models using logistic regression were fitted to investigate the association between exposure and outcome variables and will be explained within methods and results in Chapter 5 and 6.

Univariable analyses were carried out to obtain crude odds ratios (ORs) with 95% Confidence Intervals (CIs). Goodness-of-fit of nested models was assessed using the Wald test. Multivariable models were developed using a forward-fitting approach. For the analysis examining the association between a defined exposure (i.e. exposure to ARVs) and the outcome (i.e. presence of a CA), potential risk factors were identified and considered a significant risk factor if $p<0.05$ in the multivariable model.

To identify all the relevant factors independently associated with the defined outcome in order to build the risk factor analysis, the variables significantly associated with the outcome in the univariable analyses were included in the multivariable analysis.

Both Akaike's information criterion and the Bayesian information criterion are goodness-of-fit statistics which allow comparing non-nested models and penalise for model complexity (Kuha 2004). In this thesis Bayesian information criterion was used as it is more robust than AIC against overfitting (it penalized more strongly every parameters that is in the model) (Table 3.6 and Table 3.7).

Since Bayesian information criterion = -2 Log Likelihood + log($n$)×$p$ where $p$ is the number of parameters, and n is the number of independent observations Akaike's information criterion = -2LogLik + 2 ×$p$ so the penalisation factor in Akaike's information criterion is smaller than Bayesian information criterion's. In general, since the models were fitted on at most 11,197 independent observations, log(11197) x $p$ **= 9.32 x p** is a bound for the penalisation factor in Bayesian information criterion (i.e. is ~ 4.5 times higher than Akaike's information criterion's) i.e. each degree of freedom (i.e. a parameter) added to a model cost around 4.5 times more in Bayesian information criterion and Akaike's information criterion.

Several models were built and identified risk factors were added to the models and kept in the model if they improved the fit, starting with the one for which there was the strongest evidence of confounding (based on the results of the bivariable analysis, $p$<0.05 was considered significant). Sensitivity analyses (described in the relevant chapters) have been conducted to confirm the robustness of the results.

In chapter 5 and 6 to evaluate the interactions, i.e. the joint effect that being exposed to ARVs (i.e. exposure by class, by the five combinations of interest and restricted to exposure to INSTIs) and being exposed by timing (i.e. preconception vs in pregnancy) would have on the risk for CA, models were fitted (described in detail in chapter 5 and 6). However, here I will explain how I was able to obtain the 95% CIs of the ORs for the interaction terms of each model as they share the procedure. A log-odds scale was used to obtain the standard error (std error) of the multiplier for. In order to do so, it was assumed that the variance of a sum of possibly correlated variables is equal to the sum of their variances plus twice the sum of all pairwise covariances. This means that if the covariances are all zero, the variance of the sum is equal to the sum of the variances. So in logistic regression models, the standard error of the sum of estimates was obtained as the square root of the sum of the corresponding terms in the diagonal of the variance-covariance matrix of the estimates in log-odds scale, plus twice the sum of the pair-wise covariances of these terms (to account for the correlation between the estimates).

Finally the estimate for the multiplier in odds ratio scale was obtained as the exponential of the sum of estimates, and its CI was obtained by the exponential of the confidence interval in log-odds scale.

The R function `vcov` was used to extract the variance-covariance matrix of the estimates of the logistic regression models.

### *Adjusting for clustering*

Given the nature of the NSHPC and of my analysis, i.e. covering a 10-year span time, I originally wanted to consider the possibility of women contributing more than one pregnancy to the dataset through repeated pregnancies, something that can be described as being clustered at the women-level. Therefore, I tried to include one or more random effects into the models to control for multiple children from the same mother (Kirkwood et al. 2003). However, the distribution of pregnancies by mother in my dataset (Table 3.7) was as follows: of 8,373 mothers with liveborn infants, 72.1% (6,035/8,373) had only one pregnancy and 94.8% (7,940/8,373) of mothers had at most two pregnancies (i.e. 6,035 women with one pregnancy plus 1,905 with two pregnancies).

**Table 3.7 Frequencies of liveborn pregnancies by women reported to the NSHPC between 2008-2018**

| **Pregnancies** | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Women** | 6,035 | 1,905 | 387 | 39 | 7 |

Two numerical optimisers based on non-linear mixed-effects (glmer) and one penalised quasi-likelihood (`glmmPQL`) were considered. Neither of them converged to a successful maximum likelihood solution, most likely because when fitting random effects, the estimation procedure needs to include within-subject variability. In this case, the vast majority of the mothers included in the dataset did not contribute enough to this component of variability because most had information from either one or at most two pregnancies. There were therefore not enough clustered observations for the model with the random effects term to yield adequate estimates.

## 3.7 Research governance

The NSHPC (now ISOSS) collects patient data under legal permissions granted to Public Health England (PHE) under Regulation 3 of The Health Service (Control of Patient Information) Regulations 2002. PHE has permission from Parliament to collect this data without the need to seek consent from individual patients. This research is also covered by an approval from the Antenatal and Newborn Screening Programme Research Advisory Committee (ANNB_IDPS_034). EPPICC (Pregnancy) has UCL Research Ethics Committee approval (3715/007). Both projects are registered with the UCL Data Protection Office.

## 3.8 Role of the researcher

During my PhD I collaborated closely with the NSHPC team, firstly by shadowing Miss Helen Peters, the NSHPC study manager, to understand how data are collected, stored and analysed. Given my clinical background, I also contributed to the running of the NSHPC by providing input to the NSHPC team with respect to queries needing some clinical insight. For example, I was consulted to resolve medical terminology queries or doubts about treatments. I was asked to lead the re-categorization of all the CAs reported, collected and stored in the NSHPC database. I revised and re-coded all the CA cases according to the ICD-10 classification and discussed every uncertain case with Miss Helen Peters and Miss Laurette Bukasa (surveillance assistant). To solve some queries, there was a need to retrieve the original obstetric and paediatric forms, in order to access important information contained only in the notes (frequently including written notes by responders, dating from the period when paper-based forms were used). These are either stored as paper forms or available as electronic update at UCL-ICH. For example, it was necessary to go to such notes when only the affected organ (e.g. heart defect) was mentioned but not the condition (e.g. pulmonary atresia); or when additional information on the nature of the defect was necessary to define a CA (e.g. talipes is considered a CA only if it is equinovarus or adductus, but not if it is of postural origin). Notes were often helpful also to determine the cause of neonatal deaths, stillbirths and termination of pregnancies. Furthermore, I contributed to the development of two new categories for the database classification: “CAs meeting” and “CAs not meeting” the ICD-10 classification criteria. The latter are now categorized in the database as “child/infant problems” and cover a wide range of different conditions that are not CAs according to ICD-10 or are not CAs at all (e.g. infection, anemia, etc). These are important data to be collected for future

work, in light of the growing interest for the HIV-exposed uninfected children as they have an increased vulnerability to infectious disease and an altered immune response compared to HIV-unexposed children.

We decided to adopt the ICD-10 criteria since these allow for a collection of wider and more detailed range of CAs. Data collected under these criteria and stored by the NSHPC can be used within any other classification criteria for national or international analysis.

Finally, as explained in section 3.6, I performed all the data cleaning, while the statistical analyses were the result of the essential collaboration with my supervisor Prof Mario Cortina Borja. To date this work has led to one original research paper and several conference abstracts and oral presentations (see appendix 9.6).

I also contributed to the analysis evaluating DTG use in EPPICC cohorts as part of a small team managing and analysing the data reported from the participating cohorts, as well as helping to coordinate, interpret and disseminate the findings. This is part of the analysis reported in chapter 6 section 6.5 and has resulted in an abstract and accepted poster presented at CROI 2018.`

# 4 ARV in pregnancy, real-world use in the UK, and European regulatory recommendation

## 4.1 Introduction

Clinical recommendations for the use of cART have changed over time, mostly reflecting both growing availability of new ARVs and accumulating evidence with respect to their use. Additional data might confirm efficacy and safety as well as detecting new safety signals, enabling implementation of new guidance for their use. However, out of the 43 ARVs (including the boosters Cobicistat and Ritonavir) with a European marketing authorisation (as of 2018), ZDV remains the only one with a specific indication in pregnancy for its proven efficacy in the prevention of VT (Connor et al. 1994, EMA 2019e).

As mentioned in Chapter 2 section 2.4, clinical data on ARV use in pregnant women are still limited and largely generated from real-world use despite the efforts from regulatory agencies to promote, implement and better standardize post-marketing surveillance studies, spontaneous reporting and pregnancy registries, and despite providing guidance for the industry to better conduct PK-PD studies in pregnant and breastfeeding women (e.g. through scientific advice). Therefore, safety and effectiveness data often became available years after the marketing approval has been granted. This knowledge gap exposes on the one hand the risk of teratogenic and embryofoetal toxicities and on the other hand, the risk of administering ineffective treatments, jeopardizing both maternal and infants' health.

The aim of this chapter is to assess the gap between real-world use of ARVs (data from the NSHPC) and regulatory recommendations (publicly available data from the EMA) and to explore its possible impact on maternal and infant's health. The chapter is divided into two main sections: the first describes the NSHPC population and evaluates the real-world use of ARVs and their trends and patterns of changes over time (sec 4.2) including a gap-analysis to establish a correlation between periodic updates of clinical recommendations (obtained from BHIVA guidelines) and real-world use of ARVs; the second assesses the available safety data on ARVs extracted from the regulatory recommendations (sect 4.3) where data on all ARV combinations used in pregnancy and reported to the NSHPC were matched with safety and efficacy data extracted from publicly available document (mostly SmPCs) from the EMA website.

## 4.2 Real-world use of ARV agents: trends of ARV use in the NSHPC

In this section three analyses were performed using different datasets, namely:

- A descriptive analysis to characterize the main study population, i.e. all singleton pregnancies occurred between 2008-2018 and reported to the NSHPC by the 31st of December 2018 meeting the following inclusion criteria: known pregnancy outcome (i.e. livebirths, stillbirths, miscarriages and termination of pregnancies); known maternal usage of ARV combinations; and known time of ARV initiation (i.e. at conception, in the first or second-third trimesters) (section 4.2.1)

- A snapshot analysis of the patterns of ARV usage over time including all singleton pregnancies ending in a live- or still-birth occurring between 2005-2016 and reported to the NSHPC by 31st of December 2016, with a focus on women newly diagnosed in pregnancy (section 4.2.2)

- An update of the snapshot analysis above, for all pregnancies ending in a live- or still-birth occurring between 2008-2018, focusing on the most common ARV combinations reported to the NSHPC (section 4.2.2). For this analysis, the 14 most commonly used ARVs (as single agents or as FDCs) were selected. These 14 ARVs were then compared with the safety data extracted from the EMA's SmPCs and EPAR documents (addressed in section 4.3).

### 4.2.1 HIV positive pregnant women and their pregnancy outcomes: NSHPC, 2008-18

As previously discussed in Chapter 1 in resource rich settings, over the past two decades several factors have contributed to changes in the characteristics of pregnant WLWH. These include earlier antenatal engagement, earlier HIV diagnosis and cART initiation, the availability of a wide range of ARVs and increased life expectancy. The same trend can be expected in the UK and Ireland, both in terms of demographics and health status. Several previous analyses of NSHPC data have shown a continuous decline in the overall VT rates with a constant increase of women diagnosed with HIV prior to conception and the consequent increase in women conceiving on a cART regimen. Furthermore, increasing maternal age at delivery and increasing number of vaginal deliveries and sequential pregnancies have been reported (Townsend et al. 2008b, French et al. 2012, Townsend et al. 2014).

This section provides an update of these trends and patterns in the NSHPC population over a ten-year span, from 2008-2018.

*Study population*

There were 12,967 singleton pregnancies from 9,158 women living with HIV with an EDD/date of birth reported to the NSHPC between 2008-18 (Figure 4.1). Of these, 868 were excluded due to not meeting the inclusion criteria, leaving a total of 12,099 singleton pregnancies from 8,740 women, with about 1.4 pregnancies per women.

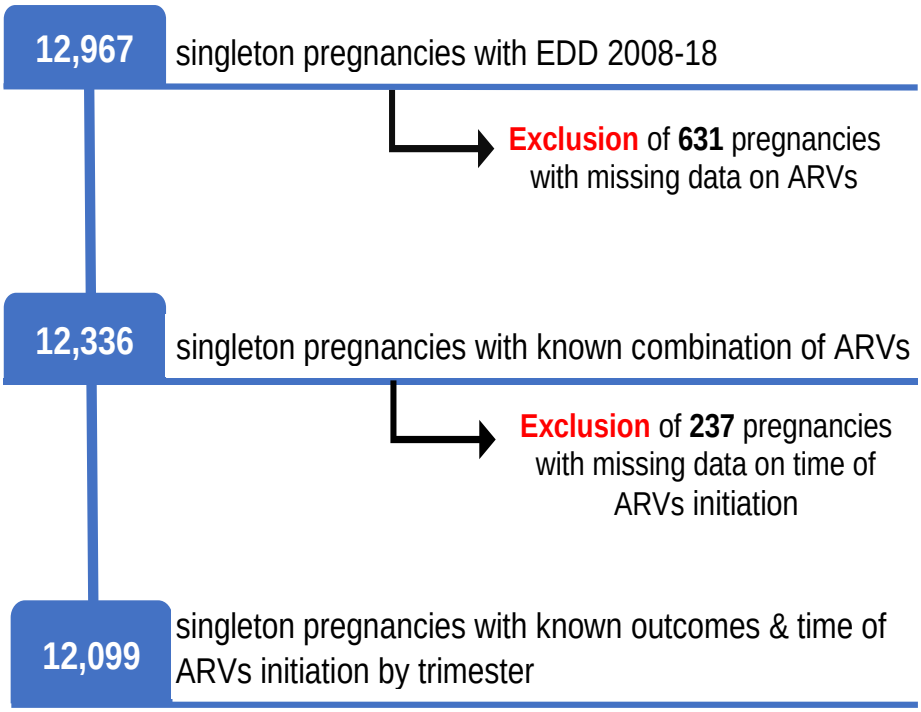
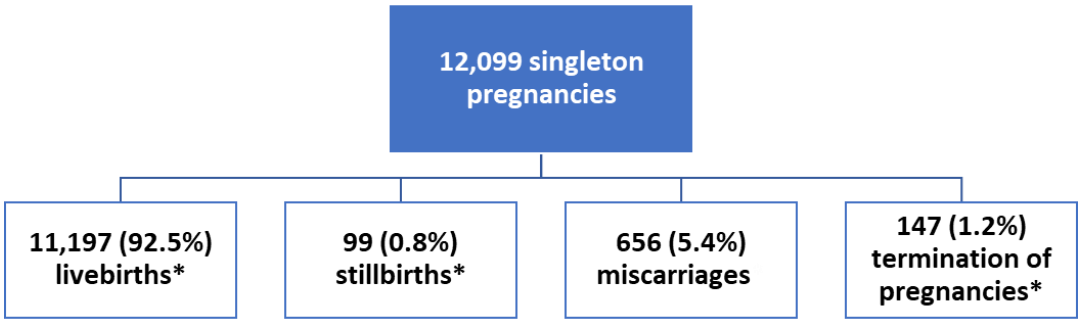



**Figure 4.1 Data selection from original NSHPC dataset**

Of the 12,099 singleton pregnancies reported to the NSHPC, 11,197 (92.5%) resulted in livebirths, 99 (0.8%) in stillbirths, 656 (5.4%) in miscarriages and 147 (1.2%) in terminations (Figure 4.2).




*For these pregnancies, outcomes and combination of ARVs by time of exposure were known

**Figure 4.2 Pregnancy outcomes of singleton pregnancies in the NSHPC, 2008-18**

Overall, the number of liveborn infants stayed at a relative steady level, just above 1,000 infants per year for the observed years, with a peak of 1,276 births in 2010. Over the study period, there was no evidence of a statistically significant trend over time in the stillbirth rate, going from 0.86% (11/1,279) in 2008 to 0.63% (4/629) in 2018 ($p$=0.787, obtained using simulations to improve the chi-squared approximation due to small sample size).

The proportion of pregnancies ending in terminations fell over time from 1.7% (22/1,279) in 2008 to 0.6% (4/629) in 2018 ($p$<0.001); while looking at the proportion of pregnancies ending in miscarriages, these have varied over time (Figure 4.3).

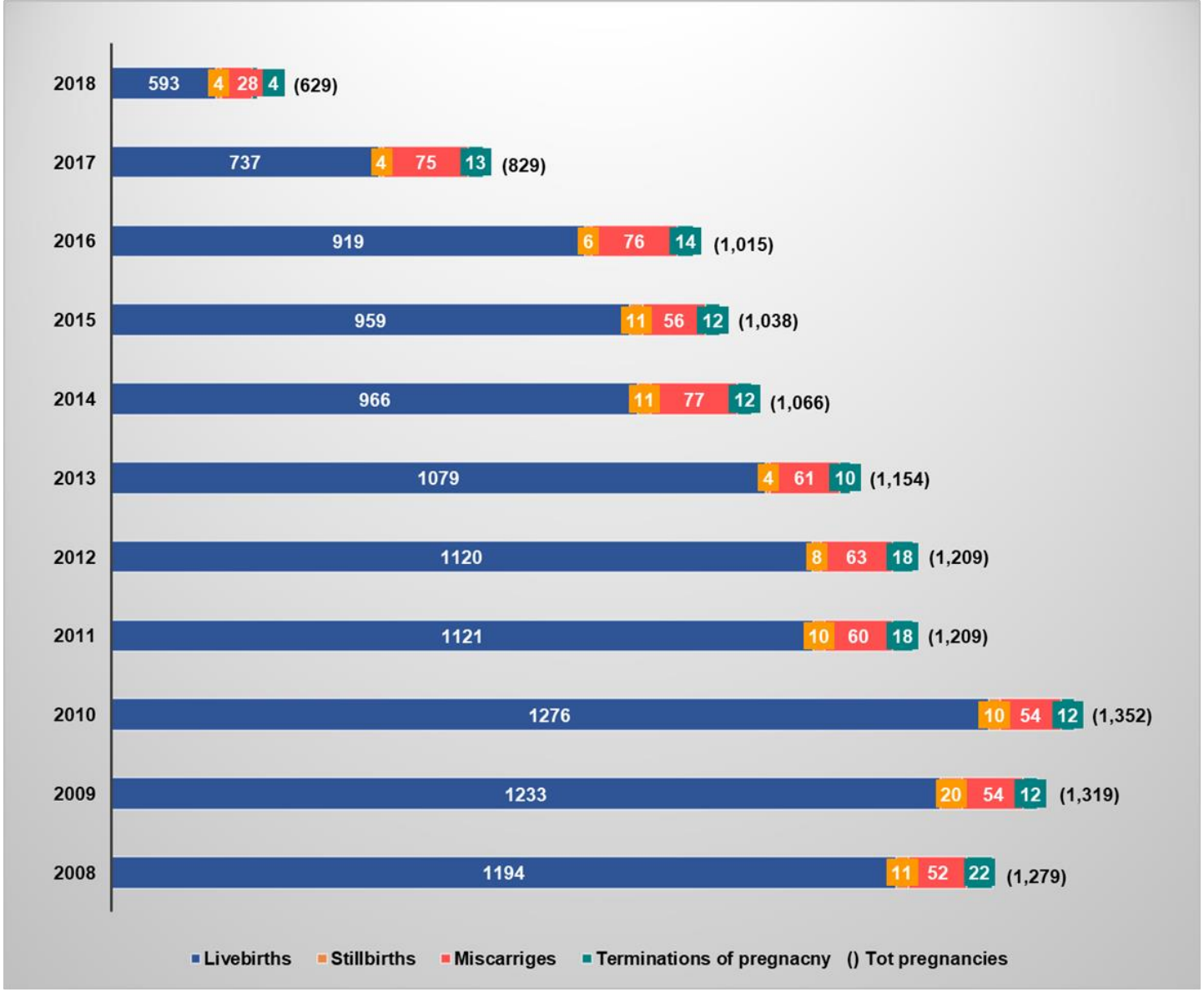



**Figure 4.3 Singleton pregnancies and their outcomes reported to the NSHPC, 2008-18**

***Maternal characteristics: all pregnancies***

Table 4.1 presents the key maternal characteristics for the reported pregnancies, stratified by pregnancy outcomes. The median age of women at conception was 33.4 years (q1=29.4, q3=37.2, IQR) overall, and 34.5 years for pregnancies ending in stillbirths (q1=30.3, q3=38.1,IQR), 36.8 years for those ending in miscarriage (q1=32.2, q3=40.0, IQR) and 34.1 years for those ending in terminations (q1=30.1, q3=39.1, IQR).

Almost three-quarters of all pregnancies were to Black African women, 72.4% of whom were born in SSA (8,763/12,099). Most of women (89.4%) acquired HIV heterosexually (10,815/12,099), while 1.5% (182/12,099) were infected vertically and 1.4% (168/12,099) via IDU. Over time, the proportion of women acquiring HIV through IDU decreased from 1.3% (17/1,279) in 2008 to 0.6% (4/629) in 2018 while the proportion of women acquiring HIV via VT increased from 0.6% (8/1,279) to 3% (19/629) over the same period.

For the vast majority (82.9%) of pregnancies, maternal HIV diagnosis was made prior to conception; the proportion of pregnancies where women knew their diagnosis before pregnancy increased from 69.0% (883/1,279) in 2008 to 90.3% (568/629) in 2018 (test-for-trend $p<0.001$).

Looking at CD4 cell count, between 2008-2018, the overall baseline median CD4 cell count was 477.0 cells/mm$^3$ (q1= 347.0, q3= 633.0, IQR), and on average every year the CD4 values increased by 10.7 cells/mm$^3$ ($p< 0.001$).

*Obstetric history*

Overall, around one fifth of pregnancies were among nulliparous women (19.6%, 2,375/12,099). Among the 99 pregnancies ending in stillbirth, in five the mother had at least one previous stillbirth, in 36 she had at least one previous termination or miscarriage, while in 52 she had at least one previous liveborn infant and in six had never previously been pregnant. Of the reported pregnancies ending in miscarriage, 45.7% (300/656) of the women had a previous miscarriage or termination and 3.5% (23/656) had a previous stillbirth. For pregnancies in which the woman reported a prior termination of pregnancy, 36.7% (54/147) also had at least one previous termination or a miscarriage, 2.0% (3/147) had a previous stillbirth and 74.1% (109/147) a previous liveborn infant.

**Table 4.1 Maternal characteristics among pregnancies reported to the NSHPC, 2008-18**

| | Livebirths (*n*=11,197) | Stillbirths (*n*=99) | Miscarriage (*n*=656) | Terminations (*n*=147) |
|---|---|---|---|---|
| **Ethnicity** | | | | |
| Black African | 8,263 (73.8%) | 77 (77.7%) | 488 (74.4%) | 106 (72.1%) |
| Black other | 418 (3.7%) | 2 (2.0%) | 13 (2.0%) | 3 (2.1%) |
| White | 1,958 (17,5%) | 10 (10.1%) | 96 (14.6%) | 25 (17.0%) |
| Other | 541 (4.8%) | 10 (10.1%) | 58 (8.8%) | 13 (8.8%) |
| Missing | 17 (0.2%) | 0 | 1 (0.2%) | 0 |
| **Region of birth** | | | | |
| SSA | 8,070 (72.1%) | 73 (73.7%) | 479 (73.0%) | 103 (70.1%) |
| UK/Ireland | 1,732 (15.5%) | 10 (10.1%) | 92 (14.0%) | 23 (15.6%) |
| Europe | 628 (5.6%) | 4 (4.0%) | 31 (4.7%) | 9 (6.1%) |
| Elsewhere | 624 (5.6%) | 8 (8.1%) | 36 (5.5%) | 6 (4.1%) |
| Missing | 143 (1.2%) | 4 (4.0%) | 18(2.7%) | 6 (4.1%) |
| **HIV acquisition route** | | | | |
| Heterosexual | 10,027 (89.5%) | 83 (83.8%) | 577 (87.9%) | 128 (87.1%) |
| IDU | 157 (1.4%) | 1 (1.0%) | 8 (1.2%) | 2 (1.4%) |
| VT | 168 (1.5%) | 1 (1.0%) | 5 (0.8%) | 8 (5.4%) |
| Other | 124 (1.1%) | 5 (5.1%) | 10 (1.5%) | 1 (0.7%) |
| Not known | 713 (6.4%) | 9 (9.1%) | 53 (8.1%) | 7 (4.7%) |
| Missing | 8 (0.1%) | 9 (9.1%) | 56 (8.5%) | 8 (5.4%) |
| **Timing of HIV diagnosis** | | | | |
| Before pregnancy | 9,184 (82.1%) | 71 (71.7%) | 643 (98.0%) | 141 (95.9%) |
| During pregnancy | 2,013 (17.9%) | 28 (26.3%) | 13 (2.0%) | 6 (4.1%) |
| **Parity** | | | | |
| Nulliparous | 2,209 (19.7%) | 29 (29.3%) | 114 (17.4%) | 23 (15.6%) |
| 1 | 3,067 (27.4%) | 29 (29.3%) | 153 (23.3%) | 40 (27.2%) |
| 2 | 2,693 (24.1%) | 18 (18.2%) | 139 (21.2%) | 37 (25.3%) |
| 3 | 1,675 (14.9%) | 10 (10.1%) | 117 (17.8%) | 21 (14.3%) |
| ≥4 | 1,553 (13.8%) | 13 (13.1%) | 133 (20.3%) | 26 (17.6%) |
| **Age at conception** | | | | |
| ≤ 25 | 774 (6.9%) | 5 (5.0%) | 20 (3.0%) | 17 (11.6%) |
| 25-30 | 2,058 (18.4%) | 15 (15.2%) | 83 (12.6%) | 19 (12.9%) |
| 30-35 | 3,581 (32.0%) | 32 (32.3%) | 145 (22.1%) | 44 (29.9%) |
| 35-40 | 3,357 (30.0%) | 27 (27.3%) | 244 (37.2%) | 37 (25.2%) |
| 40-45 | 1,312 (11.7%) | 18 (18.2%) | 148 (22.6%) | 27 (18.4%) |
| ≥45 | 115 (1.0%) | 2 (2.0%) | 16 (2.4%) | 3 (2.0%) |

*Treatment*

Of all included pregnancies, 7,249 (59.9%) were conceived on a cART regimen and with cART started during pregnancy for the remaining 4,850 (40.1%) pregnancies (Figure 4.4).

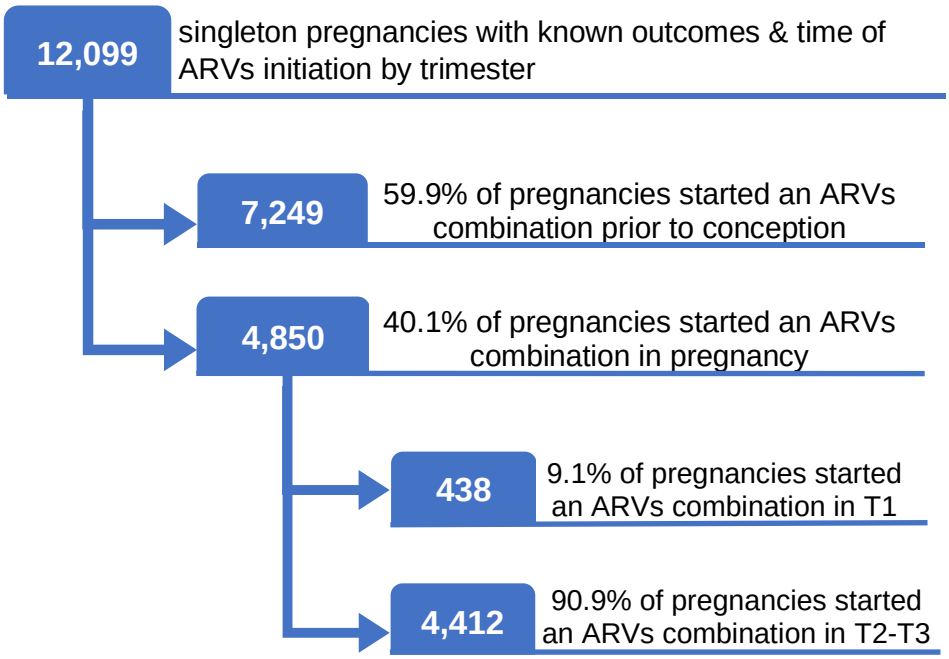



**Figure 4.4 Timing of cART initiation among singleton pregnancies, NSHPC, 2008-18**

Over time, the proportion of pregnancies conceived under cART increased from 37.7% (482/1,279) in 2008 to 80.9% (509/629) in 2018 (test-for-trend $p<0.001$) (Figure 4.5); while the proportion of women starting cART at some point in their pregnancies was 40.1%. Of the 10,039 pregnancies from women with an HIV diagnosis made before pregnancy, 72.2% (7,249/10,039) started a cART regimen prior to conception and 27.8% (2,790/10,039) started at some point during their pregnancy. Over the last decade, among women knowing their diagnosis before pregnancy, the proportion already on cART before conception increased, from 54.6% (482/883) in 2008 to 89.6% (509/568) in 2018 (test-for-trend $p<0.001$). For the 2,060 women whose HIV diagnosis was made during pregnancy, 4.5% (93/2,060) started cART early in pregnancy (i.e. T1) and 95.5% (1,967/2,060) started later in pregnancy (i.e. T2-T3).

Over time the proportion of women whose HIV diagnosis was made during pregnancy decreased from 19.2% (396/2,060) in 2008 to 2.9% (61/2,060) in 2018 (test-for-trend $p<0.001$). The proportion of women diagnosed in pregnancy starting cART in T1 increased from 1.0% (4/396) in 2008 to 14.7% (9/61) in 2018. Table 4.2 presents data on cART by time of initiation (i.e. before/during pregnancy) and baseline VL stratified by pregnancies outcomes. Of note, a high proportion of pregnancies ending in miscarriage or termination were exposed to cART in the preconception period, consistent with the finding (Table 4.1) that a higher proportion (98% and 96%, respectively) had an established HIV diagnosis before becoming pregnant compared with the livebirth group (82%).

**Table 4.2 Time of cART initiation and baseline VL for pregnancies reported to the NSHPC in 2008-18, by pregnancy outcomes**

| | Livebirths (*n*=11,197) | Stillbirths (*n*=99) | Miscarriages (*n*=656) | Terminations (*n*=147) |
|---|---|---|---|---|
| **Time of cART initiation** | | | | |
| Before pregnancy | 9,184 (82.1%) | 53 (53.5%) | 611 (93.2%) | 132 (89.8%) |
| During pregnancy | 2,013 (17.9%) | 46 (46.5%) | 45 (6.8%) | 15 (10.2%) |
| **Baseline VL, copies/μL** | | | | |
| Undetectable | 9,413 (84.1%) | 42 (42.4%) | 334 (50.9%) | 71 (48.3%) |
| Detectable | 393 (3.5%) | 8 (8.1%) | 49 (7.5%) | 18 (12.2%) |
| Missing | 1,391(12.4%) | 49 (49.5%) | 273 (41.6%) | 58 (39.5%) |

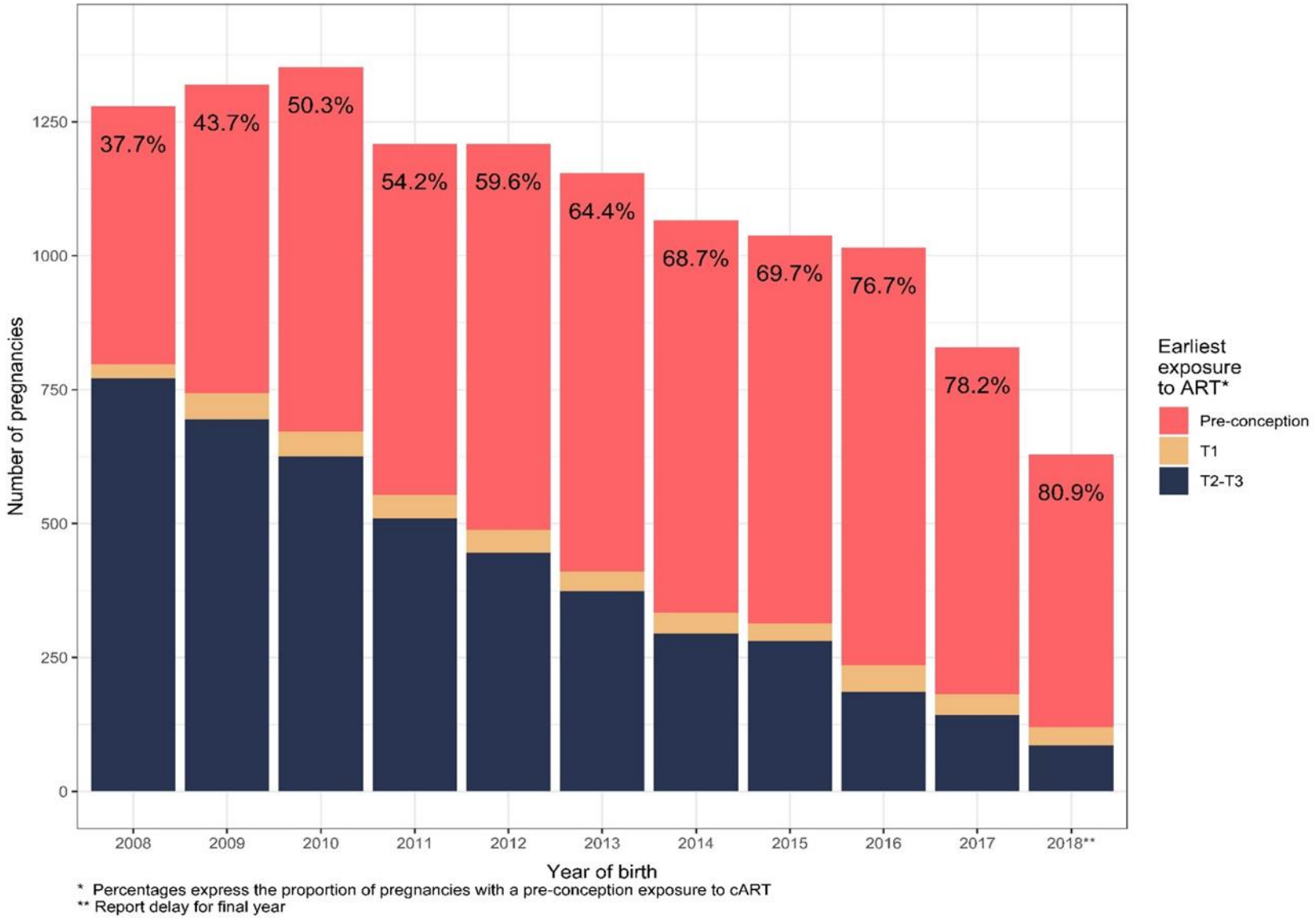



**Figure 4.5 Earliest exposure to cART in all pregnancies, NSHPC 2008-18**

***Pregnancies ending in delivery: livebirths and stillbirths (n=11,296)***

Maternal age at delivery increased over time, with some noticeable changes by age-group. For example, the proportion of pregnancies among women aged 25-30 years decreased from 29.4% (376/1,279) in 2008 to 17.3% (109/629) in 2018 (test-for-trend $p< 0.001$), while women aged 40-45 years experienced a 3-fold increase in their contribution to pregnancies, rising from 4.6% (59/1,279) in 2008 to 15.3% (96/629) in 2018 (test-for-trend $p< 0.001$).

Over the years, the proportion of pregnancies from women whose HIV diagnosis was made prior to conception rose from 67.2% (802/1,194) in 2008 to 84.8% (533/628) in 2018 (test-for-trend $p<0.001$). Furthermore, rates of pregnancies from women on cART at conception increased from 34.5% (412/1,194) in 2008 to 80.6% (478/593) in 2018 (test-for-trend *p*0.001), and so did the proportion of those knowing their HIV status and on cART prior to conception, from 51.4% (412/802) in 2008 to 89.7% (477/533) in 2018 (test-for-trend $p<0.001$). Looking at mode of delivery over the considered decade, the proportion of vaginal deliveries increased from 29.6% (379/1,279) in 2008 to 45.5% (286/629) in 2018 (test-for-trend $p<0.001$), while the elective-CS rate declined from 39.6% (507/1,279) to 27.9% (176/629) (test-for-trend $p<0.001$) as did the emergency-CS rate from 23.7% (304/1,279) to 18.1% (114/629) (test-for-trend $p=0.006$) (Table 4.3).

Overall, there were 7,111 women with effective VL suppression (i.e. undetectable VL ≤400 copies/μL) near delivery, of whom 89.2% (6,345/7,111) with values of VL ≤50 copies/μL (Table 4.3). Over time, the proportion of women delivering liveborn infants with effective suppressed VL at time of delivery increased from 60.1% (718/1,194) in 2008 to 67.4% (423/628) in 2018 (test-for-trend $p=0.003$).

**Table 4.3 cART initiation and mode of delivery for live- and still-born infants, reported to the NSHPC 2008-18, by maternal VL at delivery**

| | Maternal VL at delivery | | | Total |
|---|---|---|---|---|
| | Undetectable N=7,111 | Detectable N=335 | Missing N=3,850 | N=11,296 |
| **Timing of cART initiation** | | | | |
| Before pregnancy | 4,090 (57.5%) | 69 (20.6%) | 2,347(60.6%) | 6,506 (57.6%) |
| During pregnancy | 3,021(42.5%) | 266 (79.4%) | 1,503 (39.1%) | 4,790 (42.4%) |
| **Mode of delivery** | | | | |
| Vaginal | 3,003 (42.2%) | 33 (9.8%) | 1,728 (44.8%) | 4,764 (42.2%) |
| Elective-CS | 2,398 (33.7%) | 202 (60.3%) | 1,035 (26.8%) | 3,635 (32.2%) |
| Emergency-CS | 1,674 (23.5%) | 98 (29.2%) | 934 (24.2%) | 2,706 (23.9%) |
| Missing | 36 (0.5%) | 2 (0.6%) | 153 (3.9%) | 191 (1.7%) |

*Gestational age and birthweight in liveborn infants*

The median gestational age at delivery was 39 weeks (GW) (IQR, q1=38, q3=40). Overall, 89.7% (10,046/11,197) of liveborn infants were born at term with this proportion increasing over time from 88.0% (1,051/1,194) in 2008 to 91.1% (540/593) in 2018, though not statistically significant (test-for-trend $p$=0.064). There were 74.2% (7,452/10,046) of liveborn infants delivered at term from black African women, 17.2% (1,727/10,046) from white women and 8.5% (850/10,046) from women of other ethnicities ($p$<0.001); for 17 women data on their ethnicity was missing.

The overall proportion of preterm deliveries was 10.3% (1,151/11,197), decreasing from 11.9% (143/1,194) in 2008 to 8.9% (53/593) in 2018, though not statistically significant (test-for-trend $p$=0.064). The proportions of infants born between 34 and 36 GW and at less than 34 GW were 6.4% (713/11,197) and 3.9% (438/11,197) respectively.

Overall, 83.6% (9,362/11,197) of the liveborn infants weighed 2.5kg or more at birth. The proportion of liveborn infants with a birthweight less than 2.5kg was 11.1% (1,247/11,197); for 1.8% (211/11,197) birthweight was less than 1.5kg and the proportion of those with a weight less than 1kg was 0.7% (87/11,197); for 5.2% (588/11,197) data was missing. Over the years the proportion of infants born at ≥37GW and weighing ≥2.5kg increased from 76.7% (916/1,194) in 2008 to 84.2% (499/593) in 2018 (test-for-trend $p$<0.0003). Table 4.4 presents data on the 11,197 singleton liveborn pregnancies stratified by gestational age at delivery.

**Table 4.4 Data on singleton liveborn pregnancies reported to the NSHPC 2008-18, by gestational age at delivery**

| | Gestational age at delivery | | *P*-values |
|---|---|---|---|
| | Preterm N=1,151 | Term N=10,046 | |
| **Maternal ethnicity** | | | |
| Black African | 811 (70.4%) | 7,452 (74.2%) | 0.021* |
| White | 231 (20.1%) | 1,727 (17.2%) | |
| Other | 109 (9.5%) | 850 (8.5%) | |
| Missing | 0 | 17 (0.2%) | |
| **Median birthweight (IQR), g** | | | |
| | 2,160 (q1=1,662, q3=2,565) | 3,184 (q1=2,890, q3=3,500) | <0.001 |
| **Birthweight, g** | | | |
| <1500 | 210 (18.2%) | 1 | <0.001 |
| 1500-2499 | 536 (46.6%) | 500 (4.9%) | |
| ≥2500 | 324 (28.1%) | 9,038 (89.9%) | |
| Missing | 81 (7.1%) | 507 (5.0%) | |

* These *p*-values refer to a chi-squared test of homogeneity, missing data are not included

### 4.2.2 Snapshot analysis of ARV use

This analysis was carried out to evaluate guidelines' influence on clinical practice by addressing two objectives:

1. To generate a "snapshot" of the pattern of ARV usage in the UK and Ireland between 2005-2016, overall (1a) and among women newly diagnosed and initiating cART in pregnancy (1b); To update this snapshot for pregnancies occurring between 2008-2018 and to run the snapshot restricted to the 14 most commonly used ARV combinations (1c).

2. To evaluate whether real-world use of ARVs has changed over time among women initiating ART in pregnancy in relation to BHIVA recommendations, using the ARV usage snapshot and to explore if there is a gap between real-world use of ARVs and clinical recommendations; To assess BHIVA recommendation changes over time (2a); To investigate trends of ARV use among women newly diagnosed between 2005-2016 (2b); To analyse trends of the most common ARVs used between 2008-2018 (2c).

*Specific methods:*

To generate the snapshot on ARV use over time, data on all ARV use in pregnancies reported to the NSHPC from the 1st of January 2005 to the 31st of December 2016 were collected along with data on pregnancy outcomes (i.e. live- or still-births).

Analyses were conducted in R, with code generating all possible combinations of ARVs. Data on maternal-fetal exposure to every component of a cART regimen used during pregnancy were collected, i.e. every individual agent was the unit of analysis, except for ritonavir as booster. For example, for a combination of 3TC/ZDV, two ARVs were counted and for a combination of EFV+FTC+TDF, three ARVs were counted, while for a combination of DRV/r, one ARV was counted. In this way, every ARV received by a woman during her pregnancy was counted. For the time trends analysis, the denominator was the total number of ARVs used in the pregnancies delivering per calendar year.

To address objective 1a, data on 10,009 women and 13,757 singleton pregnancies reported to the NSHPC by the 31st of December 2016 were analysed. Data for this analysis included any ARV exposure regardless of the time of cART initiation (i.e. before or during pregnancy).

To address objective 1b, analysis was restricted to 3,496 pregnancies among women newly diagnosed with HIV (i.e. those women whose first recorded positive

HIV test was during pregnancy) starting a combination of ARVs during pregnancy.

Finally, for objective 1c, the analysis was restricted to the 14 most common ARVs used in the NSHPC between 2008-2018 to ensure that findings were reflective of the more recent epidemiological situation. Furthermore, selection of the most used ARVs increased the chance of obtaining more complete data in terms of timing of ART initiation (i.e. before/during pregnancy) and a more complete list of ARVs contained in a cART regimen rather than just the ARV class. Lastly, selection of the 14 most common ARVs used in real-life settings facilitated the extraction of recently updated safety and efficacy information from the EMA website to conduct the gap-analysis performed in section 4.3.

Figure 4.6 presents data selection from the original NSHPC dataset used to address this whole section.

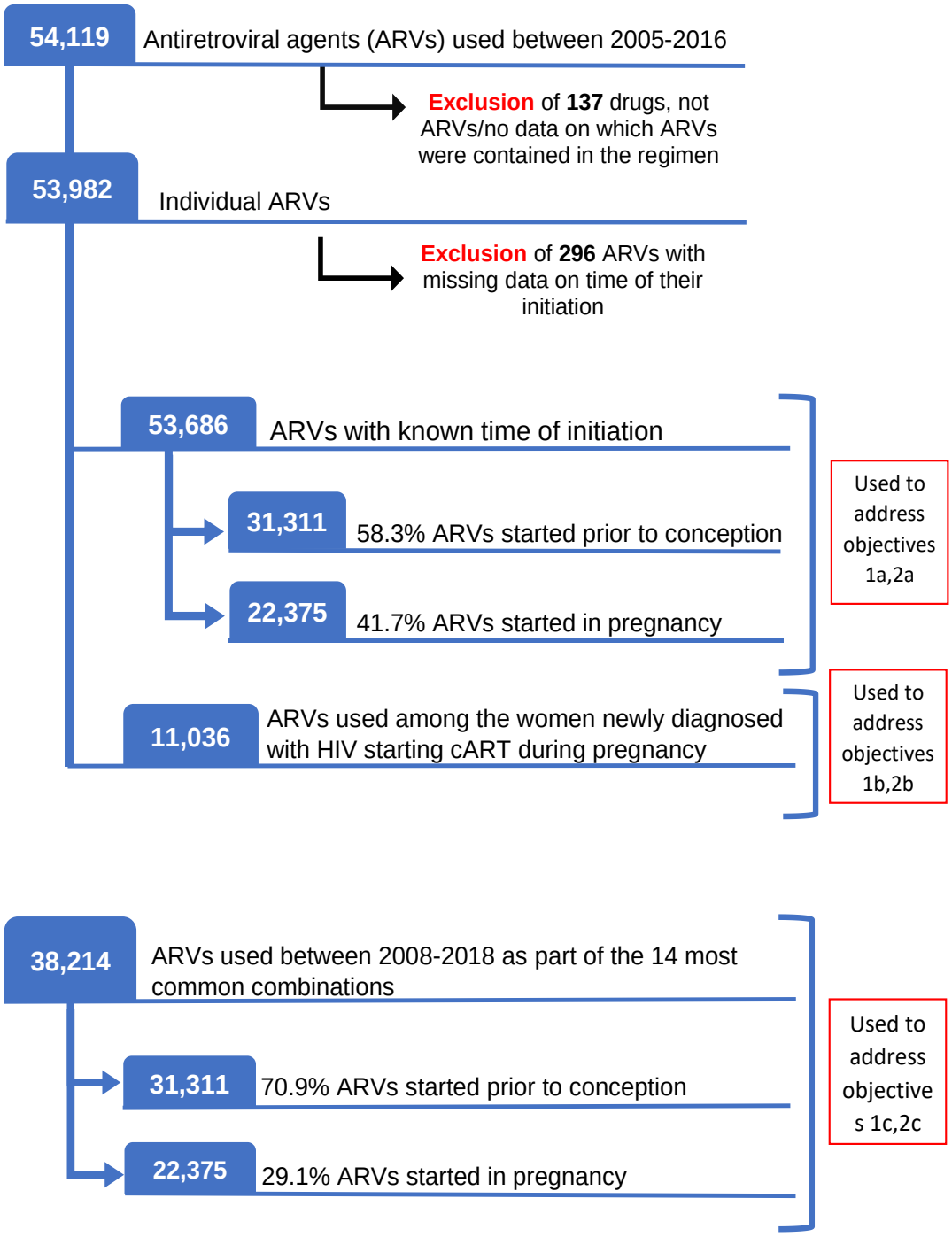



**Figure 4.6 Data selection from the original NSHPC data set and data analysis used to address section 4.2 objectives**

*Guidelines assessment*

BHIVA is the leading UK association for healthcare professionals working in HIV clinical management. It was established in 1995 aiming to provide care for people living with HV and to harmonize guidelines for HIV treatment for both adults and pregnant women. Since the 1990s BHIVA has been producing guidelines for treatment of HIV diagnosed adults and for the management of pregnant and breastfeeding women, including recommendations on the best treatment options, on VT prevention and counselling on the management of co-infections.

Guidelines are drafted by their own Writing Group and then published on BHIVA's website for public consultation with the aim of being both clinically and practically useful for healthcare professionals. Since 2010 BHIVA has adopted the modified GRADE system for the assessment, evaluation and grading of evidence and the development of recommendations (GRADE 2000, Guyatt et al. 2008). Based on a modified GRADE system, guideline advice for a "preferred" and an "alternative" cART options, where *preferred* refers to "strong recommendation most clinician and patients would want to follow unless clear rationale not to do so" and *alternative* as "conditional recommendation implies an acceptable treatment option for some patients and might be the preferred option in some selected patient".

All BHIVA guidelines for the therapeutic management of women living with HIV spanning the period from 2005-2016 were assessed to describe and evaluate the changes in recommendations for women starting cART in pregnancy(BHIVA 2005, BHIVA 2008, BHIVA 2012, BHIVA 2014).

## Snapshot results (objective 1)

### *Whole population (objective 1a)*

There was a total of 25 ARVs recorded as being in use among the pregnancies between 2005-2016. The combination of these 25 ARVs account for the total of 53,982 individual ARVs, excluding ritonavir and not restricting for the timing of ARVs initiation (i.e. includes the 296 ARVs from which data on their precise time of initiation, at conception or in pregnancy was not known). Figure 4.7 provides the snapshot of their pattern of use over time.

Looking at the trends, the more noticeable changes occurred to the four main backbone drugs, ZDV, 3TC, TDF and FTC. In 2005, ZDV was the most prescribed ARV in a cART regimen, administered 1,042 times (30.4%, 1,042/3,426) followed by 3TC given 990 times (28.8%, 990/3,426). Usage of these two drugs steadily declined over the following years, with only 29 prescriptions (1.0%, 29/2,886) of ZDV and 214 (7.4%, 214/2,886) of 3TC in 2016 (test-for-trend $p$ <0.001). On the contrary, a steady increase in FTC and TDF use was apparent, with FTC being prescribed only 8 (0.2%, 8/3,426) times and TDF 109 (3.1%, 109/3,426) times in 2005 rising to 687 (23.8%, 687/2,886) and 705 (24.5% 705/2,886) times in 2016, respectively (tests-for-trend both $p$ <0.001).

*Pregnancy outcomes live- and stillbirths*

Of the total 13,757 singleton pregnancies, 13,635 were livebirths and 122 ended in stillbirths. Stillbirths were one in 113 births at a rate of 0.8%, consistent with previous findings suggesting a higher rate of stillbirths among women living with HIV compared with the general population rate of 0.5% with one in 200 births resulting in stillbirth. Overall, the stillbirth rate among the NSHPC population declined over time, from 1.1% in 2005 to 0.7% in 2016.

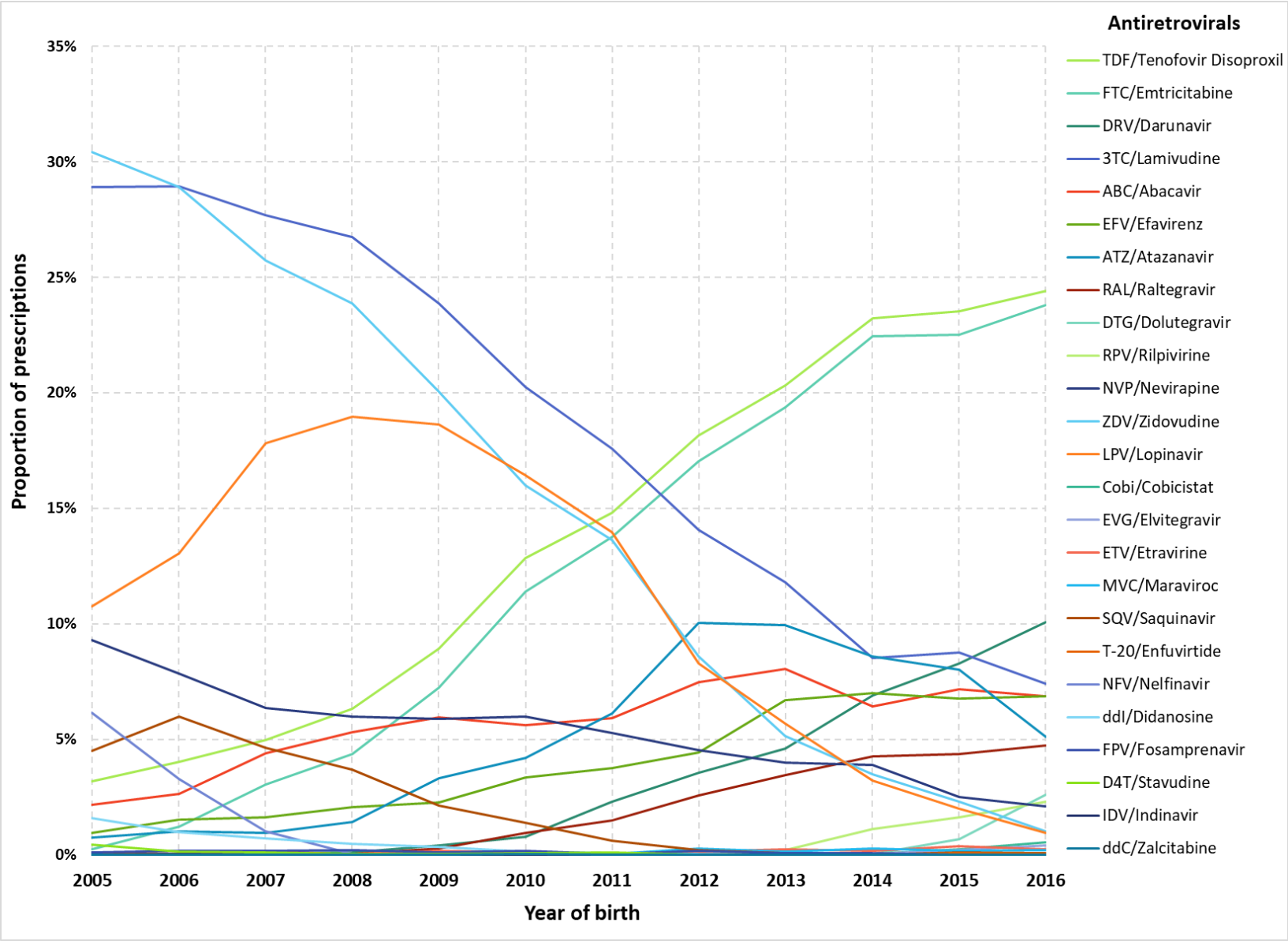



**Figure 4.7 Trends of ARV use in the NSHPC, 2005-2016**

***Women newly diagnosed with HIV starting cART during pregnancy (objective 1b)***

There were 3,496 pregnancies among newly diagnosed women initiating cART with antenatal use of 24 ARVs overall. These resulted in a total use of 11,036 ARVs between 2005-16. The general trends of ARVs usage reflect that of the overall NSHPC population reported above with distinctive changes in ARV use (Figure 4.8).

The number of pregnancies per year in this subgroup of women has declined from a peak at the start of the study period with 549 pregnancies reported in 2005 to only 87 in 2016 (Figure 4.8).

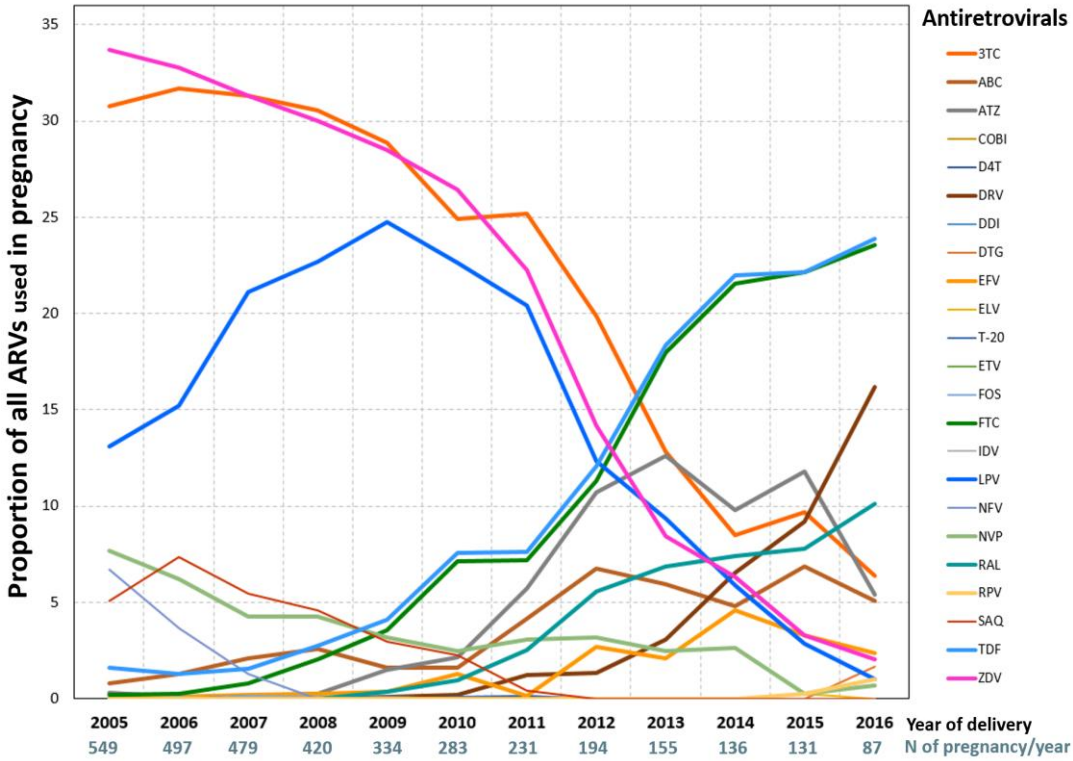



**Figure 4.8 Trends in ARVs use in newly diagnosed women with antenatal cART initiation, 2005-2016**

***Trends of the most common ARVs used between 2008-218 (objective 1c)***

There was a total of 38,214 individual usage of the 14 most common ARVs (either as single agents or as FDCs) reported to the NSHPC between 2008-18. Of those 27,099 (70.9%) were used before conception and 11,115 (29.1%) during pregnancy.

**ARV use in the NSHPC and BHIVA recommendations updates (objective 2)**

***BHIVA recommendation changes over time (objective 2a)***

There have been some substantial changes in the BHIVA recommended preferred and alternative ART regimens for pregnant women over time (Table 4.5). Some of the key changes are now addressed. In 2005 guidelines recommended ZDV monotherapy as the preferred option for pregnant women with plasma HIV RNA <10,000 copies/mL not requiring cART for maternal health or choosing to deliver by planned elective-CS. Additionally, NVP was recommended as preferred choice in combination with other ARVs for some specific scenarios (i.e. late presentation in pregnancy) due to NVP's rapid placental transfer and long half-life(Zash et al. 2016a).

In the 2008 guidelines update, a fixed-combination of ZDV/3TC was introduced as the preferred NRTI-backbone and NVP as the preferred third agent. Alternatively, any PIs boosted with ritonavir (PI/r) were introduced as alternative third agents. Guideline updates in 2012 resulted in replacing NVP with EFV as the preferred third agent. EFV was previously not recommend due to the preclinical findings suggesting increased risk of NTDs that have been previously discussed (chapter 1, section 1.5.3). NVP became an alternative agent following reports of increased hepatotoxicity associated with NVP-based regimens when started with high CD4 count (>250cells/µL) (Sanne et al. 2005).

In 2014, revisions saw the inclusion of INSTI-based preferred regimens specifically for women naïve to cART, with either RAL or a fixed-dose of EVG boosted with COBI as third agents. For ART non-naïve women, the recommendation included any boosted PI/r (mostly DRV) with EFV or NVP remaining as preferred third agents. The FDC of ZDV/3TC was still the preferred backbone but valid alternatives were TDF/FTC or ABC/3TC.

In 2016, updated guidelines advised clinicians to follow the general adult recommendations, offering TDF/FTC or ABC/3TC as preferred backbone options and DRV/r as alternative third agent; ZDV monotherapy was moved to become an alternative option.

The most recent update, the 2018 (2019 second interim update) revision, recommends ABC/3TC or TDF/FTC as preferred backbone options moving ZDV/3TC to become an alternative option. Boosted ATV (ATV/r) became the preferred third agent but with more alternatives to consider from, namely RPV, DRV/r, RAL and DTG.

**Table 4.5 BHIVA guidelines for pregnant women with updates on preferred and alternative regimens over time**

| Year | Regimen | Preferred | Alternative |
|---|---|---|---|
| 2005 | **NRTI backbone** | ZDV monotherapy | ZDV/3TC |
| | **Third agent** | – | – |
| 2008 | **NRTI backbone** | ZDV monotherapy | ZDV/3TC |
| | **Third agent** | – | Any PI/r |
| 2012 | **NRTI backbone** | ZDV/3TC | TDF/FTC or ABC/3TC or ZDV monotherapy [1] |
| | **Third agent** | EFV | NVP[2] or Any PI/r |
| 2014 | **NRTI backbone** | ZDV/3TC | TDF/FTC or ABC[3]/3TC or ZDV/3TC |
| | **Third agent** | EFV (or NVP[1] or any PI/r) | NVP[1] or any PI |
| | **Newly diagnosed** | TDF/FTC + ATZ/r, DRV/r or EFV or RAL, ELV/c | ABC/3TC + LPV/r, FOS/r or NVP[1] |
| 2016 | **NRTI backbone** | TDF/FTC or ZDV/3TC or ABC/3TC | ZDV monotherapy[1] |
| | **Third agent** | EFV or NVP or any PI | DRV/r* |
| | **Newly diagnosed** | TDF/FTC + ATZ/r, DRV/r or EFV or RAL, ELV/c | ABC/3TC + LPV/r, FOS/r or NVP[1] |
| 2018 | **NRTI backbone** | ABC/3TC or TDF/FTC | ZDV/3TC |
| | **Third agent** | EFV or ATV/r | RPV, DRV/r or RAL[4] or DTG[5] |

[1]PCS, baseline VL<10,000 HIV RNA cps/mL, CD4>350c/μL; [2]CD4<250c/μL; [3]VL<100,000cps/mL; [4]RAL 400mg twice a day; [5]after 6 weeks' gestation.
*if resistance is known; FOS: Fosamprenavir; ELV/c: Elvitegravir/cobicistat

***Trends of ARVs use among women newly diagnosed in pregnancy between 2005-16 (objective 2b)***

To better illustrate the trends of ARV usage in women diagnosed through antenatal screening reported to the NSHPC and to compare over time trends of ARVs usage with BHIVA recommendations' updates, two different sets of graphs (Figure 4.9 and Figure 4.10) and tables (Table 4.6 and Table 4.7) are presented dividing ARVs in "backbone" and "third agents".

Figure 4.9 shows the patterns of changes for NRTI backbone usage over time. At the beginning of the study period of the total 1,602 single ARVs prescribed, the most used NRTI was ZDV, accounting for 33.7% (540/1,602) of all ARVs taken in pregnancy followed by 3TC accounting for 30.7% (493/1,602), with very minimal use of TDF and FTC, accounting for 1.6% (26/1602) and 0.2% (3/1602), respectively. By 2016 the situation was quite the opposite with TDF and FTC being the most used agents accounting for approximately 24% of all the ARVs used in pregnancy (23.6% 70/297 and 23.9% 71/297, respectively) and ZDV and 3TC accounting for only 2.0% (6/297) and 6.4% (19/297), respectively.

Table 4.6 shows the updates of the BHIVA recommendations on the preferred and alternative regimens over time, considering only the "backbone".

When Figure 4.9 and Table 4.6 are compared it is interesting to notice that even though ZDV/3TC started to be recommended as preferred "backbone" with the 2012 guideline update, their usage in the real world was already declining. In particular, use of ZDV declined from 33.7% (540/1,602) in 2005 to 14.2% (94/664) in 2012, (test-for-trend $p< 0.001$).

Similar patterns but with increasing trends were seen for the backbone TDF/FTC, which started to be recommended as within preferred regimens in newly diagnosed patients by 2014 when usage for both had already increased. Noticeably, FTC usage went from less than 1% (0.2%, 3/1,602) in 2005 to 21.6% (70/297) in 2016, a 99.1% increase (test-for-trend $p< 0.001$).

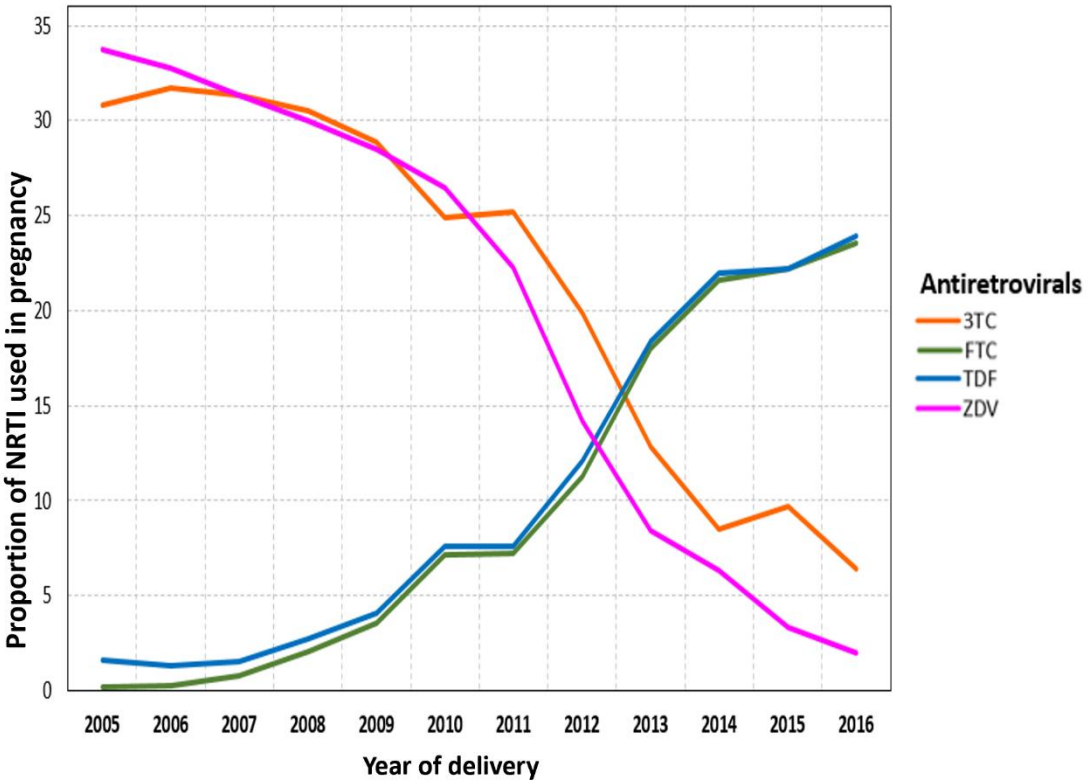



**Figure 4.9 Trends of backbone (NRTIs) usage in the NSHPC, 2005-16**

**Table 4.6 BHIVA guidelines updates on preferred and alternative backbone (NRTIs), 2005-16**

| Year | Regimen | Preferred | Alternative |
|---|---|---|---|
| **2005** | **backbone** | ZDV monotherapy | ZDV/3TC |
| **2008** | **backbone** | ZDV monotherapy | ZDV/3TC |
| **2012** | **backbone** | ZDV/3TC | TDF/FTC or ABC/3TC or ZDV monotherapy [1] |
| **2014** | **backbone** | ZDV/3TC | TDF/FTC or ABC[2]/3TC or ZDV/3TC |
| | **Newly diagnosed** | TDF/FTC | ABC/3TC |
| **2016** | **backbone** | TDF/FTC or ZDV/3TC or ABC/3TC | ZDV monotherapy[1] |
| | **Newly diagnosed** | TDF/FTC | ABC/3TC |

[1] Elective-CS, baseline VL<10,000 HIV RNA cps/mL, CD4>350c/μL;

Figure 4.10 shows the patterns of changes for the third agent usage. At the beginning of the study period, when ZDV monotherapy was still widely used in pregnancy, few third agents were prescribed and LPV, NVP and SAQ were among the most used agents. However, by 2016, following the marketing authorisation of new ARVs both from existing classes (i.e. PIs) and new classes (i.e. INSTIs), increased number of third agents' options became available and consequently their usage in pregnant women in the UK increased. For example, DRV/r was first recommended as one of the preferred options by BHIVA in 2016, even though it was licensed in 2006 and first reported to the NSHPC in 2009, from which years its usage steadily increased from less than 1.1% (1/1,074) to 16.2% (48/297) of all the ARVs prescribed in 2016 (test-for-trend $p<0.001$).

Similarly, following its license in 2008, RAL usage started to increase, with its first detection in the study population in 2009, when it accounted for less than 0.4% (4/1,074) of all the ARVs prescribed, reaching 10.1% (30/297) of them by the end of 2016 (test-for-trend $p<0.001$). Noticeable is the inverted U-shape for the use of LPV/r and ATV/r with peaks respectively reached in 2009 and 2013. Table 4.7 shows the updated BHIVA recommendation taking into consideration only the "third agents".

In this case, when Figure 4.10 and the Table 4.7 are compared, patterns of ARV use seem less associated to BHIVA's updates. For other ARVs such as ATV/r, a decline was already recorded by 2013 even though BHIVA recommendations for its use in newly diagnosed women were issued only in 2014. Meanwhile, in the same year RAL was firstly recommended by BHIVA as one of the preferred first-line option for newly diagnosed women, by which time it already accounted for 7.4% (34/459) of all the ARVs used in pregnancy reported to the NSHPC that year.

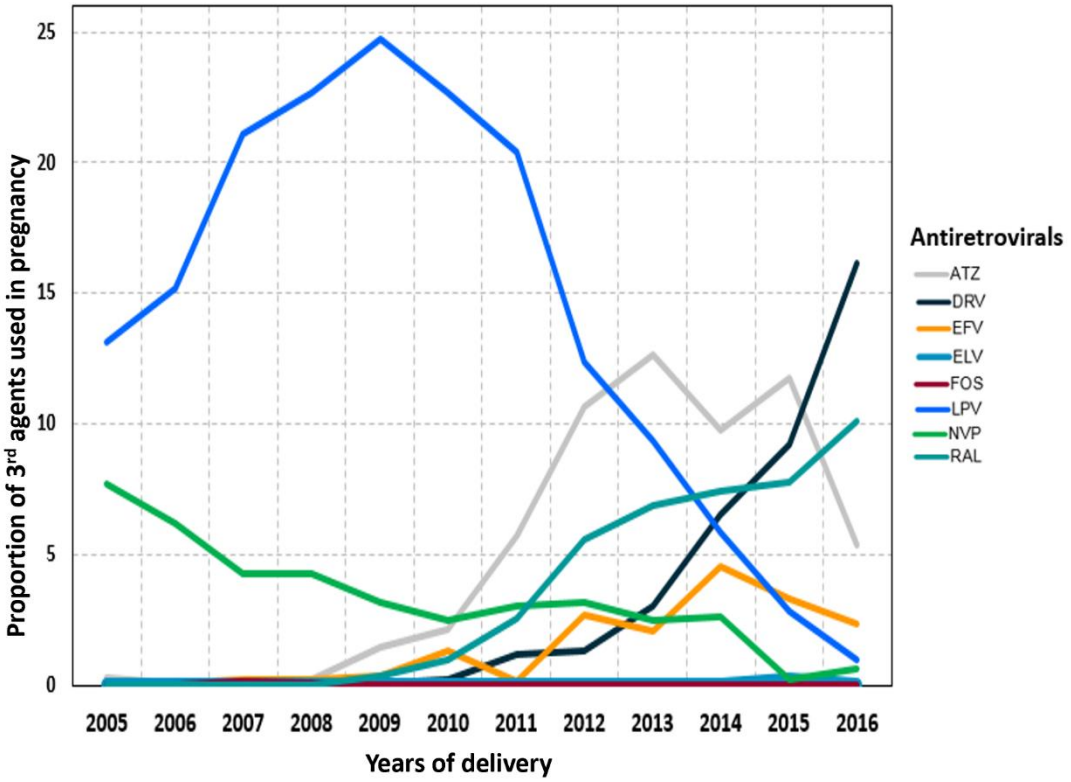



**Figure 4.10 Trends of third agents use in the NSHPC, 2005-16**

**Table 4.7 BHIVA guidelines updates on preferred and alternative third agents, 2005-16**

| Year | Regimen | Preferred | Alternative |
|---|---|---|---|
| **2005** | **Third gent** | - | NVP |
| **2008** | **Third agent** | - | Any PI/r |
| **2012** | **Third agent** | EFV | NVP[1] or any PI/r |
| **2014** | **Third agent** | EFV (or NVP[1] or any PI/r) | NVP[1] or any PI/r |
| | **Newly diagnosed** | DRV/r, ATV/r or EFV or RAL or ELV/c | LPV/r, FOS/r or NVP[1] |
| **2016** | **Third agent** | EFV or NVP[1] OR any PI/r | DRV/r* |
| | **Newly diagnosed** | ATV/r, DRV/r or EFV or RAL or EVL/c | LPV/r, FOS/r or NVP[1] |

[1]**CD4<250c/µL; *known resistance**

***Trends of the most common ARVs used between 2008-18 (objective 2c)***

Over time, patterns of ARV usage have changed, and these are displayed in Figure 4.11. Comparison between NSHPC key patterns of changes and BHIVA guidelines updates (Table 4.5) are reported below grouped by BHIVA "backbone NRTIs" and "Third agents", except for the INSTIs, which will be discussed in Chapter 6.

**Backbone NRTIs**: Looking at the trends of 3TC utilisation in the NSHPC, it can be noticed how it was widely prescribed in association with ZDV as a FDC at the beginning of the study period. However, its use, as the fixed-dose 3TC/ZDV, steady declined over time from 27.6% (1,081/3,909) of all the ARVs used in 2008 to 7.7% (254/3,284) of those used in 2016 (test-for-trend $p$ <0.01). Nevertheless, BHIVA guidelines started to recommend 3TC, in a FDC with ABC, as one of the preferred backbone option in 2016; this might explain the contrasting trends of ZDV and 3TC, the former linearly decreasing over time while the latter progressively decreasing, possibly because since 2016 it was used in combination with ABC, slowing its decline.

In 2016, also TDF/FTC were moved from alternative to preferred backbone options in a FDC, representing the most used ARVs in 2016, with TDF accounting for 24.7% (811/3,284) and FTC for 24.2% (795/3,284) of all the ARVs used; by 2018 their usage slightly decreased to 21.2% (431/2,024) and 21.8% (443/2,024) respectively, possibly reflecting a wider availability of preferred backbone options than in previous years (Table 4.5).

**Third agents:** Over time, third agents have experienced the most changes, both in terms of recommendations and in their real-world usage, possibly reflecting the increased availability of new classes and new combinations of ARVs. BHIVA guidelines started to recommend use of any boosted PI as valid alternatives to the NNRTIs EFV and NVP in 2008. Taking a closer look at some of the PI trends, for ATV/r, its usage was first reported to the NSHPC in 2008 accounting for only 1.6% (66/3,909) of all the ARVs used; with use then steadily increasing, reaching a peak of approximately 10% of all the ARVs used between 2012-13 (389/3,830 in 2012 and 358/3,701 in 2013).

In 2014 BHIVA replaced their general recommendation of "any PI/r" with the more specific recommendation of using ATV/r as preferred third agent, addressing newly diagnosed women. However, data from the NSHPC showed a steady decrease in ATV/r usage, which started from 2014 and reached the lowest level in 2018, when its usage accounted for only 3.5% (70/2,022) of all the ARVs used. Interestingly with

the 2018 BHIVA guidelines update, ATV/r started to be recommended as one of the two preferred third agent. Similarly, DRV/r usage in the NSHPC was firstly reported in 2009, accounting for only 0.5% (21/4,089) of the ARVs used, just a year after PI/r started to be recommended by the BHIVA guidelines. However, since then its use steadily increased, reaching a peak of 9.8% (323/3,284) in 2016, the same year BHIVA guidelines stared to recommend DRV/r as a valid third agent alternative.

Use of the NNRTIs EFV, NVP and RPV also changed over time. Noticeably, NVP use in the NSHPC population gradually decreased from 6.5% (253/3,909) of all the ARVs prescribed in 2008 to 1.7% (35/2,024) of those prescribed in 2018. BHIVA started to recommend NVP as alternative third agents in 2012 and as preferred in 2014, when its use in the NSHPC had already declined, accounting for 2.2% (71/3,284) of all the ARVs used. Since 2012, EFV started to be recommend and also preferred to NVP or any PI/r, however, following concerns of *in utero* exposure to EFV and increased fetal risk to develop NTDs, its usage in the NSHPC stayed at relative low rate, reaching a peak in 2013, when it accounted for 6.8% (251/3,701) of all the ARVs used. Lastly, RPV started to be recommended as an alternative third agent by BHIVA guidelines only in 2018, by which time it accounted for 3.5% (71/2,024) of all the ARVs used in the NSHPC.

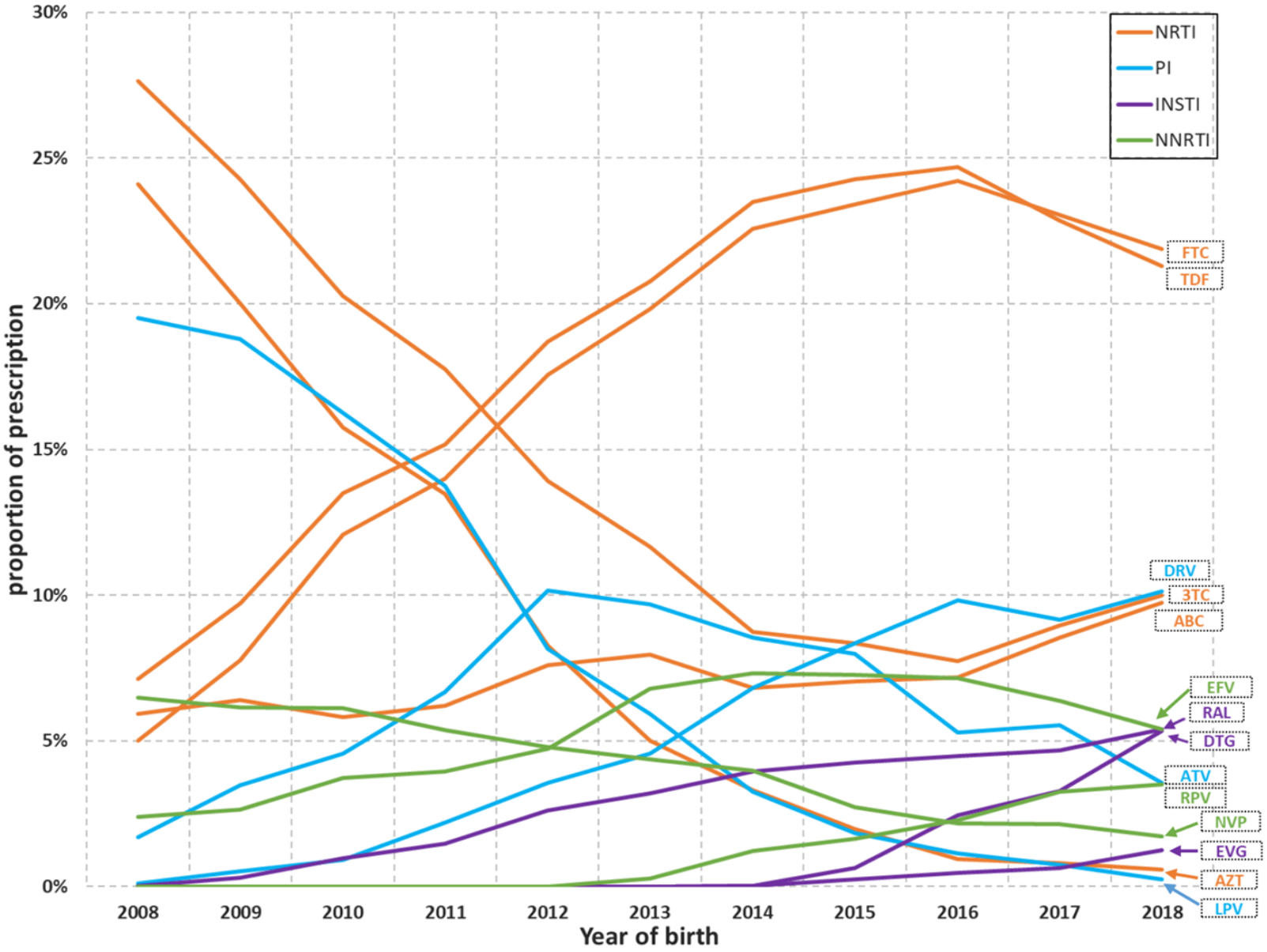



**Figure 4.11 Trends of the most common ARVs used in the NSHPC, 2008-18**

## 4.3 Antiretroviral agents and available safety and efficacy data: regulatory recommendations

In this section a synthesis of all publicly available safety data extracted from the EMA website on the 14 most common ARVs used in the NSHPC (covered in section 4.3.1) is presented, alongside a descriptive comparison of data from these regulatory recommendations with those from real-world use in pregnancy in the UK (covered in section 4.3.2). The aim was to evaluate whether inconsistencies and gaps between regulatory recommendations and real-world antenatal use of ARVs exists and to what extent. Furthermore, a further analysis on time trends was performed to evaluate changes in safety data availability over time.

### 4.3.1 Publicly available data from the EMA on safety of ARVs

*Specific methods:*

The EMA's publicly available SmPCs and EPAR documents were retrieved for each of the ARVs with a European marketing authorisation. The selected ARVs are either single agents or in a FDC and correspond to the 14 most common ARVs used in pregnancy and reported to the NSHPC between 2008-2018.

As explained in Chapter 3, section 3.3.2 and illustrated by Figure 3.3, the SmPC is divided in sections. For this chapter, specific sections of the SmPC were searched, namely Sec 4.2-*Posology and method of administration*; Sec 4.3-*Contraindications;* Sec. 4.4-*Special warnings and precaution for use*; Sec. 4.6-*Fertility, Pregnancy and lactation*; Sec. 5.1- *Pharmacodynamic properties*; 5.2- *Pharmacokinetic properties*; Sec. 5.3- *Preclinical safety data*. Data on the safe use of a medicine are usually contained in more than one section and cross-referenced across the SmPC. For example, if there is a recommendation for avoidance of a medication for a certain population or in association with certain medicines it will be outlined both in Sec 4.3 and in Sec 4.4 where more details on the targeted population can be found. Furthermore, if the information concerns pregnant and breastfeeding women and those of childbearing age further explanation of the rationale for these warnings are also given in Sec. 4.6.

Information regarding pharmacodynamics and pharmacokinetics of the product are addressed in "Sec. 5.1- *Pharmacodynamic properties"* and "5.2- *Pharmacokinetic*

*properties",* respectively. These sections present data on mechanisms of action and main CT findings that supported the marketing authorisation.

Data addressing pregnant and breastfeeding women and those of childbearing age can be found mainly in "Sec. 4.6-*Fertility, Pregnancy and lactation"* and more recently by cross-referencing with sections 4.2 and 4.4. In sec. 4.6 all the available clinical data are usually reported and frequently categorised as "large" (>3,000 or >1,000), "moderate" (300-1,000), "discrete" (>800), "limited" (<300), "no/limited, no-adequate and well-controlled" data from human/clinical studies. Usually, preclinical results are briefly described in this section and cross-referenced with the more detailed "Sec. 5.3- *Preclinical safety data"* to support the recommendations. Furthermore, Sec. 4.6 can specifically address women of childbearing age, for example with special conditions such as requiring a pregnancy test before starting the treatment and/or compliance with contraceptive methods while on treatment.

As previously mentioned, since 2005 RMPs are part of the EPAR (chapter 3, section 3.3.2), therefore I have included all the available RMPs retrieved from updated EPAR and PSUR addressing pregnancy and lactation "important potential" or "important identified" risks and missing information, i.e. all the available additional pharmacovigilance activities (defined in chapter 2 sections 2.3.3 and 2.4.2).

Search criteria for SmPC, EPAR and PSUR were any of the following words: "pregnancy", "pregnant", "pregnancies", "pregnant women", "women", "women of childbearing potential (WCBP)", "WCBP", "teratogenicity", "embryo-foetal toxicity", "embryo-foetal development", "placenta transfer", "placenta", "power to cross the placenta", "infants", "toxicity", "foetal toxicity", "developmental toxicity", "reproductive toxicity", "breastfeeding", "lactation", "lactating" "breast milk", "safety in pregnancy".

Furthermore, to overcome the lack of homogeneity in the terminology used across different SmPCs, I have harmonized these recommendations as follow:

- Whenever "human studies", "clinical studies", "clinical findings" and "trials" were found these were considered synonyms of clinical studies/CT
- "should not be used" or "not recommended" were considered as *clear recommendations* restricting the usage of the relevant agent or FDC in pregnant and breastfeeding women
- "should/may be used only if benefits outweigh risks", "the malformative risk is unlikely…", "as precautionary measure", "caution should be used…", "may be used if necessary" were all considered as *not clear recommendations* for the relevant ARV's usage in pregnant and breastfeeding women.

*Results:*

Tables 4.8 summarizes data extrapolated from the most recently updated SmPCs, EPAR and PSUR documents for each ARV included in the analyses. A detailed list of all the data collected is presented in appendix 9.3. There were 27 ARVs with a European marketing authorisation, 14 as single agents and 13 as FDCs matching the 14 most common ARVs used in the NSHPC over the study period. It is noteworthy that no SmPC had a recommendation for the safe and effective use of the relevant agent/FDC in pregnancy and breastfeeding women.

For 70.4% of the SmPCs (19/27) *no clear recommendation* for use in pregnancy was provided, with more than a third (36.8%, 7/19) stating that use in pregnancy should occur "only if the potential benefit justifies the potential risk for the fetus". The remaining 29.6% (8/27) had *clear recommendation* to avoid use in pregnancy, either by clearly stating that the agent/FDC was not recommended or that it "should not be used or initiated in pregnancy". Furthermore, for 44.4% (12/27) of the most recently updated SmPCs (i.e. for those ARVs with safety or efficacy signals recently detected) *clear recommendation* for avoidance of such ARVs were also addressed in sections 4.2 and 4.4.

For recommendations on using ARVs while breastfeeding, 44.4% (12/27) of SmPCs had *no clear recommendation* and 55.5% (15/27) had *clear recommendation* restricting use while breastfeeding on the basis of limited safety data on ARVs' effects in newborns/infants. However, all 27 ARVs had a clear recommendation to avoid breastfeeding, "as a general rule", to prevent VT.

Recommendations specifically addressing women of childbearing age were reported in only 33.3% (9/27) of the SmPCs. These were mostly advising to undergo a pregnancy test before treatment initiation and/or to use contraceptive measures. Three SmPCs raised concerns on the effectiveness of oral contraception and consequently recommended a second (barrier) method, two of which cross-referenced with section 4.4.

In all SmPCs, section 4.4 specified the robustness and the quality of the data from clinical studies on pregnant women, (i.e. if the amount of data gathered over time was large, moderate or limited; and in 55.5% (15/27) SmPCs the trimester of first exposure to the ARVs was reported, either as single agents or as FDC when data concerned exposure to the whole cART regimen. However, not all the SmPCs were clear in their wording, with some using the term "limited data" to say no data and others using the same term to indicate "less than 300 observations".

Furthermore, for most of the newly authorised combinations of ARVs, defined as those with a marketing authorisation obtained since 2014, 75% (6/8) did not have adequate nor well-controlled studies on pregnant women. All the SmPCs also reported data on preclinical findings and the type of studies, namely reproductive toxicity and embryofetal developmental studies. Nevertheless, assessment of the potential teratogenic effects of ARVs on the animal's offspring were reported for half 51.8% (14/27) of the SmPCs (these data are provided in appendix 9.3 not in Table 4.8). Special warnings and potential dose adjustments for ARV use, specifically addressing pregnancy and breastfeeding women were reported in 85.2% (23/27) of SmPCs. These were mostly warnings addressing the risk for mitochondrial dysfunction following *in utero* exposure to NRTIs or warnings about the increased risk for viral failure and thus increased risk of VT, as consequence of reduced exposure to ARVs (e.g. ATV/COBI as consequence of the reduced levels of the booster Cobicistat). The remaining 14.8% (4/27) did not report any special warnings nor requirements for dosage adjustments.

Pharmacokinetics and pharmacodynamics studies in pregnancy and breastfeeding women were reported in 44.4% (12/27) of the SmPCs, with the remaining 55.5% (15/27) lacking such studies. The ability for ARVs to cross the placental barrier was reported in 33.3% (9/27) of the SmPCs; however when assessing the EPARs, data on 11 more ARVs and their ability to cross the placental barrier were retrieved, bringing the total number of ARVs with such data to 74.1% (20/27). Of these 20 ARVs, 20% (4/20) reported such ability only from animal models not from CT findings.

There was also a lack of consistency in how these data are reported across the different SmPCs. For example, for DRV as single agent with the booster Cobicistat, DRV's ability to cross the placental barrier was only reported in the EPAR, while DRV as single agent in combination with the booster Ritonavir did not report such data in the EPAR. Furthermore, when FTC was given in the FDC of FTC/TAF, FTC/TAF/EVG/c, FTC/TDF/EVG/c and FTC/TAF/DRV/c there was no mention in either the SmPC or the EPAR about FTC's ability to cross the placental barrier, while as single agent FTC and as the FDC of FTC/TDF, EFV/FTC/TDF, FTC/RPV/TDF and FTC/RPV/TAF such information is clearly stated in the EPAR.

Furthermore, 29.6% of the ARVs (8/27) had available updated RMPs. These RMPs addressed missing information such as lack of safety data in pregnancy and/or lactating women; two RMPs addressed important identified risk (i.e. NTDs) for the FDC of EFV/FTC/TDF and ABC/3TC/DTG. For these ARVs detailed a-PhV and

PASS activities were stated, namely the APR, the DOLOMITE-EPPICC study and the DOLOMITE NEAT ID Network study.

The APR to collect any data on the risk for CAs/NTDs with exposure to ARVs in pregnancy and the other two studies to assess safety and effectiveness of DTG, pregnancy outcomes and NTDs detection. For the remaining 70.4% (19/27), RMPs did not addressed safety concerns for ARV use in pregnant and breastfeeding women.

## Table 4.8 Publicly available data on the most common ARV combinations taken from the EMA website

| ARVs | Pregnancy recommendation | | Breastfeeding recommendation | | Recommendation for WCBA | Clinical studies | Preclinical studies | Special warnings/ posology | PK/PD studies | Placental barrier | RMPs |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unclear | Clear | Unclear | Clear | | | | | | | |
| EFV/FTC/TDF | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓* | ✓ |
| 3TC/ZDV | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓* | |
| FTC/TAF | ✓ | | | ✓ | | ✓ | ✓ | ✓ | | ✓* | ✓ |
| RPV | ✓ | | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| FTC | ✓ | | | ✓ | | ✓ | ✓ | ✓ | | ✓* | |
| 3TC | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓ | |
| FTC/RPV/TDF | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓* | |
| ATV/c | | ✓ | | ✓ | | ✓ | ✓ | ✓ | | ✓* | ✓ |
| FTC/TAF/EVC/c | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ |
| RAL | ✓ | | | ✓ | | ✓ | ✓ | | | ✓ | |
| LPV/r | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ | | |
| ABC/3TC | ✓ | | ✓ | | | ✓ | ✓ | ✓ | | ✓ | |
| FTC/RPV/TAF | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓* | |
| DRV/r | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ | | |
| ATV/r | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ | | |
| DRV/c | | ✓ | ✓ | | | ✓ | ✓ | ✓ | ✓ | | |
| FTC/TDF/EVG/c | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | |
| FTC/TAF/DRV/c | | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓* | |
| EFV | | ✓ | | ✓ | ✓ | ✓ | ✓ | | | ✓ | |
| DTG | ✓ | | ✓ | | ✓ | ✓ | ✓ | | | ✓ | |
| COBI/c | | ✓ | | ✓ | | ✓ | ✓ | ✓ | | | |
| ABC/3TC/DTG | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |
| ZDV/3TC/ABC | ✓ | | ✓ | | | ✓ | ✓ | ✓ | | | |
| FTC/TDF | ✓ | | | ✓ | | ✓ | ✓ | ✓ | | ✓* | ✓ |
| NVP | ✓ | | ✓ | | ✓ | ✓ | ✓ | | | ✓ | |
| TDF | ✓ | | | ✓ | | ✓ | ✓ | ✓ | | ✓* | ✓ |
| ABC | ✓ | | ✓ | | | ✓ | ✓ | ✓ | | ✓ | |

WCBA: women of childbearing age; PK/PD studies: pharmacokinetics/pharmacodynamics studies; RMPs: risk minimisation plans; *data extracted from the EPAR

***Trends in regulatory recommendations***

Over time SmPCs and EPAR have been updated whenever new real-world data on ARV use in pregnancy became available. Out of the 27 ARVs analysed, for 15 ARVs either as single agents or in a FDC I was able to retrieve the original SmPCs at time of marketing authorisation, while for the remaining 12 ARVs, I could not retrieve the original SmPCs, but only access older versions of published EPARs (namely "EPAR procedural steps taken and scientific information after authorisation") and collect updated data on their safety and efficacy and if changes in recommendation over time had occurred.

Of the 15 with the SmPC at time of marketing approval, almost all (93.3%, 14/15) did not report data from pregnancy PK studies at time of marketing authorisation and 53.3% (8/15) did not mention the agent/FDC's ability to cross the placental barrier. However, by 2018, 46.7% (7/15) SmPCs reported PK-studies and incorporated changes in recommendations for pregnancy use due to these new findings. Over time, data on ARVs' ability to cross the placental barrier have also been updated; however this information is mostly (in 46.7% (7/15) cases) contained in the EPARs rather that in the more accessible SmPCs in which such data was stated in only 26.7% (4/15) with the remaining 73.3% (11/15) not stating such property.

Recommendations for all agents/FDCs use while breastfeeding remained constant since time of their approval, advising not to breast-feed to prevent VT. In regard to specific recommendations to avoid breastfeeding while taking certain ARV combinations, there were five FDCs (ABC/3TC, ABC/3TC/ZDV ABC/3TC/DTG, FTC/TDF and EFV/FTC/TDF) or which recommendations were updated once data on one of the ARV's ability to be excreted in human milk became available.

Of the remaining 12 ARVs with no SmPCs from the time of marketing approval, for four, namely DRV/r, DRV/c, ATV/r and ATV/c, there were updated sections 4.2, 4.4, 4.6, 5.2 of their SmPCs after years of marketing approval due to new findings concerning their efficacy in pregnancy (see section 4.3.2). Furthermore, for 83.3% (10/12) of the ARVs changes in recommendations for their use were made and cross-references were added. Most of the recommendations became more restrictive and clearer, i.e. changing from "the use may consider in pregnancy only in the potential benefits justifies the potential risks" to "it should not be used in pregnancy". Cross-referencing with section 4.2 and 4.4 to justify the recommendations and the warnings were added to strength the recommendations.

### 4.3.2 Safe should also be effective

In this section ARVs either as single agents or in FDC with identified PK studies showing concerns for ARVs' effectiveness in pregnancy were analysed. The aim of this analysis was to evaluate if there is a time gap between specific ARV's marketing approval and the first time data from PK studies on pregnant women are mentioned in regulatory recommendations and to compare such data with real-world use of ARVs by collecting data from the NSHPC.

*Methods:*

The analysis was restricted to ARVs with data from PK studies reported to the EMA and thus publicly available from the SmPCs and EPAR documents. Therefore, sections 4.2, 4.4, 4.6, 5.1 and 5.2 of the SmPC and the EPAR document *"EPAR-Procedural steps taken and scientific information after authorisation*" were assessed and any data on ARVs efficacy, PK studies and new findings concerning ARVs' efficacy were collected.

DRV, RPV, COBI and ATV were the ARVs, either as single agents or in a FDC with concerns in their usage in pregnancy following PK-studies.

For the real-world use of the same ARVs, data on all pregnant women (regardless of pregnancy outcome) exposed to any cART containing such ARVs with an EDD between 2008-18 reported to the NSHPC by 31st of December 2018 were collected.

A preliminary study including only pregnancies with EDD from 1st January 2013 to 31st March 2017 were used in an accepted poster presentation, which won the best poster award at the 9th International Workshop on HIV Paediatrics held in Paris 21-22 July 2017.

*Results:*

**From the EMA documents:** There were 11 cART containing DRV, RPV, COBI/c and ATV. DRV is marked either as single agent in combination with the boosters COBI/c (Rezolsta) or the booster Ritonavir (Prezista) or in the FDC of FTC/TAF/DRV/c (Symtuza); RPV is marketed either as single agent (Edurant) or in the FDCs of FTC/TDF/RPV (Eviplera) and FTD/TAF/RPV (Odefsey); COBI/c is marked as single agent (Tybost) and in the FDC as booster to DRV (Rezolsta) and to the INSTI EVG in the FDC FTC/TAF/EVG/c (Genvoya) and FTC/TDF/EVG/c (Stribild); ATV is marked as single agent with the booster ritonavir (Reyataz) or with

the booster COBI/c (Evotaz). For each of these combinations, PK studies found reduced plasma concentrations as a consequence of pregnancy-induced PK changes, which can possibly lead to viral failure and increased risk of VT.
Therefore, for 63.6% (7/11) ARV combinations, changes in their recommendations were made, strongly advising against their use during pregnancy and to switch to other regimens whenever possible (as for latest access to EMA data; 20/05/2020). Furthermore, for 45.5% (5/11) of these ARVs, specific warnings and recommendations for women of childbearing age were inserted, recommending the use of effective contraceptive to avoid pregnancy while taking such ARVs.

Evaluation of the time-gap showed a median lag of 6 years between European marketing authorisation and publication of data from PK studies on pregnant women (Table 4.9).

## Table 4.9 Time-lines of marketing authorisation and availability of pregnancy PK data for cART containing DRV, RPV, COBI/c and/or ATV

| ARVs | Year of marketing authorisation | Year of PK data availability in pregnancy[1] | Key findings of PK studies | Product information affected[2] |
|---|---|---|---|---|
| **DRV/r** | 2007 | 2018 | **TMC114HIV3015 study**: PK, efficacy and safety data from DRV/r arm Phase 3b: 6/7 women completed study & showed $AUC_{12h}$ of DRV/r 600/100mg twice/die 26% and 16%, respectively lower in T2 and T3 vs PP (6-12w) and for DRV/r 800/100mg once/die $AUC_{24h}$ was 31% and 32% respectively lower in T2 and T3 vs PP | SmPC sections 4.2, 4.4, 4.6, 5.2 |
| **DRV/c** | 2014 | | **TMC114HIV3015 study on DRV/c:** PK, efficacy and safety data from DRV/c arm Phase 3b: lower DRV exposure in T2 (56% reduction in AUC) and in T3 (50% reduction in AUC) vs PP. Main cause of low exposures is marked reduction in COBI as consequence of preg-associated enzyme induction | |
| **DRV/c/FTC/TAF** | 2017 | | | |
| **RPV** | 2011 | 2017 | **TMC114HIV301 study on RVP**: exposure to RPV was lower in T2-T3 vs PP (6-12W); RPV 25mg once/die in T2 had mean intra-individual values for RPV $C_{max}$, $AUC_{24h}$, $C_{min}$ of respectively, 21%, 29%, 35% lower than in PP and in T3 of respectively, 20%, 31%, 42% lower than in PP | SmPC sections 4.2, 4.4, 4.6, 5.1, 5.2 |
| **RPV/FTC/TDF** | 2011 | | | |
| **RPV/FTC/TAF** | 2016 | | | |
| **COBI/c** | 2013 | 2019 | **PK-studies:** reduced levels of COBI in pregnancies results in lower ATV or DRV exposure compared to PP. | SmPC sections 4.2, 4.4, 4.6 |
| **FTC/TAF/EVG/c** | 2013 | | **IMPAACTP-1026s study on EVG/c**: exposure to EVG was lower in T2-T3 vs PP; in T2 mean intra-individual values for EVG $C_{max}$, $AUC_{24h}$, $C_{24}$ were respectively, 8%, 24%,81% lower than in PP and in T3 were respectively, 28%, 44%, 89% lower than in P; while for COBI reduction in T2 were respectively of 28%, 44%, 60% vs PP and in T3 38%, 59% 76% vs PP. | SmPC sections 4.2, 4.4, 4.6, 5.2 |
| **FTC/TDF/EVG/c** | 2013 | | | |
| **ATV/r** | 2004 | 2011 | **PK-studies**: ATV peak concentration and AUC were found approx. 26-40% higher during PP period (4-12w) compared to those observed historically in HIV non-preg patients | SmPC sections 4.2, 4.6, 5.2 |
| **ATV/c** | 2015 | 2019 | **PK-studies:** reduced levels of COBI in pregnancies results in lower ATV exposure compared to PP. | SmPC sections 4.2, 4.4, 4.6 |

[1]Commission decision (CD) issued/amended is for procedures that affect the terms of the marketing authorisation (e.g. SmPC, labelling, etc).

[2]Which section will be updated (e.g. SmPC, package leaflet, etc.) For this analysis only the updated SmPC section are reported.

**From the NSHPC:** Of the 12,099 pregnancies reported to the NSHPC between 2008-18, 4,530 (37.4%) were exposed to one of the four ARVs: 1,710 (14.1%) to DRV as DRV/r or DRV/c, 335 (2.7%) to RPV as RVP/FTC/TDF or RVP/TDF/TAF, 148 (1,2%) to COBI as FTC/TAF/EVG/c or FTC/TDF/EVG/c and 2,337 (19.3%) to ATV as ATV/c or ATV/c.

RPV obtained a European marketing authorisation in 2007 but it was not until 2013 that it was first prescribed in the UK and Ireland. RPV use increased >13-fold from 2013 to 2018, being used in only 0.8% (10/1,154) of the total pregnancies in 2013 and increasing to 11.1% (70/629) in 2018 (test-for-trend $p<0.001$).

Between 2008-18 the number of pregnancies exposed to DRV increased from 0.3% (4/1279) to 30.1% (189/629), while the number of pregnancies exposed to ATV went from 5.2% (66/1,279) to 11.1% (70/629) (test-for-trend for both $p< 0.001$).

COBI was first used in pregnancy in 2014, following its marketing authorisation in 2013; the number of pregnancies exposed to COBI as FTC/TAF/EVG/c or FTC/TDF/EVG/c rose from only one pregnancy, accounting for 0.1% (1/1,066) of all the ARVs used in 2014 to 11.4% (72/629) of those used in 2018 (test-for-trend for both $p< 0.001$) (Figure 4.12).

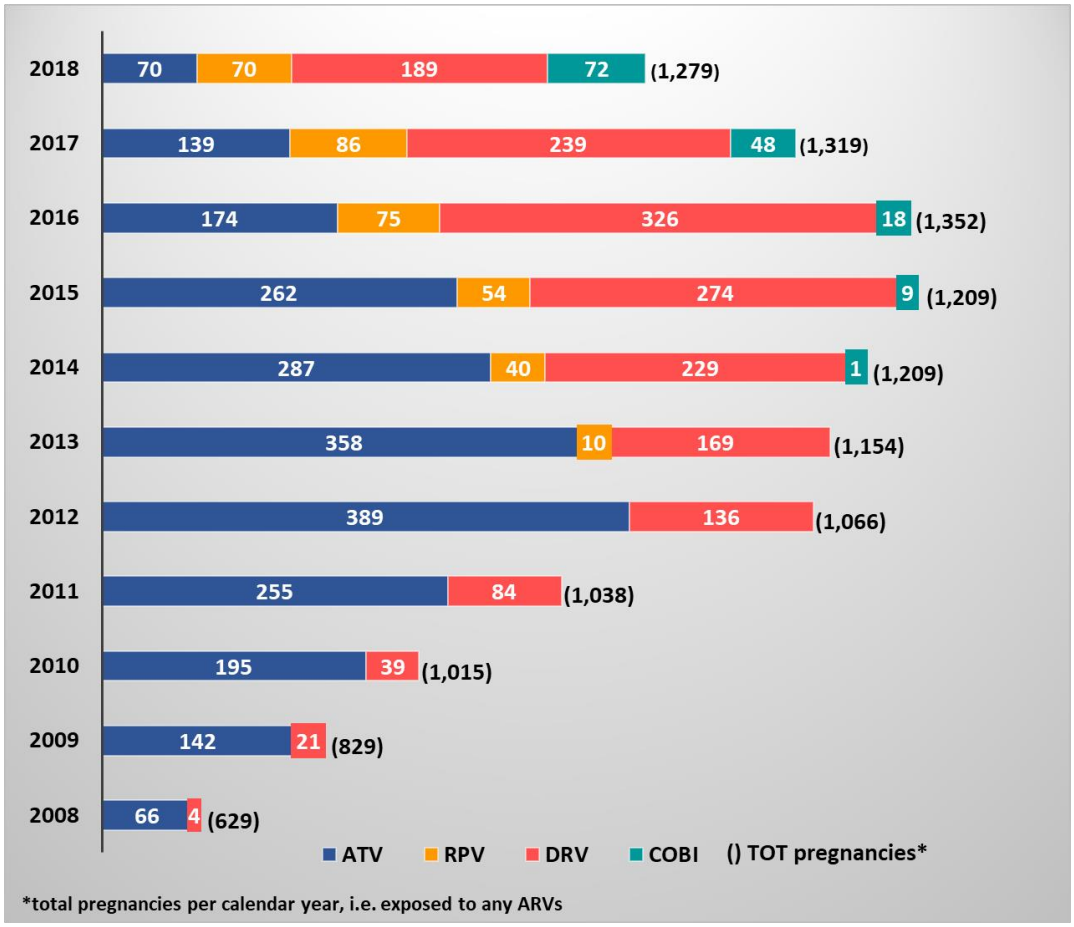



**Figure 4.12 Number of pregnancies exposed to ATV, RPV, DRV and COBI, by calendar year**

## 4.4 Conclusion

Over the past decade demographics and health status of HIV positive pregnant women living in the UK and Ireland have changed. Overall, there was an increase in the number of women who knew their HIV status before becoming pregnant and a consequent increase in the number of women starting treatment before becoming pregnant. Antenatal testing and early cART initiation also allowed for the overall increased proportion of women with effective VL suppression at delivery. Looking at pregnancy outcomes, over the years the overall number of liveborn infants per year was relatively steady, with the proportion of infants born at term with a weight at birth ≥2.5kg increasing over time. On the contrary, over time the number of pregnancies ending in stillbirths and of those delivering preterm liveborn declined even if not reaching statistical significance.

The "snapshot analysis" allowed evaluation of the complex relationship between real-world use of ARVs and guidelines. Results have shown how clinical guidelines are updated approximately every two years, whenever the clinical evidence base has been expanded. However, where there is limited evidence, guidelines appear to be driven mostly by clinical practice via observational studies. The main analysis focused on newly diagnosed women starting cART in pregnancy and my findings suggest that for NRTIs, changes in usage often precede guideline recommendations, while the increased use of specific third agents appears to be accelerated by specific recommendations, as one might expect; though, other factors might contribute to prescribing patterns, such as changes in commissioning.

The gap-analysis comparing real-world use of ARVs and regulatory recommendations evaluated available safety and effectiveness data and identified two main issues. Firstly, no ARVs up to 2018 have had clear regulatory recommendation on their use in pregnancy, regardless of accumulating data on their use for maternal health, prevention of VT and adverse pregnancy outcomes. Secondly, most of the restrictive recommendations for the use of ARVs in pregnancy are either the result of unavailability of data rather than the result of identified toxicity risks for the fetus or are the consequence of efficacy and/or safety risks only identified years after marketing authorisation was granted. Additionally, results identified a lack of heterogeneous and standardized wording across different SmPCs, for example, the word "limited" meaning "no data" in some cases but also "less than 300 observations" in others. This can cause ambiguity and confusion for

healthcare providers, policy makers and women themselves when following the recommendations. However, in recent years the structure of the SmPCs has been improved, possibly to reflect the fast pace at which new data has become available and the consequent necessity to update in a timely and correct way each section of the SmPC. For example, following identified pregnancy-induced PK-changes for EVG/c, recommendations for its two combinations FTC/TAF/EVG/c and FTC/TDF/EVG/c have been updated using consistent wording for their recommendations, proving that regulatory agencies do take seriously efficacy warnings.

In regard to the identified time-gap between year of ARVs approval and first time data on human pregnancy become available, results have shown that 75% of newly authorised ARVs combination do not have adequate or well controlled studies on pregnant women, despite real-life settings (e.g. data from the NSHPC) showing clear evidence of widespread use of ARVs. This is in line with previous studies (Colbers et al. 2019, Wickremsinhe et al. 2019) showing how safety and efficacy data arrives with considerable delays (median lag time off 6 years in the NSHPC) from time of medicine approval and PK data from pregnant women.

## 4.5 Key points

**HIV positive pregnant women reported to the NSHPC from 2008-18:**

- Of 12,099 singleton pregnancies 11,197 (92.5%) resulted in livebirths, 99 (0.8%) in stillbirths, 656 (5.4%) in miscarriages and 147 (1.2%) in terminations. Almost all (89.7%) liveborn infants were delivered at term, weighing 2.5kg or more 83.6%) and of Black African mothers (74.2%).
- For 82.9% of women, HIV diagnosis was made prior to conception with rates significantly increasing over the study period from 69% to 90%.
- Overall, 59.9% of women started cART prior to their pregnancy with numbers significantly rising from 37.6% in 2008 to 80.9% in 2018; 72.2% of women who knew their HIV status prior to conception had started cART prior to their pregnancy.
- Overall, in 62.9% of pregnancies from women delivering live- and still-born infants effective suppression of VL (≤400 copies/μL) near delivery was reported;

of those almost all 89.2% reported VL values of ≤50 copies/μL around time of delivery with 57.5% of those women already on cART at time of conception.

**Snapshot analysis of ARV use**:

- Between 2005-16 25 different ARVs were used by pregnant WLWH in the UK, with a total of 53,686 individual ARVs used within 13,757 singleton pregnancies, 58.3% of which were started before conception and 41.7% during pregnancy.
- In the NSHPC, noticeable changes occurred in the usage of the four NRTI backbones: with ZDV and 3TC being the most prescribed in 2005, each accounting for approximately 30% of all the ARVs, but then drastically declining to 1% and 7%, respectively by 2016 (test-for-trend $p< 0.001$). FTC and TDF experienced a steady increase, accounting for <1% and 3%, respectively of all the ARVs used in 2005 but increasing to approximately 24% by the end of the period.
- ZDV/3TC as a FDC started to be recommended by BHIVA as preferred backbone from 2012, when its usage in NSHPC was already declining (ZDV went from 33.7% (540/1,602) in 2005 to 14.2% (94/664) in 2012, (test-for-trend $p< 0.001$)). A noticeable change was the inverted U-shape trend for the use of LPV/r and ATV/r with peaks respectively reached in 2009 and 2013.
- Looking at the 14 most common ARV combinations used between 2008-18, there was a total of 38,214 individual ARV usages; third agents experienced the most noticeable changes, both in terms of recommendations and their real-world use, most likely reflecting the increased availability of new classes and new combinations of ARV.

**EMA data on safety of ARVs:**

- There were 27 ARVs with a European marketing authorisation; for 70.4% and 44.4% of SmPCs there was *no clear recommendations* respectively for usage in pregnancy and breastfeeding; only 33.3% of SmPCs specifically addressed women of childbearing age (mostly advising to undergo pregnancy testing before treatment initiation and/or to comply with contraceptive measures).
- For 75% of newly authorised medicines, no adequate or well-controlled studies on pregnant women were stated and only half of the SmPC reported teratogenic effect of ARVs on animal's offspring; for 44.4% of the SmPCs, PK-PD studies in pregnant and breastfeeding women were reported; the ARV's transplacental

passage was reported in 33.3%, a proportion that rose to 74.1% if data from EPAR are included.

- Only 29.6% of ARVs had an available updated RMP that addressed pregnancy and breastfeeding missing information or identified/potential risks.
- Regulatory recommendations have been updated when new data have become available. At the time of marketing authorisation, 93.3% of the original SmPCs had no PK data reported and 53.3% did not mention transplacental passage, but by 2018, data from PK studies and on transplacental passage were reported in 46.7% and 26.7% of SmPCs, respectively.

**Safe should be also effective:**

- For DRV, RPV, COBI/c, and ATV, results showed a median lag of 6 years between European marketing authorisation and publication of data from PK studies on pregnant women; reporting decreased COBI levels and consequent reduced ARVs plasma concentrations in pregnancy vs postpartum; and low exposure has been associated with increased risk of failure to suppress VL and therefore increased risk for VT.
- Evaluation of DRV, RPV, COBI/c, and ATV use in real-world (i.e. from NSHPC data) showed how these were widely and increasingly used over time, e.g. RPV-based regimen increased by more than thirteen times from 2013 to 2018 (test-for-trend $p<0.001$).

# 5 Congenital anomalies in pregnant women living with HIV in the UK

## 5.1 Introduction

As described in Chapter 1 section 1.5.3, there is evidence suggesting exposure to ARVs increases the risk for adverse pregnancy outcomes. Furthermore, an increasing number of women are now conceiving on cART, resulting in additional concerns as to whether *in utero* exposure to ARVs may increase the risk for embryofetal toxicity and teratogenic effects, particularly for newly authorised ARVs, which as shown in Chapter 4 section 4.3, usually come with less safety data.

Over the years, studies have compared frequency of CAs in cART-exposed infants with that in the general population and reported similar CAs prevalence, with some reporting no association between first trimester exposure to any ARVs and increased risk for CAs (Sanne et al. 2005, Ford et al. 2014, Phiri et al. 2014) and others identifying potential safety signal such as increased risk for CHD, musculoskeletal and skin defects requiring continued monitoring (Sibiude et al. 2015, Williams et al. 2015). In this chapter, the association between *in utero* ARV exposure and CAs, using data from the NSHPC is explored.

The first section describes CA prevalence and evaluates exposure to ARVs used in the UK during the study period by earliest exposure and risk for CAs among liveborn infants addressing four objectives. In this section risk factors potentially associated with increased risk of CAs have also been evaluated (section 5.2.3). The second section describes other adverse pregnancy outcomes, namely stillbirths, miscarriages and terminations of pregnancy with reported CAs.

## 5.2 Singleton livebirths pregnancies with reported CAs

This analysis was carried out using data from the NSHPC for the period 2008-2018 addressing four objectives:

1. To describe the prevalence of CAs overall and according to timing of cART exposure (categorised as periconception or later pregnancy), including trends over time

2. To describe the type of CAs by organ/system and multiple defects within infants (if reported) (objective 2a); to explore patterns of CAs for infants exposed to five ARV combinations of specific interest, using “the rule of three” (objective 2b)

3. To assess risk factors for CAs, including the association between exposure to ARVs and CAs (logistic regression models) by class of ARV (objective 3a) and by the five ARV combinations above (objective 3b)

4. To characterise infants with CAs and to compare them to infants without CAs with particular focus on other adverse birth outcomes and survival.

*Special definitions:*

In this chapter periconception exposure is defined as maternal ART use started before conception and includes the first trimester (T1), while “exposure in pregnancy” or “T2-T3” indicates that earliest exposure to cART to have occurred in the second and third trimesters of pregnancy.

*Special methods:*

Analyses are focused on the 11,197 liveborn singleton infants with an EDD/ date of delivery reported to the NSHPC between 2008-18 described in chapter 4 (see also section 4.2 and table 4.1) unless otherwise specified. For this analysis as explained in chapter 3, the earliest exposure to ARVs was considered as the first exposure to any combination of ARVs, regardless of the duration of their use.

To address objective 1, CA rates and 95% CI were calculated, overall and by timing of cART exposure.

To address objective 2, as explained in Chapter 3 section 3.4.1, all the CAs reported to the NSHPC were re-classified according to the EUROCAT classification criteria

following the *"EUROCAT Prevalence Data Table*" (EUROCAT 2018). Consequently, all the anomalies not meeting EUROCAT criteria or included in the EUROCAT list of *"minor anomalies for Exclusion"* have been excluded from the analyses.

To explore patterns of CAs following exposure to the five most commonly prescribed cART regimens over time, the "rule of three" was applied. The definition of the rule of three used is that 3/*n* is the upper 95% CI bound for binomial probability *p* when in *n* independent trials no event occur (Jovanovic et al. 1997). The rule of three is often used by clinicians in safety evaluation of clinical procedures. In this case it was used to evaluate whether three or more CAs of the same organ and system occurred in infants with *in utero* exposure to the same combination of ARVs at a particular time (i.e. those infants with periconception exposure).

To address objective 3, a regression model was fitted to evaluate the association between *in utero* exposure to ARVs and identification of CAs. ARVs were evaluated first by class (i.e. NNRTI, NRTI, PI and INSTI) and then by five selected ARV combinations of interest (i.e. TDF/FTC +EFV; AZT/3TC +LPV/r; TDF/FTC +ATV/r; TDF/FTC +DRV/r; TDF/FTC+ RPV).

### 5.2.1 Prevalence and trends of CAs in the study population (objective1)

Of the total 11,197 livebirths from singleton pregnancies there were 227 infants presenting with at least one CA and ten with more than one reported CA to the NSHPC between 2008-18, giving a prevalence of 2.03% (95% CI 1.77, 2.31).

The proportion of infants with periconception exposure to cART was 61.2% (6,857/11,197); of these, 147 had at least one CA, giving a prevalence of 2.14% (95% CI 1.81, 2.51). Among the 4,340 (38.8%) infants with earliest exposure to cART in T2-T3, 80 had at least one CA, a prevalence of 1.84% (95% CI 1.46, 2.29).

Over calendar time, there have been no major trends in CA prevalence, which was 2.3% (28/1,194) in 2008 and 2.7% (16/593) in 2018 (test-for-trend $p$=0.367) (Figure 5.1).

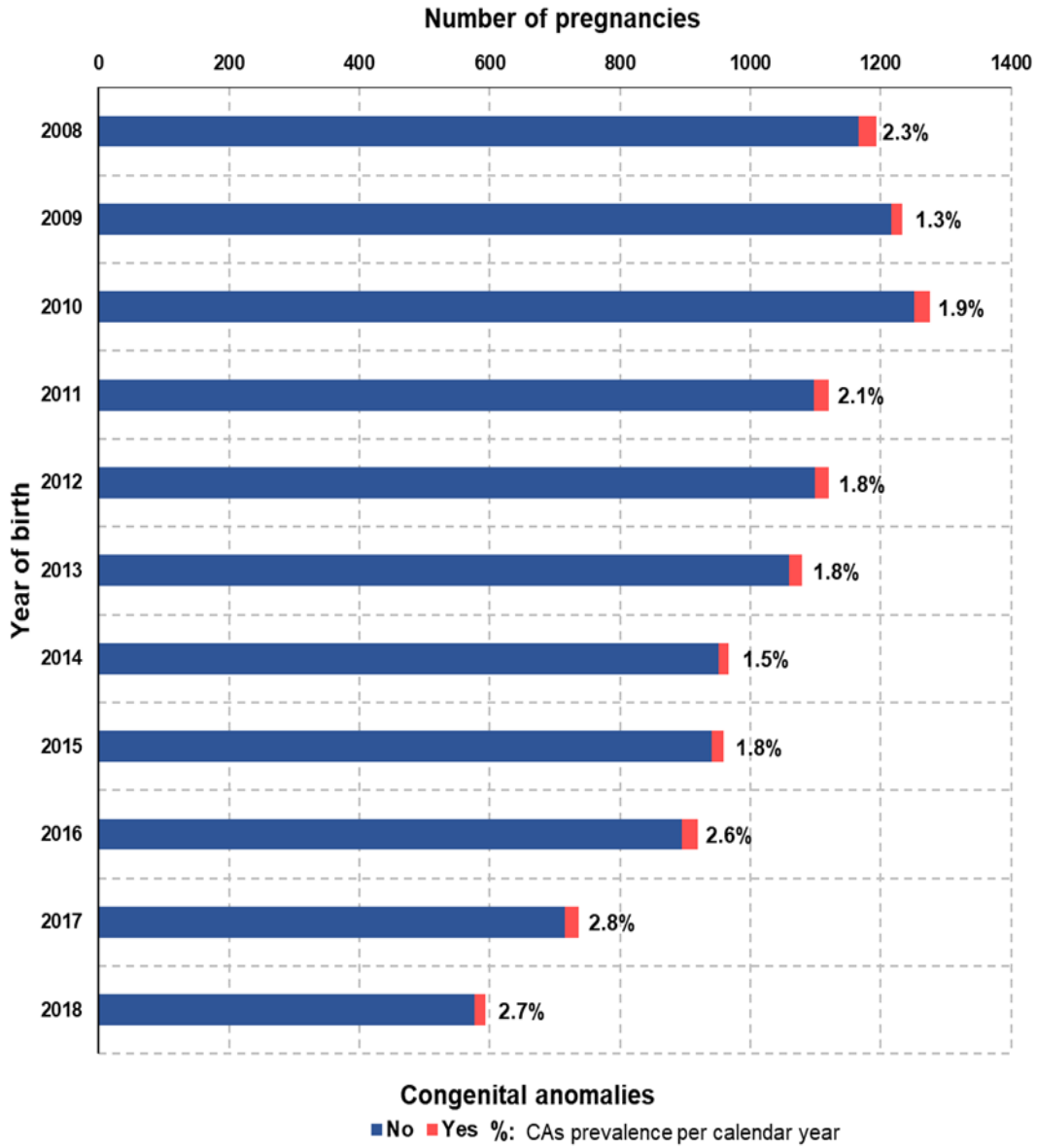



**Figure 5.1 Prevalence of CAs reported to the NSHPC, per calendar year**

Figure 5.2 displays ARV-specific CA rates and 95% CI for the 14 most commonly used ARVs reported between 2008-18 and described in chapter 4 section 4.2.2; of note, as ARVs are used in combinations, each CA may contribute to multiple numerators in the Figure.

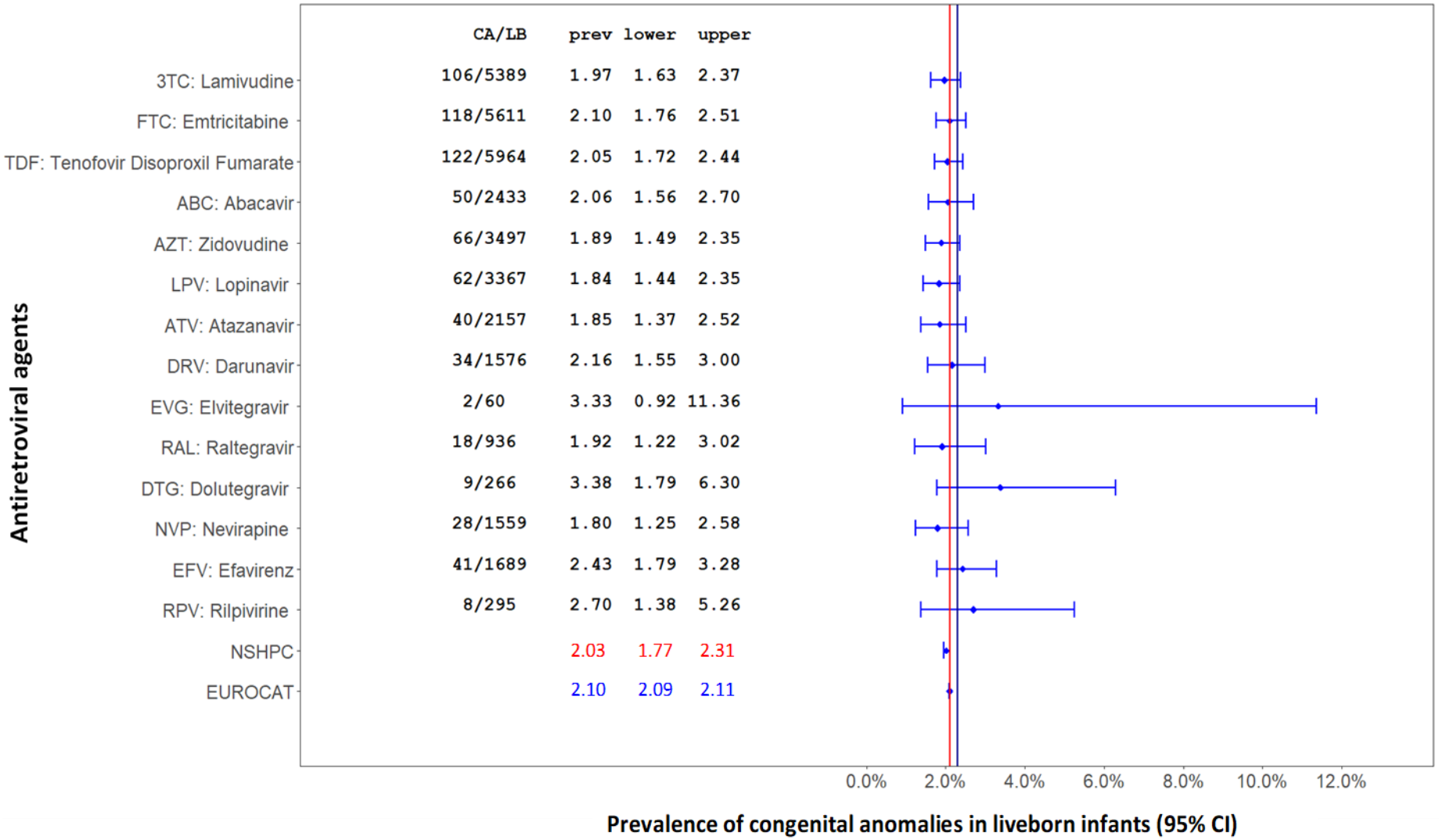



**Figure 5.2 ARVs-specific CA rates and 95% CI for the 14 most commonly used ARVs reported to the NSHPC, 2008-18**

## 5.2.2 Type of CAs by EUROCAT criteria (objective 2)

**Description of CAs by organ/system (objective 2a):**

Table 5.1 was adapted from the EUROCAT table of prevalence (EUROCAT 2018) and reflects the total number of CAs (i.e. 227) reported to the NSHPC during the study period.

The three most common CAs were limb anomalies accounting for 31.3% of all the reported CAs, with over three-quarters being polydactyly; followed by chromosomal anomalies (17.2%), with Down's syndrome been the most commonly recorded chromosomal syndrome (79.5%, 31/39); and CHD (12.7%), particularly ventricular and atrial septal defects accounting for over half of the CHDs (51.7%, 15/29). Genital system anomalies accounted for an overall 7.9% of all the anomalies, with hypospadias being the most frequent anomaly within this group (77.8%, 14/18) (Table 5.1).

Six infants presented with more than one CA, four of whom had a CA plus a conditional/associated CA (i.e. "pattern of anomalies that occur often together and at least two of which are morphologic" (Hennekam et al. 2013)). These are also reported in Table 5.1 as footnotes.

Two infants presented with more than one CA, one considered as main CA by the EUROCAT classification and the second not meeting the full criteria, i.e. one had Hirschsprung's disease and club foot-talipes, though not defined equinovarus and hence meeting EUROCAT *"minor anomalies for Exclusion" criteria*; and the other infant had a congenital hydronephrosis and undescended testicle, which is classified among "other genital malformations" .

Of the four infants presenting with a CA plus a conditional/associated CA, three had a chromosomal anomaly with associated manifestations, namely one trisomy 21 associated with the digestive anomaly tracheal stenosis and two CHDs, namely atrio-ventricular septal defects (AVSD) and coartation of aorta which are relatively rare conditions in the general population but more frequently seen in patients with Down's syndrome (Shapiro et al. 2000); one trisomy 18 associated with Dandy Walker syndrome along with vetriculomegaly and agenesis of corpus callosum, these are often described within the syndrome (Kollias 2014); and one trisomy 13 associated with cleft palate and/or hare lip. The last infant presented with a CHD, namely coarctation of aorta associated with ventricular septal defect, a frequently seen association (Plunkett et al. 2014).

**Table 5.1 Description of CAs meeting EUROCAT criteria, reported to the NSPHC in 2008-18, by cART earliest exposure**

| Organ system classification | Total (N) | Earliest exposure to cART | |
|---|---|---|---|
| | | periconception | T2-T3 |
| **All anomalies** | **227 (2.03%)** | **147 (2.14%)** | **80 (1.84%)** |
| **Nervous system** | **17** | **10** | **7** |
| Neural Tube Defects: | | | |
| Encephalocele | 1 | 0 | 1[1] |
| Spina Bifida | 1 | 1 | 0 |
| Hydrocephalus | 3 | 2[1] | 1 |
| Microcephaly | 7 | 3 | 4 |
| Malformation of/agenesis of corpus callosum | 2 | 2 | 0 |
| Other malformations/ unspecified: ventriculomegaly | 3 | 2 | 1 |
| **Eye, Ear, Face, Neck** | **2** | **2** | **0** |
| Congenital cataract | 1 | 1 | 0 |
| Congenital glaucoma | 1 | 1 | 0 |
| **Congenital heart defects** | **29** | **21** | **8** |
| Ventricular septal defect (VSD) | 9 | 6 | 3 |
| Atrial septal defect (ASD) | 3 | 3 | 0 |
| Atrioventricular septal defect (AVSD) | 3 | 1 | 2[1] |
| Tetralogy of Fallot | 2 | 2 | 0 |
| Ebstein's anomaly | 1 | 1 | 0 |
| Pulmonary valve stenosis | 3 | 2 | 1 |
| Pulmonary valve atresia | 1 | 1 | 0 |
| Aortic valve atresia/stenosis | 1 | 0 | 1 |
| Hypoplastic left/right heart | 1 | 1 | 0 |
| Coarctation of aorta | 3 | 2[2] | 1 |
| Total anomalous pulmonary venous return (TAPVR) | 2 | 2 | 0 |

| Organ system classification | Total (N) | Earliest exposure to cART | |
|---|---|---|---|
| | | periconception | T2-T3 |
| **Respiratory** | **5** | **2** | **3** |
| Cystic adenomatous malformation of lung | 5 | 2 | 3 |
| **Oro-facial clefts** | **6** | **3** | **3** |
| Cleft palate and/or hare lip | 6 | 3 | 3 |
| **Digestive system** | **15** | **11** | **4** |
| Oesophageal atresia with or without trachea-oesophageal fistula | 3 | 1 | 2 |
| Duodenal atresia or stenosis | 5 | 4 | 1 |
| Atresia or stenosis of other parts of small intestine | 1 | 1 | 0 |
| Ano-rectal atresia and stenosis | 2 | 1 | 1 |
| Hirschsprung's disease | 3 | 3[3] | 0 |
| Diaphragmatic hernia | 1 | 1[1] | 0 |
| **Abdominal wall defects** | **2** | **2** | **0** |
| Gastroschisis | 1 | 1 | 0 |
| Other malformation: OEIS- Cloacal Exstrophy | 1 | 1 | 0 |
| **Urinary** | **14** | **10** | **4** |
| Bilateral renal agenesis including Potter syndrome | 2 | 1 | 1[1] |
| Multicystic renal dysplasia | 2 | 1 | 1 |
| Congenital hydronephrosis | 3 | 3[4] | 0 |
| Posterior urethral valve and/or prune belly | 1 | 0 | 1[1] |
| Other malformations/unspecified | 6 | 5 | 1 |
| **Genital** | **18** | **12** | **6** |
| Hypospadias | 14 | 10 | 4 |
| Other malformations: Undescended testicle | 4 | 2 | 2 |

| Organ system classification | Total (N) | Earliest exposure to cART | |
|---|---|---|---|
| | | periconception | T2-T3 |
| **Limb** | **71** | **40** | **31** |
| Club foot- talipes equinovarus | 6 | 4 | 2 |
| Hip dislocation and/or dysplasia | 6 | 4 | 2 |
| Polydactyly | 54 | 31 | 23 |
| Syndactyly | 5 | 1 | 4 |
| **Other anomalies/ syndromes** | **9** | **7** | **2** |
| Craniosynostosis | 1 | 1 | 0 |
| Congenital constriction bands/amniotic band | 1 | 1 | 0 |
| Situs inversus | 1 | 1 | 0 |
| Congenital skin disorders | 1 | 1 | 0 |
| Foetal alcohol syndrome | 1 | 0 | 1 |
| Genetic syndrome+ microdeletions | 3 | 3 | 0 |
| Other anomalies/syndrome | 1 | 0 | 1 |
| **Chromosomal** | **39** | **27** | **12** |
| Down syndrome/ trisomy 21 | 31 | 20[5,1] | 11 |
| Patau syndrome/ trisomy 13 | 4 | 4[6,1] | 0 |
| Edward syndrome/ trisomy 18 | 2 | 1[1] | 1[7,1] |
| Turner syndrome | 1 | 1 | 0 |
| Other chromosomal syndrome | 1 | 1[1] | 0 |

*Adapted from EUROCAT prevalence table; [1]Neonatal/infants deaths; [2]Multiple anomalies in one LB: coarctation of aorta & VSD; [3]Multiple anomalies in one LB: Hirschsprung's disease & club foot talipes; [4]Multiple anomalies in one LB: Congenital hydronephrosis & undescended testicle; [5]Multiple anomalies in three LB: Down Syd & tracheal stenosis & AVSD & coartation of aorta; [6]Multiple anomalies in one LB: Trisomy 13 & cleft palate and/or hare lip; [7]Multiple anomalies in one LB: Trisomy 18 & Dandy Walker Syd & ventriculomegaly & agenesis of corpus callosus

### 5.2.3 Pregnancies exposed to the five ARV combinations of interest (objective 2b):

These five ARV combinations represent the four most commonly prescribed combinations between 2008 and 2018, namely TDF/FTC+EFV, AZT/3TC+LPV/r, TDF/FTC+ATV/r, TDF/FTC+DRV/r, with the addition of TDF/FTC+ RPV which was chosen in light of the analyses performed in Chapter 4 (sections 4.2.2 and 4.3.2) and over the course of my PhD to assess RPV's effective and safe use in pregnant women.

For this analysis only liveborn singleton pregnancies exposed to the five combinations of interest have been included.

*Trends of use over time*

There were 1,228 pregnancies exposed to TDF/FTC+EFV between 2008-18. Over time the proportion of pregnancies exposed to the combination increased from 3.1% (17/541) in 2008 to 33.6% (77/229) in 2018 (test-for-trend $p<0.001$), with trends of use progressively increasing, reaching a peak in 2014, then dropping (Figure 5.3).

The combination AZT/3TC+LPV/r was used overall in 1,974 pregnancies; rates of pregnancies exposed to the combination over time progressively decreased from 94.1% (509/541) in 2008 to 0.8% (2/229) in 2018 (test-for-trend $p<0.001$).

Looking at the combination of TDF/FTC+ATV/r, there were 1,060 pregnancies exposed, with use increasing over time from 2.8% (15/541) in 2008 to 15.3% (35/229) in 2018 (test-for-trend $p<0.001$).

There were 832 pregnancies exposed to TDF/FTC+DRV/r between 2009-18 (DRV/r was licensed in EU in 2007). Over time the percentage of pregnancies exposed to this combination increased from 0.5% (3/576) in 2009 to 31.4% (72/229) in 2018 (test-for-trend $p<0.001$), reaching a peak in its use in 2016 (Figure 5.3).

For the combination of TDF/FTC+RPV, there were 215 pregnancies exposed between 2013-18 (RPV was licensed in EU in 2011). Rates of pregnancies exposed to the combination steadily increased from 0.8% (4/502) in 2013 to 18.8% (43/229) in 2018 (test-for-trend $p<0.001$).

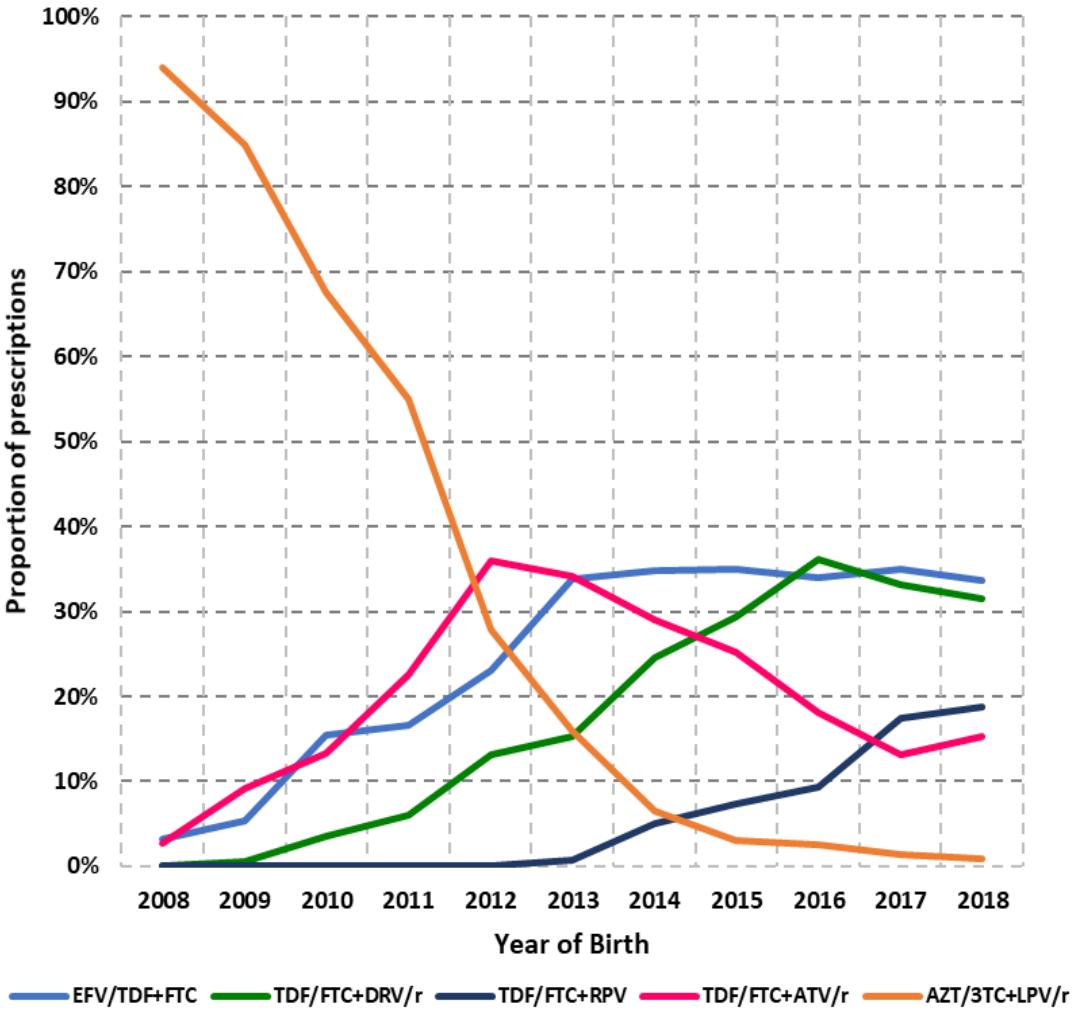



**Figure 5.3 Trends of use of the five ARV combinations of interest, by calendar years, NSHPC 2008-18**

*Pregnancies ending in livebirths*

Table 5.2 summarizes key characteristics for the liveborn pregnancies exposed to the five combinations of interest. The majority of pregnancies were exposed to the combinations from before periconception, with the exception of AZT/3TC+LPV/r which was started in almost 85% of the pregnancies in T2-T3.

**Table 5.2 Comparison of characteristics of liveborn infants exposed to the five ARV combinations of interest, NSHPC 2008-1**

| | TDF/FTC +EFV | AZT/3TC+LPV/r | TDF/FTC+ATV/r | TDF/FTC+DRV/r | TDF/FTC+RPV |
|---|---|---|---|---|---|
| **Total, N** | **1,228** | **1,974** | **1,060** | **832** | **215** |
| **Timing of earliest exposure** | | | | | |
| Preconception | 91.4% (1,123/1,228) | 15.5% (305/1,974) | 65.5% (694/1,060) | 72.2% (601/832) | 92.6% (199/215) |
| T2-T3 | 8.6% (105/1,228) | 84.5% (1,669/1,974) | 34.5% (366/1,060) | 27.8% (231/832) | 7.4% (16/215) |
| **% Preterm among livebirths** | | | | | |
| | 8.7% (107/1,228) | 10.9% (216/1,974) | 8.4% (89/1,060) | 12.9% (108/832) | 9.3% (20/215) |
| **% LBW among livebirths** | | | | | |
| | 9.3% (114/1,228) | 12.9% (255/1,974) | 8.8% (93/1,060) | 11.8% (98/832) | 8.4% (18/215) |
| **Overall CA prevalence (95% CI)** | | | | | |
| | 2.77% (1.29, 3.85) | 1.67% (1.15, 2.34) | 1.79% (1.08, 2.78) | 1.56% (0.83, 2.66) | 1.86% (0.51, 4.69) |
| **CA prevalence by timing** | | | | | |
| Preconception | 2.94% (2.03, 4.10) | 1.97% (0.73, 4.23) | 0.10% (0.41, 2.07) | 1.83% (0.92, 3.25) | 2.01% (0.55, 5.06) |
| T2-T3 | 0.95% (0.02, 5.19) | 1.62% (1.07, 2.34) | 3.28 % (1.71, 5.66) | 0.87% (0.10, 3.09) | - |

### *Congenital anomalies*

**TDF/FTC+EFV**

There were 34 liveborn infants with at least one CA, these are reported in Table 5.3. Three infants presented with multiple CAs most of which have been already presented in section 5.2.2 objective 2a. One infant presented with congenital hydronephrosis associated with undescended testicle; one presented with Trisomy 13 associated with orofacial cleft; and one had four defects, the chromosomic anomaly Down's syndrome associated with tracheal stenosis, and two CHD (i.e. AVSD and coartation of aorta). Three infants died, two within the first 28 days of life both presenting with chromosomal anomalies, one was affected by Wolf-Hirschhorn syndrome and one had Trisomy 13 associated with orofacial cleft. The infant who died after 28 days of life was the infant with the four defects described above.

**AZT/3TC+LPV/r**

Infants with reported CAs are presented in Table 5.4. Of the 33 infants with CAs, one had multiple CAs, namely trisomy 18 associated with Dandy Walker syndrome, ventriculomegaly and agenesis of corpus callosum who died within the first 28 days of life. Two other infants died within the first 28 days of life, both preterm, and presenting with urinary system anomalies.

**TDF/FTC + ATV/r**

Table 5.5 shows the 19 infants presenting with one CA (none presented with more than one); one infant with the reported chromosomal anomaly Trisomy 13, died within the first 28 days of life.

**TDF/FTC + DRV/r**

There was a total of 13 infants with one CA and no reports of multiple anomalies (Table 5.6). Three infants died within the first 28 days of life reporting CAs: the chromosomic anomaly Trisomy 18, one with diaphragmatic hernia, and one with hydrocephalous.

**TDF/FTC + RPV**

The four infants presented in Table 5.7 had one CA, no reports of multiple anomalies; with one infant affected by Down's syndrome reported to have died after 28 days of life.

**Table 5.3 Description of CAs meeting EUROCAT criteria reported to the NSPHC, by earliest exposure to TDF/FTC+EFV**

| Organ system classification | Total (N) | Earliest exposure to cART | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **All anomalies** | **34** | **33** | **1** |
| **Nervous system** | **5** | **5** | **0** |
| Neural Tube Defects: Spina Bifida | | 1 | 0 |
| Microcephaly | | 2 | 0 |
| Other malformations/ unspecified | | 2 | 0 |
| **Eye, Ear, Face, Neck** | **1** | **1** | **0** |
| Congenital glaucoma | | 1 | 0 |
| **Congenital heart defects** | **2** | **2** | **0** |
| Atrio-ventricular septal defect (AVSD) | | 1 | 0 |
| Ventricular septal defect (VSD) | | 1 | 0 |
| **Oro-facial clefts** | **1** | **0** | **1** |
| Cleft palate and/or hare lip | | 0 | 1 |
| **Digestive system** | **2** | **2** | **0** |
| Atresia or stenosis of other parts of small intestine | | 1 | 0 |
| Hirschsprung's disease | | 1 | 0 |

| | | | |
|---|---|---|---|
| **Urinary** | **2** | **2** | **0** |
| Other congenital malformation of the Kidney (Duplex Kidney) | | 1 | 0 |
| Congenital hydronephrosis[1] | | 1 | 0 |
| **Genital** | **3** | **3** | **0** |
| Hypospadias | | 3 | 0 |
| **Limb** | **8** | **8** | **0** |
| Hip dislocation and/or dysplasia | | 1 | 0 |
| Polydactyly | | 7 | 0 |
| **Chromosomal** | **10** | **10** | **0** |
| Down's syndrome[2,4] | | 8 | 0 |
| Patau syndrome/ trisomy 13[3,4] | | 1 | 0 |
| Other chromosomal syd[4] | | 1 | 0 |

[1]Multiple anomalies in one LB: Congenital hydronephrosis & undescended testicles; [2]Multiple anomalies in one LB: Down Syd associated with tracheal stenosis & AVSD & coartation of aorta; [3]Multiple anomalies in one LB: Trisomy 13 & cleft palate; [4]Neonatal/infant deaths

**Table 5.4 Description of CAs meeting EUROCAT criteria, reported to the NSPHC, by earliest exposure to AZT/3TC+LPV/r**

| Organ system classification | Total (N) | Earliest exposure to cART | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **All anomalies** | **33** | **6** | **27** |
| **Nervous system** | **2** | **0** | **2** |
| Hydrocephalus | | 0 | 1 |
| Other congenital malformation | | 0 | 1 |
| **Congenital heart defects** | **3** | **0** | **3** |
| Atrioventricular septal defect (AVSD) | | **0** | 1 |
| Ventricular septal defect (VSD) | | | 1 |
| Coartation of aorta | | 0 | 1 |
| **Respiratory** | **2** | **0** | **2** |
| Congenital Cystic Adenomatoid Malformation (CCAM) | | 0 | 2 |
| **Digestive system** | **2** | **1** | **1** |
| Duodenal atresia and stenosis | | 1 | 0 |
| Ano-rectal atresia and stenosis | | 0 | 1 |
| **Urinary** | **4** | **1** | **3** |
| Bilateral renal agenesis including Potter syndrome[1] | | 0 | 1 |
| Congenital hydronephrosis | | 1 | 0 |

| | | | |
|---|---|---|---|
| Other congenital malformation of the kidney[1] | | 0 | 2 |
| **Genital** | **2** | **1** | **1** |
| Other malformations: Undescended testes | | 1 | 1 |
| **Limb** | **12** | **2** | **10** |
| Hip dislocation and/or dysplasia | | 1 | 0 |
| Club foot- talipes equinovarus | | 0 | 1 |
| Syndactyly | | 0 | 1 |
| Polydactyly | | 1 | 8 |
| **Other anomalies/ syndromes** | **1** | **0** | **1** |
| Skeletal dysplasias (moebius syndrome) | | 0 | 1 |
| **Chromosomal** | **6** | **1** | **5** |
| Down's syndrome | | 1 | 4 |
| Edward syndrome/Trisomy 18[1,2] | | 0 | 1 |

[1]Neonatal/infant deaths; [2]Multiple anomalies in one LB with neonatal death: Trisomy 18 associated with Nervous system anomalies: Dandy Walker syndrome, ventriculomegaly and agenesis of corpus callosum

**Table 5.5 Description of CAs meeting EUROCAT criteria, reported to the NSPHC, by earliest exposure to TDF/FTC+ATV/r**

| Organ system classification | Total (N) | Earliest exposure to cART | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **All anomalies** | **19** | **7** | **12** |
| **Nervous system** | **1** | **1** | **0** |
| Malformation of/agenesis of corpus callosum | | 1 | 0 |
| **Congenital heart defects** | **1** | **0** | **1** |
| Ventricular septal defect (VSD) | | 0 | 1 |
| **Digestive system** | **2** | **1** | **1** |
| Duodenal atresia and stenosis | | 1 | 0 |
| Oesophageal atresia with or without trachea-oesophageal fistula | | 0 | 1 |
| **Genital** | **3** | **0** | **3** |
| Hypospadias | | 0 | 2 |
| Undescended testicle | | 0 | 1 |
| **Limb** | **7** | **2** | **5** |
| Syndactyly | | 1 | 0 |
| Polydactyly | | 1 | 5 |
| **Other anomalies/ syndromes Chromosomal** | **1** | **0** | **1** |
| Fetal alcohol syndrome | | 0 | 1 |
| **Chromosomal** | **4** | **3** | **1** |
| Down's syndrome | | 1 | 1 |
| Patau syndrome/trisomy 13[1] | | 2 | 0 |

[1]Neonatal/infant deaths

**Table 5.6 Description of CAs meeting EUROCAT criteria, reported to the NSPHC, by earliest exposure to TDF/FTC+DRV/r**

| Organ system classification | Total (N) | Earliest exposure to cART | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **All anomalies** | **13** | **11** | **2** |
| **Nervous system** | **1** | **1** | **0** |
| Hydrocephalus[1] | | 1 | 0 |
| **Congenital heart defects** | **3** | **3** | **0** |
| Ventricular septal defect (VSD) | | **2** | 0 |
| Tetralogy of Fallot | | 1 | 0 |
| **Digestive system** | **1** | **1** | **0** |
| Diaphragmatic hernia[1] | | 1 | 0 |
| **Genital** | **2** | **2** | **0** |
| Hypospadias | | 1 | 0 |
| Undescended testicle | | 1 | 0 |
| **Limb** | **5** | **3** | **2** |
| Hip dislocation and/or dysplasia | | 1 | 0 |
| Polydactyly | | 2 | 2 |
| **Chromosomal** | **1** | **1** | **0** |
| Edward syndrome/trisomy 18[1] | | 1 | 0 |

[1]neonatal/infant deaths

**Table 5.7 Description of CAs meeting EUROCAT criteria, reported to the NSPHC, by earliest exposure to TDF/FTC+RPV**

| Organ system classification | Total (N) | Earliest exposure to cART | |
|---|---|---|---|
| | | periconception | T2-T3 |
| **All anomalies** | **4** | **4** | **0** |
| **Congenital heart defects** | **1** | **1** | **0** |
| Pulmonary valve stenosis | | 1 | 0 |
| Digestive system | **1** | **1** | **0** |
| Oesophageal atresia with or without trachea-oesophageal fistula | | 1 | 0 |
| **Limb** | **1** | **1** | **0** |
| Polydactyly | | 1 | 0 |
| **Chromosomal** | **1** | **1** | **0** |
| Down's Syndrome[1] | | 1 | 0 |

[1]neonatal/infant deaths

### 5.2.4 Evaluation of risk factors associated with CAs (objective 3)

To address this objective, logistic regression models were fitted to investigate the association between *in utero* exposure to ARVs and presence of CAs. This section in divided into two main investigations: the first included all ARVs by class and the second evaluated exposure to the combination analysed in section 5.2. Findings related to exposure to specific INSTI drugs are addressed in Chapter 6.

*Specific methods*

The outcome of interest was the presence of at least one CA meeting the EUROCAT classification criteria. The primary exposure of interest was *in utero* exposure to ARVs in relation to timing (i.e. periconception exposure). ARV exposure was assessed first as "any exposure to ARVs"; then by ARV class and lastly as cART, using the five combinations of interest (i.e. TDF/FTC+EFV, AZT/3TC+LPV/r, TDF/FTC+ATV/r, TDF/FTC+DRV/r, TDF/FTC+ RPV).

Time of exposure was assessed as the earliest exposure to a combination of ARVs (i.e. periconception vs T2-T3), regardless of the duration.

Bivariable analyses including all liveborn infants with *in utero* exposure to ARV combinations were used to measure: 1) association between overall CAs and time of exposure (i.e. periconception vs T2-T3) to ARV by class and 2) association between presence of at least one CA and infant sex; For these investigations, Fisher's exact test was used to obtain OR and *p*-values. Then analyses were carried out including the variables found statistically significant in the previous analyses.

Risk factors were evaluated using prior knowledge acquired from literature (Sibiude et al. 2014, Williams et al. 2015, Martinez de Tejada et al. 2019) and analyses as described in Chapter 3. Risk factors investigated were maternal ethnicity (defined as black African, White and other) and maternal age at delivery (defined by 2 categories <35 and ≥35 years old). Each potential risk factor was evaluated and if found significant added to the model and kept if improving the model fit. The best model was investigated using Bayesian information criterion (for more details see appendix 9.4).

Logistic regression analyses were used to evaluate association between *in utero* exposure to the ARVs as described above, i.e. by class and by the five combinations of interest. A sensitivity analysis was conducted excluding both the limb anomaly and all chromosomal anomalies. In the logistic regression model, NRTIs were excluded from the risk factor analysis with the rationale that this exposure was universal.

### *Results:*

#### *Unadjusted analysis for all liveborn infants reported to the NSHPC,* 2008-18 (*n*= 11,197)

Investigation of the association between time of exposure (i.e. periconception vs T2-T3) and presence/absence of CAs, and of the association between infant sex and presence/absence of CAs are reported in Table 5.8 and 5.9, respectively. Results showed that neither were statistically significant.

**Table 5.8 Liveborn infants with/without CAs, by time of first exposure to ARVs**

| | Congenital anomalies | | | | |
|---|---|---|---|---|---|
| | No | | Yes | | Total |
| **First time of exposure to ARVs:** | ***n*** | **%** | ***n*** | **%** | ***n*** |
| **Periconception** | 6,313 | 97.83 | 140 | 2.17 | 6,453 |
| **T2-T3** | 4,657 | 98.17 | 87 | 1.83 | 4,744 |
| **Total** | 10,970 | 97.9 | 227 | 2.03 | 11,197 |
| **OR (95% CI)** | 0.84 (0.63, 1.11) | | | | |

**Table 5.9 Liveborn infants with/without CAs, by sex**

| | Congenital anomalies | | | | |
|---|---|---|---|---|---|
| | No | | Yes | | Total |
| **Sex:** | ***n*** | **%** | ***n*** | **%** | ***n*** |
| **Female** | 5,312 | 98.19 | 98 | 1.81 | 5,410 |
| **Male** | 5,472 | 97.78 | 124 | 2.22 | 5,596 |
| **Missing** | 186 | 97.38 | 5 | 2.62 | 191 |
| **Total** | 10,970 | 97.9 | 227 | 2.03 | 11,197 |
| **OR (95% CI)** | 1.23 (0.93, 1.62) | | | | |

Association between maternal age at delivery and CAs was firstly investigated using a regression spline. Two degrees of freedom were deemed appropriate based on Bayesian information criterion, and both terms in the spline were significant ($p < 0.034$). Looking at the predicted probabilities from this model, as shown in Figure 5.4, it is interesting to note similarly increased probabilities of CAs both for the youngest (i.e. <20 years old) and oldest mothers (i.e. 40-45 years old).

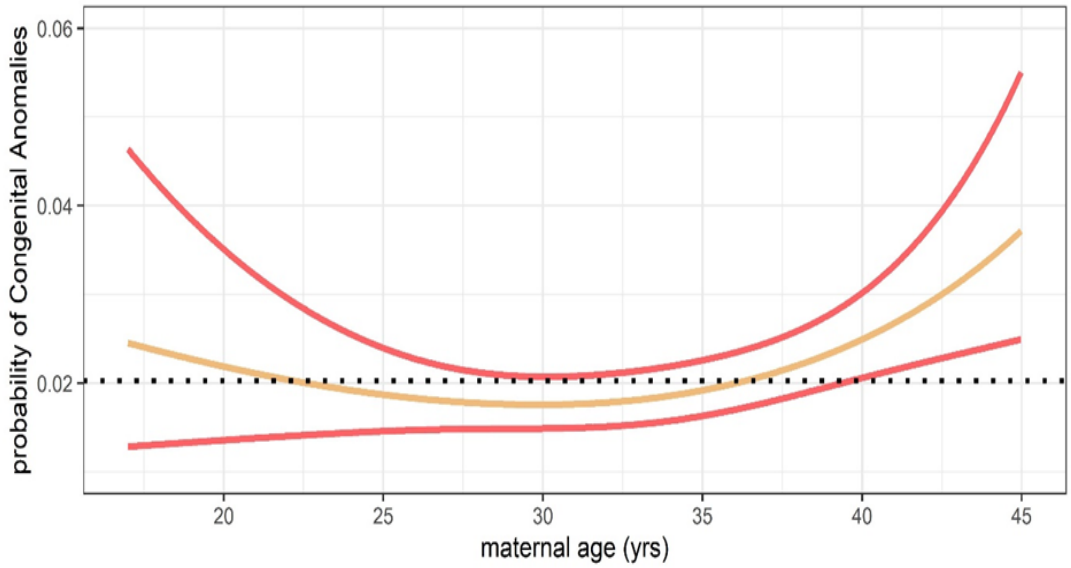



**Figure 5.4 Probability of CA by maternal age at delivery, NSHPC 2008-18**

**Association between *in utero* exposure to ARVs and CAs, by ARV class (objective 3a)**

*Univariable analyses*

For the univariable analyses all pregnancies ending in livebirths (i.e. *n*=11,197) were included. Assessment of the risk factors which are reported in Table 5.10 showed maternal age at delivery to be the only statistically significant risk factor.

A sensitivity analyses was performed to look at the effect of maternal age at delivery on the risk for CAs by comparison of two datasets, the unrestricted dataset which included all CAs (*n*=227) and a restricted which excluded chromosomal anomalies and polydactyly (*n*=134). Infants born to women aged over 35 years were 1.85 times more likely to develop a CA than those born to younger mothers (95%CI 1.85-1.06; *p*=0.003). However this effect was lost once chromosomal anomalies and polydactyly were excluded (OR=1.14, 95% CI 0.50-2.66, *p*=0.75), most likely reflecting the known influence of maternal age in the development of chromosomal anomalies, as also illustrated by Figure 5.4 (Andrews et al. 1985).

*Multivariable model*

For this analysis ethnicity was excluded given that in the univariable analysis it was not found to be statistically significant nor to improve the fit of the model based on Bayesian information criterion, thus the final model included maternal age at delivery, ARV class, time of earliest exposure to ARVs, and an interaction between ARV class and time of exposure. There was a statistically significant increased risk of CA for

women aged over 35 compared to those aged less than 35 years at delivery ($p$=0.04) as one would have expected from the spline regression function in Figure 5.4.

Looking at exposure to ARV by classes, INSTIs were the only class associated with an increased risk of presence of CAs (aOR 1.67, $p$=0.05), though this will be further commented in Chapter 6, as part of a more detailed analysis on INSTIs. When considering time of first exposure to ARVs, there was no statistically significant association between periconception exposure versus later exposure in pregnancy (aOR= 1.68; $p$=0.18) (Table 5.10).

The interaction effects, i.e. the joint effect that being exposed to a specific ARV class (i.e. INSTIs, PIs and NNRTIs) and timing of this first exposure (i.e. at periconception vs later) have on the risk for CAs are reported in Table 5.10. The aORs reported in the table are defined *multipliers* to the aORs of exposure to ARV classes and by time of first exposure. Therefore, to obtain the actual measure of the risk for CAs, the three aORs need to be multiplied. For example, looking at INSTIs, the aOR of exposure to INSTIs is 1.67, (i.e. risk of CA is 67% higher when INSTIs are used) and the aOR of first exposure from periconception to any ARVs was 1.68, (i.e. risk is 68% higher with first exposure from periconception versus later in pregnancy); 0.28 is the multiplier to the ORs of exposure to INSTIs and first exposure from periconception when both are present, hence the effect of the interaction is 0.80 (95% CI 0.29, 2.21) (1.67*1.68*0.28=0.80) in OR scale. This means that risk of CAs when exposure to INSTIs occurred around periconception time was reduced by approximately 20% with respect to the absolute baseline (i.e. not being exposed to INSTIs and not being exposed from periconception time).

Similarly, being exposed to NNRTI from periconception reduced the risk for CAs by approximately 17% with respect to the absolute baseline (i.e. not taking NNRTI and not from periconception time), whereas being exposed to PIs from periconception increased by approximately 27% the risk for CAs with respect to the absolute baseline (i.e. not being exposed to PI and not being exposed from periconception).

## Table 5.10 Risk factors for CAs in liveborn infants exposed to ARVs

| | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| **Explanatory variables** | **OR** | **95% CI** | ***p*-values** | **aOR** | **95% CI** | ***p*-values** |
| **Maternal ethnicity** | | | | | | |
| Black African | Baseline | | | | | |
| White | 0.91 | 0.63 - 1.28 | 0.60 | | | |
| Others | 0.89 | 0.44 - 1.60 | 0.72 | | | |
| **Maternal age at delivery (years)** | | | | | | |
| <35 | Baseline | | | Baseline | | |
| ≥35 | 1.33 | 1.02 - 1.74 | 0.03* | 1.32 | 1.00 - 1.72 | 0.04* |
| **ARV class** | | | | | | |
| INSTIs (vs no INSTI) | 1.09 | 0.71 -1.61 | 0.66 | 1.67 | 0.97 - 2.72 | 0.05* |
| PIs (vs no PI) | 0.88 | 0.67 - 1.15 | 0.34 | 1.14 | 0.67 - 1.90 | 0.61 |
| NNRTIs (vs no NNRTI) | 1.10 | 0.83 - 1.45 | 0.48 | 1.37 | 0.79 - 2.33 | 0.25 |
| **Time of earliest exposure to ARVs** | | | | | | |
| Later pregnancy | Baseline | | | Baseline | | |
| Periconception | 0.86 | 0.65 - 1.12 | 0.27 | 1.68 | 0.76 - 3.56 | 0.18 |
| **Time of earliest exposure*ARV class** | | | | | | |
| INSTIs | | | | 0.28 | 0.09 - 0.77 | 0.02* |
| PIs | | | | 0.66 | 0.31 -1.48 | 0.30 |
| NNRTIs | | | | 0.36 | 0.10 - 1.06 | 0.07 |

*$p$-value reaches the level of significance (<0.05)
OR: Odds Ratio; aOR: adjusted OR; CI confidence interval

**Association between *in utero* exposure to ARVs and CAs, by the five ARV combinations of interest (objective 3b)**

Association between exposure to the five ARV combinations of interest and CAs were investigated both in univariable and multivariable analyses on a total of 5,309 infant-mother pairs (Table 5.11). In univariable analyses, older maternal age (≥35 years vs younger) was associated with an increased risk of CA (OR= 1.89, *p*=0.003), whilst exposure to AZT/3TC+LPV/r was associated with significantly lower risk of CA versus TDF/FTC+EFV (OR= 0.55, *p*=0.002).

After adjusting for maternal age at delivery, ARV combinations and time of first exposure to ARVs, results showed that the significant association with maternal age persisted, with infants whose mothers were aged ≥35 years 1.80 times more likely to develop a CA than those whose mothers were younger than 35 years at delivery (*p*=0.008) (Table 5.11).

Infants with exposure to AZT/3TC+LPv/r showed a probability of being protective, though no longer statistically significant in the adjusted model (*p*=0.07). As for the univariable analysis, no statistically significant association between time of earliest exposure to the five ARV combinations and identification of CAs was found.

## Table 5.11 Risk factors for CAs in liveborn infants exposed to the five most common ARV combinations

| | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| **Explanatory variables** | **OR** | **95% CI** | ***p*-values** | **aOR** | **95% CI** | ***p*-values** |
| **Maternal age at delivery (years)** | | | | | | |
| <35 | Baseline | | | | Baseline | |
| ≥35 | 1.89 | 1.24 - 2.89 | 0.003* | 1.80 | 1.16 - 2.79 | 0.008* |
| **ARV combinations** | | | | | | |
| TDF/FTC+EFV | Baseline | | | | Baseline | |
| AZT/3TC+LPV/r | 0.55 | 0.32 - 0.94 | 0.02* | 0.53 | 0.26 - 1.05 | 0.07 |
| TDF/FTC+RPV | 0.69 | 0.20 - 1.80 | 0.50 | 0.71 | 0.21 - 1.84 | 0.53 |
| TDF/FTC+ATV/r | 0.72 | 0.39 - 1.31 | 0.29 | 0.71 | 0.37 - 1.31 | 0.27 |
| TDF/FTC+DRV/r | 0.64 | 0.30 - 1.27 | 0.22 | 0.65 | 0.30 - 1.28 | 0.23 |
| **Time of earliest exposure to ARVs** | | | | | | |
| Later pregnancy | Baseline | | | Baseline | | |
| Periconception | 0.84 | 0.55 – 1.28 | 0.43 | 1.31 | 0.74 - 2.30 | 0.35 |

**p*-value reaches the level of significance (<0.05)
OR: Odds Ratio; aOR: adjusted OR; CI confidence interval

### 5.2.5 Characterisation of infants with and without CAs (objective 4)

Of the total 11,197 liveborn infants reported to the NSHPC, 227 presented with at least one CA and 10,970 with no reported CAs. Comparison of infants presenting with and without CAs was performed (Table 5.12). Results showed that infants with at least one CA were more often males compared with infants without CAs, though not reaching statistical significance, that a significantly higher proportion of preterm births occurred among infants with a CA compared to those without and that a higher proportion of infants with a weight at birth of less than 2.5kg had a CA compared to those without CAs.

The median gestational age for infants with a CA was 38 GW (q1=25, q3=42), while for those without a CA was 39 GW (q1=22, q3=44). Looking at the association between gestational age at delivery and the risk for CAs, the proportion of CA among infants delivered at term was 1.8% and among preterm infants was 3.3%, with an OR of 1.88 (95% CI 1.59, 3.17) for infants born preterm versus those born at term ($p$<0.001). Median birthweight for infants presenting with a CA was 2950g (q1=2542, q3=3370) and for infants without a CA was 3130g (q1=2800, q3=3466). The higher rate of LBW among infants presenting with a CA is to be expected given their preterm rate and the fact that preterm deliveries and LBW are often a consequence of having a CA (Rasmussen et al. 2001, Honein et al. 2009).

Additionally, findings showed a higher proportion of infant deaths (i.e. neonatal and infant deaths) among infants presenting with at least one CA compared with those without, with rates of 0.7 deaths per 1,000 (95% CI 0.41, 1.12) and 0.05 deaths per 1,000 (95% CI 0.04, 0.07), respectively; this difference is expected considering that CA are a known leading cause for infant mortality.

**Table 5.12 Characteristics of liveborn infants, by presence of CA, NSHPC 2008-2018**

| Variables | With CA | Without CA | *p*-values* |
|---|---|---|---|
| Tot, *n* (%) | 227 | 10,970 | |
| **Sex** | | | |
| **Male** | 124 (54.6%) | 5,472 (49.9%) | 0.150 |
| **Female** | 98 (43.2%) | 5,312 (48.4%) | |
| **Missing** | 5 (2.2%) | 186 (1.7%) | |
| **Gestational age at delivery** | | | |
| **At term (≥37 GW)** | 181 (79.7%) | 9,865 (89.9%) | <0.001 |
| **Preterm (<37 GW)** | 46 (20.3%) | 1,105 (10.1%) | |
| **Weight at birth** | | | |
| **≥2.5 Kg** | 165 (72.7%) | 9,197 (83.8%) | <0.001 |
| **<2.5 kg** | 50 (22.0%) | 1,197 (10.9%) | |
| **Missing** | 12 (5.3%) | 576 (5.3%) | |
| **Mortality** | | | |
| **Neonatal/infant death** | 16 (7.0%) | 59 (0.5%) | <0.001 |

*** These *p*-values refer to a chi-squared test of homogeneity excluding missing data**

## 5.3 CAs in stillbirths, miscarriages and termination of pregnancies

As previously mentioned in Chapter 1 (section 1.5.1), pregnancy outcomes such as miscarriage and stillbirth carry some intrinsic difficulties in their detection and monitoring, including under-ascertainment of early and very early pregnancy losses and lack of uniform classification systems for stillbirths. Furthermore, stillbirths and miscarriages might occur because of CAs and termination of pregnancies might be planned because of the detection of CAs. Therefore, and particularly for early miscarriages and early stillbirths, there might be an under-reporting of CAs, especially for malformations that might not be detectable by ultrasonography (early pregnancy scan), might not be obvious externally (early stillbirths) or where pathological examinations and/or histological test might be necessary.

The NSHPC seeks to collect information on all pregnancies, not only livebirths, including rationales for termination of pregnancy and whether CAs were identified. In this section I briefly describe pregnancies ending in stillbirths, miscarriages and terminations with reported CAs.

*Stillbirths*

Of the 99 singleton pregnancies ending in stillbirths and reported between 2008-18, eight had at least one CA meeting EUROCAT definitions, giving a prevalence of 8.1% (95% CI 3.55, 15.30). Most of the infants were delivered vaginally (7/8), preterm (6/8), born to mothers aged 40 to 45 years (7/8), and had periconception exposure to different cART regimens, although most of them were PI-based (6/8).

There were four infants with chromosomal anomalies from women who had a periconception exposure to cART, all of whom were aged 40-45 years at time of delivery. These were trisomy 13, Down's Syndrome and two infants with trisomy 18. Three further infants presented with a nervous system anomaly: one with multiple CAs, namely a NTD associated with ventriculomegaly, one with a NTD and one had microcephaly. The last infant presented with a congenital malformation on the kidney (renal fusion).

*Miscarriages*

Of the 656 pregnancies ending in miscarriage, six reported a CA meeting the EUROCAT criteria, giving a prevalence of 0.9% (95 CI 0.34, 1.98). Of these, two were identified chromosomal syndromes, namely Down's Syndrome and trisomy 18; two nervous systems anomalies (both NTDs); and one respiratory anomaly, namely Congenital Cystic Adenomatoid Malformation, all in infants whose mothers started

different cART regimen before periconception; while the last CA, an abdominal wall defect, namely omphalocele, occurred in an infant whose mother started cART in T2-T3. Most of the women were aged 35 to 40 years (5/6).

*Termination of pregnancy*

Of the 147 reported terminations of pregnancy, 26 reported at least one CA meeting EUROCAT criteria, giving a prevalence of 17.7% (95% CI 11.89, 24.83). Most of the CAs (17/26) were chromosomal syndromes, followed by nervous system anomalies (5/26 were NTDs). Two fetuses had multiple anomalies, one affecting mostly the nervous system and one the digestive and genital systems.

Additionally, there were five terminations of pregnancy where severe genetic disorders and/or multiple organ deficiencies non-compatible with life not meeting EUROCAT criteria were reported. One foetus presented with a severe brain malformation; one had a genetic condition known as quadruple X; one was affected by Edward's syndrome (trisomy 18); while for the remaining two unspecified multiple organs deficiencies were reported. Most of the mothers were aged 40 to 45 years (19/26) and were exposed to PI-based regimen from before periconception (14/26).

***Neural tube defects (NTDs)***

NTDs are a group of CAs of particular interest in light of recent concerns regarding periconception exposure to DTG and an increased observation of such anomalies reported from the Tsepamo Study. Here I present all the identified NTDs reported to the NSHPC, while a detailed analysis on DTG and the issue of its use and the increased risk for NTDs is presented in Chapter 6. There was a total of 11 NTDs among the 12,099 pregnancies reported to the NSHPC between 2008-18, giving a prevalence of 0.09% (95% CI 0.04, 0.16) (Table 5.13).

This prevalence is consistent with both European estimates, i.e. EUROCAT prevalence of NTDs not associated with chromosomic anomalies was reported to be at around 0.09% between 2002-2015 (Khoshnood et al. 2015) and with UK prevalence, estimated to affect around 0.1% of pregnancies (Morris et al. 2016).

All women started cART before periconception, with the exception of two women who started later in pregnancy. There were eight spina bifida cases, one in a liveborn infant; two in stillborn infants; four were terminations of pregnancy; and one ended in a miscarriage. One mother contributed to three of the eight spina bifida (one miscarriage, two terminations).

**Table 5.13 Neural tube defects reported to the NSHPC, 2008-18**

| Pregnancy outcome | Time of exposure | Nervous system defect |
|---|---|---|
| Miscarriage | Periconception | Spina Bifida |
| Termination of pregnancy | Periconception | Spina Bifida |
| Termination of pregnancy | Periconception | Spina Bifida |
| Livebirth | Periconception | Spina Bifida |
| Termination of pregnancy | Periconception | Spina Bifida |
| Stillbirth | Periconception | Spina Bifida associated with ventriculomegaly |
| Livebirth | T2-T3 | Encephalocele |
| Stillbirth | T2-T3 | Spina Bifida |
| Miscarriage | Periconception | Encephalocele |
| Termination of pregnancy | Periconception | Spina Bifida associated with ventriculomegaly and limb defect reduction of lower limb |
| Termination of pregnancy | Periconception | Holoprosencephaly |

## 5.4 Key points

- Overall, 227 liveborn infants presented with at least one CA, a prevalence of 2.03% (95% CI 1.77, 2.31); 147 had a periconception exposure to cART (2.14%; 95% CI 1.81, 2.51) and 80 (1.84%;95% CI 1.46, 2.29) in T2-T3. These prevalences have stayed relatively steady over time.
- Assessment of the five ARV combinations of interest by the rule of three did not identify any particular pattern of CAs by system/organ criteria.
- Analysis using a regression spline showed highest predicted probabilities of CA for infants whose mothers were either very young (<20 years) or in the oldest category (40-45 years) ($p$<0.01).
- In the main adjusted model exploring risk factors for CA, ($n$=11,197) and assessing *in utero* exposure to third agent class of ARV, maternal age at delivery ≥35 years (vs <35 years) was found to increase the risk of CA ($p$=0.04); INSTIs were the only class associated with an increased risk of CA (aOR 1.67, $p$=0.05); while time of first exposure to ARV was not found to increase the risk for CAs.

- From the joint effect of being exposed to a specific ARV class and timing of first exposure, a reduced risk for CA of approx. 20% and 17%, respectively was found when first exposure to INSTI ($p$=0.02) or to NNRTI ($p$=0.07) occurred in periconception period compared with not being exposed to INSTIs (or NNRTI) and not being exposed from conception; exposure to PIs from periconception increased CA risk by approx. 27%.
- In an adjusted analysis restricted to 5,309 pregnancies with receipt of one of five ARV regimens of interest, maternal age at delivery ≥35 (vs <35 years) was associated with increased risk of CA ($p$=0.008); no association between first time of exposure and the risk of CA, and some evidence of reduced risk of CA with AZT/3TC+LPV/r (vs TDF/FTC+EFV)(aOR 0.53, $p$=0.07) was found.
- A higher proportion of both LBW and preterm births was found among infants with a CA vs without CA ($p$<0.001).
- Deaths rates among the 227 infants with CA were higher than those among the 10,970 infants without CA (0.7 per 1,000 vs 0.05 per 1,000 death rates, respectively, p<0.001).
- Among the 99 stillborn infants, the prevalence of CA was 8.1% (95% CI 3.55, 15.30) and among the 147 pregnancies ending in terminations the prevalence of CA was 17.7% (95% CI 11.89, 24.83).
- Of the total 12,099 pregnancies reported, 11 resulted in resulted in an infant with a NTD (prevalence of 0.09%; 95% CI 0.04, 0.16).

# 6 The INSTI class: assessing regulatory recommendations, trends in use in pregnancy in the UK, and congenital anomalies following *in utero* exposure

## 6.1 Introduction

As presented in Chapter 4, trends of ARVs use over time have changed with an increasing number of new available options, particularly among the third agents. For example, use of the INSTIs over time has steadily increased in the UK. Several properties of INSTIs have made this class of ARVs a very effective and valuable third agent option (Kandel et al. 2015, Brenner et al. 2017) to the point that DTG and RAL are currently both recommended as alternative third agents by the UK BHIVA guidelines for pregnant and breastfeeding women (Chapter 4, sec 4.2.2, Table 4.5, (BHIVA 2019a).

INSTIs are a relatively new class of ARV characterised by rapid viral suppression, minimal toxicity, antiviral activity against strains of viruses resistant to other drug classes, a strong transplacental transfer and consequent rapid and effective capacity to reduce maternal VL (Dow et al. 2014, Cecchini et al. 2017, Elliot et al. 2017, Rimawi et al. 2017). RAL is a first-generation INSTI initially with a main indication for women presenting late to antenatal care and/or with a high VL in late pregnancy (Bailey et al. 2018, Gilleece et al. 2018, Orrell et al. 2018). EVG is another first-generation INSTI co-formulated with the booster Cobicistat (EVG/c) sharing the same promising profile of RAL. DTG is a second-generation INSTI with several unique properties such as limited cross-resistance with the first-generation INSTIs (Dow et al. 2014) and high barrier to resistance, meaning that pregnant women on DTG-based regimens have lower probability to develop HIV drug-resistance, to transmit drug-resistant HIV infection to their infants and to pass on drug-resistant strains to their sexual partners (Brenner et al. 2017).

INSTIs in general, and DTG in particular, have also shown a higher tolerability and lower reports of discontinuation due to adverse events compared to other third agents, as observed in studies comparing DTG to other INSTIs (i.e. RAL), to PIs (i.e. DRV/r,

ATV/r) (Molina et al. 2015) and to NRTIs such as EVF (Walmsley et al. 2015). For example, the study SINGLE, an ongoing phase III, multicentre, randomized, double-blind, non-inferiority study that compared DTG-based to EFV-based regimen, reported lower discontinuation rates due to adverse events, i.e. 4% (16/414) vs 14% (58/419) at week 144, respectively (Patel et al. 2014, Walmsley et al. 2015). Furthermore, results from the same study reported better rates of viral suppression (VL<50copeis/mL) at week 144 of therapy showing how of the 883 participants, 71% exposed to DTG-based vs 63% exposed to EFV-based regimen maintained viral suppression ($p$=0.01); a difference driven by the low rates of drug discontinuation due to adverse effects (Walmsley et al. 2015).

With such a promising profile and its numerous benefits, DTG was a much awaited new drug to include in the existing arsenal of antiretrovirals.

When I started my PhD in 2016, I had selected DTG as a case-study given the increasing usage of INSTIs among pregnant and breastfeeding women to evaluate the association between *in utero* exposure to this medication and pregnancy outcomes. Two years later, a surveillance study reported a safety signal (see later in this section) concerning the use of DTG from time of conception and a potential increased risk for NTDs, reinforcing the rationale for the choice of DTG as my case-study.

This chapter is structured into four main sections; in section 6.3 available safety data for use of INSTIs in pregnancy from the EMA are synthesized and presented to address the gap between real-world use and regulatory recommendations with the same methodology as explained in Chapter 4.

In section 6.4 the analyses on DTG conducted over the years of my PhD are presented including 1) a preliminary analysis which was accepted as a poster presentation and won the best poster award at the 9$^{th}$ International Workshop on HIV Paediatrics held in Paris July 2017, 2) a final analysis on pregnancies exposed to DTG reported to the NSHPC by 31$^{st}$ December 2018 and 3) a recent pooled analysis from Dolomite-EPPICC presented as a poster at the 2020 Conference on Retroviruses and Opportunistic Infections (CROI).

In section 6.5 an analysis on RAL and EVG-containing cART evaluating their safe use in pregnant women is presented, including evaluation of their trends of use over time, and identification of CAs.

In section 6.6, an analysis developed following the DTG safety signal is presented - a logistic regression model was fitted to evaluate the risk for CAs in infants whose mothers started INSTIs in the periconception period.

This chapter addresses the following three objectives:

1) To evaluate the DTG safety signal (i.e. increased risk of NTDs with periconception use) using data from multiple sources; one analysis to evaluate EMA recommendations in order to identify the gap between real-world use of DTG and regulatory recommendations (objective 1a); three analyses to evaluate trends of DTG usage over time and to collect data on pregnancy outcomes, particularly on CAs; of these three analyses, two used the NSHPC data (objectives 1b and 1c), and one used real-world European data (EPPICC) (objective 1d)

2) To assess the potential for a drug class-safety signal by analysing *in utero* exposure to RAL and EVG by describing their trends of use over time in the UK (objective 2a); by describing the prevalence of CAs in exposed fetus/infants overall, according to time of first exposure (periconception/ pregnancy) and by type of CAs (i.e. organ/system) (objective 2b)

3) To investigate the association between *in utero* exposure to INSTIs and CAs, by timing of first exposure (periconception vs T2-T3) and by exposure to the single INSTIs agents reported in the UK by 31$^{st}$ December 2018.

## 6.2 Safety signal

DTG was granted a European marketing authorisation from the EMA in 2014, the same year that the Botswana Tsepamo Study was launched (Figure 6.1). The study is an observational prospective birth outcomes surveillance designed to compare birth outcomes by maternal HIV-status (i.e. WLWHvs women living without HIV-) and, among WLWH, to compare different cART regimens and their time of initiation (i.e. preconception vs during pregnancy). The study was set up following introduction of Option B+ in Botswana and with the initial aim to evaluate whether preconception exposure to EFV-based regimen was associated with increased risk for NTDs (a safety signal discussed in chapter 1 section 1.5.3).

In 2016, after the Botswana government changed their national guidelines, modifying preferred the first-line ART regimen from TDF/3TC+EFV to TDF/3TC+DTG for all adults, the Tsepamo Study added objectives to evaluate pregnancy outcomes in women receiving DTG. Preliminary results on the safety of DTG-based regimen started during pregnancy were reassuring, showing similar birth outcomes for women on DTG- vs EFV-based regimens (risk for any adverse birth outcome for women on DTG vs EFV was 33.2% vs 35.0%, respectively; aRR 0.95 (95%CI 0.88 to 1.03; and the risk of any severe birth outcome was 10.7% vs 11.3%, respectively (95%CI 0.94 0.81 to 1.11) (Zash et al. 2018a).

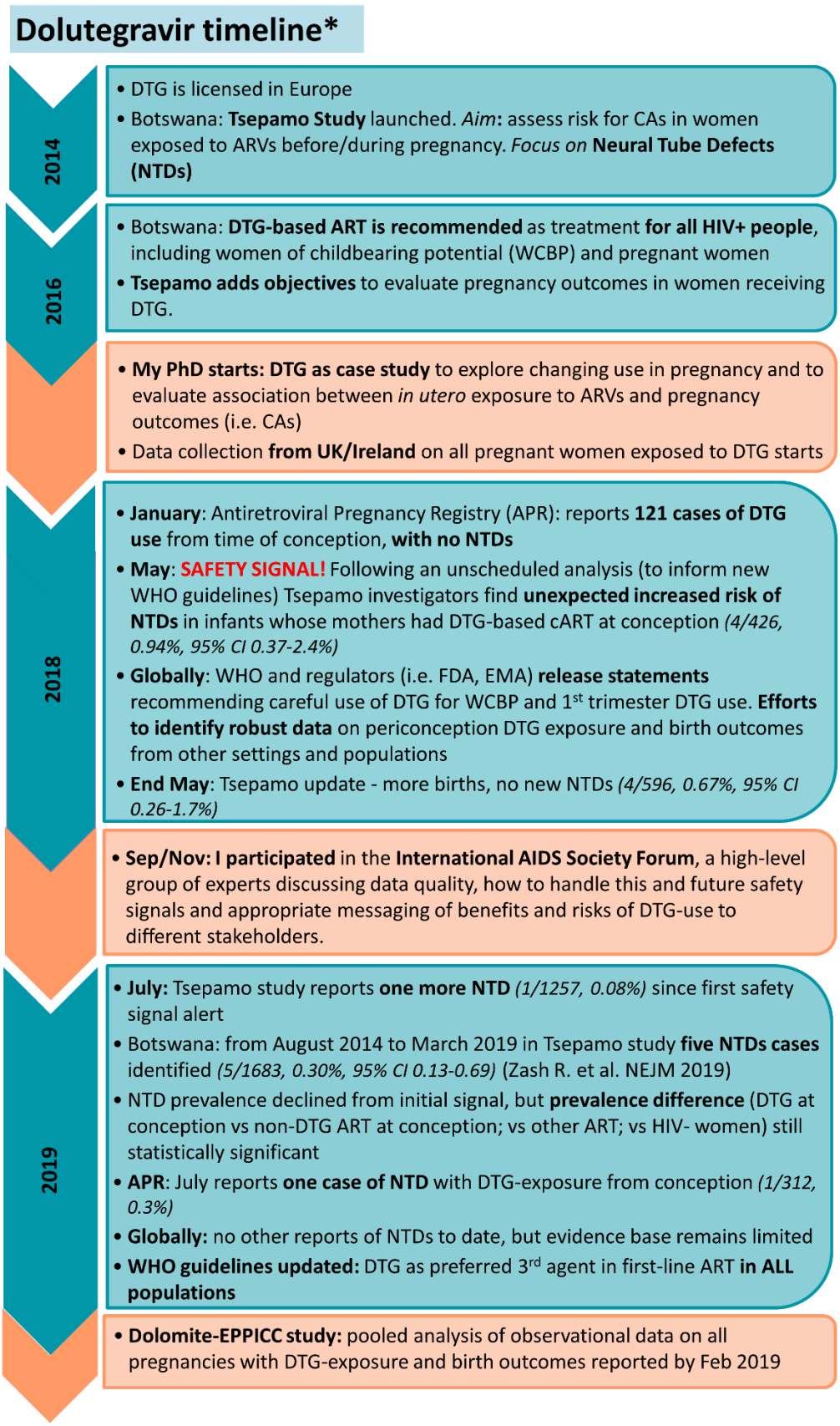
In April 2018, the Tsepamo Study performed an early unscheduled analysis of pregnancy outcomes to inform the WHO Guideline Development Group whose intention was to transition most people living with HIV to DTG-based regimens. Given that their previous analysis evaluated DTG use in pregnancy, this one focused on DTG-based regimen exposed at preconception. Preliminary results reported a small yet statistically significant increase in the absolute risk for NTDs in infants whose mothers started a DTG-based regimen from before time of conception, reporting 4 NTDs in 426 pregnancies with a prevalence of 0.94% (95% CI 0.37%, 2.4%) compared to 0.12% (95% CI 0.07%, 0.21%) in infants whose mothers received non-DTG-based regimen from time of conception (14 NTDs in 11,300); to 0.05% (95%CI 0.02%, 0.15%) in those exposed to EFV-based combinations (3 NTDs in 5,787 and to 0.0% (95%CI 0.0%, 0.13%) in those exposed to maternal DTG during pregnancy (0 NTDs in 2,812 (Zash et al. 2018b).

On May 2018, WHO issued a statement followed by several others including regulatory agencies such as the FDA and EMA, the US President's Emergency Plan for AIDS Relief (PEPFAR), as well as a Dear doctor letter from the originator company ViiV Healthcare, all recommending varying degrees of caution for DTG use for any

women of childbearing potential and for those in their first trimester of pregnancy(EMA 2018, FDA 2018, GSK Dear Doctor letter 2018, PEPFAR 2018, BHIVA 2018b, WHO 2018b).

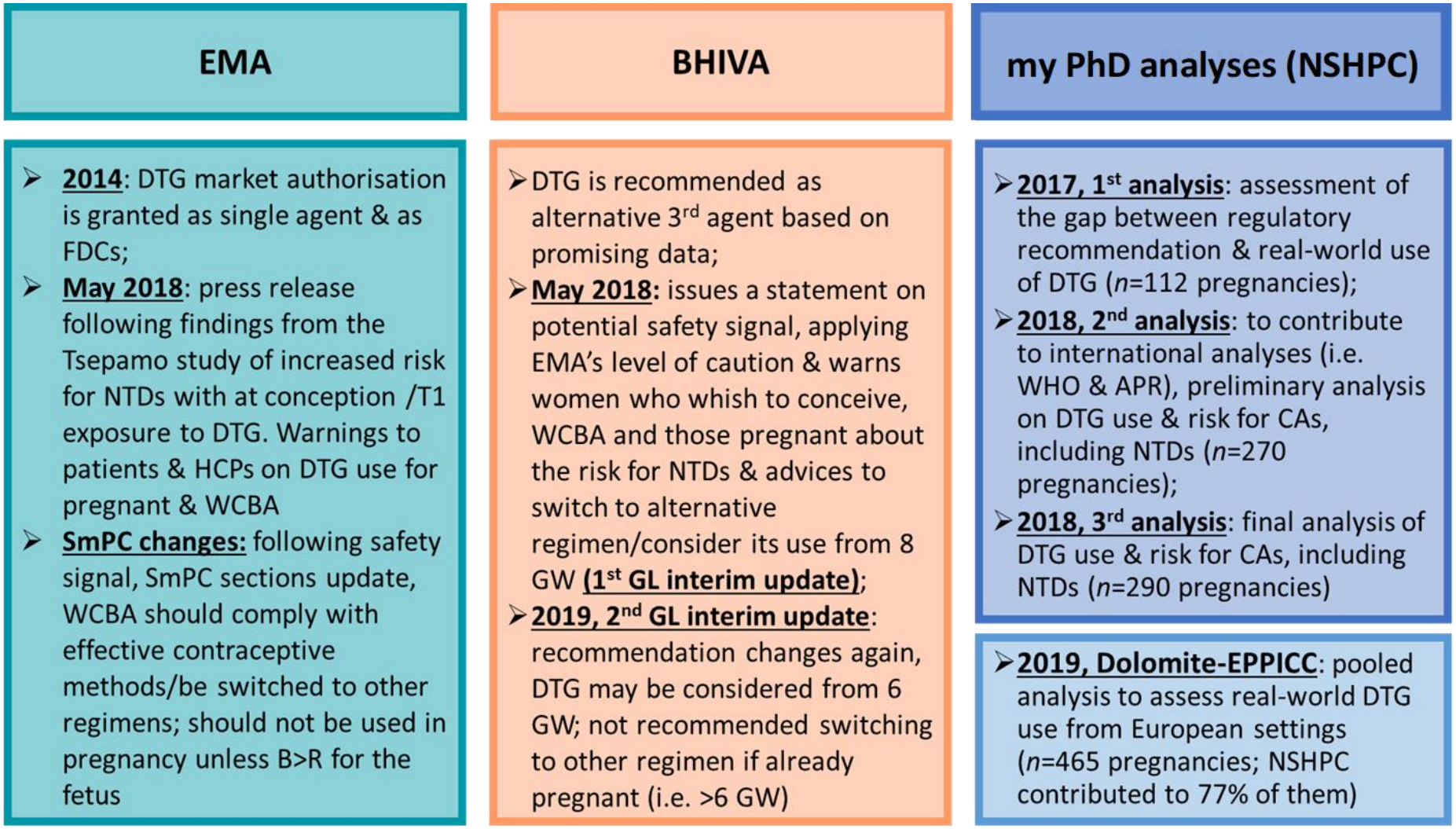
Since the signal was first reported on the 1st of May 2018 until the 31st of March 2019, one additional NTD was identified by the Tsepamo Study (which in the meantime expanded the participating sites from 8 covering about 45% of all births in Botswana to 18, covering about 72% of all births), bringing the total number of NTDs to 5 among 683 infants born to women receiving DTG at conception, a prevalence of 0.30% (95% CI 0.13, 0.69). Even though the NTDs prevalence had diminished, it still remained greater than all other comparison groups, i.e. of women receiving non-DTG regimen at conception (15/14,792 NTDs, prevalence 0.10%; 95%CI, 0.06 to 0.17) or for those taking EFV-based regimens at conception (3/7,959 NTDs, prevalence 0.04%; 95%CI, 0.01 to 0.11); and with an absolute difference in prevalence between DTG-based and non–DTG-based regimen exposure from conception of 0.20% (95%CI, 0.01 to 0.59) (Zash et al. 2019).

In the same year, following the release of the July APR report, one NTD was reported among 312 periconception exposures to DTG-containing regimens (prevalence 0.30%), while no other NTDs associated with periconception exposure to DTG were reported from any other studies which started to investigate the signal (Orrell et al. 2017, Bornhede et al. 2018, Grayhack et al. 2018, Money et al. 2018, APR 2019, Chouchana et al. 2019, Nomathemba et al. 2019).




***More recent development will be discussed in chapter 7**

**Figure 6.1 Summary of key events in the DTG issue up to end of 2019**




**HCPs: health care professionals; WCBA: women of childbearing age; B>R benefits outweigh risks; GL guidelines; GW: gestation week**

**Figure 6.2 summary of UK's key events for DTG issues up to the end of 2019, alongside the analyses performed over the years of my PhD research**

## 6.3 Synthesis of available safety and efficacy data from EMA: regulatory recommendations, 2014-2018

In this section, assessment of publicly available data on INSTIs extracted from the EMA website are presented. The aim was to evaluate changes in regulatory recommendation over time with the same methodology presented in Chapter 4.

*Special methods:*

Over the study period recommendations have been updated according to new findings (as for the latest access to EMA; 20/05/2020).

### 6.3.1 Evaluation of EMA recommendations for DTG use (objective 1a)

To address the gap between real-world use and regulatory guidelines for the use of DTG, an analysis on EMA recommendations was conducted. DTG was authorised as Tivicay (DTG) and as Triumeq (a FDC of DTG/ABC/3TC) in 2014. Below are described the most relevant changes that occurred over time for the SmPCs for these formulations; further details are reported in Table 6.1 and Figure 6.2.

Looking at the relevant sections of the SmPCs of both formulations at time of authorisation, *"Sec. 4.6-Fertility, Pregnancy and lactation"* reported limited data from DTG use in human pregnancy and no data from breastfeeding, hence no knowledge as to whether DTG was secreted in breast human milk. There was no report of PK-PD data in "Sec. 5.1- *Pharmacodynamic properties"* and "5.2- *Pharmacokinetic properties",* respectively. Based on these data, recommendations for DTG use, both as single agent and as FDC, were to use these products "*only if the expected benefit justified the potential risk to the fetus"*. No report of any toxicities, particularly no developmental or reproductive toxicity nor teratogenicity from animal models (both rats and rabbits) were reported in "Sec. 5.3- *Preclinical safety data"* for either formulation. For both formulations, the SmPC mentioned DTG transplacental passage observed in animal models, but human data were lacking (Table 6.1).

In 2018 evaluation of the SmPCs for DTG as single agent and as FDC included updated recommendations. For both formulations, no additional preclinical findings were reported, hence "Sec. 5.3- *Preclinical safety data"* remained unchanged, as did "Sec. 5.1- *Pharmacodynamic properties"* and "5.2- *Pharmacokinetic properties"* with no additional PK-PD data.

However, “Sec. 4.6-*Fertility, Pregnancy and lactation”* of both formulations was updated following the Tsepamo Study findings, with a brief report included to justify the changes (with slightly different wording in each), advising for avoidance of DTG use during first trimester of pregnancy unless there is no alternative, and similarly in T2-T3 stating that *“it should only be used if the benefit justifies the potential risk to the fetus”.* In the <Tivicay or Triumeq> sec 4.6, it was reported that despite more than 1,000 pregnancy outcomes from women with T2-T3 exposure to DTG with no evidence of increased risk for malformative and/or other fetal/neonatal negative effects, the safety of DTG use during T2-T3 cannot be confirmed.

Furthermore, sec. 4.6 stated that the mechanism by which DTG may interfere in human pregnancy is unknown; therefore, due to the potential risk for NTDs with early pregnancy exposure (i.e. T1) and the impossibility to confirm its safe use later in pregnancy (i.e. T2-T3), the stated recommendations in the section advise HCPs to perform a B-R evaluation when prescribing DTG for women of childbearing age and those pregnant.

The recommendations in Sec. 4.6 were also updated for women of childbearing age, advising women to undergo a pregnancy test before treatment initiation and to comply with effective contraceptive methods to avoid becoming pregnant whilst on treatment.

**Table 6.1 Summary of DTG characteristics extracted from the relevant SmPC sections of both DTH authorised formulations**

| ARV/ Trade name | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Fertility, Pregnancy and lactation (Clinical data) | Sec 5.1 PD & Sec 5.2 PK properties | Sec 5.3 Preclinical safety data |
|---|---|---|---|---|---|
| **DTG (Tivicay)** | 2014 | **2014:** No data | **2014:** **Preg:** Limited data in human pregnancy and no data from breastfeeding. **Recommendation**: Should be used in pregnancy only if the expected the benefit justifies the potential risk to the fetus. | No data on preg/B-F<br><br>DTG: t ½ ~14 h | In rats & rabbits no developmental toxicity, nor teratogenicity<br><br>Placenta crossing: in animal models |
| | | **Current:** No data | **Current: WCBA:** to undergo preg test & comply with contraceptive measure. **Preg**: Preliminary data from a surveillance study suggests increased incidence of NTDs (0.9%) vs (0.1%) in non-DTG regimens. As NTDs occur within the first 4 weeks, the potential risk would concern women exposed to DTG at time of conception/early preg; >1,000 outcomes from T2-T3 in human preg indicate no malformative nor fetal/neonatal negative effect. However, as the mechanism by which may interfere in human preg is not known, the safety in its use in T2-T3 cannot be confirmed. **B-F**: not known in human milk. **Recommendation:** Due to potential risk of NTD, Tivicay should not be used in T1 unless there is no alternative, similarly in T2-T3 should only be used if the benefit justifies the potential risk to the fetus. | | |
| **DTG + ABC/3TC (Triumeq)** | 2014 | **2014: Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | **2014:** **Preg**: No data on Triumeq use in human preg. Limited data on DTG use. Moderate (>400) data in human preg T1 on ABC/3TC and >3,000 data on 3TC from T1 and >600 outcomes from T1 on ABC indicates no malformative toxicity. Mitochondrial dysfunction reports in HEU infants exposed to NRTI. **B-F:** 3TC, ABC and its metabolites excreted in human milk, DTG not known. **Recommendation:** Should be used in preg only if the expected benefit justifies the potential risk to the fetus. | No data on preg/B-F<br><br>DTG: t ½ ~14 h<br>ABC: t ½ 1.5 h<br>3TC: t ½ 5-7.5 h | **ABC:** toxicity to developing embryo & fetus of rats*****, no rabbits**.**<br>**3TC:** increased early embryonic deaths, only rats.<br>**DTG**: no developmental toxicity, nor teratogenicity.<br><br>Placenta crossing: in animal models |
| | | **Current: Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | **Current:** **WCBA**: as per Tivicay. **Preg:** >1,000 data from T2-T3 for Triumeq in human preg indicate no evidence of increased risk of malformative and foetal/neonatal effects. DTG update as per Tivicay. **Recommendation:** should only be used during T2-T3 when the expected benefit justifies the potential risk to the fetus | | |

WCBA: women of childbearing age; Preg: pregnancy; B-F; breast-feeding; HEU infants: HIV exposed uninfected infants; t ½: half-life

*****Decreased fetal body weight, fetal oedema, increased skeletal variation, early intrauterine death & stillbirth

### 6.3.2 Evaluation of EMA recommendation for RAL and EVG use

#### *Raltegravir*

RAL was licensed as the single agent Isentress in 2007. Looking at the relevant sections, when first authorised, the lack of adequate data on human pregnancy or on its use in breastfeeding women was highlighted in "Sec. 4.6-*Fertility, Pregnancy and lactation"*; there were no pregnancy PK-PD data reported (Table 6.2). Preclinical studies showed some reproductive toxicities which were mentioned in section 4.6 and addressed in depth in "Sec. 5.3- *Preclinical safety data"* reporting no teratogenicity but a slight increase of supernumerary ribs only in pups of dams exposed to a 4.4-fold of RHD. The section also reported evidence of RAL's transplacental transfer in animal models, but no data on human pregnancies. Therefore, recommendations in 2007 were to avoid its use in pregnant and breastfeeding women. Further details are reported in Table 6.2.

Assessing the most recent SmPC (2018), data on RAL use in pregnant women had accumulated by a moderate amount (300-1,000 reports in human pregnancy) and did not suggest malformative nor fetal/neonatal toxicity. Therefore, there was a slight change in the wording of recommendations, now advising to prescribe RAL in pregnancy "*only if the expected benefits justify the potential risk to the fetus"* (Table 6.2).

#### *Elvitegravir*

EVG is marketed within two FDCs, namely Stribild (FTC/TDF+EVG/c) licensed in 2013 and Genvoya (FTC/TAF+EVG/c) authorised in 2015. At time of first authorisation, there were limited data from clinical studies on pregnant women (<300 for both FDCs) and no data from PK-PD studies nor information on transplacental passage. Data from preclinical studies on animal models reported no reproductive toxicity, but an increased post-implantation loss, a decrease in pups' weight and a significant decrease in maternal body weights at 125mg/kg/day but only in rats.

Therefore, the recommendations for both FDCs were to use EVG "*only if the potential benefits justified the potential risk for the fetus".* Further details are shown in Table 6.2. By the end of the study period, the SmPCs for both formulations had been updated with respect to pregnancy due to findings of reduced EVG/c exposure (already reported in chapter 4, section 4.3.2 and Table 4.9).

Three sections (namely sec. 4.2; 4.4; 4.6) were updated and each reported the same recommendation not to start EVG during pregnancy and to switch to an alternative regimen if becoming pregnant whilst treated with EVG because the substantial reduction of EVG exposure may result in failure to suppress viral replication and increased risk for VT. Sec. 4.6 also addressed women of childbearing age strongly advising to comply with effective contraceptive measures while on treatment.

Additionally, Sec 4.4. warns of the requirement to comply with specific dosages of the oral contraceptives ethynyl-oestradiol drospirenone (D) (i.e. at least 30µg) or to switch to reliable contraceptive method alternatives due to potential drug-drug interactions. PK-PD data are now reported in the relevant sections (i.e. Sec. 5.2 and 5.3), however there is still no mention of EVG's ability to cross the placenta (Table 6.2).

**Table 6.2 Summary of RAL and EVG characteristics extracted from the relevant SmPCs' sections of authorised formulations**

| ARV/ Trade name | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Fertility, Pregnancy and lactation (Clinical data) | Sec 5.1 PD & Sec 5.2 PK properties | Sec 5.3 Preclinical safety data |
|---|---|---|---|---|---|
| **RAL (Isentress)** | 2007 | **2007**: no data | **2007**: **Preg**: No adequate data in human preg, preclinical studies have shown some reproductive toxicities. **B-F**: not if is excreted in human milk. **Recommendation:** Should not be used during pregnancy. | No data on preg/B-F<br><br>RAL: t ½ 9 h | In rats & rabbits no teratogenicity, a slight increase of supernumerary ribs only in rats of dams exposed to 4.4-fold at RHD<br><br>Placenta crossing: in animal models |
| | | **Current**: no data | **Current**: Moderate (300-1,000) data in human pregnancy indicate no malformative nor fetal/neonatal toxicity. **B-F**: not known if is excreted in human milk. **Recommendation:** Should be given in preg only if the expected benefit justifies the potential risk to the fetus | | |
| **EVG/c + FTC/TDF (Stribild)** | 2013 | **2013: Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | **2013: WCBA:** contraceptive measure required. **Preg:** No/limited data in preg; moderate data (300-1,000) with FDC/TDF in human preg indicate no malformation or fetal/ neonatal toxicity. **B-F:** EVG/c not known if is excreted in human milk; FTC & TDF are excreted. **Recommendation:** Should be used in preg only if the potential benefit justifies potential risk. | No data on preg/B-F<br><br>FTC: t ½ 10h<br>TDF: t ½ 12-18h<br>COBI: t ½ 3.5h<br>EVG: t ½ 12.9h | **EVG, FTC**: no special hazard.<br>**COBI:** No reProdTox in rats & rabbits but increased post-implantation loss and decreased fetal weights in rats associated with a significant ↓ maternal body weights at 125mg/kg/day<br>**TDF**: no reProdTox, but reduced viability index/weight of pups.<br><br>Placenta crossing: not known |
| | | **Current**: **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs.<br>**Sec 4.2** same warnings as per sec. 4.6 for WCBP and preg, same recommendation | **Current**: **WCBA:** contraceptive measure must be used. **Preg**: >1,000 human preg outcomes with FTC, TDF indicates no malformation or fetal toxicity. Sec 4.2, 4.4, 4.6 updated: EVG/c in T2-T3 shown substantial reduction in EVG exposure, the substantial reduction may result in Viral suppression failure, and warning re risk for hyperkalaemia. **B-F**: as 2013. **Recommendation:** Should not be initiated in preg, and women who become preg during therapy should be switched to alternative regimen | **IMPAACT P1026s,** showed lower EVG exposure in T2-T3 vs PP for EVG/c-containing regiments. | |
| **EVG/c + FTC/TAF (Genvoya)** | 2015 | **2015:** as per Stribild 2013 | **2015:** as per Stribild 2013 + **Preg**: no data on TAF use & >1,000 preg outcomes with FTC indicates no malformation or fetal toxicity | As per Stribild 2013 | |
| | | **Current**: as per Stribild | **Current:** as per Stribild + **WCBA**: an effective contraceptive methods (oral +other) should be used. Plasma concentration of drospirenone might be increased, hence monitoring du to potential for hyperkalaemia. | **IMPAACT P1026s,** same findings of Stribild | |

WCBA: women of childbearing age; Preg: pregnancy; B-F; breast-feeding; HEU infants: HIV exposed uninfected infants; t ½: half-life, h: hour; PP: post-partum; reProdTox: reproductive toxicities studies

## 6.4 Dolutegravir

### 6.4.1 Dolutegravir in the NSHPC (objectives 1b and 1c)

***First analysis (objective 1b):***

The first analysis on DTG was part of a focused study where DTG was evaluated together with two other newly approved ARVs, namely RPV and COBI (results for these agents are presented in Chapter 4 section 4.3.2) used in pregnant women and the regulatory recommendations on their administration, as a preliminary analysis of my PhD. As per chapter 5, exposure "from preconception" or "from time of conception" defines the earliest exposure to DTG to have occurred before conception and includes T1.

*Specific methods:*

For DTG real-word use, data on the selected ARVs were collected from all pregnancies (including ongoing pregnancies) with an EDD reported to the NSHPC from 1st January 2013 to 31st of March 2017. To estimate trends of DTG use, all pregnancies reported over the considered study period were included as denominator. Data collection started from 2013 as this was the first year of marketing authorisation for COBI.

*Results:*

There were 4,831 pregnancies reported to the NSHPC between 2013 and 2017, of which 343 (7%) were exposed to RPV-, DTG- or COBI-based regimens. A total of 112 pregnancies were exposed to a DTG-based regimen by the time of this analysis. The proportion of pregnancies exposed to DTG from time of conception was 46.4% (52/112) with 53.6% (60/112) exposed in T2-T3.

The first time a pregnancy was reported to the NSHPC with exposure to a DTG-based regimen was in 2015, just a year after European marketing-authorisation was granted. The use of DTG increased more than 10-fold between 2015 and 2016, from 0.3% (3/1,136) to 3.3% (33/1,002) of the total ARVs used (test-for-trend p<0.001).

Looking at pregnancy outcomes, of the 112 pregnancies exposed to DTG, there were outcomes available for 36 (32.1%) pregnancies at the time of the analysis with the remainder of reported pregnancies ongoing; of these 33 (91.6%) ended in a livebirth, one (2.7%) was a stillbirth and two (5.5%) were miscarriages, all of which were exposed from preconception (Table 6.3).

Among the 33 liveborn infants, one presented with a CA, namely a polydactyly, giving a prevalence of 3.03% (95% CI 0.76, 15.70).

**Table 6.3 Number of pregnancies exposed to DTG and their outcomes, NSHPC 2014-2017**


*Gap analysis between real-world use and regulatory recommendations*

Comparing data from the NSHPC with the evidence and recommendations reported in the SmPCs of both formulations of DTG, a gap was identified between regulatory recommendations and real-world use for women of childbearing age and for those pregnant and breastfeeding at the time of the first analysis (section 6.3.1).

EMA data reported distinct warnings to avoid DTG use in pregnancy on the base of no preclinical findings showing specific hazards, reflecting the limited availability of relevant data from clinical trials (i.e. “no/limited data”). Data from the NSHPC showed a substantial increase in DTG use over time in pregnancy and in women of childbearing age who then become pregnant, demonstrating as mentioned before, how much awaited DTG was given the many benefits associated with its use.

### *Second analysis (objective 1c):*

For this analysis, the same population described in Chapter 4 was considered (i.e. *n*= 12,099 pregnancies reported to the NSHPC between 2008-18). As per chapter 5, periconception exposure defines a first exposure (to DTG) occurring prior to conception and includes early T1, while exposure in T2-T3 defines an exposure occurring later in pregnancy.

Before this main analysis, a rapid one in response to DTG safety signal was performed to contribute to international analyses (i.e. WHO and APR), which is not included here, but reported in Figure 6.2.

*Results:*

By the end of 2018, of the 12,099 singleton pregnancies reported to the NSHPC there was a total of 290 (2.4%) pregnancies exposed to DTG-based regimens from 273 women. Table 6.4 displays key maternal characteristics for the reported DTG-exposed pregnancies. The median age of women at conception was 33.1 years (q1=28.7, q3=38.1, IQR). The majority of pregnancy were to Black African women, 93.6% of whom were born in SSA (176/188). Most women (80.3%) acquired HIV heterosexually, 8.9% were infected vertically and 2.7% via IDU.

For the vast majority (93.1%) of pregnancies, maternal HIV diagnosis was made prior to conception, with the proportion of women knowing their diagnosis before pregnancy increasing from 0.1% (1/1,066) in 2014 to 15.3% (96/629) in 2018 (test-for-trend $p<0.001$). Looking at mode of delivery, of the 268 pregnancies ending in deliveries, 43.0% were delivered vaginally; over time the proportion of vaginal deliveries increased from 0.1% (1/1,066) in 2014 to 7.8% (49/629) in 2018 (test-for-trend *p<0.001*).

Regarding CD4 cell count, between 2014-2018, the overall baseline median CD4 cell count was 455.5 cell/mm$^3$ (q1=319.0, q3=650.0, IQR) with 26 missing data. Overall, there were 199 women with undetectable VL (≤400 copies/μL) near delivery, of whom 91.0% (181/199) with values of VL ≤50 copes/μL. Over time the proportion of women with effective suppressed VL by the time of delivery increased from 0.1% (1/1,066) in 2014 to 12.1% (76/629) in 2018 (test-for-trend $p<0.001$).

**Table 6.4 Maternal characteristics for the pregnancies exposed to DTG-based regimen reported to the NSHPC, 2008-18**

| Maternal characteristics (*n* =290) | | | | | |
|---|---|---|---|---|---|
| **Ethnicity** | | | | | |
| Black African<br>188 (64.8%) | Black Other<br>20 (6.9%) | White<br>69 (23.8%) | Other<br>13 (4.5%) | | |
| **Region of birth** | | | | | |
| SSA<br>180 (62.1%) | UK/Ireland<br>64 (22.1%) | Europe<br>22 (7.6%) | Elsewhere<br>19 (6.5%) | Missing<br>5 (1.7%) | |
| **HIV acquisition route** | | | | | |
| Heterosexual<br>233 (80.3%) | IDU<br>8 (2.7%) | VT<br>26 (8.9%) | Other<br>6 (2.1%) | Missing<br>17 (5.8%) | |
| **Parity** | | | | | |
| Nulliparous<br>57 (19.6%) | 1<br>75 (25.9%) | 2<br>64 (22.1%) | 3<br>44 (15.2%) | ≥4<br>50 (17.2%) | |
| **Mode of delivery*** | | | | | |
| Vaginal<br>115 (43.0%) | Elective-CS<br>89 (32.9%) | Emergency-CS<br>60 (22.4%) | Missing<br>10 (3.7%) | | |
| **Age at conception grouped (years)** | | | | | |
| ≤ 25<br>35 (12.1%) | 25-30<br>59 (20.3%) | 30-35<br>73 (25.2%) | 35-40<br>84 (28.9%) | 40-45<br>35 (12.1%) | ≥45<br>4 (1.4%) |
| **Timing of HIV diagnosis** | | | | | |
| Before pregnancy<br>270 (93.1%) | | During pregnancy<br>20 (6.9%) | | | |
| **VL at delivery, copies/μL (within 30 days of delivery)** | | | | | |
| Detectable<br>10 (3.4%) | Undetectable<br>199 (68.6%) | Missing<br>81 (27.9%) | | | |

***For the *n*=268 pregnancies ending in live- and still-births

*Time of DTG exposure*

Looking at time of first exposure to DTG there were 243 (83.8%) pregnancies with periconception exposure to DTG, and 47 (16.2%) exposed from T2-T3. Over time the number of pregnant women using DTG-based regimens increased from 0.1% (1/1,066) in 2014 to 16.8% (106/629) in 2018 (test-for-trend p<0.001); similarly, the proportion of pregnancies with periconception exposure to DTG increased from 55.0% (11/20) in 2015 to 83.0% (88/106) in 2018 (test-for-trend p<0.001).

Furthermore, among women knowing their HIV diagnosis from before pregnancy, 82.2% (222/270) were already on a DTG-based regimen, a proportion that increased over time, from 0.1% (1/1,066) in 2014 to 14.0% (88/629) in 2018 (test-for-trend *p*<0.001).

*Pregnancy outcomes*

Overall, of the 290 pregnancies exposed to DTG, there were 266 (91.7%) singleton liveborn pregnancies, two pregnancies ended in stillbirth, 14 (4.8%) in miscarriages and eight (2.7%) in terminations (Table 6.5). Looking at pregnancy outcomes stratified by time of exposure to a DTG-based regimen, the majority (219/266, 82.3%) of the liveborn infants were born to mothers with periconception exposure to DTG while 17.7% (47/266) started DTG during T2-T3. For all the remaining pregnancy outcomes (i.e. stillbirths, miscarriages and termination of pregnancies) women were exposed to DTG from periconception (Table 6.5).

**Table 6.5 Singleton pregnancy outcomes reported to the NSHPC between 2008-18, by earliest DTG exposure**

| | Total DTG exposed | Earliest exposure to DTG | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **Tot outcomes, N** | 290 | 243 | 47 |
| **Livebirths** | 266 (91.7%) | 219 (82.3%) | 47 (17.7%) |
| **Stillbirths** | 2 (0.7%) | 2 | 0 |
| **Miscarriages** | 14 (4.8%) | 14 | 0 |
| **Terminations** | 8 (2.7%) | 8 | 0 |

*Gestational age and birthweight among liveborn infants*

Of the 266 liveborn infants the overall proportion of those born at term was 87.6% (233/266); of these term infants, the proportion exposed to DTG-based regimens from before periconception and from T2-T3 were respectively 84.1% and 11.6%. The overall proportion of preterm deliveries (i.e. <34 and between 34-36GW) was 12.4% (33/266), with almost 70.0% (23/33) exposed to DTG from before periconception (i.e. 14 preterm deliveries at <34 weeks plus the 9 preterm deliveries between 34-36 weeks) (Table 6.6).

Looking at birthweights, the proportion of liveborn infants with a weight of 2.5kg or more at birth was 80.1% (213/266) of which 82.2% were exposed to DTG-based regimen from before periconception (Table 6.6). The proportion of infants delivered at term exposed to DTG from before periconception with a weight of 2.5kg or more at birth was 87.7% (157/179). The overall proportion of liveborn infants with a birthweight less than 2.5kg (i.e. <1500 and between 1500-2499 g) was 12.8% (34/266), of which 85.3% (29/34) were exposed to DTG from before periconception (i.e. the 7 infants with a weight <1500 plus the 22 infants with a weight between 1500-2499g at delivery) (Table 6.6).

**Table 6.6 Neonatal outcomes for the singleton livebirths pregnancies exposed to DTG reported to the NSHPC, 2008-18**

| | Total DTG exposed | Earliest exposure to DTG | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **Total, N** | 266 | 219 | 47 |
| **Gestational age** | | | |
| <34 weeks | 17 (6.4%) | 14 (82.4%) | 3 (17.6%) |
| 34-36 weeks | 16 (6.0%) | 9 (56.2%) | 7 (43.7%) |
| ≥37 weeks | 233 (87.6%) | 196 (84.1%) | 37 (11.6%) |
| **Birth weight (g)** | | | |
| <1500 | 8 (3.0%) | 7 (87.5%) | 1 (12.5%) |
| 1500-2499 | 26 (9.7%) | 22 (84.6%) | 4 (15.4%) |
| ≥2500 | 213 (80.1%) | 175 (82.2%) | 38 (17.8%) |
| Missing | 19 (7.1%) | 15 (78.9%) | 4 (21.1%) |

*Congenital anomalies among liveborn infants*

Of the 266 liveborn infants exposed to a DTG-based regimen, there were nine reported CAs, a prevalence of 3.38% (95% CI 1.56, 6.32). Stratifying by earliest exposure to DTG, of the 219 infants with periconception exposure to DTG, eight presented with a CA, a prevalence of 3.65% (95% CI 1.59, 7.07); while among the 47 infants exposed to DTG in T2-T3, one had a CA, a prevalence of 2.13% (95% CI 0.05, 11.29). There was no report of NTDs over the considered time period.

Of the nine infants with a reported CA none had multiple defects, and most were males (6/9), with a birth weight of 2.5kg or more (7/9) and delivered at term (7/9). Table 6.7 provides a detailed list of the CAs reported meeting the EUROCAT classification.

**Table 6.7 List of CAs meeting EUROCAT classification among the n=266 liveborn infants, by earliest DTG exposure reported to the NSHPC 2008-18**

| Organ system classification | Earliest exposure to DTG | |
|---|---|---|
| | Periconception (*n*=219) | T2-T3 (*n*=47) |
| **All anomalies** | **8 (3.29%)** | **1 (%)** |
| **Congenital heart defects** | | |
| Atrial septal defect (ASD) | 1 | 0 |
| **Urinary** | | |
| Congenital hydronephrosis | 1 | 0 |
| Ectopic Kidney | 1 | 0 |
| **Genital** | | |
| Hypospadias | 3 | 0 |
| **Digestive system** | | |
| Duodenal atresia and stenosis | 0 | 1 |
| **Abdominal wall defects** | | |
| Gastroschisis | 1 | 0 |
| **Limb** | | |
| Polydactyly | 2 | 0 |

### 6.4.2 Dolutegravir in the Dolomite-EPPICC study (objective 1d):

This analysis was carried out to assess pregnancy and neonatal outcomes following DTG use during pregnancy in real-world European settings, addressing the following two objectives:

a) To assess the characteristics of pregnant women receiving DTG-based regimens

b) To evaluate the frequency of adverse pregnancy and birth outcomes, by earliest timing of DTG exposure

*Special methods:*

EPPICC-Dolomite was set up in 2017 to address use and safety of DTG in pregnant women and their exposed infants in Europe. The study involves pooled analysis of observational data, following periodic data mergers, as described in chapter 3, section 3.3.1. For this analysis, data from six participating countries, namely UK and Ireland, Spain, Switzerland, Italy and Romania were merged to perform an analysis on prospectively collected individual patient data on all pregnancies with a prenatal exposure to a DTG-based regimen and with birth outcomes reported by February 2019.

UK contributes to Dolomite-EPPICC through the provision of NSHPC's data and is the major contributor to the study, hence the previous two analyses (i.e. section 6.4.1) presented and the related data described on DTG are included in this EPPICC analysis. Spain contributes through two cohorts, the Madrid cohort of HIV-infected mother-infant pairs and the NENEXP Study (Catalonia). Switzerland contributes to EPPICC through the Swiss Mother and Child HIV Cohort Study (MoCHiV); Italy through the Italian Group on Surveillance of Antiretroviral Treatment in Pregnancy; and Romania though the Victor Babies Hospital Cohort.

EPPICC collects data on any congenital anomaly identified and reported by the participating studies (i.e. no classification system is applied at time of data extraction/merger). However, to be consistent with the classification criteria I have used across my thesis, I re-classified the CAs reported to Dolomite-EPPICC to comply with EUROCAT classification for inclusion/exclusion criteria as explained in chapter 3, section 3.4.1. Consequently, four CAs reported to EPPICC-Dolomite were excluded from this analysis being part of the EUROCAT list of *"minor anomalies for exclusion", i.e.* not compliant with EUROCAT inclusion criteria.

*Special definitions:*

For this analysis periconception exposure was defined as an exposure to DTG within the first 6 weeks of estimated gestation (WG); later T1 as initial exposure started after 6 of estimated WG; T2-T3 as initial exposure after 12 estimated WG. Induced abortion was defined as a voluntary termination of pregnancy before 22 estimated gestational weeks; spontaneous abortion as a death of a fetus or expulsion of the products of conceptions before 22 WG of age.

*Results:*

There was a total of 453 pregnancies from 428 women from the six cohorts, namely 347 (76.6%) from the UK and Ireland, 45 (9.9%) from Spain, 29 (6.4%) from Switzerland, 29 (6.4%) from Italy, and 3 (0.7%) from Romania. Of these, 443 were singleton pregnancies and ten were twin pregnancies (Table 6.8).

Looking at time of DTG-exposure, among the 453 pregnancies, 325 (70.2%) started DTG-containing regimen from before periconception, 31 (8.5%) started later in T1 and 106 (22.9%) in T2-T3 (Table 6.10).

**Table 6.8 Total pregnancies reported to Dolomite-EPPICC study, 2017-19**

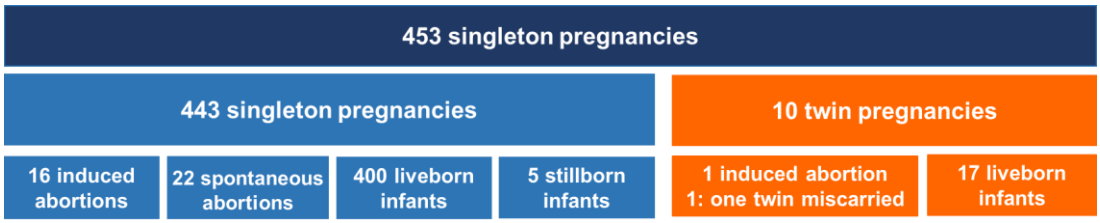



Table 6.9 presents key maternal characteristics. Overall, women were mainly of back African origin (54%), most acquired HIV heterosexually (82.7%) and had periconception DTG exposure (70.0%).

**Table 6.9 Dolomite-EPPICC maternal characteristics**

| **Maternal characteristics (*n* =428)** | | | | |
|---|---|---|---|---|
| **Ethnicity** | | | | |
| Black African<br>229 (53.5%) | Black Other<br>35 (8.2%) | White<br>129 (30.1%) | Other<br>35 (8.2%) | Missing<br>0 |
| **HIV acquisition route** | | | | |
| Heterosexual<br>326 (82.7%) | IDU<br>11 (2.8%) | VT<br>42 (10.6%) | Other<br>15 (3.8%) | Not known<br>34 (7.9%) |

*Pregnancy outcomes*

There was a total of 463 infants (453 singletons and 10 twins) exposed to a cART containing DTG (Table 6.10). Of these, 417 (90.1%) were liveborn infants, 5 (1.1%) were stillborn, 23 (5.0%) were pregnancies terminating in spontaneous abortions and 18 (3.7%) in induced abortions. Overall, of the 417 pregnancies ending in in livebirths, 67.1% (280/417) were in women starting DTG-based regimen before periconception and 25.4% (106/417) in women starting DTG in T2-T3 (Table 6.10). For all the remaining pregnancy outcomes (i.e. stillbirths, spontaneous and induced abortions) earliest exposure to DTG-based regimens occurred mostly before periconception, with only one pregnancy terminating in an induced abortion being exposed to DTG from later T1 (Table 6.10).

Looking at the liveborn infants, there were 229 males, 185 females and for three data was missing. Of the five stillbirths, four were female, one of unknown sex, one was preterm and one born at term. Two infants died, one being extremely preterm (at 23 GW) and one after 48h of life, neither of which presented with a CA and with both exposed to DTG from before periconception.

**Table 6.10 Pregnancy outcomes reported to the Dolomite-EPPICC study, by earliest DTG exposure**

| | Total DTG exposed | Earliest exposure to DTG | | |
|---|---|---|---|---|
| | | Periconception | Later T1 | T2-T3 |
| **Tot outcomes, N** | 463* | 325 (70.2%) | 31 (6.7%) | 106 (22.9%) |
| **Livebirths** | 417 (90.1%) | 280 (86.1%) | 30 (96.8%) | 106 (100%) |
| **Stillbirths** | 5 (1.1%) | 5 (1.5%) | 0 | 0 |
| **Spontaneous abortions** | 23 (5.0%) | 23 (7.2%) | 0 | 0 |
| **Induced abortions** | 18 (3.7%) | 17 (5.2%) | 1 (3.2%) | 0 |

*Includes outcomes from the 10 twin pregnancies

*Gestational age and birthweight in singleton liveborn infants*

Of the 400 livebirth singleton pregnancies, the majority (66.5%, 266/400) started DTG-based regimen before periconception, with the overall majority of liveborn infants being born at term and with a weight at birth of 2,500g or more (Table 6.11).

**Table 6.11 Neonatal outcomes for the 400 singleton livebirths pregnancies exposed to DTG and reported to the Dolomite-EPPICC study**

| | Total DTG exposed | Earliest exposure to DTG | | |
|---|---|---|---|---|
| | | Periconception | Later T1 | T2-T3 |
| **Total, N** | 400 | 266 | 30 | 104 |
| **Gestational age** | | | | |
| <34 weeks | 12 (3.1%) | 8 (3.0%) | 1 (3.3%) | 3 (2.8%) |
| 34-36 weeks | 39 (9.7%) | 24 (9.0%) | 2 (6.7%) | 13 (12.5%) |
| ≥37 weeks | 334 (83.5%) | 222 (83.5%) | 26 (86.7%) | 86 (82.7%) |
| Missing | 15 (3.7%) | 12 (4.5%) | 1 (3.3%) | 2 (1.9%) |
| **Birth weight (g)** | | | | |
| <1500 | 12 (3.0%) | 8 (3.1%) | 1 (3.3%) | 3 (2.9%) |
| 1500-2499 | 36 (9.0%) | 23 (8.6%) | 2 (6.7%) | 11 (10.6%) |
| ≥2500 | 342 (85.5%) | 230 (86.5%) | 26 (86.7%) | 86 (82.7%) |
| Missing | 10 (2.5%) | 5 (1.8%) | 1 (3.3%) | 4 (3.8%) |

*Congenital anomalies*

Among the 417 liveborn infants there were 14 identified CAs reported to EPPICC meeting the EUROCAT inclusion criteria (which, as mentioned in methods, was applied for this analysis), giving a prevalence of 3.36% (95% CI 1.85, 5.57); these are reported in Table 6.12. Among the 280 liveborn infants with a periconception exposure to DTG, 11 reported a CA, a prevalence of 3.93% (95% CI 1.97, 6.92); while among the 106 liveborn infants exposed in T2-T3, three reported a CA, giving a prevalence of 2.8% (95% CI 0.59, 8.05)

There was no report of NTDs and applying the “rule of three” for infants whose mothers started DTG-based regimen from before periconception, there was no report of CAs affecting the same organ/system. One infant with periconception exposure to DTG was reported with two defects, namely hypospadias and polydactyly.

There were no reported CAs for pregnancies ending in stillbirths or spontaneous abortions, while of the 18 induced abortions, there was one carried out due to an identified CA (neural migration disorder and severe microcephaly) at 29 GW with periconception exposure to DTG-based regimen.

**Table 6.12 List of CAs meeting EUROCAT inclusion criteria for the liveborn infants reported to the Dolomite-EPPICC study, by earliest DTG exposure**

| Organ system classification | Tot, *n* (%) | Earliest exposure to DTG | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **All anomalies** | **14 (3.3%)** | **11 (3.9%)** | **3 (2.8%)** |
| **Congenital heart defects** | **2** | | |
| Atrial septal defect (ASD) | | 1 | |
| Ventricular septal defect (VSD) | | 1 | |
| **Urinary** | **3** | | |
| Congenital hydronephrosis | | 2 | |
| Ectopic Kidney | | 1 | |
| **Genital** | **4** | | |
| Hypospadias[1] | | 3 | 1 |
| **Digestive system** | **1** | | |
| Duodenal atresia and stenosis | | | 1 |
| **Abdominal wall defects** | **1** | | |
| Gastroschisis | | 1 | |
| **Limb** | **3** | | |
| Polydactyly[1] | | 2 | 1 |

[1]one infant had hypospadias and polydactyly

## 6.5 The other INSTIs in the NSHPC: Raltegravir and Elvitegravir

Following the report of the DTG safety signal, concerns as to whether a class effect for the entire INSTIs arose. As explained in Chapter 1, RAL and EVG share similar properties to DTG such as strong trans-placental transfer and rapid and effective reduction of maternal VL. Therefore, I conducted an analysis on RAL and EVG use in pregnant women in the UK and assessed the presence of CAs and particularly for NTDs, following *in utero* exposure.

A first analysis in response to the signal was conducted covering pregnancies with EDD between September 2008 and April 2018 and reported to the NSHPC by May 2018, looking at RAL and EVG exposure and presence of CAs (not classified according to EUROCAT criteria). This was published as a brief report and is not discussed here but reported in appendix 9.5. A second analysis was conducted to evaluate trends of RAL and EVG use over time (section 6.5.1), and to describe the prevalence of CAs in the exposed fetus (section 6.5.2) from the same population analysed in Chapter 4, i.e. the 12,099 pregnancies reported to the NSHPC by 31$^{st}$ December 2018.

### 6.5.1 Trends of RAL and EVG use over time, 2008-18 (objective 2a)

***Pregnancies exposed to RAL***

Of the 12,099 singleton pregnancies reported to the NSHPC, there were 978 (8.1%) exposed to RAL-containing regimen from 894 women. Table 6.13 reports key maternal characteristics for the reported RAL-exposed pregnancies. The median age at conception was 34.0 years (q1=29.4, q3=37.9, IQR). The majority of pregnancy (66.8%) were to Black African women, 93.7% of whom were born in SSA (613/654). The majority (86.6%) of women acquired HIV heterosexually, while 3.4% were infected vertically and 1.7% via IUD.

For the vast majority (73.5%) of pregnancies, maternal HIV diagnosis was made prior to conception, with the proportion of women knowing their diagnosis before pregnancy increasing from 0.1% (1/1,066) in 2008 to 13.2% (83/629) in 2018 (test-for-trend $p$<0.001).

Overall baseline median CD4 cell count was 440.0 cell/mm$^3$ (q1=290.0, q3=63.5, IQR), with 47 missing data. Overall, the proportion of women with effective VL suppression (≤400 copies/μL) near delivery was 67.9% (664/978), of whom 83.0% (551/664) with values of VL ≤50 copes/μL

**Table 6.13 Maternal characteristics for the pregnancies exposed to RAL-based regimen reported to the NSHPC, 2008-18**

| Maternal characteristics (*n* =978) | | | | | |
|---|---|---|---|---|---|
| **Ethnicity** | | | | | |
| Black African<br>654 (66.8%) | Black Other<br>22 (2.2%) | White<br>245 (25.1%) | Other<br>57 (5.8%) | | |
| **Region of birth** | | | | | |
| SSA<br>625 (63.9%) | UK/Ireland<br>185 (18.9%) | Europe<br>96 (9.8%) | Elsewhere<br>56 (5.7%) | Missing<br>16 (1.6%) | |
| **HIV acquisition route** | | | | | |
| Heterosexual<br>847 (86.6%) | IDU<br>17 (1.7%) | VT<br>33 (3.4%) | Other<br>11 (1.1%) | Missing<br>70 (7.2%) | |
| **Parity** | | | | | |
| Nulliparous<br>236 (24.1%) | 1<br>267 (27.3%) | 2<br>193 (19.7%) | 3<br>147 (15.0%) | ≥4<br>135 (13.8%) | |
| **Age at conception grouped (years)** | | | | | |
| ≤ 25<br>87 (8.9%) | 25-30<br>174 (17.8%) | 30-35<br>293 (29.9%) | 35-40<br>285 (29.1%) | 40-45<br>117 (11.9%) | ≥45<br>22 (2.2%) |
| **Timing of HIV diagnosis** | | | | | |
| Before pregnancy<br>719 (73.5%) | | During pregnancy<br>259 (26.5%) | | | |
| **VL at delivery, copies/μL (within 30 days of delivery)** | | | | | |
| Detectable<br>10 (3.4%) | Undetectable<br>199 (68.6%) | Missing<br>81 (27.9%) | | | |

*Time of exposure*

The proportion of pregnancies with first exposure to RAL from before periconception was 54.7% (535/978), and the proportion of those exposed from T2-T3 was 45.3% (443/978) (Table 6.14). Over time the proportion of pregnancies exposed to RAL significantly increased from only 0.1% (1/1,279) in 2008 to 17.3% (109/629) in 2018 (test-for-trend $p$<0.001); and most of the pregnancies in 2018 had been exposed to RAL prior to conception 61.5% (67/109).

Furthermore, among women knowing their diagnosis from before pregnancy, the proportion of those already on a cART containing RAL was 64.1% (461/719); this proportion increased over time, from 0.1% (1/1,319) in 2009 to 10.6% (67/629) in 2018 (test-for-trend $p$<0.001).

Overall, there were 936 (95.7%) singleton liveborn pregnancies, nine (1%) ended in stillbirths, 24 (2.4%) in miscarriages and nine (1%) in terminations. Table 6.14 shows pregnancy outcomes stratified by time of exposure to RAL-based regimen.

**Table 6.14 Singleton pregnancy outcomes reported to the NSHPC between 2008-18, by earliest RAL exposure**

| | Total RAL exposed | Earliest exposure to RAL | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **Tot outcomes, N** | 978 | 535 (54.7%) | 443 (45.3%) |
| **Livebirths** | 936 (95.7%) | 504 (94.2%) | 432 (97.5%) |
| **Stillbirths** | 9 (1%) | 4 (0.8%) | 5 (1.1%) |
| **Miscarriages** | 24 (2.4%) | 21 (3.9%) | 3 (0.7%) |
| **Terminations** | 9 (1%) | 6 (1.1%) | 3 (0.7%) |

*Pregnancies ending in delivery: livebirths and stillbirths (n=945)*

Of the 945 singleton pregnancies there were 443 females and 476 males (26 infants with missing data). The overall proportion of women delivering live- and still-born infants knowing their HIV diagnosis prior to becoming pregnant was 73.0% (690/945) and among them the proportion of those also on a RAL-based regimen before becoming pregnant was 63.3% (437/690).

Over the years, the proportion of pregnancies from women whose HIV diagnosis was made prior to conception increased from 0.1% (1/1,279) in 2008 to 12.7% (80/629) in 2018 (test-for-trend $p<0.001$). Furthermore, rates of pregnancies in women knowing their HIV diagnosis and on a RAL-based regimen from before periconception rose from 0.3% (4/1,319) in 2009 to 10.3% (65/629) in 2018 (test-for-trend $p<0.001$).

Overall, for 651 women undetectable VL (≤400 copies/μL) near delivery was reported, of whom 83.1% (541/651) with values of VL ≤50 copies/μL (Table 6.15). Of the women who started RAL before conception with available data on VL at time of delivery, 95.4% (350/367) had effective suppressed VL, while of the women starting RAL during pregnancy 84.3% (301/357) reported undetectable VL, underscoring the effectiveness of RAL in reducing VL even when is started in pregnancy.

The majority of women with detectable VL had elective CS as per national guidelines, while the relatively high rates of emergency CS might reflect preterm labour and concerns about the risk for VT. Rates of elective CS are still relatively high among women with undetectable VL, possibly reflecting obstetric indications/maternal choice.

**Table 6.15 RAL initiation and mode of delivery for live- and still-born infants, reported to the NSHPC 2008-18, by maternal VL near delivery**

| | Maternal VL near delivery | | | Total |
|---|---|---|---|---|
| | Undetectable N= 651 | Detectable N=73 | Missing N=221 | N=945 |
| **Timing of RAL initiation** | | | | |
| Before pregnancy | 350 (53.7%) | 17 (23.3%) | 141 (63.8%) | 508 (53.7%) |
| During pregnancy | 301 (46.2%) | 56 (76.7%) | 80 (36.2%) | 437 (46.2%) |
| **Mode of delivery** | | | | |
| Vaginal | 251 (38.5%) | 5 (6.8%) | 94 (42.5%) | 350 (37.0%) |
| Elective CS | 227 (34.9%) | 46 (63.0%) | 60 (27.2%) | 333 (35.2%) |
| Emergency CS | 169 (26.0%) | 22 (30.2%) | 52 (23.5%) | 243 (25.7%) |
| Missing | 4 (0.6%) | 0 | 15 (6.8%) | 19 (2.0%) |

*Gestational age and birthweight in liveborn infants*

Overall, the median gestation age was 39 GW (q1=38, q3=40, IQR). Almost all infants had a weight at birth of 2.5kg or more (760/838) (Table 6.16) and over half of these were exposed to RAL-based regimen from before periconception (409/760).

**Table 6.16 Neonatal outcomes for the singleton livebirths pregnancies exposed to RAL reported to the NSHPC, 2008-18**

| | Total RAL exposed | Earliest exposure to RAL | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **Total, N** | 936 | 504 (53.8%) | 432 (46.2%) |
| **Gestational age** | | | |
| <34 weeks | 38 (4.1%) | 22 (4.4%) | 16 (3.7%) |
| 34-36 weeks | 60 (6.4%) | 32 (6.3%) | 28 (6.5%) |
| ≥37 weeks | 838 (89.5%) | 450 (89.3%) | 388 (89.8%) |
| Missing | 0 | 0 | 0 |
| **Birth weight (g)** | | | |
| <1500 | 18 (1.9%) | 10 (2.0%) | 8 (1.8%) |
| 1500-2499 | 73 (7.8%) | 36 (7.1%) | 37 (8.6%) |
| ≥2500 | 800 (85.5%) | 433 (86.0%) | 367 (85.0%) |
| Missing | 45 (4.8%) | 25 (4.9%) | 20 (4.6%) |

### *Pregnancies exposed to EVG*

There were 66 (0.5%) singleton pregnancies exposed to EVG-based regimens in 60 women of the 12,099 reported to the NSHPC in the study period. The median age at conception was 35.2 years (q1=30.4, q3=38.4, IQR). The majority of pregnancies were to Black African women, 97.4% of whom were born in SSA (37/38). Most women acquired HIV heterosexually and for almost all women HIV diagnosis was made before conception, with the proportion of women knowing their diagnosis before conception increasing from 0.1% (1/10,66) in 2014 to 3.9% (25/629) in 2018 (test-for-trend $p$<0.001) (Table 6.17).

Baseline median CD4 cell count was 508.0 cell/mm$^3$ (q1=391.0 q3=637.0, IQR), with five missing data. The proportion of women with effective suppression of VL (≤400 copies/µL) near delivery was 71.2%, of whom 93.6% (44/47) had values below 50 copies/µL.

**Table 6.17 Maternal characteristics for the pregnancies exposed to EVG-based regimen reported to the NSHPC, 2008-18**

| **Maternal characteristics (*n* =66)** | | | | | |
|---|---|---|---|---|---|
| **Ethnicity** | | | | | |
| Black African<br>38 (57.6%) | Black Other<br>2 (3.0%) | White<br>21 (31.8%) | Other<br>5 (7.6%) | | |
| **Region of birth** | | | | | |
| SSA<br>37 (56.1%) | UK/Ireland<br>16 (24.2%) | Europe<br>7 (10.6%) | Elsewhere<br>5 (7.6%) | Missing<br>1 (1.5%) | |
| **HIV acquisition route** | | | | | |
| Heterosexual<br>59 (89.4%) | IDU<br>0 | VT<br>1 (1.5%) | Other<br>1 (1.5%) | Missing<br>5 (7.6%) | |
| **Parity** | | | | | |
| Nulliparous<br>11 (16.7%) | 1<br>16 (24.2%) | 2<br>13 (19.7%) | 3<br>11 (16.7%) | ≥4<br>15 (22.7%) | |
| **Age at conception grouped (years)** | | | | | |
| ≤ 25<br>4 (6.1%) | 25-30<br>12 (18.2%) | 30-35<br>17 (25.7%) | 35-40<br>19 (28.8%) | 40-45<br>11 (16.7%) | ≥45<br>3 (4.5%) |
| **Timing of HIV diagnosis** | | | | | |
| Before pregnancy<br>64 (97.0%) | | | During pregnancy<br>2 (3.0%) | | |
| **VL at delivery, copies/µL (within 30 days of delivery)** | | | | | |
| Detectable<br>1 (1.5%) | | Undetectable<br>47 (71.2%) | | Missing<br>18 (27.3%) | |

*Time of exposure*

The majority (87.8%) of singleton pregnancies with exposure to an EVG-based regimen were in women starting this before conception, with the remaining 12.1% exposed in T2-T3 (Table 6.18). Over time the proportion of pregnancies exposed to EVG significantly increased from only 0.1% (1/1,066) in 2014 to 3.9% (25/629) in 2018 (test-for-trend $p$<0.001), as did the proportion of women aware of their diagnosis from before conception, and already on an EVG-based regimen, increasing from 0.1% (1/1,066) of pregnancies in the NSHPC in 2014 to 3.8% (24/629) in 2018 (test-for-trend $p$<0.001).

Overall, there were 60 (91.0%) singleton liveborn pregnancies, three (4.5%) ending in miscarriage, and three (4.5%) in terminations. Table 6.18 shows pregnancy outcomes stratified by time of exposure to EVG-based regimen.

**Table 6.18 Singleton pregnancy outcomes reported to the NSHPC between 2008-18, by earliest EVG exposure**

| | Total EVG exposed | Earliest exposure to EVG | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **Tot outcomes, N** | 66 | 58 (87.8%) | 8 (12.1%) |
| **Livebirths** | 60 (91.0%) | 52 (89.6%) | 8 |
| **Miscarriages** | 3 (4.5%) | 3 (5.2%) | 0 |
| **Terminations** | 3 (4.5% | 3 (5.2%) | 0 |

*Singleton liveborn pregnancies*

Almost all of the 60 liveborn infants (32 female, 28 male) had periconception exposure to EVG (Table 6.18). The 60 infants were born at a median gestational age of 39 GW (q1=37.7, q3=39, IQR), with 11.7% born preterm (7/60). Looking at mode of delivery, 48.3% (29/60) of pregnancies were delivered vaginally, 33.3% (20/60) via elective CS and 18.3% (11/60) via emergency CS. Overall, 15.0% (9/60) of infants had a low birthweight; among 53 infants born at term, four had a low birthweight (7.5%).

### 6.5.2 Evaluation of CAs following *in utero* exposure to RAL and EVG (objective 2b)

***Prevalence of CAs by time of exposure to RAL and EVG and by type of CA***

Of the 1,044 pregnancies exposed to RAL and EVG with known pregnancy outcomes, there were 21 infants with identified CAs meeting EUROCAT criteria. Looking at exposure to these agents singularly, there were 18 liveborn infants exposed to RAL reported to have a CA, giving a prevalence of 1.92% (95% CI 1.14, 3.02). For the remaining pregnancy outcomes no CAs were reported.

When stratifying by time of exposure to RAL-based regimen, of the 504 liveborn infants with periconception exposure, 14 presented with a CA, a prevalence of 2.78% (95% CI 1.53, 4.62); while among the 432 liveborn infants exposed from T2-T3, four reported a CA, a prevalence of 0.9% (95% CI 0.25, 2.35) (Table 6.19). No multiple CAs nor particular patterns of CA affecting the same organ/system with periconception exposure to RAL were observed and no detection of NTDs reported. However, there were two nervous system anomalies: one microcephaly in a liveborn infant and one hydrocephaly in a reported neonatal death of an infants delivered preterm (at 30 GW) (Table 6.19).

Looking at pregnancies exposed to EVG-based regimens, there were three pregnancies with a CA, giving a prevalence of 4.55% (95% CI 0.95, 12.71); two of these were in liveborn infants and one in a termination of pregnancy (Table. 6.20). Stratifying by time of exposure to EVG, one infant was exposed from the periconception period and one in T2-T3. There was no report of multiple CAs nor of NTDs, but one nervous system anomaly, namely microcephaly was reported. The termination of pregnancy occurred at 14 GW, in a pregnancy with periconception exposure to EVG, following identification of the chromosomal anomaly trisomy 18, (Table 6.20).

**Table 6.19 Distribution of CAs by EUROCAT organ/system criteria in liveborn infants reported to the NSHPC 2008-18, by timing of RAL exposure**

| Organ system classification | Total, *n* | Earlies exposure to RAL | |
|---|---|---|---|
| | | Periconception | T2-T3 |
| **All anomalies** | **18** | **14** | **4** |
| **Nervous system** | **2** | | |
| Hydrocephalus[1] | | 1 | 0 |
| Microcephaly | | 0 | 1 |
| **Congenital heart defects** | **5** | | |
| Atrial septal defect (ASD) | | 1 | 0 |
| Ventricular septal defect (VSD) | | 2 | 0 |
| Ebstein's anomaly | | 1 | 0 |
| Total anomalous pulmonary venous return | | 1 | 0 |
| **Respiratory** | **1** | | |
| Congenital Cystic Adenomatoid Malformation (CCAM) | | 0 | 1 |
| **Oro-facial clefts** | **2** | | |
| Cleft palate and/or hare lip | | 2 | 0 |
| **Digestive** | **1** | | |
| Duodenal atresia and stenosis | | 0 | 1 |
| **Urinary** | **2** | | |
| Multicystic dysplastic kidney | | 0 | 1 |
| Other cystic kidney disease | | 1 | 0 |

| | | | |
|---|---|---|---|
| **Abdominal wall defects** | **1** | | |
| Omphalocele | | 1 | 0 |
| **Limb** | **1** | | |
| Club foot-talipes equinovarus | | 1 | 0 |
| **Other anomalies/syndromes** | **1** | | |
| Congenital constriction bands/amniotic bands | | 1 | 0 |
| **Chromosomal** | **2** | | |
| Down's syndrome | | 2 | 0 |

[1]Neonatal/infant deaths

**Table 6.20 Distribution of CAs by EUROCAT organ/system criteria in pregnancies reported to the NSHPC 2008-18, by timing of EVG exposure**

| Organ system classification | Total, N | Earliest exposure to EVG | |
|---|---|---|---|
| | | At conception | T2-T3 |
| **All anomalies** | **3** | **2** | **1** |
| **Nervous system** | **1** | | |
| Microcephaly | | 0 | 1 |
| **Chromosomal** | **2** | | |
| Down's syndrome | | 1 | 0 |
| Edward syndrome/ Trisomy 18 | | 1 | 0 |

## 6.6 Modelling risk for CAs in infants exposed to INSTIs (objective 3)

In chapter 5 the association between use of ARVs, timing of their initiation and the probability of CA was investigated through a multivariable logistic regression model.

As already described in section 5.2.4 no statistically significant association between CAs and exposure to ARV by class was found, with the exception of the INSTIs (OR= 1.67, 95% CI 1.00- 1.72, *p*=0.05) (Table 5.12, chapter 5). However, when assessing the interaction effects, (i.e. the joint effect of exposure to specific ARV class and timing of this first exposure), being exposed to INSTIs from periconception time reduced the risk for CAs by approximately 20% with the absolute baseline (i.e. not being exposed to INSTIs and not being exposed from periconception time). Therefore, I conducted a focused statistical analysis on the use of INSTIs as described below.

*Special methods:*

The association between exposure to INSTIs, time of this exposure and the probability of developing CAs was assessed using cross-tabulation tables and fitting a logistic regression model. The unit of analysis for the model were all liveborn infants with *in utero* exposure to a cART containing INSTIs or not containing INSTIs (i.e. exposed to one of the other third agents) reported by 31$^{st}$ December 2018 (i.e. *n*=11,197).

For this model, the risk factors that were statistically significant in the original bivariable analyses reported in section 5.2.3, i.e. maternal age at delivery ( <35 years, ≥35 years) and time of first exposure to INSTIs (periconception vs later in pregnancy) were included. Also here, the best model was investigated using Bayesian information criterion (for more details see appendix 9.4).

***Results:***

*Observed Probability of CAs*

The number of pregnancies exposed to INSTIs vs exposure to any other ARVs stratified by time of first exposure is reported in Table 6.21. There were 1,232 singleton liveborn pregnancies exposed to INSTIs-based regimen, of which 53.5% (659/1,232) were exposed from the periconception period, and 9,965 pregnancies were exposed to other regimens not containing INSTIs, 58.1% (5,794/9,965) of which started in the periconception period.

**Table 6.21 Pregnancies ending in livebirths exposed/not exposed to INSTIs, by time of first exposure**

| | Time of first exposure | | |
|---|---|---|---|
| **Exposure to INSTIs** | Preconception | Pregnancy | total |
| **No** | 5,794 | 4,171 | 9,965 |
| **Yes** | 659 | 573 | 1,232 |
| **Total** | 6,453 | 4,744 | 11,197 |

I first looked at the probability of having a CA in relation to the time of first exposure to any ARV class. The probabilities of CA by first exposure to any ARV class were 2.17% (95% CI 1.84, 2.56) when first exposure occurred from preconception and 1.83% (95% CI 1.49, 2.23) when ARVs were started in pregnancy (Table 6.22).

**Table 6.22 Liveborn infants with/without CAs, by time of exposure to any ARVs**

| | Time of first exposure | | |
|---|---|---|---|
| **Congenital anomalies** | Preconception | Pregnancy | total |
| **No** | 6,313 | 4,657 | 10,970 |
| **Yes** | 140 | 87 | 227 |
| **Total** | 6,453 | 4,744 | 11,197 |

The probability of having a CA with exposure to INSTIs vs any other class of ARV was investigated, firstly regardless of the time of exposure (Table 6.23) and then taking into consideration timing of first exposure (Table 6.24 and Table 6.25).

The probability of developing a CA with exposure to any class of ARVs was found to be 2.01% (95% CI 1.75, 2.30), which is close to the overall CA prevalence of 2.03%. Being exposed to INSTIs at any time was found to increase the probability of CA to 2.19% (95% CI 1.51, 3.17). The difference between these two probabilities shows an increased risk in developing a CA as a result of exposure to INSTIs (i.e. 2.19% - 2.01% = 0.18%).

**Table 6.23 Liveborn infants with/without a CA, exposed or not to INSTIs**

| | Exposure to INSTI | | |
|---|---|---|---|
| **Congenital anomalies** | No | Yes | total |
| **No** | 9,765 | 1,205 | 10,970 |
| **Yes** | 200 | 27 | 227 |
| **Total** | 9,965 | 1,232 | 11,197 |

Table 6.24 is restricted to infants with preconception exposure to any cART, stratified by receipt of INSTIs vs any other ARV class; a larger probability for CAs was found with exposure to INSTIs, 3.34% (95% CI 2.21, 5) than with exposure to any other ARV class, 2.04% (95% CI 1.70, 2.43).

**Table 6.24 Presence of a CA by exposure to INSTIs among liveborn infants with first exposure to ART from preconception**

| | Exposure to INSTI | | |
|---|---|---|---|
| **Congenital anomalies** | No | Yes | Total |
| **No** | 5,676 | 637 | 6,313 |
| **Yes** | 118 | 22 | 140 |
| **Total** | 5,794 | 659 | 6,453 |

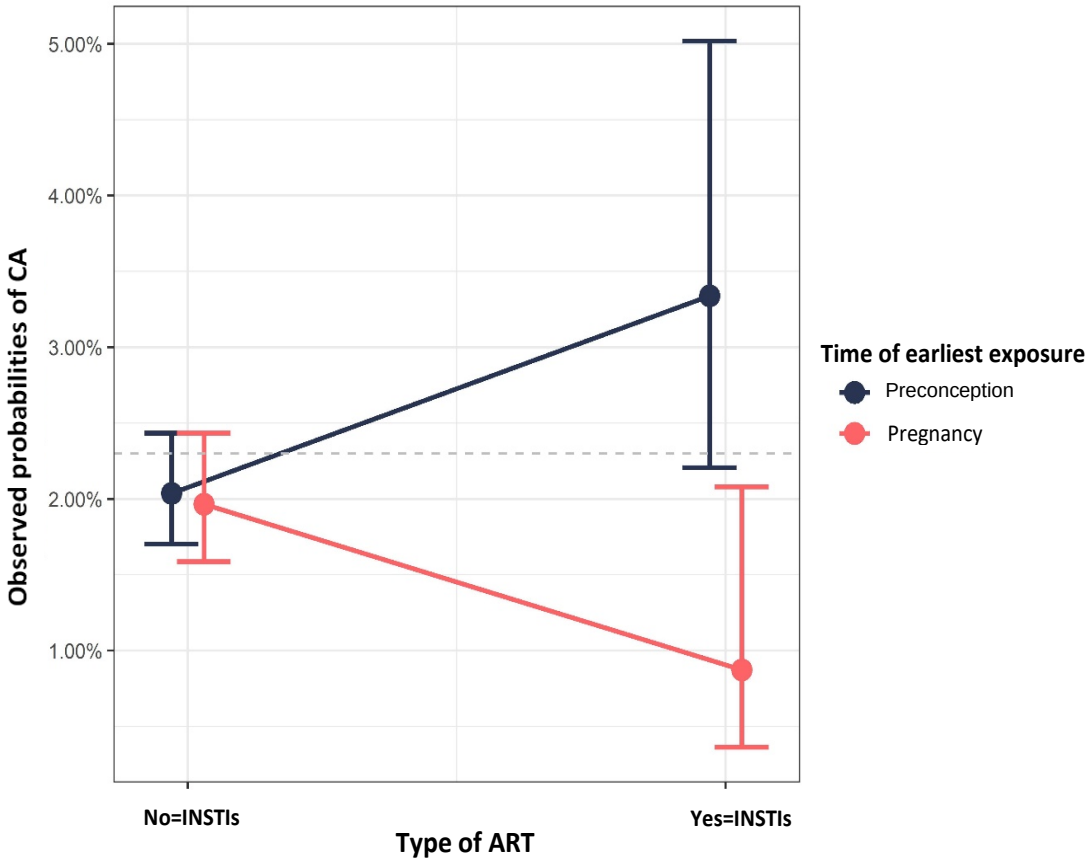
On the contrary, among infants whose mothers started cART during pregnancy the observed probability for CA was 0.87% (95% CI 0.37, 2.03) among the INSTI group, smaller than the probability observed among infants whose mothers started cART with any other class of ARV during pregnancy (1.97%; 95% CI 1.59, 2.43 vs 0.87%; 95% CI 0.37, 2.03) (Table 6.25).

**Table 6.25 Presence of a CA by exposure to INSTIs among liveborn infants with first exposure to ART in pregnancy**

| | Exposure to INSTI | | |
|---|---|---|---|
| **Congenital anomalies** | No | Yes | total |
| **No** | 4,089 | 568 | 4,657 |
| **Yes** | 82 | 5 | 87 |
| **Total** | 4,171 | 573 | 4,744 |

Figure 6.3 includes as covariates both exposure to INSTIs (versus any other ARV class) and the timing of first exposure. When exposure to any ARV class other than INSTIs (No=INSTIs) occurred there was no difference in the probability of developing a CA, regardless of the time of first exposure. In other words, the probability for an infant to have a CA when exposed to non-INSTI-based cART is close to the overall observed prevalence of CA of 2.03 (expressed by the dotted line).

On the contrary, when looking at exposure to INSTIs (Yes=INSTIs) the time of first exposure makes a significant difference: first exposure to INSTIs from preconception increases the probability to develop a CA, while first exposure to INSTIs later in gestation shows a marked decrease in the probability to develop a CA.




Dotted line shows overall prevalence of CA in the population

**Figure 6.3 Observed probabilities of CAs by exposure and time of earliest exposure to INSTIs among liveborn infants**

Given the important findings and the interpretation of Figure 6.3, I decided to investigate further the interaction between exposure to INSTIs, intercepted by time of exposure, and the probability of developing a CA adjusting for other covariates, e.g. maternal age at delivery. To do so, a logistic regression models was fitted, and the results are described below.

*Logistic regression models*

The results from the adjusted risk factor analyses are presented in Table 6.26. Infants whose mothers were aged ≥35 years were found at a statistically significant higher risk of CA than those aged <35 years at delivery ($p$=0.04). Exposure to INSTIs increased by 1.46 times the odds of having a CA compared to not being exposed to INSTIs, though not statistically significant ($p$=0.11).

I then looked at the interaction effects, i.e. the joint effect that being exposed to INSTIs and that timing of first exposure to INSTIs have on the risk for CAs which is reported in Table 6.26. Similarly to what already explained in chapter 5 section 5.2.4, in order

to obtain the actual measure of the risk for CAs, the three aORs need to be multiplied. The aOR of exposure to INSTIs was 1.46 and the aOR of first exposure from preconception period to INSTIs was 1.01; while 0.35 is the multiplier to the ORs of exposure to INSTIs and first exposure from periconception period when both are present, hence the effect of the interaction was 0.52 (95% CI 0.17, 1.48) (1.46*1.01*0.35=0.52) in OR scale. This means that when exposure to INSTIs occurred around the preconception period the risk for CA was reduced by approximately 48% with respect to the absolute baseline (i.e. not being exposed to INSTIs and not being exposed from periconception).

**Table 6.26 Risk factors for CAs in pregnancies ending in livebirths exposed to INSTIs**

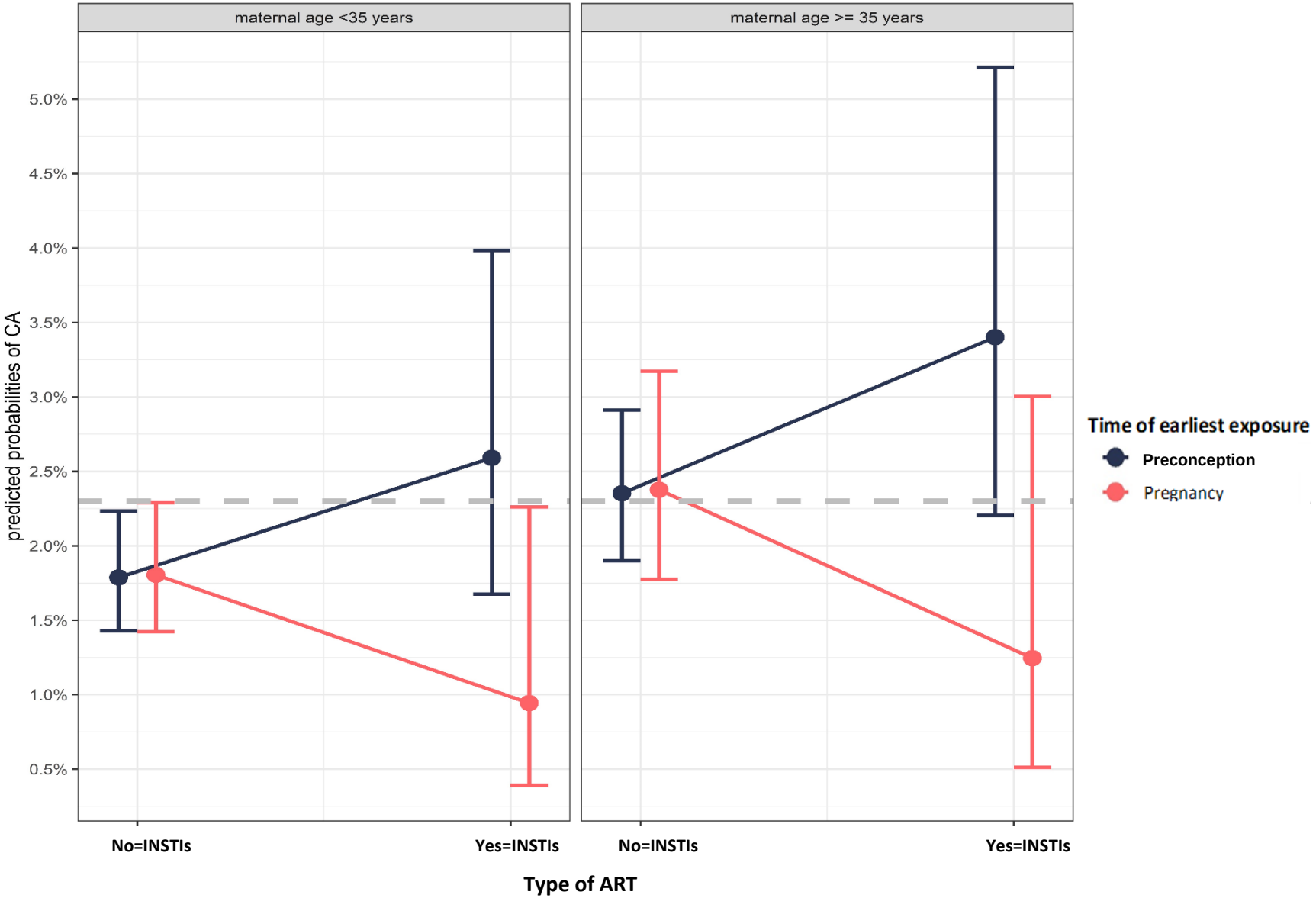
| Explanatory variables | aOR | 95% CI | *p*-value |
|---|---|---|---|
| **Maternal age at delivery (years)** | | | |
| <35 | Baseline | | |
| ≥35 | 1.32 | 1.01 - 1.73 | 0.04* |
| **ARV class as third agent** | | | |
| Any other ARV | Baseline | | |
| INSTIs | 1.46 | 0.90 - 2.27 | 0.11 |
| **Time of earliest exposure to INSTIs** | | | |
| Later in pregnancy | Baseline | | |
| Periconception | 1.01 | 0.75 - 1.35 | 0.95 |
| **Time of earliest exposure*INSTIs** | | | |
| INSTIs | 0.35 | 0.11 - 0.92 | 0.04* |

**p*-value reaches the level of significance (<0.05); aOR: adjusted odds ratio, CI confidence interval

It is interesting to note how the interaction between exposure to INSTIs and being exposed from the preconception period to INSTIs had a similar value to that in the model presented in chapter 5 (exploring ARVs by class), i.e. a difference in OR of 0.28 (95% CI 0.09, 0.77; $p$=0.02) and of OR of 0.35 (95% CI 0.11, 0.92; $p$=0.04), respectively.

A further analysis was carried out in light of the results from the observed probabilities of CAs and those reported in chapter 5, i.e. the fact that being exposed to INSTIs was associated with a slight increase in the risk of CA but being exposed to INSTIs from periconception period seemed to reduce the risk for CA. A possible interpretation for this is that a substantial proportion of the INSTIs were taken or were started later in pregnancy and therefore women might have conceived on some other third agents, and then over time these trends might have changed. Thus, I have evaluated the impact of calendar time of deliveries as a potential contributing factor to the risk of CA. This was evaluated with deliveries grouped into those occurring in the years before versus after the licencing of DTG (i.e. 16/01/2014). However, from univariable unadjusted analysis, there was no statistically significant difference in the risk of CAs when considering this calendar period variable (OR 1.30; 95% CI 0.98, 1.70; $p$=0.06 for deliveries before DTG licensing versus after).

In Figure 6.4 the predicted probabilities from the logistic regression model presented in Table 6.26 are shown in relation to maternal age at delivery. The four points in each panel correspond to the predicted probabilities of developing CA in relation to exposure to INSTIs and timing of first exposure, while the line next to the four points represents the 95% prediction (confidence) interval. The blue and pink lines identify first exposure to INSTIs from preconception and during pregnancy, respectively.




Dotted line shows overall prevalence of CA in the population

**Figure 6.4 Predicted probabilities of CA adjusted for maternal age at delivery, first exposure and exposure to INSTIs among liveborn infants with in utero exposure to ARVs reported to the NSHPC, 2008-18**

## 6.7 Key points

**Evaluation over time of available safety and efficacy data on INSTIs from the EMA**

- At the start of the study period no INSTI formulations had reports of any specific toxicities from preclinical studies, limited data on human pregnancy from clinical studies, and no reports on PK-PD data in pregnancy. By the end of the period, recommendations for DTG both for women of childbearing age and those pregnant had changed, recommending to avoid DTG use during T1 "*unless there is no alternative",* and in T2-T3, due to the risk for NTDs.
- EVG recommendations also become more restrictive advising for avoidance of EVG during pregnancy; to switch to another regimen for women becoming pregnant; and to comply with contraceptive methods for women of childbearing age, due to reported reduced EVG/c concentrations,
- On the contrary, RAL recommendations become more permissive, due to the moderate amount of accumulated data in pregnancy not suggesting any malformative nor fetal/neonatal toxicity.

**DTG analyses in the NSHPC**

- First analysis on DTG reported an increase in the proportion of pregnancies exposed to DTG over time (2014-17) and an increased number of women conceiving whilst taking DTG, with no reports of NTDs. This analysis also identified a discordance between real-world use of DTG (substantially increasing over time) and regulatory recommendations (restrictive and with warnings to avoid its use).
- Second analysis (2008-18) reported 84% of pregnancies with DTG use were conceived on DTG, with this proportion having significantly increased over time (test-for-trend $p<0.001$). Most of the pregnancies resulted in livebirths (91.7%), of which the majority had periconception exposure to DTG. There were nine CAs, giving a prevalence of 3.38% (95% CI 1.56, 6.32), with no reports of NTDs.

**DTG in the Dolomite-EPPICC study**

- To date this is the largest European study on DTG use in pregnancy; data were collected on 453 pregnancies from six cohorts, of which 70% had periconception exposure to DTG.

- There was a total of 14 CAs meeting EUROCAT criteria, a prevalence of 3.36% (95% CI 1.85, 5.57), with no reports of NTDs nor of CAs affecting the same organ/system by the “rule of three”. One infant had multiple defects and there was no report of CAs for pregnancies ending in stillbirth or spontaneous abortion, while one induced abortion was carried out due to an identified CA.

**The other INSTIs: RAL and EVG**

- Over half of all pregnancies with use of RAL-based regimens had exposure from the periconception period, with this significantly increasing over time ($p$<0.001). By 2018, one in six pregnancies in women with HIV in the UK were exposed to RAL.
- Among women who started RAL during pregnancy, for 84% undetectable VL by delivery was reported, underscoring the effectiveness of RAL in reducing VL even when stared in pregnancy.
- EVG was rarely used in pregnancy – although there was a significant increase from 2014, by the end of 2018, less than 4% of all pregnancies were exposed; of the total of 66 pregnancies exposed to EVG, 88% were in women on EVG from before conception.
- Among liveborn infants with *in utero* exposure to RAL or EVG, 1.92% (95% CI 1.14, 3.02) and 4.55% (95% CI 0.95, 12.71) respectively had a CA. For both agents there was no report of NTDs, with no specific patterns of CA for RAL-exposed infants; for EVG-exposed infants the small numbers preclude any meaningful interpretations.

**Risk for CAs in infants exposed to INSTIs**

- There was an increased observed probability of CA with any exposure to INSTIs vs any other class of ARVs as third agent (2.19% vs 2.01%, respectively). Stratifying by time of first exposure, there was a higher probability of CA with preconception exposure to INSTIs vs to any other ARV class (3.34% vs 2.04%) and a lower probability of CA with exposure to INSTIs vs any other class of ARV during pregnancy (0.87% vs 1.97%).
- Results from the adjusted risk factor analysis showed that infants whose mothers were aged ≥35 years at delivery were at higher risk of developing a CA than those delivered from mother aged <35 years (p=0.04).
- From the interaction effect of being exposed to INSTIs and time of first exposure, a reduced risk for CA of approx. 48% was found when first exposure to INSTIs occurred in the periconception period compared with not being exposed to INSTIs and not being exposed from preconception period.

# 7 Discussion

## 7.1 Introduction

This thesis explores a number of issues pertinent to the contemporary management of pregnant women living with HIV in terms of safe and effective use of available ARVs.

In this final chapter I will integrate my findings with those from other studies, evidencing inconsistencies between real-world use of ARVs in pregnancy, guidelines and regulatory recommendations, and suggest how to fill the gaps and improve access to more safe and effective treatment for pregnant and breastfeeding women and those of childbearing age.

I will discuss the current paradigm where pregnant and non-pregnant women are regularly excluded from registrational drug trials with the consequent lack of safe and effective data on use in these populations. I will review existing proposed options along with my own to fill the current gap.

The chapter is structured into a first section presenting an overview of the current situation (section 7.2); a section about what the DTG safety signal has taught us (section 7.3); followed by a discussion of the proposed actions to shift the current paradigm (section 7.4), and finally a conclusion section where I will draw my final conclusions and provide an overview of ongoing and future work to move into the new paradigm.

## 7.2 Status quo

Access to safe and effective cART is key to prevent and reduce VT and maternal disease progression (PHASES 2020). However, pregnancy-related changes and sex related differences might alter drugs' efficacy and safety. These are not properly studied and there is paucity of these essential data because being pregnant is often an exclusion criterion for enrolment in registrational trials (Sheffield et al. 2014, Abrams et al. 2020, Eke et al. 2020). Therefore, pregnancy data are usually limited at the time of a drug's approval. Most of data on pregnant and breastfeeding women are

carefully extrapolated from pre-clinical reproductive toxicities studies from animal models and from post-marketing phase when ARV combinations have been already approved and widely used in the general population. However, data extracted from pre-clinical reproductive toxicities studies are not always applicable to the complex pregnancy-related changes in humans. Consequently absence of reproductive toxicities/teratogenic effects in animal models cannot completely predict the absence of such in humans; equally the identification of reproductive toxicities/teratogenic effects in animal models does not mean that a toxic/teratogenic effects in humans might be expected (Carney et al. 2011, Mofenson et al. 2019a).

Furthermore, often completion of pre-clinical reproductive and toxicities studies are not required until phase III CTs have started and therefore these data are not available until late in the drug's development (Mofenson et al. 2019a, Abrams et al. 2020). My findings presented in chapter 4, demonstrate this by showing how, of the 27 ARVs with an EU marketing authorisation, only half had information in their SmPC reports with respect to teratogenic effects from animal models.

Therefore, post-marketing studies represent the main source of data on ARVs use in pregnancy, also because data on rare defects and toxicities such as teratogenicity will inevitably be detected only by post-marketing studies, given that preclinical studies and CTs cannot provide the necessary amount of data. However, the current conduct of post-marketing studies inevitably results in delays in the availability of safety and efficacy data. This was demonstrated by the gap-analysis in chapter 4 that showed 75% of the newly authorised ARVs (i.e. those with a marketing authorisation obtained since 2014) lack adequate or well-controlled studies in pregnant women.

### 7.2.1 PK-PD studies

Pregnancy-related changes greatly alter both the maternal and feto-placental interface and the maternal PK process of drug's ADME (as already described in chapter 1 and 2). For women living with HIV the physiological changes induced by pregnancy can also profoundly alter the PK of ARVs resulting in lower concentration and reduced exposure. This potentially increases the risk for treatment failure and as a consequence may increase the risk for VT, for maternal HIV diseases progression and for HIV-drug resistance development (Ngarina et al. 2015, Onoya et al. 2017, Eke et al. 2019, Eke et al. 2019). ARV's transplacental transfer and breast milk concentration are also important determinants of fetal exposure to drugs; surrogate markers to evaluate both the risk for embryo-fetal developmental disruption and the

risk for infant's exposure through the milk to toxic drug effects should be assessed, but often are not evaluated in either animal models or CT.

Currently, PK-PD studies are mostly conducted in the post-marketing phase and data are generated from small post-approval opportunistic PK studies (i.e. enrolling pregnant and breastfeeding women already on a treatment of interest into a PK study mostly performed by academic independent research groups) (Eke et al. 2019, Eke et al. 2019). As mentioned in chapter 2, there are two main ongoing PK studies in pregnant women living with HIV, both open-label, parallel-groups, multicentre with opportunistic design studies, namely PANNA and IMPAACT. From these studies it was recently found that ARVs co-administered with COBI had a marked reduction in their plasma concentration due to very low concentrations of COBI in pregnant women taking the drug at standard doses (i.e. the dosage used for the general non-pregnant population) (Colbers et al. 2014, Crauwels et al. 2016, Colbers et al. 2017, Colbers et al. 2019, Crauwels et al. 2019). This provides an example of the risk of not conducting early PK-PD studies that include pregnant women, i.e. leaving women for years exposed to underdosing also potentially increasing the risk for maternal HIV disease progression and for VT. Therefore, even though in this particular case regulators promptly changed their recommendations (as reported in chapter 4), showing an encouraging step toward a proactive pharmacovigilance, these still came with too many years of delay. Furthermore, there was not an equally prompt request for more data on the adequate (i.e. both safe and effective), dosing for pregnant women. Hence women are once more left with the difficult choice of switching to other (older) regimens if they are planning a pregnancy or to comply with pregnancy testing and contraceptive measure to prevent the pregnancy if of childbearing age.

Colbers et al. reported a median time lag of six years between ARV's FDA approval and first published PK data in pregnancy (Colbers et al. 2019). I found the very same time lag of six years between EMA marketing approval of DRV, RPV, ATV, COBI and publication of data from PK studies on pregnant women. Additionally, I found that only 44% of the SmPC reported PK-PD studies conducted in pregnant and breastfeeding women and only 33% reported on transplacental passage, a proportion that rose to 74% only if data from the EPAR were included. Furthermore, 93% of the original SmPC (i.e. the SmPC at the time of marketing authorisation) did not have any PK-PD data on pregnant and breastfeeding women and for 53% of the original SmPC there was no mention of ARVs transplacental passage.

### 7.2.2 Detection of rare defects such as NTDs

Evaluation of the association between *in utero* exposure to ARVs and CA requires post-marketing surveillance study. This are particularly needed to detect rare defects such as NTDs given that CT cannot provide the necessary conditions, not having the necessary sample size to detect such rare events. It was estimated that to rule out a 2-fold increase in the overall risk for CAs, with a 3% prevalence in the general population, 200 preconception/early first trimester exposures are required and for rare events such as NTDs, with an approximate 0.1% prevalence (varies by countries) at least 2,000 preconception/early first trimester exposures are required to rule out a 3-fold increase in the risk (Watts 2007).

Furthermore, because disruption to the physiological organogenesis occurs within the first trimester of pregnancy (for example, with respect to NTDs, the physiological closure of the neural tube occurs within the first 28 days from the day of conception), to detect the causality between exposure to a given drug and its ability to induce the defect, women should be enrolled in CT in very early stages of their pregnancies, potentially even before conceiving and prolonging the follow-up to the post-marketing phase to reach the necessary sample size.

The current pharmacovigilance post-marketing databases such as the WHO's ViGiAccess, the FDA's AERS and the EMA's EudraVigilance do not offer much of support either due to several limitations, such as retrospective enrolment, lack of a denominator (i.e. the actual number of patient exposed to a given drug), risk for duplicates (i.e. multiple sources reporting the same event), risk for selection bias (i.e. risk to fuel false alarm/signals) and a frequent lack of collection of background information that could contribute or be a co-cause for the observed anomaly (i.e. confounders and risk factors) (Hill et al. 2019).

Therefore, most of the current surveillance systems for ARVs' safe use and detection of CA originate from registries based on spontaneous voluntary reporting. These are useful tools used mainly in HICs but limited by the long-time required to accumulate a sufficient number of observations with preconception/first trimester exposure. Alternatively data can be obtained from large, prospective observational studies, mostly conducted in LMICs. These carry limitations stemming from the challenges to collecting sufficient data in countries with lack of a systematic surveillance system for drug safety in pregnancy, with high prevalence of home-births and with insufficient training to recognise CAs (Zash et al. 2016a, Bailey et al. 2018).

***The importance of collecting all adverse pregnancy outcomes***

Most of the current registries and birth surveillance systems do not routinely collect information on early stillbirths, miscarriages or termination of pregnancies. However, CA might be the cause of such adverse pregnancy outcomes or lead to planned termination of pregnancies, therefore collection of all pregnancy outcomes (i.e. termination of pregnancy, miscarriages and stillbirths) is essential to truly understand drugs' safety and their collection should become standard procedure (Lechat et al. 1993, Zaganjor et al. 2016, Zash et al. 2016a, Mofenson 2018). For example, of the 11 NTDs reported to the NSHPC only two occurred in liveborn infants (chapter 5), information that would have been missed if collection were limited to liveborn infants leading to an underestimation of the true rates of NTDs. In addition, as noted in Chapter 4, there are some important difference in periconception exposure to cART between pregnancies that end in live- or still-births and those that end in terminations or miscarriages - i.e. the latter are more likely to have such exposure. This is also important to consider when interpreting CAs prevalence and risk factor analyses that are based only on livebirths with no inclusion of other pregnancy outcomes; for example, from Chapter 5, among the 99 stillborn infants a CA prevalence of 8.1% (95% CI 3.55, 15.30) and among the 147 termination of pregnancies a CA prevalence of 17% (95% CI 11.89, 24.83) were respectively reported. However, as previously discussed, collection of adverse pregnancy outcomes such as very early-miscarriages might be under-estimated in the NSHPC because they happen before a women engages with antenatal care.

Furthermore, a balance is needed with respect to collection of maternal information. On the one hand requiring collection of too many data items could both hamper the quality and overwhelm the staff reporting. On the other hand, information on factors such as nutrition, vitamins and folic acid supplements may be considered important because genetic factors and nutrition habits are also important contributing factors to certain defects such as NTDs (MRC 1991, Budhiraja et al. 2002, Dunlap et al. 2011, Atta et al. 2016). For example, looking at the NSHPC data, I was able to detect a single women contributing three of the total 11 NTDs, suggesting a possible genetic-nutrition aetiology rather than the exposure to potentially teratogenic effects of ARVs.

### 7.2.3 Hurdles in B-R in pregnancy

As already mentioned in chapter 2, B-R assessment of medicine use in pregnant women carries several levels of complexity. The lack of sufficient safety and efficacy data on pregnancy makes it difficult to strike the balance between the unknown risks of a new ARV and the potential benefits over older agents. Furthermore, even when data become available from real-world use and post-marketing studies, the B-R re-assessment is often further delayed by the regulatory authorities. In addition B-R assessment predominantly focuses on the risk of taking a medicine and its potential toxicities on the developing fetus (the 'innocent bystander') without an equal focus on the benefits for both the mother and the fetus.

My gap-analysis assessing the 27 ARVs with EU marketing-authorisation found no clear recommendation for their use in pregnancy in over 70% of the SmPCs. Only 33% specifically addressed women of childbearing age, but mostly to recommend pregnancy testing before treatment initiation and to comply with contraceptive measures whilst being on treatment. These observations indicate regulators are too conservative and restrictive in their recommendations, as also recently highlighted in a workshop held by the EMA itself on *benefit-risk of medicines used during pregnancy and breastfeeding*, where most of the HCP and patient participants shared the view that recommendations included in SmPC are still too conservative and risk averse, and do not provide meaningful information for prescribing decisions (EMA 2020). These results also underlie the default position taken by many pharma companies which recommend not using a drug in pregnancy because they failed to collect sufficient data prior to licencing, as this was not required by regulators, while others were more proactive, but only with post-marketing safety studies in pregnant women.

The gap-analysis also showed a trend of increasing real-world use of cART, particularly of newer drugs, increasingly started from before the time of conception regardless of the recommendations. This is illustrated by the increased proportion of pregnancies in the NSHPC conceived under cART, from 37.7% (482/1,279) in 2008 to 80.9% (509/629) in 2018 (test-for-trend $p<0.001$). This trend also reflects the decreasing proportion of pregnant women diagnosed with HIV in pregnancy, with most having established diagnosis from before pregnancy, and thus already on treatment as a result of the "Treat All" era. There is also a high proportion of unplanned pregnancies among women living with HIV (Sutton et al. 2014, Salters et al. 2017). These factors underscore the disconnect between real-world use of ART and SmPC recommendations and the need for women of childbearing age to have preconception counselling with their HIV doctors to discuss the cART regimen they are on and its safety in view of a potential pregnancy.

The historic EFV and NTDs signal provides evidence of the disconnect between what is included in the SmPC and what data are available from reliable sources (e.g. research publications, registries, etc.). As discussed earlier in this thesis, the signal was based only on preclinical findings and case reports. This signal and the fact that TDF/XTC+EFV was the WHO first-line regimen between 2012 and 2018 provided a rationale to include the regimen in my NSHPC analysis and regulatory recommendation analysis.

Looking at the most recent SmPC (last accessed 06/02/2021), it still includes very restrictive recommendations for women of childbearing age, pregnant and breastfeeding. These recommendations are based on the results from 2013 APR report on 904 pregnancies with first trimester exposure to EFV-containing regimen detecting only one NTD, for an expected prevalence of 0.01%. Recommendations are also based on a total of nine reported cases of NTDs with no mention of the denominator, hence prevalence cannot be determined (EMA 2020b).

In the meantime, data accumulated from several studies, including systematic reviews and meta-analyses commissioned by the WHO (Ford et al. 2010, Ford et al. 2011, Ford et al. 2014), a pooled analysis from the EPPICC study (Martinez de Tejada et al. 2019) and a surveillance study (Tsepamo study) all demonstrating that EFV does not carry increased risk for NTDs (Zash et al. 2019). Furthermore, the APR regularly updates its reports, now with a total of 1,040 pregnancies with first trimester exposure to EFV and still only one NTD reported (Scheuerle et al. 2019). In addition the Tsepamo study also updated its reporting, now with 2,999 pregnancies exposed to EFV at conception and five NTDs observed (Zash et al. 2020). Thus, none of the above were incorporated into the Pharma databases nor in the SmPC.

Turning to the real world, data from the NSHPC showed an increasing use over time of EFV-based regimens, e.g. for TDF/FTC+EFV from 3.1% (17/541) in 2008 to 33.6% (77/229) in 2018 (test-for-trend $p<0.001$), a high rate (>91%) of pregnancies staring the combination from before conception. Furthermore, there was no report of NTDs in the 1,228 liveborn infants exposed to EFV-based regimen (and this data also contributed to the EPPICC analysis above). From the gap-analysis it emerges that SmPC typically report a standardised section on pregnancy, but very rarely this provides useful indication to help HCPs in their prescription. This is due to both the lack of initial data and the inertia in updating the pregnancy related sections whenever data from real-world use become available, because real-world use of ARVs happens regardless of the SmPC recommendation, as I have shown with the UK experience.

### 7.2.4 Other missed opportunities to include pregnant in research: Tuberculosis and COV-19

In chapter 2 I gave the example of the Ebola vaccines to demonstrate missed opportunities to include pregnant and nonpregnant women in registrational studies. Pregnant women have also been excluded from recent registrational trials for new interventions including trials to evaluate PrEP, and for the development of drugs against Tuberculosis (TB) and malaria, two frequent HIV co-infections in LMICs (Abdool Karim et al. 2010, Baeten et al. 2012, Gupta et al. 2016c, Moore et al. 2019, Gupta et al. 2019a).

TB is another infectious disease greatly affecting pregnant women, who are therefore a critical population to protect against both infection and active TB. Since 2011 the WHO recommended isoniazid preventive therapy (IPT) to be used as prophylaxis for all population at a greater risk of active TB (i.e. including pregnant women living with HIV) (WHO 2011). However, pregnant women were not included in any CT assessing safe and effective use of IPT (Gupta et al. 2019a). It was only in 2019 that a large, randomised study, the TB APRISE trial, compared the standard recommended IPT for women living with HIV during pregnancy vs IPT given at 12 weeks after delivery and found that infants with *in utero* exposure had worse outcomes (i.e. higher rates of stillbirths, miscarriages and LBW) than those infants whose mother started the regimen after delivery (Gupta et al. 2019b).

Currently we are facing a pandemic caused by a novel virus and once again pregnant and breastfeeding women have been mostly excluded (inclusion criteria in CT are still negative pregnancy test/compliance with contraceptive methods) or removed from the trials once becoming pregnant. Smith et al. evaluated all international registrational trials related to COVID-19 and found 927 CT in the WHO international clinical trials registry related to COVId-19 research, of which 46% explicitly excluded pregnant women or failed to address pregnancy at all and only 16 (1.7%) were pregnancy related (Smith et al. 2020). Looking at the trials registered in ClinicalTrials.gov of the 388 COVID-19 related trials only 5 (1.3%) were pregnancy related. The study demonstrated that <2% of all COVID-19 registered trials included pregnant women and only three were RCTs for the evaluation of drugs/supplement use (Smith et al. 2020). Furthermore, looking at Moderna and Pfizer latest press releases related to safety studies, it is stated that DART studies on animal models (i.e. developmental and reproductive toxicities studies) were, once again, only conducted at the verge of completion of phase III in nonpregnant individuals (ModernaTX 2020, Pfizer-BioNTech press release 2021).

Meanwhile, the first vaccine trial involving pregnant women was only just now announced (late February 2021). This is a phase 2/3, randomized, placebo-controlled, observer-blind study that will test the safety, tolerability and immunogenicity of the Pfizer-BioNTech COVID-19 vaccine (BNT162b2) in preventing COVID-19 in healthy pregnant women (Pfizer-BioNTech. 2021). The CT will also evaluate safety of infants with *in utero* exposure to vaccine through maternal vaccination and mother-to-child transfer of the potentially protective antibodies, with infants monitored for approximately six months of age. The aim is to conduct a worldwide CT, including countries such US, Brazil, Canada, Chile, Mozambique, Spain and UK with the intention to enrol about 4,000 pregnant women aged over 18 and between 24-34 GW by January 2023 (Pfizer-BioNTech. 2021).

## 7.3 An update since detection of the Dolutegravir safety signal

The experience with the DTG safety signal gives a sense of *déjà vu* with respect to what happened with the EFV safety signal, particularly as both agents were suspected to increase the risk of NTDs when taken at around periconception period.

Following the 2018 safety signal, several studies started to evaluate DTG use in pregnancy, with a particular focus on those women with exposure to DTG from periconception period (Figure 7.1). For example, from the Global North data on around 500 pregnancies with pre/peri-conception exposure to DTG have been collect by several prospective cohorts (i.e. France's EPF, two US sites), registries (i.e. APR) and surveillance studies (i.e. UK's NSHPC, Canada's CPHSP) (Grayhack et al. 2018, Money et al. 2018, Albano et al. 2019, Money et al. 2019, Sibiude et al. 2019) and none reported increased risk of NTDs. Also a recent study from Brazil, a retrospective observational national cohort study of 382 women exposed to DTG from periconception did not report any NTDs (Pereira et al. 2021).

Over the course of my PhD, whilst assessing DTG use in the UK, there have not been any reports of NTDs. Following the signal, in my second analysis I evaluated *n*=266 singleton liveborn pregnancies exposed to DTG and found an overall CA prevalence of 3.38% (95% CI 1.56, 6.32) and no NTDs in infants whose mothers were exposed to DTG-based regimen starting before conception. Also from the pooled analysis with data from the Dolomite-EPPICC study, to date the largest in Europe to evaluate DTG use in pregnancy, there was no report of NTDs and 70% of pregnancies were exposed from periconception with an overall CA prevalence 3.36% (95% CI 1.85, 5.57) (according to EUROCAT inclusion criteria). However, none of the above mentioned studies nor mine allow any safety conclusions to be drawn given the small number of observations that preclude ruling out the risk for NTDs.

The Tsepamo Study also provided updated findings, reporting two more cases of NTDs with DTG-exposure from time of conception. These brought the total number of observed NTDs since the first report in May 2018 to seven (7/3,591 NTDs) with a prevalence of 0.19% (95% CI 0.09, 0.4), while women receiving non-DTG cART at conception had a prevalence of 0.11% (95%CI 0.07, 0.17); those exposed to EFV-based regimen at conception of 0.07% (95%CI 0.03, 0.17); those HIV-negative of 0.07% (95%CI 0.06, 0.09) and for those starting DTG in pregnancy of 0.04% (95%CI 0.01, 0.16) (Zash et al. 2020). Importantly, the prevalence difference between women

exposed from conception to DTG-based regimen and all the other comparison groups has decreased to 0.09%, though not statistically significant (95%CI -0.03, -0.30).

In the meantime, data supporting the advantages of taking DTG have been accumulating. A recent meta-analysis on five clinical trials, namely DolPHIN-1, DolPHIN-2, IMPAACT 2010, ADVANCE and NAMSAL jointly providing data on 1,074 pregnant women showed a significant superiority of DTG-based regimen's efficacy in reducing VL over EFV-based regimen, with 90% vs 72% of pregnant women having viral suppression at delivery (OR 2.90; 95% CI 1.54-5.46; *p*=0.001). The study also evaluated the risk for preterm deliveries and found an increased risk for women exposed to EFV- vs DTG-based regimen (EFV vs DTG: 12% vs 8%, *p*=0.04) (Asif et al. 2020). Recently a modelling study to inform treatment guidelines by Phillips et at. was used to evaluate the B-R ratio of ART initiation policies (Phillips et al. 2020). The study utilized an individual-based model with several parameters such as rates of HIV testing, ART adherence, resistance, extent of VL monitoring, etc. included to create different epidemic setting scenarios, reflecting the diversity of epidemic and programmatic situations in SSA. For example, they modelled drug activity and resistance, VT and risk of NTDs, and the potential effect of weight gain due to exposure to DTG. For each scenario they considered the situation in 2018 (i.e. DTG safety signal) and compared ART initiation policies with EFV-based regimen vs DTG-based regimen in women intending pregnancy. The authors found that a policy of ART initiation with DTG in women intending pregnancy was predicted to produce more healthy life years compared to the policy of ART initiation with EFV-based regimen (DALY were averted) in 83% of the setting scenarios and was cost-effective (net DALY averted) in 87% of the setting scenarios (Phillips et al. 2020). This study was among others utilised by the WHO to support the strong recommendation for DTG use as preferred first-line option for ART initiators, including women intending pregnancy (Dugdale et al. 2019, WHO 2019b).

***National and international guidelines updates***

The WHO updated its guidelines in July 2019 recommending DTG as the preferred first-line third agent for all adults, adolescents and children (following approved DTG dosing) with HIV(WHO 2019b). This recommendation was marked as "strong" meaning that evidence now supports DTG-based regimen as first-and second-line ART regimen also acknowledging the decline in the estimate of NTD risk associated with periconception use. Therefore, the current recommendations are for TDF/FTC or 3TC +DTG, as the first-line preferred regimen followed by EFV-based regimens as the alternative first-line regimen at a lower dosage (i.e. 400mg vs the previous 600mg

(Encore1 Study Group 2014, Mulenga et al. 2019)). This new recommendation lifts any previous restrictions on DTG for women of childbearing age and recognises the importance of a women-centred approach and were announced at the 10th International AIDS Society Conference on HIV science (IAS 2019) and at that time it was also reported that a total of 123 countries had introduced DTG-based regimens, including 41 LMIC and most of the high-burden countries of SSA (WHO 2019b). More recently a systematic review and network meta-analysis comparing efficacy, tolerability and safety of the first-line regimens DTG-and EFV-based ART was conducted and further supported the current WHO recommendation of choosing DTG as the preferred regimen followed by the low dose EFV regimen (Kanters et al. 2020).

BHIVA guidelines in the meantime have been updated twice since 2018 (i.e. since the signal). In March 2019 (second interim update), it was recommended to use DTG only from 6 GW ("which must be confirmed") until further data on the use of DTG in pregnancy became available (BHIVA 2019b); data from the Tsepamo Study, two other studies assessing NTDs and the APR 2019 report were listed as the rationale (Raesima et al. 2019, Zash et al. 2019, BHIVA 2019b, Mofenson et al. 2019b). The more recent update in 2020 (third interim update) reported further data from the Tsepamo Study but kept the same recommendations as before (Zash et al. 2019, BHIVA 2020, Chinula et al. 2020, Zash et al. 2020).

EMA recommendations changed twice since the safety signal. There was a first prompt response in May 2018. As previously discussed, this was very restrictive, recommending women of childbearing age to undergo pregnancy testing and to comply with contraceptive measures in order to access DTG-based treatment as well as recommending avoiding use of DTG-based regimen in T1 unless there are no alternatives, alongside a careful evaluation of the benefits and the risk for the fetus before prescribing DTG in T2-T3. These are reported in section 6.3.1, Table 6.1 accordingly to the latest access to EMA data on the 20/05/2020. However, at the beginning of 2021, following the updates of the Tsepamo Study, the SmPCs of both DTG formulations were further updated and now recommendations have a more favourable B-R assessment as shown in Table 7.1 (EMA 2021a, EMA 2021b).

For women of childbearing age, counselling is now recommended to discuss the potential risk for NTDs and the possibility to take effective contraceptives measures, with no more wording such as "must comply" or "have to" stated in the SmPC. For pregnant women there is now a note addressing those who wish to plan their pregnancies, and a report of the most recent data from the Tsepamo Study and the APR, from which the new recommendation is based. These take into consideration

the limited window within which NTDs can occur and therefore acknowledge the risk of switching to other regimens if a pregnancy has been already confirmed (i.e. in T1 or in later trimesters).

A potential increased risk for NTDs when used in pregnancy from periconception period was identified for both EFV and DTG, in each case based only on preliminary findings. However, the response from regulators regarding recommendations for their use developed differently. This is partly due to the different calendar time periods in which the signals emerged. As discussed, there is growing emphasis for inclusion of pregnant women in research and drug development, with an intense engagement of regulators who are acknowledging the need to shift from the current paradigm. This might partly explain why EFV recommendations have remained the same since 2013 despite the accumulating evidence against the increased risk for NTDs, while DTG recommendations have been promptly updated as soon as data have become available. It is reasonable to hypothesize that the different assessment approach for DTG is the consequence of the new cultural environment, where awareness of including pregnancy is finally present. This, however, seems to be applied only to the most recent authorised medicine, while older agents such as EFV are not considered for an update.

In reality, it is unlikely that older formulations will be revised unless potential or identified risk are signalled. This was the case for COBI-based formulations following the identified reduced effectiveness of COBI due to pregnancy-induced PK changes. This also demonstrates the current attitude of focusing predominantly on the risk of medicine use, rather that applying a balanced B-R assessment.

Nevertheless, reviews and updates of drugs should be applied to all agents, regardless, if “old” or “new”, whenever new data on both safety and effectiveness become available. In fact even if EFV is considered an “old” drug it is still a second-line recommended third agent, hence still widely used. Therefore it is unreasonable to maintain the dichotomy of “old” and “new”, since this will aggravate the difficulties to generate evidence in pregnancy for less used agents (e.g. third-line recommended agents), which are likely to require even more time to reach the necessary numbers of early pregnancy exposures and consequently to detect rare health outcomes. Therefore, there is a general need for a proactive review and update of the older products to enable HCP’s informed decisions.

**Table 7.1 Updated summary of DTG characteristics extracted from the relevant SmPC sections of both DTG authorised formulations**

| ARVs/ Trade name | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Fertility, Pregnancy and lactation (Clinical data) | Sec 5.1 PD & Sec 5.2 PK properties | Sec 5.3 Preclinical safety data |
|---|---|---|---|---|---|
| **DTG (Tivicay)** | 2014 | **Current:** No data | **Current: WCBA:** should be counselled about the potential risk of NTDs, including consideration of effective contraceptive measures. If a women plans preg, the B-R of continuing treatment should be discussed **PREG**: data from birth surveillance study in Botswana show a small ↑ of NTDs 7/3,591 deliveries (0.19%, 95%CI 0.09, 0.40) to mothers taking DTG at the time of conception vs 21/19,361 deliveries (0.11%, 95%CI 0.07,0.17) to women exposed to non-DTG at the time of conception. The incidence of NTDs in the general population ranges from 0.5-1 case per 1,000 LBs (0.05-0.1%). Most NTDs occur within the first 4 weeks of embryonic development after conception (approx. 6 weeks after the last menstrual period). Data from APR do not indicate increased risk of major defects in >600 women exposed to DTG in preg, but insufficient data to address risk of NTDs. >1,000 outcomes from T2-T3 exposure indicate no evidence of increased risk of feto/neonatal toxicity**. B-F**: excreted in human milk is small amounts. **Recommendation:** If a preg is confirmed in T1 while on DTG, B-R of continuing DTG vs switching to another cART should be discussed. Taking the GA and the critical time period of NTDs development into account. DTG may be used in T2-T3 when the expected B justifies the potential R to the fetus. | No data on preg/B-F<br>DTG: t ½ ~14 | In rats & rabbits no developmental toxicity, nor teratogenicity<br>Placenta crossing: in animal models |
| **DTG + ABC/3TC (Triumeq)** | 2014 | **Current: Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | **Current: WCBA, PREG. B-F, Recommendation**: as per Tivicay. | No data on preg/ B-F<br>DTG: t ½ ~14 | **DTG**: no developmental toxicity, nor teratogenicity.<br>Placenta crossing: in animal models |

WCBA: women of childbearing age; Preg: pregnancy/pregnant; B-F; breast-feeding; pt.: patient; LBs: live births; B-R: benefits and risks HEU infants: HIV exposed uninfected infants; t ½: half-life expressed in hours ; APR: Antiretroviral Pregnancy Registry; ↑: increased/increase

*****Decreased fetal body weight, fetal oedema, increased skeletal variation, early intrauterine death & stillbirth

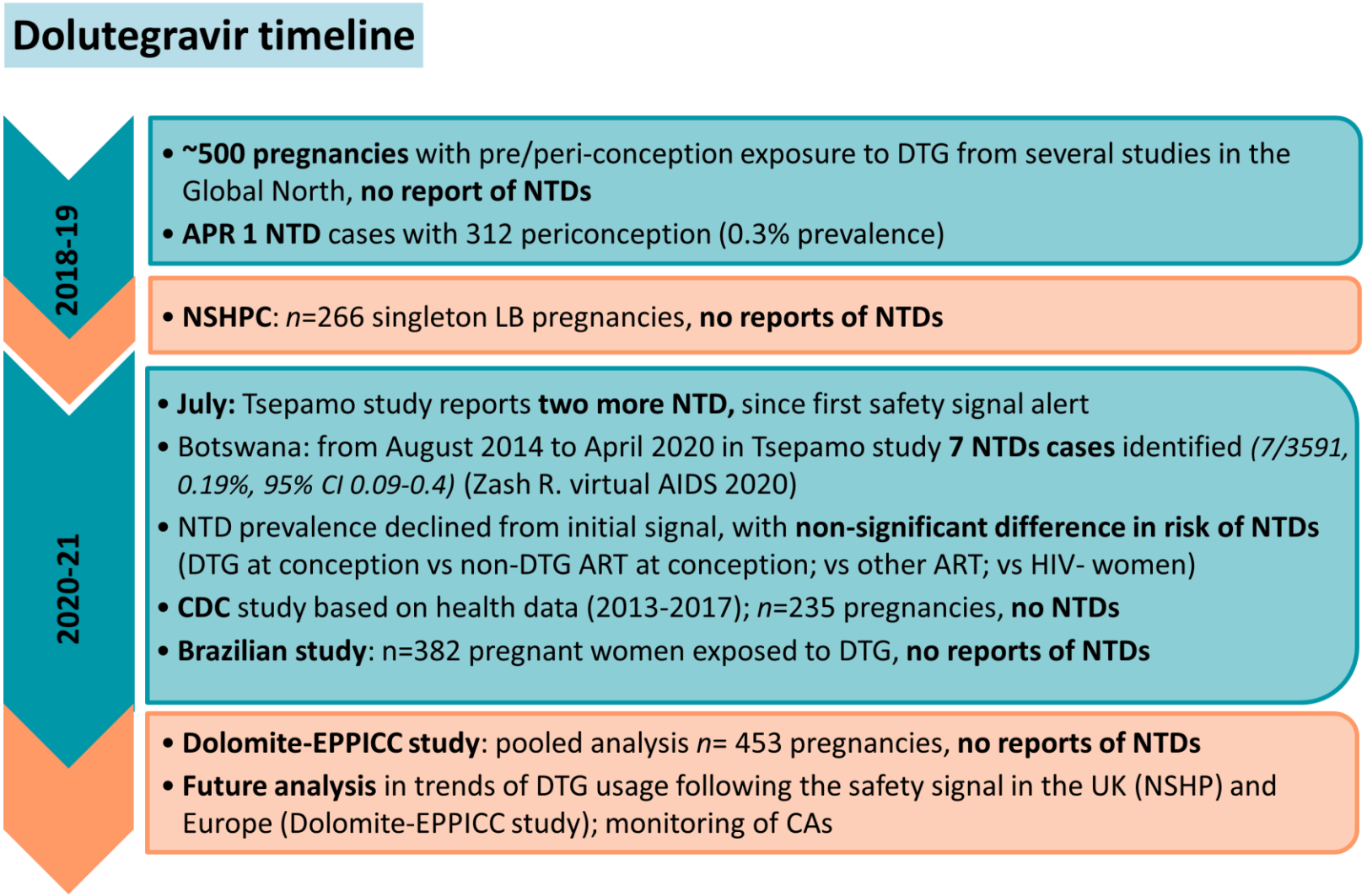



**Figure 7.1 Summary of key events in the DTG issue, latest update**

### 7.3.1 Single agent of class effect?

The DTG safety signal resulted in additional scrutiny of the entire class of INSTIs and raised the question of whether there might be a class effect. Therefore, to contribute to real-world evidence on the safety on INSTIs use in pregnancy I evaluated RAL and EVG use in the UK and assessed the risk for CA in general and particularly for NTDs. The analysis provided real-world data on 978 pregnancies exposed to RAL- and 66 to EVG-based regimens. Over half of all the pregnancies exposed to RAL-based regimen had exposure from periconception period, with this trend significantly increasing over time ($p$<0.001). Initial clinical recommendations of RAL use were mostly intended for late-pregnancy presenters given its rapid viral suppression, and this has been supported by the higher rates (>84%) of women in the NSHPC who started RAL in pregnancy and still had undetectable VL by delivery, underscoring the effectiveness of RAL in viral suppression (BHIVA 2020, Joao et al. 2020). The increasing trends of periconception use of RAL over time, possibly reflect an overall increase in the use of RAL, including among women of childbearing age and those newly diagnosed with HIV. This is supported by my findings from the snapshot analysis assessing trends of ARV use among women newly diagnosed with HIV in pregnancy, showing an increased use of RAL over time, from 0.4% in 2009 to 10.1% in 2016 (test-for-trend $p$<0.001),

The overall CA prevalence for infants with *in utero* exposure to RAL or EVG in the UK was 1.92% (95% Cl 1.14, 3.02) and 4.55% (95% CI 0.95, 12.71), respectively and with no reports of NTDs for either agent. From the logistic regression model after adjusting for maternal age at delivery and evaluating the joint effect of being exposed to INSTIs and time of first exposure, a reduced risk for CA of approximately 48% was found when first exposure to INSTIs occurred in the periconception period compared with not being exposed to INSTIs and not being exposed from periconception period. This is an interesting finding considering the initial safety signal identified an increased risk for NTDs for infants whose mother started DTG-based regimen from before conception.

In the meantime, other studies have evaluated the risk for NTDs as a class effect and also found none. For example, the French Perinatal Cohort firstly evaluated 309 infants exposed to INSTIs at conception, 224 to RAL and 44 to EVG, and more recently evaluated 808 women exposed to INSTIs during pregnancy, with 218 women exposed to RAL- and 48 to EVG-based regimen from conception, with neither analysis

reporting identification of NTDs (Sibiude et al. 2019, Sibiude et al. 2021). Similarly, no NTDs were identified among 231 RAL- and 155 EVG-livebirth outcomes exposed from conception in the APR (Albano et al. 2019).

These are reassuring findings, however for EVG-exposed infants the small numbers preclude any meaningful interpretations, and also RAL-exposed infants are not enough to detect rare events such as NTDs. This finding also highlights on the one hand the value of population-based studies of prospective nature such as NSHPC and also the Tsepamo Study in providing the necessary birth surveillance, and on the other hand, the need for pooled analysis combining data from different real-world studies in order to reach the statistical power required to assess rare events. For example, Merck the manufacturer company of RAL used published data from multiple sources to evaluate the safe use of RAL in pregnancy, including data from the NSHPC that I presented as a poster at CROI 2018 (Sconza et al. 2018). This allowed synthesis of a total of 2,426 pregnancies with a reported outcome, however once again the number of observations were not enough to rule out the risk for NTDs (i.e. 927 pregnancies exposed to RAL during T1, including 557 in the preconception period) (Shamsuddin et al. 2019). EPPICC is also planning to evaluate safe use of RAL in all participating European cohorts. However, while the necessary numbers of observation accumulate, a systematic review and meta-analysis could provide a meaningful evaluation of the current evidence of real-world use of RAL.

### 7.3.2 Birth surveillance following in utero exposure to combinations of ARV in the NSHPC between 2008-2018

This analysis was carried out in consideration of the growing literature concerning adverse pregnancy outcome and potential increased risk for CA for infants with *in utero* exposure to ARV. Exposure to ARV was assessed first as “any exposure to ARVs”; then by ARV class and lastly as cART, using the five combinations of interest (i.e. TDF/FTC+ EFV, AZT/3TC+ LPV/r, TDF/FTC+ ATV/r, TDF/FTC+ DRV/r, TDF/FTC+ RPV). The time of exposure was assessed as the earliest exposure to a combination of ARVs (i.e. periconception vs T2-T3), regardless of the duration. Furthermore, risk factors for CAs were assessed with a logistic regression model to evaluate the association between exposure to ARVs first by class of ARV then by the five ARV combinations.

Overall, 227 of the 11,097 liveborn infants reported to the NSHPC between 2008-2018 presented with at least one CA, a prevalence of 2.03% (95% CI 1.77, 2.31).

These rates are consistent with both national population estimates for 2008-2016 in the UK (e.g. 2.0% among livebirth in 2010) (EUROCAT 2014) and with historical prevalence in the NSHPC of 2.8% (95% CI 2.5, 3.2) in 1990-2007 (Townsend et al. 2009). Applying EUROCAT classification criteria, I did not detect any particular pattern of CA affecting the same organ/system. My findings are consistent with those of other studies previously conducted and generally reassuring (Williams et al. 2015, Uthman et al. 2017, Rough et al. 2018, Veroniki et al. 2018, Nguyen et al. 2019), including a meta-analysis evaluating preconception ART use (Uthman et al. 2017). Also the recent EPPICC study evaluating the association between exposure to EFV-based regimens and the likelihood of CAs and the French study evaluating the risk of CA for infants with *in utero* exposure to RAL, did not find increased risk for CAs. (Sibiude J 2017, Martinez de Tejada et al. 2019).

However, there are some studies suggesting specific toxicities with exposure to particular ARVs, such as the recent report of a potential increased risk for microcephaly in infants with *in utero* exposure to EFV-based regimens from the SMARTT study (Williams et al. 2020). SMARTT is a study specifically designed to evaluate the safety on ARVs exposure longitudinally in HIV-exposed uninfected children in the USA. Reassuringly, the overall prevalence of microcephaly was within the expected range for the general population (Williams et al. 2020).

Meanwhile, two studies have evaluated AZT exposure and the risk for CHD: a French study which identified an association between T1 exposure to AZT and increased risk for CHD, particularly for VSD (Sibiude et al. 2015) and the SMARTT study which identified a subclinical difference in left ventricular structure and function (Lipshultz et al. 2015). Nevertheless, the APR found no significant difference evaluating over 13,000 pregnancies exposed to AZT in any trimester (Vannappagari et al. 2016a). I also evaluated AZT as part of the combination AZT/3TC+LPV/r and found no increased risk for CHD (3/1,974, 0.15% 95%CI 0.03, 0.44) when compared with national estimates for general population (i.e. 8 in every 1,000 infants born in the UK have a CHD).

The majority of studies (also those I have cited above) have been conducted to investigate each ARV contained in a regimen separately in order to assess their individual toxicity and the potential for teratogenic effect, however ARVs are mostly administered in regimens of at least three different agents combined. Therefore it is quite difficult to determine which one might be associated with the identified toxicity or detected/suspected CA and whether they result from a periconception period exposure to one particular ARV or if the combined effect of the agents might contribute

to the toxic effect (Zash et al. 2016a). Some studies have tried to evaluate ARV as combinations such as Williams et al. who looked at prenatal ARV exposure as any ARV, HAART (i.e. regimen containing two or more drug classes), ARV by class and ARV by single agent, but still not focusing on specific ARV combinations (Williams et al. 2015). A more recent study evaluated the risk of adverse pregnancy outcomes (i.e. PTD, LBW) among infants *in utero* exposed to combinations of AZT/3TC+LPV/r, TDF/FTC+ATV/r or TDF/FTC+LPV/r (Rough et al. 2018), as did Zash et al., who evaluated the risk of adverse birth outcomes (i.e. SB, PTD, SGA and neonatal death) for infants exposed from and after conception to several ART regimens, including TDF/FTC+EFV, and AZT/3TC+LPV/r (Zash et al. 2017); however none of these studies evaluated the risk for CAs with exposure to specific regimens. The APR too, at the moment does not evaluate data on CA prevalence for specific regimens, but only for individual agents.

However, an Italian study has recently highlighted the importance of choosing for pregnant women a regimen both safe and effective, addressing the issue of limited comparative information on ARVs from different classes used in pregnant women. The analysis evaluated all pregnancies reported between 2008 and 2018, ending in livebirths and exposed within 32 GW to a three-drug regimen, and since all regimens contained a backbone of two NRTI, comparison was made between third agent by class (i.e. a PI, NNRTI, INSTI) and compared these three options in terms of pregnancy outcomes including risk for CAs. The authors reported absence of any major difference between use of the three drug classes in pregnancy and outcomes of interest (Floridia et al. 2020).

In my analysis I investigated five ARV combinations, four of which were the most commonly used in the UK over the last ten years; and the fifth (i.e. RPV) an ARV with an identified reduced effectiveness due to PK-PD pregnancy-induced changes (i.e. reduction up to 50% of RPV exposure in pregnancy, particularly T3 vs postpartum period) (Colbers et al. 2017). These were firstly assessed by the rule of three to evaluate whether exposure to them was associated with any particular pattern of CA by system/organ criteria, and none was found. Then restricting the analysis on the 5,309 pregnancies with receipt of one of the five ARV regimens, a logistic regression model was fitted, and after adjusting for maternal age at delivery, no association between time of first exposure and the risk of CA was found. Furthermore, some limited evidence of a reduced risk of CA with use of AZT/3TC+ LPV/r versus TDF/FTC+ EFV ($p$=0.07) was found. These are reassuring results considering both the prolonged time of observation (ten years) and the overall numbers with exposure

to the combination (i.e. >800 pregnancies for each combination except RPV-based regimen that had >200 pregnancies), however as previously explained, over 2,000 observation are needed to rule out the risk for rare events. Finally, in line with previous studies (French et al. 2012, Townsend et al. 2014, EUROSTAT 2015, Lean et al. 2017, Townsend et al. 2017), I found maternal age at delivery to have increased over time, e.g. there was a 3-fold increase in the contribution of women aged 40-45 years to all pregnancies, rising from 4.6% (59/1,279) in 2008 to 15.3% (96/629) in 2018 (test-for-trend p< 0.001). Furthermore, I found that maternal age at delivery over >35 year was associated with increased risk for the development of CAs in all my analyses (i.e. regardless of the type of ARV, class, or combination of ARV evaluated). This is in line with previous findings of advanced maternal age conferring increased risk for CAs, particularly for chromosomal disorders (Allen et al. 2009, Stothard et al. 2009, Hill et al. 2018, Moorthie et al. 2018). This is important information for women planning a pregnancy or following the discovery of a pregnancy underlying the importance for women to engage as early as possible with antenatal care and screening programmes in order to manage the risks.

### 7.3.3 What have we learnt from DTG?

The reported observation on DTG experience taught different stakeholders, including clinicians, pharma and policy-maker some lessons.

One limitation stems from studies with small sample sizes, which are underpowered for detection of rare events such as NTDs (e.g. for DTG, NSHPC/Dolomite-EPPIC exposure were in <300 pregnancies, for RAL & EVG exposure were in >1,000 jointly). This underscores the need for better planned post-marketing surveillance studies and in settings where there are large populations of pregnant women living with HIV in order to generate timely and robust evidence.

Regulatory recommendations were promptly updated in the relevant SmPCs sections, showing encouraging trends of prompt amendments of regulatory guidelines when a signal is reported as well as encouraging progress in the post-marketing pharmacovigilance. However, this also highlights the current limitations in terms of pre-marketing safety data (i.e. DTG safety was evaluated in preclinical studies and no teratogenic, nor embryo-foetal or fertility toxicity was found in animal models) and underlines once again the presence of a time gap between real-world use and regulatory recommendations.

The DTG experience propelled institutions such as International AIDS Society (IAS) to convene an International Forum *"IAS Forum on Dolutegravir Safety"* of high-level experts committed to issue a set of actions aiming to optimize access to DTG-containing regimens, even if uncertainties regarding the specific risk of NTDs remained, and how to respond to and manage future safety signals. The Forum, in which I participated as a rapporteur, focused on data collection, data quality, data interpretation and the appropriate messaging of the risk and the benefits for DTG use in women of childbearing age with HIV and the imperative need to involve the affected community in the decision-making process (AfroCAB 2018, IAS Forum 2018, Mofenson et al. 2019a).

The DTG safety signal also underpins the need for clear messaging, one that "*needs to provide a range of levels of simplicity/complexity depending on the audience. Messaging should achieve uniformity across agencies, including messaging for country Ministries of Health to help governments implement strategies and avoid ambiguity in interpretation. Patients and healthcare providers need to have appropriate and clear information and materials. They need to be able to assess patient-specific treatment options, communicate risk and benefits including levels of certainty, and ways to potentially mitigate risk as well as support women-centered decision-making*", as stated by the IAS forum (AfroCAB 2018, Mofenson et al. 2019a). Therefore, clear messages should be provided within clinical guidelines and from regulators to strongly support HCP delivering care and to support women living with HIV to make informed decisions.

## 7.4 Going further: a need for a paradigm shift

All the above considerations sustain the necessity to shift the current paradigm of justifying the inclusion of women of childbearing age and those pregnant and breastfeeding in CT.

The DTG safety signal highlighted several of the issues of the current situation, such as the need for a different approach to B-R assessment, one that should take into consideration drug's access, availability and toxic/tolerability profiles. For example, DTG-based regimens have been shown to rapidly reduce VL and have fewer side effects than EFV-based regimens. In countries where concerns about viral drug resistance to NNRTI are increasing, withholding a drug such as DTG should be really carefully evaluated. Furthermore, not all women of childbearing age might desire pregnancy and others might want to plan their pregnancies, therefore effective contraceptive measures for them should be available and should be offered but not required as the condition to access DTG (AfroCAB 2018).

Without appropriate research on women, the risk of exposing women either to excessive and potentially toxic dosage or to a suboptimal and potentially ineffective dose will always persist. Equally, the risk of exposing the developing fetus to potentially toxic and teratogenic effects of the new drugs will also persist. Excluding pregnant women from research does not remove the risks but simply shifts the risks, moving from the well-controlled setting provided by CT, with informed consent and intensive safety monitoring to the less controlled and less monitored setting of the clinical care/hospitals, as recently stated by Dr Lockman at the latest Conference on Retroviruses and Opportunistic Infections (Lockman 2021), ultimately resulting in a "off-label use" of medicines in pregnancy. Several steps of the current path to generate, collect and accumulate data for women of childbearing age and those pregnant and breastfeeding need to change in order to shift the paradigm. These are displayed in Figure 7.2 and discussed in the following three sections.

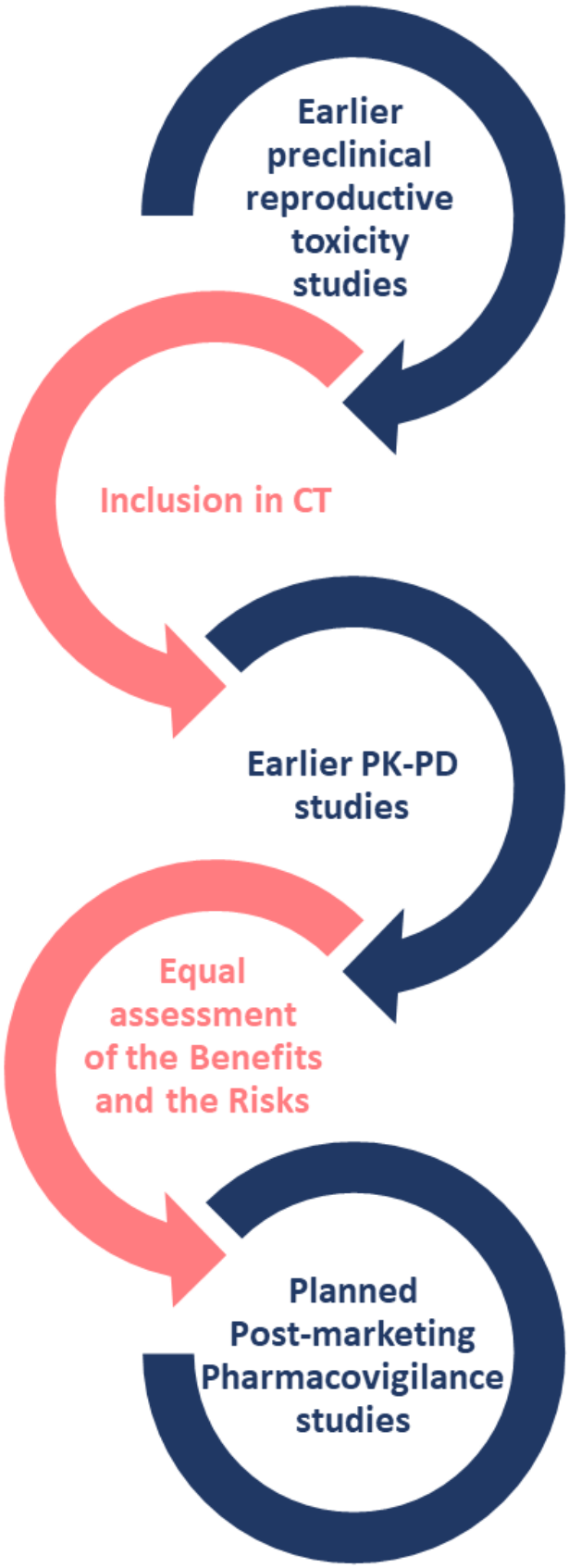



**Figure 7.2 Proposed steps to shift the current paradigm to include pregnant and nonpregnant women in research**

### 7.4.1 Early preclinical data availability and inclusion of pregnant women in CTs

There is a need for early availability of preclinical reproductive toxicity studies. Ideally reproductive toxicities studies should be conducted and completed during early preclinical stages of a drug development, given that globally women of childbearing age make up more than half of the population living with HIV, and consequently every new developed agent will be inevitably used by a woman of childbearing age (UNAIDS 2019a, UNAIDS 2019c).

These studies should also be improved and standardized. As reported in Chapter 2 and further supported by my findings in Chapter 4, often there is a lack of standardization in preclinical studies' design with no mention of the tested dosage or of the number of animals tested nor of the types of toxicities explored (i.e. lack of teratogenic studies).

Application of innovative methodology and new technologies such as in silico, *in vitro* and *in vivo* models should also be considered. For example, transplacental transfer models or modelling methods such as physiologically-based pharmacokinetic (PBPK) as suggested by Eke et al. could help characterize or rule out drug's possible risks enhancing the opportunity to enrol pregnant women in CTs (Eke et al. 2020, PHASES 2020). The EMA also has recently supported and emphasized the need for new methods to predict medicines' effects and to generate data in earlier phases of drug development in order to also enable better post-marketing evaluation later on (EMA 2020).

Several authors have suggested methods and proposed frameworks to include more women, particularly pregnant women in CTs from earlier stages. For example, Roes et al. in 2018 proposed a practical framework for responsible inclusion of pregnant women in registrational trials, with a question-based approach and practical suggestions of key CT design features (Roes et al. 2018). According to Roes et al. enrolment of pregnant women in CT could start after phases I and II are completed in non-pregnant individuals, just before entering phase IIb. The WHO ART expert groups PADO and CADO recently adopted these principles as the "Roes framework" and proposed several actions to include and retain women in registrational trials (Abrams et al. 2020).

It was also suggested that PK-PD studies should be performed during drug development instead of waiting for the post-marketing phase and for the necessary number of observations to accumulate. Hence, enrolment of pregnant women should start during phase IIb of CTs whenever the B-R assessment is favourable and by

enrolling women who are treatment-experienced and with no other treatment options (Roes et al. 2018, Abrams et al. 2020). Another successful strategy to include pregnant women in CT is provided by the Microbicide Trials Network where women are enrolled as late in pregnancy as possible, to minimize fetal exposure to the investigational drug, and once treatment has proven safe and effective, women are enrolled earlier in their pregnancies (Mhlanga et al. 2018, Rubin 2018).

In addition to actively including pregnant women in CT from earlier stages, it is important to foresee in the study design how to retain women who might become pregnant after enrolment (typically removed from the trial) and how to capture and analyse the outcomes of these pregnancies. A first step could be to maintain their participation in the trial as long as a favourable B-R ratio is proven. In regard to capturing pregnancy outcomes, this could be done within the trials, either envisaging an *a priori* protocol or as a separate prospective observational study (PHASES 2020).

These proposed new approaches have been either already set in place in recently launched studies or are being considered for planned new trials. Tenofovir Alafenamide (TAF), CAB LA and two recently authorised ARVs, Bictegravir (INSTIs) and Doravirine (NNRTI) will be investigated in pregnant women within IMPAACT 2026 and PANNA to evaluate PK-PD changes in pregnancy and post-partum (IMPAACT 2026 2020, PANNA 2020). Additionally, a phase IIb study performed by Gilead Sciences is evaluating Bictegravir use in pregnancy (NCT03960645) (Gilead Sciences 2020). The ARIA study (as mentioned in chapter 1 a trial enrolling specifically women) enrolled ART-naive women and randomly assigned them to either a first-line DTG-based regimen or to ATV/r ones and allowed women who became pregnant to remain in the study (NCT01910402) (Orrell et al. 2017).

Lastly, several studies to evaluate Dapivirine (NNRTI) use for prevention have been planned or are currently recruiting. Dapivirine is expected to be used for PrEP as a vaginal ring and to be used by breastfeeding women, particularly in those countries where breastfeeding is supported. Therefore, there is a planned study to look at the safety of Dapivirine in breastfeeding mother-infant pairs (NCT04140266), whilst the DELIVER trial, a phase III study currently recruiting (NCT03965923), will evaluate efficacy and safety of Dapivirine as vaginal ring vs TDF/FTC as daily tablets in 750 HIV-uninfected pregnant women and their infants.

### 7.4.2 New B-R assessment

Three main actions are needed to shift the current paradigm: to equally focus on the benefits and on the risks; to equally evaluate the fetal and the maternal risk, and fetal and maternal benefits; to generate data earlier, ideally before any safety signal emerges from real-world settings, by imposing regulatory proactive post-marketing studies.

The current focus is mainly on the risk of taking a medicine. As supported by my previous findings, when new data suggest that a drug may be unsafe or ineffective if used in pregnancy, regulatory recommendations are updated in a timely way; however whenever there is data supporting safe and effective use of a drug in pregnancy there is no equally prompt update of the recommendations. Furthermore, currently the main focus is on the risks for the "innocent bystander" but not for the benefit of treating the mother. This was recently highlighted by a survey conducted by the EMA where participants were asked if they would have liked more information on the benefits for the mother and their infants using a given medicine versus not using it, to which 62% of the 156 respondents said yes, underlying the need for a better understanding of the consequences of not being treated (EMA 2020).

Women and infant should be considered as two individuals with separate and different risks and benefits which however are interlinked. The risks for the fetus should be carefully balanced with the benefits of treating the mother's condition effectively but also with the most tolerable regimen too (e.g. health improvements, reduction of disease progression) and the potential secondary benefits for the fetus. Risk varies in different gestational ages, and this needs to be included in the B-R assessment. For example, for women infected with TB administration of DAA or IPT could simply be delayed after 12 GW when the teratogenic risk is reduced (Freriksen et al. 2019). Furthermore, not all women of childbearing age will desire a pregnancy and others might want to plan their pregnancies, therefore effective contraceptive measures for them should be available and offered, but not required, as the condition to access DTG.

Another consideration regarding B-R assessment for medicine use in pregnancy relates to maternal drug-related toxicities and the impact of these on both women's health and pregnancy outcome. For example, a 2015 UK study reported among women on cART those pregnant had an increased risk of liver enzyme elevation by 70% and a more than tripled risk for severe liver enzyme elevation compared with nonpregnant women (Huntington et al. 2015). Additionally, after years of ARV usage, several studies have identified maternal hypertension is an important risk factor for

adverse pregnancy outcomes for women living with HIV on cART possibly due to a direct toxic effect of cART on the placenta (Chen et al. 2012, Shapiro et al. 2012, Machado et al. 2014, Zash et al. 2016b). More recently several studies have reported maternal weight gain for women using INSTI-based regimen whilst being pregnant, and obesity is another known risk factor for adverse pregnancy outcomes. Recent data from the Tsepamo Study evaluated weight gain in pregnant women initiating either DTG or EFV during pregnancy and showed significant lower risk of insufficient weight gain and weight loss for those starting a DTG-based regimen vs EFV-based regimen (<0.8kg/week between GW 18-36) (Caniglia et al. 2020). Additionally, the study compared WLWH with those HIV-uninfected and neither women initiating DTG nor those starting EFV gained as much weight as these HIV-uninfected pregnant women; these findings suggest a possible impact of HIV or ART (or both) on the ability to gain weight in pregnancy(Caniglia et al. 2020). However, these recent results are preliminary with insufficient long-term data collection to draw stronger conclusions and geographic difference that might need consideration (Eckard et al. 2019, Bengtson et al. 2020, Chinula et al. 2020, Jao et al. 2020, Malaba et al. 2020). Once again emerges the importance of conducting studies that include pregnant women enabling to generate the evidence to support HCP take a balanced decision.

### 7.4.3 Recent initiatives on the right path

The past decade witnessed increasing efforts from different stakeholders to address the lack of inclusion of pregnant and non-pregnant women in CT and to provide solutions.

Both the FDA and EMA have recently specifically addressed the need to facilitate inclusion of women, both pregnant and non-pregnant in CT and both institutions have issued guidance on conducting PK-PD studies in pregnant and breastfeeding women (FDA 2018, Eke et al. 2020, EMA 2020). My findings (chapter 4 and 6) demonstrate that regulatory recommendations in recent years have been updated in a timely way whenever a signal was detected, possibly reflecting the faster pace at which new data has become available and the consequent necessity to update each section of the SmPC promptly and accurately. Furthermore, the EMA has recently launched, in collaboration with the ConcePTION consortium (established under the EU's Innovative Medicine Initiative), a project that builds on existing initiatives such as EUROmediCAT to develop an "European knowledge bank" to facilitate generation and dissemination of evidence that could speed up changes and updates of medicine labels and SmPCs with respect to pregnancy (ConCEPTION 2020, EMA 2020).

Trials such as DolPHIN-2, PROMISE, VESTED and several from the IMPAACT Network and more recently the Tsepamo Study have all provided compelling examples of well-designed studies with fair and ethical inclusion of pregnant women giving equal significance to maternal and fetal outcomes, demonstrating that research with pregnant women is possible and feasible (Fowler et al. 2015, Fowler et al. 2016, Zash et al. 2018a, Kintu et al. 2019, IMPAACT 2010/VESTED 2020) . Also studies such as PANNA and IMPAACT provide examples of international cooperation given

their similar design and thus the chance of conducting joint data analyses (Mofenson et al. 2019a, Abrams et al. 2020, IMPAACT P1026s 2020, PANNA 2020).

Other institutions and stakeholders have also been active in contributing to the needed paradigm shift. A Task Force on research Specific to Pregnant Women and Lactating Women (PRGLAC) established by the 21st Century Cures Act in the US has recently issued 15 recommendations on how to facilitate research and develop safe and effective therapies for pregnant and breastfeeding women in general (NIH 2018). PHASES, established in 2013 and focusing on ethical issues related to conducting and including pregnant women with HIV and co-infections (e.g. malaria, TB) in CT has released a guidance with 12 recommendations to obtain better, earlier and in a more systematic way evidence for pregnant women directed to multiple stakeholders (PHASES 2020). In 2019 the WHO organized a workshop with IMPAACT (IMPAACT/WHO) to reach a consensus on the appropriate design, analysis and interpretation of pharmacology studies in pregnant women living with HIV entitled “Approaches to Optimize and Accelerate Pharmacokinetic Studies in Pregnant and Lactating Women” (WHO et al. 2019). Additionally, **i**n 2019 the Pregnancy Research Ethics for Vaccines, Epidemics, and New Technologies **(**PREVENT) working group, an international team of multidisciplinary experts issued 22 recommendations to promote equity for pregnant women and their infants in epidemic vaccine development and response against emerging and re-emerging pathogens, including infection from Zika virus, influenza and Ebola (Krubiner et al. 2021). Among these recommendations, the need to plan urgently and proactively for the evaluation of vaccine candidates in pregnancy and to identify trials that meet ethical standards for fair inclusion of pregnant women based on B-R assessment are central (Krubiner et al. 2021).

### 7.4.4 Proposed actions to move forward

Regulators, pharma industries, academia, HCPs, activist and women themselves should all contribute to shift the paradigm. This process will most likely be an iterative rather than a radical shift, with step changes, some of which are already happening (section 7.4.3), with others discussed here.

Safety information could be captured by robust birth surveillance systems and studies. These should be standardized to be able to collect high-quality data and should be conducted in settings where most women are likely to use the medicine in order to get safety data as rapidly as possible. For example, very few post-authorisation pharmacovigilance studies are conducted in SSA, yet safety signal such as the DTG have and will inevitably come out from settings where the medicine will be widely prescribed and used by a large population. At the time of this thesis no EU-US study has reached the necessary number of observations to being able to rule-out the risk for NTDs since the signal was detected, however the Tsepamo Study reached, over the same time span, the necessary 2,000 observation to rule out the risk.

This is the current paradox of HIV high burden settings (usually LMIC) where well designed studies could identify signals of potential teratogenic risk, given the big number of observations, but where there are several limitations due to the settings (i.e. limited antenatal care access, limited ultrasound screening, difficulties in retention in care, high proportion of deliveries outside healthcare settings, etc.) and the fact that only the national/WHO guidelines recommended drugs can be evaluated (i.e. usually fixed combinations for the whole population with limited alternative options). On the contrary, settings with low burden of HIV (usually HIC) could identify with more precision signals given the wider availability and accessibility to new drugs with fewer barriers due to the setting (i.e. more accurate pregnancy dating, better access to antenatal care, access to ultrasound and prenatal diagnosis, better ascertainment of CA), yet safety data for specific drugs are very slow to accumulate mostly due to the smaller number of observations.

In the context of clinical recommendations, perhaps HCP could also implement preconception counselling. It will be important to understand if preconception counselling is provided to women of childbearing age by their HIV doctors, and whether this covers the important issue of ART safety, i.e. if the regimen a woman is currently on is a safe / appropriate option when she becomes pregnant.

The BHIVA guideline group were frontrunners in recommending a prior discussion with women of childbearing age on cART options, taking into account women's concerns and preferences and the possibility of individualised treatment and of switching after delivery to another regimen, preferable for long-term use based on toxicity and tolerability information (BHIVA 2020)

A recent qualitative study investigated reproductive counselling in the US and found preconception counselling is inconsistently integrated into primary/HIV healthcare (Simone et al. 2018). However, this and other studies identified a need to integrate preconception counselling into the primary/HIV care with the aim to provide the necessary information for a planned pregnancy (e.g. effective treatment to prevent/reduce VT, maintain supressed VL, contraceptive methods, PrEP for serodiscordant couple, etc.), but did not address the importance of also discussing the safety of treatment in the event of pregnancy (Steiner et al. 2013, Boelig et al. 2015, Coll et al. 2016, Simone et al. 2018).

Encouragingly, a recent Italian study by Floridia et al. acknowledges the importance of evaluating safety and efficacy of ARV combinations prescribed in pregnancy, particularly in the context of preconception counselling when therapeutic options should be discussed between pregnant women and their clinician (Floridia et al. 2020). Furthermore, the Centers for Disease Control and Prevention (CDC) and American College of Obstetricians and Gynaecologist (ACOG) have recently updated a joint guideline concerning *preconception counselling and care for women of childbearing age with HIV*, addressing the importance of educating and counselling women about factors that might affect the selection of a given ARV combination based on their different status (i.e. trying to conceive, pregnant women, or postpartum) addressing, for example, the case of DTG to explain toxicity and teratogenicity (CDC 2020b). Similarly, the European AIDS Clinical Society (EACS) guidelines have a designated section for "*Treatment of pregnant women living with HIV or women considering pregnancy*" listing ARVs not recommended in women who wish to conceive and with a note regarding reproductive health and the importance of reproductive counselling (EACS 2020).

Therefore, proactive preconception counselling from HCPs about safety of available ART regimen in pregnancy should be one of the recommended steps towards the paradigm shift. This will become even more relevant with the roll-out of long acting regiments like CAB LA, if women in HIV care are not being asked about their childbearing intentions, which means that the possibility of a planned or unplanned pregnancy is not considered by the physician.

Regulators need to be proactive both in pre-marketing and in post-marketing phases, addressing equally the benefit and the risk. In the pre-marketing phase they should impose study designs allowing inclusion of pregnant women and planning ahead for

for the post-marketing surveillance studies. As previously mentioned, this could start with retaining women with incident pregnancies in trials as long as a favourable B-R ratio is proven, by following them up and collecting pregnancy outcomes. In the post-marketing phase, regulators should update their guidelines and SmPCs, both addressing risk with warnings and cautions notes, and benefits confirming the favourable B-R ratio. Furthermore, post-marketing safety studies should be carried out routinely whenever data on a given population are not generated in earlier phases of drug development. Without planned evidence generation on drug usage, data will only become available when signals are either detected from follow-up in prospective observational studies or emerge from the general population usage, which includes women of childbearing age and those pregnant and breastfeeding.

It is important to remark that SmPCs and guidelines are meant to help HCP to make prescribing decisions. The passive approach of "banning" drugs based on absence of evidence should be replaced by the proactive approach of generating the data necessary for strong evidence-based recommendation and not only on a precautionary approach which inevitably will relegate women to use suboptimal and often older regimen.

The most recent SmPCs updates for both DTG formulations (i.e. 26/02/2021) discussed in section 7.3 demonstrate that regulators are moving in the right direction to fill the knowledge gap between real-world use and recommendations. This is an encouraging example of how B-R assessments for women of childbearing age and those pregnant should be conducted, i.e. taking into consideration the specifics of a safety signal such as that of NTDs (i.e. window of causality), the benefits and risks for the mother balanced with those of the fetus (i.e. risk for NTDs vs the risk of switching to other option), and offering counselling and effective contraceptive measures instead of imposing compliance with them in order to access the treatment.

I propose that a shift is needed from the current hierarchical structure of first generating data on safety and efficacy and then updating and "catching up" with real-world use/evidence-generation to a new continuous cycle where data can be better and more rapidly generated in the pre-marketing phase and better and more rapidly accumulated in the post-marketing phase, so that the identified gap between real-world use and regulatory recommendation can be filled, something that inevitably requires all the stakeholders involved to jointly cooperate as proposed by Figure 7.3.

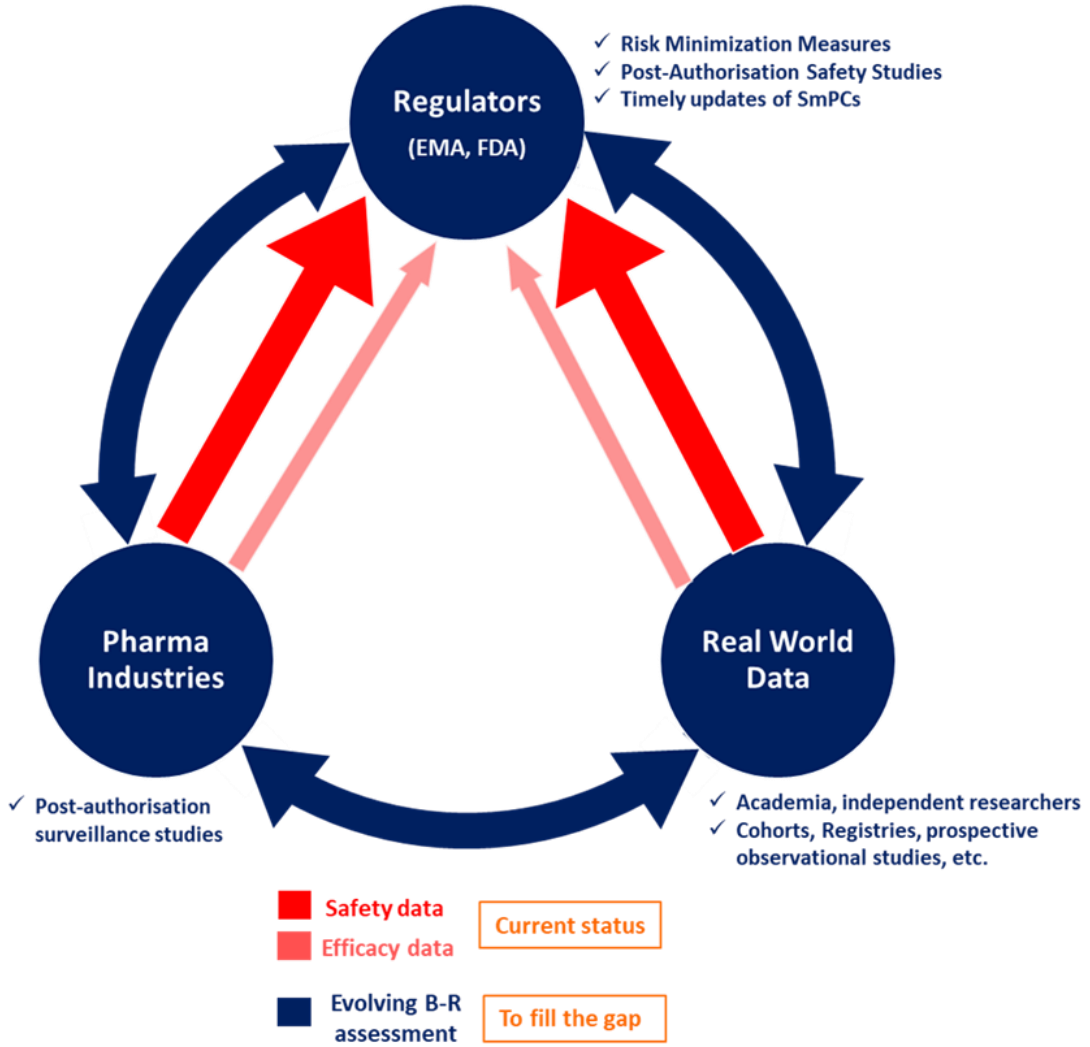



**Figure 7.3 Proposed actions in post-marketing phase to move towards a new paradigm**

Figure 7.3 illustrates the current (red and pink arrows) and the proposed process (blue cycle). The thick red arrows represent safety data flows, while the thin pink arrows represent the efficacy data flows. The difference in arrow size reflects the difference in the speed and the likelihood for safety and efficacy data to reach the decision-making level (i.e. regulators). The thick blue cycle arrows represent the proposed change to always integrate safety and efficacy data, whichever the source, for a continuous B-R assessment.

## 7.5 Strengths and Limitations

This thesis used data from three main sources: individual patient data from the NSHPC, a well-established population-based prospective surveillance study and from EPPICC, an international network of cohort studies, and publicly available data from the SmPC and EPAR to assess safe and effective data, along with regulatory recommendations from the EMA.

Two main strengths of the NSHPC are carried within its acronym: National and Surveillance; hence being nationally representative and being an active long-term surveillance programme collecting data on all HIV positive women diagnosed by the time of delivery and their infants engaging with maternity units across the UK. Furthermore, it is based on an unselected and unconsented population, hence it does not carry selection bias. It is characterised by very high (>90%) rates of reporting (Townsend et al. 2008b, Townsend et al. 2014) with a good ascertainment by both obstetric and paediatric respondents within routine clinical care (this parallel data collection system ensures the high case ascertainment rate). Good ascertainment is also guaranteed by the web-based data collection that makes reporting easier; the fact that responders are sent notification requests and have to also send null returns if no woman was seen (i.e. active surveillance); and the fact that maternity reporting is part of the NHS service specification, meaning is part of the responder's job to report (PHE/NHS. 2019).

The NSHPC collects data on any pregnancy outcome, hence I was able to include pregnancies ending in stillbirths, miscarriages and termination of pregnancies, resulting in a less biased estimates. Collection of these outcomes, and not just of livebirths is important to identify CAs which could be the cause of stillbirths and miscarriages or lead to planned termination of pregnancies. For example and as previously mentioned (section 7.2.2 and chapter 5) of the 11 NTDs reported to the NSHPC, only two occurred in liveborn infants, with the rest identified in other pregnancy outcomes, data that would have been lost if the NSHPC only collected data on livebirths.

Another strength of NSHPC data is the mother-infant pair linkage system and the consequent possibility to identify sequential pregnancies in the same women and therefore evaluate the potential individual contribution of each women in certain trends and patterns. For example, it was through this system that identification of one women contributing to three of the 11 reported NTDs was possible.

The NSHPC also carries some intrinsic limitations. It is a national study representing only one country, though looking at the demographics of the women, many (>77%) of

whom are African and consequently some aspects of the interaction between genetics and drugs may well be of generalisability to other settings.

The NSHPC is also a surveillance study, hence lacks the in depth collection of certain data such as background medical and obstetric history, and clinical measures in pregnancy. Therefore, medical underlying conditions such as epilepsy or comorbidities such as hypertension or diabetes are not routinely collected. Similarly, are not collected lifestyle information such as vitamin supplements assumption (e.g. folic acid) or smoking habits and alcohol/drug intake. In addition, no information on other medications (e.g. antidepressant, antivirals or antibiotics, etc.) is routinely collected, and it was therefore not possible to explore their potential effects and/or contribution to adverse events or potential for drug-drug interactions.

The NSHPC collects information on CAs both through maternal respondents (i.e. those identified at or shortly after delivery) and also through paediatric respondents (i.e. following follow-up visits). However, it is not primarily set up to collect information on CAs, hence there is not a routine standardized assessment of CAs by a study embryologist within the NSHPC The EUROCAT classification system was mainly used to overcome this limitation, this is also why I have decided to re-classify all the CAs reported to the NSHCP accordingly to this well-established standardized and increasingly used classification criteria that also allows sharing and comparison with other studies.

Another limitation of the NSHPC is that children HIV exposed but uninfected are not followed-up after infection status is confirmed and therefore there might be an under-ascertainment of late diagnosed congenital anomalies in some cART-exposed children. As children are meant to be followed up to 18 months, this duration should be sufficient to detect most late diagnoses of CAs. However, in more recent years, some paediatricians have been discharging children prior to this age, which might compromise assessment of CA with late manifestations.

As already discussed, for rare defects such as NTDs, at least 2,000 preconception/early first trimester exposures are required to rule out a 3-fold increase in the risk (i.e. for NTDs from 0.1% to 0.3%) (Watts 2007). This means that, even a national surveillance like the NSHPC is limited in regard to detection of rare defects. The number of pregnancies per year over the considered time period ranged from 1,279 in 2008 to 629 in 2018, and even though over time an increasing proportion of pregnancies were conceived under cART (i.e. from 38% in 2008 to 81% in 2018), these are still well under the required 2,000 preconception exposures, demonstrating the limitations of my analyses. Even when I evaluated the association between specific ARVs, such as RAL or commonly used combination such as TDF/FTC+EFV,

and risk for CA, numbers were still under the 2,000 observations. Even using EPPICC data to evaluate DTG use across the European participating countries, as reported in chapter 6 and here in section 7.3, with a pooled analysis, small numbers still limited the ability to draw stronger safety conclusions.

An analytical limitation was the impossibility to adjust for clustering by woman i.e. the fact that some women contributed more than one pregnancy to the dataset. As explained in chapter 3, I originally planned to account for this women-level clustering particularly when fitting regressions model to evaluate the association between ARV, time of exposure and the risk for CA by introducing one or more random effects, but this was not possible.

Lastly, this thesis mainly focused on safety and evaluation of CAs and the risk for CA in infants with *in utero* exposure to combinations of ARVs. However, there is another important aspect that will be interesting to further explore in the future that I have just briefly touched upon, that of the effectiveness of regimens (in terms of viral load suppression) and under-dosing. Women have been potentially exposed to under-dosage for years for some specific ARVs before studies were conducted that observed PK-PD pregnancy-related changes, as supported by the case of DRV, AT and, RPV co-administered with COBI.

With respect to the EMA data, I was limited to accessing only publicly available data. This is, for example, why of the 27 ARVs analysed, I was unable to retrieve the original SmPC at time of marketing authorisation for 12 ARVs and had to rely on extrapolating the necessary data on their safety and efficacy by accessing older version of published EPARs. Current versions of EPARs and SmPC are usually the only publicly available documents, with older versions or the original version usually archived at the EMA for access only by EMA staff. I was able to find older versions for some products in publicly-accessible archives, but since the EMA website was updated, this was no longer possible. Furthermore, because of an internal policy that anonymised the original sources of data, I could not clarify whether some of the data presented might have been duplicates, for example, collected from both the NSHPC and the APR and merged in a cumulative total to fill the predefined categories (e.g. “large amount of data” (>3,000 or >1,000, “moderate (300-1,000) etc.), considering that the NSHPC contributes to APR data on ARV use in pregnancy.

## 7.6 Future directions

This thesis identified three main findings: an increased earlier use of ARVs in pregnancy and from before conception and a wider range of available ARV combinations for pregnant women living with HIV in the UK between 2008-2018; a gap between real-world use of ART and the regulatory/clinical recommendations for pregnant women living with HIV; no evidence of increased risk of CAs in infants with *in utero* exposure to ART nor any particular patterns of CAs affecting the same organs/systems by the rule of three.

In this chapter I have briefly touched on the current *status quo*, on how my studies contributed to the existing literature, and how they are in line with recent strengthening efforts to support a paradigm shift to include women of childbearing age, pregnant and breastfeeding in research, at every level. There is an urgent need to ethically include women in CT, to tailor study designs, to enable proactive pharmacovigilance and to improve the post-marketing surveillance studies for a faster detection of rare events such as NTDs.

As mentioned at the beginning of my thesis, over the past two decades under-reporting of women in biomedical research has been recognised and progressively addressed, for example by initiatives such as the US “Women's Health Initiative” and “the Second Wave” aimed to promote ethically and responsible research in pregnant women. In the meantime, increasing evidence has accumulated proving that pregnant and non-pregnant women need to be included in CTs in order to generate relevant safe and effective data in a timely manner. Two major CTs led the way, namely WAVES and ARIA by enrolling only women, then two major studies followed, namely IMPAACT and PANNA evaluating pregnancy induced PK-changes in women exposed to ARV during pregnancy (T2/T3 vs postpartum period). However, it was the DTG signal that really propelled the discussion about the need to include pregnant and non-pregnant women in biomedical research and this new wave is already showing its effects toward a new paradigm, as supported by the ongoing and future studies reported in section 7.4.1. As discussed in section 7.2.4 the issue has received further attention during the current COVID-19 pandemic with respect to CTs of treatments and vaccine. The DTG safety signal was reported while I was already working on my PhD, having already selected DTG as a case study as new promising third agent, therefore I felt I could really contribute to this “new wave”.

All the above will be particularly relevant to future strategies for care, development of ARVs and for ART regimens such as long acting injectables and 2-drug ART regimens. New formulations such as CAB LA bring the challenge of an exposure from

before the time of conception potentially lasting throughout the whole pregnancy even if injections are stopped as soon as pregnancy is identified. It will be equally important to evaluate both safety and efficacy also for the 2-drug ART regimens. In fact, the potential increased B-R ratio produced by the decreased toxicity/ increased tolerability given the exposure to two instead of three drugs might be annulled by the potential decreased effectiveness. This in pregnancy could lead to lack of viral suppression and therefore to prevent VT.

Moving forward, it is also important to achieve the standardization of CT protocols, to allow comparison and encourage sharing and pooling data from different national and international studies in order to timely address any future potential signals. Collaborations and investment for studies in settings where most women with HIV live can maximize data aggregation, increasing the power of the findings whilst reducing the limitations coming from smaller studies.

Finally, collaboration and proactive cooperation from all the involved stakeholders should be achieved. Pharma industries should proactively propose ways to include pregnant women in CT protocols and regulatory guidelines should be flexible and easily adaptable to any new findings also coming from academia, real world evidence, independent research and surveillance studies such as the NSHPC. Surveillance studies have the potential to provide evidence on drug safety, even if their primary purpose is to monitor HIV burden and to provide a framework for service evaluation. In this way the system will move from a hierarchical structure to a continuous cycle of generated, accumulated and shared data among all the involved stakeholders to achieve the goal of providing the best and safest treatment for all women living with HIV.

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# 9 Appendices

## 9.1 NSHPC reporting forms (2019 editions)

### 9.1.1 Obstetric notification form

## NSHPC confidential pregnancy notification

MREC approval ref: MREC/04/2/009 — form date 10/19 — www.ucl.ac.uk/nshpc

**CONFIDENTIAL**

*Please complete all sections of this form.*

**PART 1: WOMAN'S DETAILS**

Date of birth: ___/___/___ | Hospital no.: | NHS/CHI no.: | Soundex: .....

Ethnic origin:
☐ White ☐ Black other
☐ Black African ☐ Asian, Indian Subcontinent
☐ Black Caribbean ☐ Other Asian / Chinese
☐ Mixed or other, specify: .....

Country of birth: .....
If not UK/Ireland, date arrived: ___/___/___

Postcode (leave off last letter): ☐☐☐☐ ☐☐■

Previous pregnancies:
*Please indicate below numbers of previous livebirths/stillbirths/miscs/terms and dates of livebirths where known.*
..... livebirth(s), date(s) if known: _____ ..... stillbirth(s) ..... misc(s)/term(s)

**PART 2: INFECTION HISTORY**

Maternal infection probably acquired: ☐ In UK/Ireland ☐ Abroad, specify: ..... ☐ Not known

Likely exposure:
☐ Heterosexual, specify partner's likely risk factor if known: .....
☐ Vertical transmission, specify place and age at diagnosis: .....
☐ Injecting drug use
☐ Other, specify: .....

Date of 1st positive test: ___/___/___ | CDC stage C disease ever? ☐ No ☐ Yes, date of onset: ___/___/___ (specify details overleaf) | If type 2 only, tick here: ☐

Diagnosed *when*: ☐ During this pregnancy or ☐ Before this pregnancy

Diagnosed *where*: ☐ Antenatal ☐ GUM clinic ☐ Other, specify: .....

Any evidence of seroconversion in this pregnancy? ☐ No ☐ Yes (specify details overleaf) ☐ Not known

**PART 3: PREGNANCY DETAILS**

Antenatal booking date: ___/___/___ | EDD: ___/___/___ and/or LMP: ___/___/___

Pregnancy status:
☐ Continuing to term – planned mode of delivery: ☐ Vaginal ☐ CS ☐ Not yet decided
☐ Miscarriage* – date: ___/___/___ at ..... weeks gestation
☐ Termination* – date: ___/___/___ at ..... weeks gestation
*If miscarriage or termination, any congenital abnormality? ☐ No ☐ Yes: .....

Infant feeding intention at booking: ☐ Breastfeeding ☐ Artificial (formula) feeding ☐ Not yet decided

**PART 4: DRUG TREATMENT DURING THIS PREGNANCY**

Was this woman on antiretrovirals when she became pregnant? ☐ No ☐ Yes

Did she receive antiretrovirals during pregnancy? ☐ No ☐ Yes ☐ Not yet ☐ Declined

| Antiretroviral drugs | Before preg? | Date started (or gest. week) | Date stopped (or gest. week) |
|---|---|---|---|
| Drug 1 ..... | Yes / No | ___/___/___ ____ | ___/___/___ ____ |
| Drug 2 ..... | Yes / No | ___/___/___ ____ | ___/___/___ ____ |
| Drug 3 ..... | Yes / No | ___/___/___ ____ | ___/___/___ ____ |
| Drug 4 ..... | Yes / No | ___/___/___ ____ | ___/___/___ ____ |
| Drug 5 ..... | Yes / No | ___/___/___ ____ | ___/___/___ ____ |

**PART 5: MATERNAL CLINICAL STATUS**

Symptomatic in this pregnancy? ☐ No ☐ Yes, specify: .....
Sexual health screening test in this pregnancy? ☐ No ☐ Yes, 1st screen date this pregnancy: ___/___/___
Concurrent maternal infection(s)? ☐ None ☐ HBV ☐ HCV ☐ Syphilis ☐ Other, specify: .....

**PART 6: MATERNAL TEST RESULTS**

*Please provide the first test results available in this pregnancy.*
Viral load: _____ copies/ml Date: ___/___/___ CD4: _____ (____%) Date: ___/___/___

Form completed by: Name: _____ Date: ___/___/___
Position: _____ Telephone: _____ Email: _____

**Please return to: CONFIDENTIAL, FAO H Peters, Surveillance Studies Group PPP Programme, UCL GOS Institute of Child Health, 30 Guilford St, London WC1N 1EH. Telephone 020 7905 2739 or email nshpc@ucl.ac.uk for any queries (helen.peters2@nhs.net for those with identifiers).**

## 9.1.2 Obstetric Outcome form

# NSHPC outcome of notified pregnancy

MREC approval ref: MREC/04/2/009 form date 10/19 www.ucl.ac.uk/nshpc

**CONFIDENTIAL**

*Please complete all required sections of this form.*

Your ref: EDD: Hospital of delivery:

**PART 1: CHILD INFORMATION**

☐ Livebirth or ☐ Stillbirth
If twins*, tick here: ☐
*Please give details of second twin overleaf

Date of birth: ___/___/___ Gest.: ..... wks ☐ Male or ☐ Female

Birthweight: ..... kg Birth head circumference: ..... cm

Hospital no. .....
NHS/CHI no. .....
Paediatrician: .....

Congenital abnormalities? ☐ No ☐ Yes: .....
Other perinatal problems? ☐ No ☐ Yes: .....
Planned mode of infant feeding?
☐ Planning to formula feed only ☐ Planning to breastfeed*
*please give details in part 8 of form

**PART 2: PREGNANCY AND DELIVERY DETAILS**

Postcode at delivery (leave off last letter): ☐☐☐☐ ☐☐■

Pregnancy complications:
☐ None
☐ Pre-eclampsia*
☐ Gestational diabetes
☐ Other: .....
.....
*please give details overleaf

Invasive procedures in pregnancy:
☐ None ☐ Amniocentesis
☐ CVS ☐ Cordocentesis
If yes, date of procedure: ___/___/___
Viral load at time of procedure:
..... copies/ml Date: ___/___/___

Mode of delivery:
☐ 1. Elective CS to prevent mother-to-child transmission
☐ 2. Planned vaginal delivery
☐ 3. Elective CS for any other reason
☐ 4. Unplanned vaginal delivery
☐ 5. Emergency CS
Reason for delivery by 3, 4, or 5: .....
.....
*Planned* mode of delivery: ☐ Vaginal ☐ Elective CS ☐ Not known

Invasive procedures at delivery (tick all that apply):
☐ None
☐ Ventouse
☐ Forceps, type: .....
☐ Scalp monitor
☐ FBS
Symptomatic at delivery?
☐ No ☐ Yes: .....
.....
If died, date of death: ___/___/___

Rupture of membranes? ☐ No / Only at delivery ☐ Yes, duration: ..... hours ..... minutes

**PART 3: DRUG TREATMENT DURING PREGNANCY**

Antepartum treatment? ☐ No ☐ Yes
If yes, reason for treatment: ☐ Prevention of mother-to-child transmission ***only***
☐ Maternal health ***and*** prevention of mother-to-child transmission

| Antiretroviral drugs: | Date started (or gest. week) | Date stopped (or gest. week) |
|---|---|---|
| Drug 1 ..... | ___/___/___ ____ | ___/___/___ ____ |
| Drug 2 ..... | ___/___/___ ____ | ___/___/___ ____ |
| Drug 3 ..... | ___/___/___ ____ | ___/___/___ ____ |
| Drug 4 ..... | ___/___/___ ____ | ___/___/___ ____ |
| Drug 5 ..... | ___/___/___ ____ | ___/___/___ ____ |

Any other significant drugs (e.g. PCP prophylaxis, TB treatment, methadone, illicit drugs):
Drug 1 ..... Date: ___/___/___ Drug 2 ..... Date: ___/___/___
Additional treatment intra-partum:
☐ None ☐ IV AZT ☐ Single dose nevirapine ☐ Other oral antiretrovirals: .....
Post-partum for infant:
☐ None ☐ Oral AZT ☐ IV AZT ☐ Triple, specify: .....

**PART 4: MATERNAL TEST RESULTS NEAR DELIVERY**

Viral load: _____ copies/ml Date: ___/___/___ CD4: _____ (____%) Date: ___/___/___
Clade of virus if known: _____

Form completed by: Name: _____ Date: ___/___/___
Position: _____ Telephone: _____ Email: _____

**Please return to: CONFIDENTIAL, FAO H Peters, Surveillance Studies Group PPP Programme, UCL Institute of Child Health, 30 Guilford St, London WC1N 1EH. Telephone 020 7905 2739 or email nshpc@ucl.ac.uk for queries (helen.peters2@nhs.net for those with identifiers).**

Please enter any additional relevant information in the space below.

**PART 5: ADDITIONAL INFORMATION**

Please complete parts 6 and 7 in the case of a twin pregnancy.

**PART 6: CHILD INFORMATION FOR SECOND TWIN**

☐ Livebirth or ☐ Stillbirth

Date of birth: ___/___/___ Gest ..... wks ☐ Male or ☐ Female

Birthweight ..... kg Birth head circumference ..... cm

Hospital no. .....
NHS/CHI no. .....
Paediatrician: .....

Congenital abnormalities? ☐ No ☐ Yes: .....
Other perinatal problems? ☐ No ☐ Yes: .....
Planned mode of infant feeding?
☐ Planning to formula feed only ☐ Planning to breastfeed*
*please give details in part 8 of form

**PART 7: TWIN CHORIONICITY AND AMNIONICITY**

Chorionicity: ☐ Monochorionic ☐ Dichorionic ☐ Chorionicity not known

Amnionicity: ☐ Monoamniotic ☐ Diamniotic ☐ Amnionicity not known

Please complete part 8 ***if this mother is planning to breastfeed.***

**PART 8: BREASTFEEDING CIRCUMSTANCES**

Is breastfeeding being managed in line with current BHIVA Guidelines*? ☐ No ☐ Yes ☐ Not known

*Please be aware that breastfeeding is not recommended in the current BHIVA Guidelines. See BHIVA Guidelines 8.4 Infant Feeding: https://www.bhiva.org/file/FCUpXrfVqWsYi/BHIVA-Pregnancy-guidelines-update-2014.pdf.

What are the reasons for wanting to breastfeed? *Please tick boxes that most closely fit this case.*

☐ Bonding
☐ Health benefits for baby/mother
☐ Financial concerns
☐ Concerns about disclosure of HIV status
☐ Breastfed previously (before diagnosis)
☐ Breastfed previously (after diagnosis)
☐ Family/friends expectations/pressure
☐ Other, details: .....
.....

What is the intended duration of breastfeeding? ..... weeks / months (circle) or ☐ Not known

GP aware of mother's HIV status? ☐ No ☐ Yes ☐ Not known
Partner aware of mother's HIV status? ☐ No ☐ Yes ☐ Not known

Please provide any other information related to management of breastfeeding here:

**Please return to: CONFIDENTIAL, FAO H Peters, Surveillance Studies Group PPP Programme, UCL Institute of Child Health, 30 Guilford St, London WC1N 1EH. Telephone 020 7905 2739 or email nshpc@ucl.ac.uk for queries (helen.peters2@nhs.net for those with identifiers).**

## 9.1.3 Paediatric notification form

# NSHPC confidential paediatric notification

MREC approval ref: MREC/04/2/009 form date 10/18 www.ucl.ac.uk/nshpc

CSTU MSTU SU PAED HOSP

**PART 1: CHILD INFORMATION**

Date of birth: ___/___/___ ☐ Male or ☐ Female Initials: ..... Soundex: .....

Hospital no. .....
NHS/CHI no. .....

Ethnic origin:
☐ White ☐ Black other
☐ Black African ☐ Asian, Indian Subcontinent
☐ Black Caribbean ☐ Other Asian / Chinese
☐ Mixed or other, specify: .....

Home postcode (leave off last letter): ☐☐☐☐ ☐☐■
Home postcode at birth: ☐☐☐☐ ☐☐■

Place of birth:
☐ UK/Ireland – hospital of birth: .....
☐ Abroad – country of birth: .....

How was this child identified as infected or at risk of infection?
☐ Mother known to be infected in pregnancy
☐ Mother diagnosed after the birth of this child
☐ Child symptomatic
☐ Other family member diagnosed
☐ Other, specify: .....

Siblings?
If you are aware of any *siblings* reported to us, please give dates of birth or other ref. below:
.....
.....
.....

**PART 2: DETAILS OF EXPOSURE TO INFECTION (MATERNAL OR OTHER)**

Exposed to maternal infection? ☐ No* ☐ Yes (if yes, complete all of part 2) ☐ Not known
*If no, other exposure risk for child? ☐ No ☐ Yes, specify: .....

Mother's date of birth: ___/___/___
Mother's country of birth: .....
If not UK/Ireland, date arrived: ___/___/___

Mother's no. of previous livebirths: ..... stillbirths: ..... miscarriages/terminations: .....

Mother diagnosed *when*:
☐ Before this pregnancy
☐ During this pregnancy
☐ At delivery
☐ After the birth of this child

Mother's likely source of infection:
☐ Heterosexual, specify partner's likely risk factor if known: .....
☐ Vertical transmission, specify place and age at diagnosis: .....
☐ Injecting drug use
☐ Other, specify: .....

Maternal infection probably acquired: ☐ In UK/Ireland ☐ Abroad, specify: ..... ☐ Not known

**PART 3: DETAILS FOR CHILDREN BORN IN UK/IRELAND (for children born abroad, skip to part 4)**

I. Perinatal details

Gest ..... weeks Birthweight ..... kg
Birth head circumference ..... cm

Mode of delivery:
☐ Vaginal
☐ Elective CS
☐ Emergency CS
☐ Not known

Concurrent maternal infection(s)?
☐ None
☐ HBV
☐ HCV
☐ Syphilis
☐ Other, specify: .....

Congenital abnormalities?
☐ No ☐ Yes, specify: .....

Other confirmed infection(s) in infant?
☐ No ☐ Yes, specify: .....

Other problems? ☐ None ☐ Necrotising enterocolitis
☐ Other, specify: .....

Infant required ventilation?
☐ No ☐ Yes, details: .....

Was the infant breastfed? ☐ No ☐ Yes, specify duration: ..... ☐ Not known
If yes, this was: ☐ Before maternal diagnosis
☐ By diagnosed mother on fully suppressive therapy
☐ By diagnosed mother in other circumstances, specify: .....
.....

II. Treatment details

Antiretrovirals given for mother and/or infant to reduce risk of vertical transmission? ☐ No ☐ Yes

-ART antenatally? ☐ None ☐ Yes, specify: ..... ☐ Not known

-ART at delivery? ☐ None ☐ IV AZT ☐ Not known
☐ Other, specify: .....

-ART post-partum for infant? ☐ None ☐ Not known
☐ Oral AZT ☐ IV AZT Date started: ___/___/___ Duration .....wks
☐ Triple, specify: ..... Date started: ___/___/___ Duration .....wks

III. Laboratory investigation results

Please indicate this child's current infection status: ☐ Infected ☐ Presumed uninfected* ☐ Indeterminate

*We regard a child as **a) presumed uninfected** on the basis of two negative PCR results over the age of 1 month (with one test at age ≥3 months, if not breast feeding. If breast feeding, need to have two negative PCR results 4 and 8 weeks after stopping) and **b) definitively uninfected** based on a negative antibody result over the age of 18 months.

Diagnostic test results:
Please provide results and sample dates of all diagnostic tests *including earliest (+ or -) PCR result for infected infants.*

| | + - | sample date | + - | sample date | + - | sample date |
|---|---|---|---|---|---|---|
| Antibody: | ☐☐ | ___/___/___ | | | | |
| PCR (type below): | ☐☐ | ___/___/___ | ☐☐ | ___/___/___ | ☐☐ | ___/___/___ |
| PCR test type: | ☐ DNA ☐ RNA ☐ N/K | | ☐ DNA ☐ RNA ☐ N/K | | ☐ DNA ☐ RNA ☐ N/K | |

Viral load (if detectable): _____ copies/ml Date: ___/___/___ If type 2 infection, tick here: ☐

Any laboratory or clinical side effects of ART in exposed infant (eg anaemia, neutropenia, adrenal dysfunction, lactic acidosis)? ☐ No ☐ Yes, specify: .....

**PART 4: DETAILS FOR INFECTED CHILDREN BORN ABROAD**

I. Diagnosis and treatment details

Date of arrival in UK/Ireland: ___/___/___ Date of first clinical presentation in UK/Ireland: ___/___/___

Diagnosed *when*: ☐ Before arrival in UK/Ireland, year: ..... & country: .....
☐ After arrival in UK/Ireland

If diagnosed abroad, any ARVs before arrival in UK/Ireland?
☐ No
☐ Yes, specify drugs and dates if known: .....
.....
☐ Not known

II. Laboratory investigation results

Diagnostic test results:
Please provide results and sample dates of all diagnostic tests undertaken in UK/Ireland.

| | + - | sample date | + - | sample date | + - | sample date |
|---|---|---|---|---|---|---|
| Antibody: | ☐☐ | ___/___/___ | ☐☐ | ___/___/___ | ☐☐ | ___/___/___ |
| PCR (type below): | ☐☐ | ___/___/___ | ☐☐ | ___/___/___ | ☐☐ | ___/___/___ |
| PCR test type: | ☐ DNA ☐ RNA ☐ N/K | | ☐ DNA ☐ RNA ☐ N/K | | ☐ DNA ☐ RNA ☐ N/K | |

Viral load (if detectable): _____ copies/ml Date: ___/___/___ If type 2 infection, tick here: ☐

**PART 5: TREATMENT AND CLINICAL DETAILS FOR *ALL INFECTED CHILDREN***

Date of last examination: ___/___/___

Current antiretroviral treatment? ☐ No ☐ Yes ☐ Not known
If yes, specify drugs: .....

Any CDC stage C symptoms? ☐ No ☐ Yes, specify details and dates below:
1. ..... Date (mm/yy): /
2. ..... Date (mm/yy): ___/___

**PART 6: FOLLOW-UP STATUS *FOR ALL CHILDREN***

Date of last contact: ___/___/___

Any other serious conditions diagnosed? ☐ No ☐ Yes, specify: .....
.....

**Current status:**
☐ Still in follow-up at this unit
☐ Discharged (uninfected)

**If not seen:**
☐ Follow-up elsewhere, details: .....
☐ Lost to follow-up, details.....
☐ Known to have left UK/Ireland
☐ Dead, date of death: ___/___/___ & cause of death: .....

Form completed by: Name: _____ Date: ___/___/___

Position: _____ Telephone: _____ Email: _____

**Thank you for your help. Please return this form to: CONFIDENTIAL, FAO H Peters, Surveillance Studies Group PPP Programme, UCL GOS Institute of Child Health, 30 Guilford St, London WC1N 1EH. Telephone the NSHPC on 020 7905 2815 or email nshpc@ucl.ac.uk if you have any queries. For emails containing identifiers, please contact: kate.francis3@nhs.net**

## 9.1.4 Paediatric outcomes form

# NSHPC follow-up to establish infection status

MREC approval ref: MREC/04/2/009 form date 10/18 www.ucl.ac.uk/nshpc

CSTU MSTU HOSP

**PART 1: CHILD INFORMATION**

Date of birth: ___/___/___ | Sex: ..... | Initials: ..... | Soundex: .....

NHS/CHI no. .....

**PART 2: INFECTION STATUS AND LABORATORY INVESTIGATIONS**

Has an antibody test been carried out at ≥18months?

☐ No, details ..... ☐ Yes, please provide below:

| | + | - | sample date | + | - | sample date | + | - | sample date |
|---|---|---|---|---|---|---|---|---|---|
| Antibody (≥18months): | ☐ | ☐ | ___/___/___ | | | | | | |
| PCR (type below): | ☐ | ☐ | ___/___/___ | ☐ | ☐ | ___/___/___ | ☐ | ☐ | ___/___/___ |
| PCR test type: | ☐ DNA ☐ RNA ☐ N/K | | | ☐ DNA ☐ RNA ☐ N/K | | | ☐ DNA ☐ RNA ☐ N/K | | |

If 18 month antibody not done please provide any PCR results (with dates) undertaken since ___/___/___.

*We regard a child as **a) presumed uninfected** on the basis of two negative PCR results over the age of 1 month (with one test at age ≥3 months, if not breast feeding. If breast feeding, need to have two negative PCR results 4 and 8 weeks after stopping) and **b) definitively uninfected** based on a negative antibody result over the age of 18 months.

**Part 3: Infant feeding**

Was the infant breastfed? ☐ No ☐ Yes, specify duration: ..... ☐ Not known

If yes, this was: ☐ Before maternal diagnosis
☐ By diagnosed mother on fully suppressive therapy
☐ By diagnosed mother in other circumstances, specify: .....
.....

**PART 4: ART EXPOSURE SIDE EFFECTS**

Any laboratory or clinical side effects of ART in exposed infant (e.g. anaemia, neutropenia, adrenal dysfunction, lactic acidosis)? Update if any additional side effects since

☐ No ☐ Yes, specify: .....

**PART 5: FOLLOW-UP STATUS**

Date of last contact: ___/___/___

Any other serious conditions diagnosed?
☐ No ☐ Yes, specify: .....

Current status:
☐ Still in follow-up at this unit
☐ Discharged (uninfected)

If not seen:
☐ Follow-up elsewhere, details: .....
☐ Lost to follow-up, details.....
☐ Known to have left UK/Ireland
☐ Dead, date of death: ___/___/___ & cause of death: .....

Thank you for completing this form. If you have any further details, please write them on the back of this form.

Form completed by: Name: _____ Date: ___/___/___

Position: _____ Telephone: _____ Email: _____

Thank you for your help. Please return this form to: CONFIDENTIAL, FAO H Peters, Surveillance Studies Group PPP Programme, UCL Institute of Child Health, 30 Guilford St, London WC1N 1EH. Telephone the NSHPC on 020 7905 2815 or email kate.francis3@nhs.net if you have any queries.

## 9.2 EPPICC-DOLOMITE reporting forms

# EPPICC INSTI Drug Safety Studies

## Data Merger

Standard Operating Procedure for data transfer (v 1.0)

**1. Introduction**

This document provides guidance on the preparation of data files for the DOLOMITE-EPPICC Study and for the RAL Pregnancy Study.

It is expected that each cohort will be responsible for gathering, computerizing and submitting its own data. Subsequently it will be electronically merged by the DOLOMITE-EPPICC Data Manager.

**The deadline for data submission for this merger is 11 February 2019.** Cohorts are welcome to send data in advance of this date.

After the submission of data we will be working closely with studies to clean the data. This will involve:

- sending out data consistency checks in the form of a discrepancy report
- processing responses and sending further checks where necessary

**2. Eligibility criteria**

All pregnant women with any exposure to **Dolutegravir** (TIVICAY®, TRIUMEQ®, JULUCA and D3) AND/OR to **Raltegravir** (RAL, ISENTRESS®) at any time during the pregnancy, regardless of pregnancy outcome, including pregnancies that are still ongoing at the time of the merger.

In addition to individual patient data on eligible mother-infant pairs, participating studies will be required to provide aggregate data on **all** pregnancies (not only those exposed to Dolutegravir or Raltegravir) via the CONTEXT table. These aggregate data will provide a context for the interpretation of study results (e.g. rates of adverse birth outcomes) and allow us to describe trends in use of INSTIs in pregnancy over time.

**3. General data considerations**

Formats based on the HIV Cohorts Data Exchange Protocol (HICDEP) will be used for all data submissions for this study. The HICDEP format is based on a relational structure, and the data for this study will be collected in a series of tables, which are described in the next section. Look-up tables for the codes to be used may be specified within the table descriptions or in the appendices.

**Please refer to the HICDEP specification** (http://hicdep.org/Wiki/v/8/pt/2) for further clarification of variable definitions and formats, or contact the EPPICC Data Manager.

The pregnancy data requested will generally refer to the entire period from conception until delivery, unless otherwise indicated. Please provide as complete information as possible.
Please provide us with your raw data for numeric values; please do not round these values up or down.

If data are missing, please ensure that the field is left blank.

Regarding dates, ideally we would like precise dates (ie YEAR-MONTH-DAY), but it might be that only the month or year is known for some dates.

- Where the date day is unknown, the date should be coded as the 15th of the month, so that 1999-12-?? becomes 1999-12-15. This enables the date to be no more than 15 days away from the actual date.
- In case both the month and day are unknown, the date should be coded from the mid-point of the year, so that 1999-??-?? becomes 1999-07-01.
- If the year is unknown please code as 1911-11-11.

**4. Data transfer procedure**

We are happy to accept files in either Access or Excel. Files should all be sent together. Please do not send incomplete files or different files on different dates. Files may be sent via email or we can arrange to do this via FTP, on request.

**Email:** The initial data files should be zipped and password-protected (preferably using WinZIP AES encryption) and emailed to [redacted].

The password for the zip file (and the name of the zip software used, eg 7-zip www.7-zip.org ) should be sent in a separate email.

**5. Definitions**

| Pregnancy Outcome | Definition |
|---|---|
| Induced abortion | Voluntary termination of pregnancy before 22 weeks gestation |
| Spontaneous abortion | Death of a fetus or expulsion of the products of conception before 22 weeks gestation |
| Low birth weight | Birth weight of <2500 grams |
| Very low birth weight | Birth weight of <1500 grams |
| Preterm birth | Birth of live infant at <37 weeks gestation |
| Stillbirth | Death of a fetus occurring at 22 weeks of gestation or more, or for situations in which the gestational age is unavailable, a fetus weighing at least 500 g |

## 5. Description of the data tables

**Table 1 - BAS table - Demographic data for each mother**

Demographic data for the mother (1 row per patient)

Each woman should appear once in this table.

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID for the mother (unique and anonymous) |
| BIRTH_D | yyyy-mm-dd | Birth date of mother |
| ETHNIC | 10 = White<br>20 = Black<br>21 = Black African<br>22 = Black Caribbean<br>30 = Hispanic<br>40 = Asian<br>50 = Indigenous from Americas<br>60 = Indigenous from other continents<br>1020 = White/Black<br>1040 = White/Asian<br>2030 = Black/Hispanic<br>3040 = Hispanic/Asian<br>102040 = White/Black/Asian<br>97 = other<br>98 = Prohibited<br>99 = Unknown | Ethnicity of woman |
| ORIGIN | Numeric with codes<br>http://hicdep.org/Wiki/v/8/pt/4/Table/49/FieldID/637 | Country of birth *(if country of birth not available, please provide region)* |
| HIV_D | yyyy-mm-dd | Date HIV was diagnosed. |
| AIDS_Y | 0=No<br>1=Yes<br>9=unknown | Has patient been given an AIDS diagnosis? (i.e. WHO stage 3 or 4, or CDC stage C) |
| AIDS_D | yyyy-mm-dd | Date AIDS was diagnosed. |
| HCV_COINF | 0=No<br>1=Yes<br>9=unknown | Is the woman seropositive for HCV? |
| HBSAG_Y | 0=No<br>1=Yes<br>9=unknown | Is the woman hepatitis B surface antigen positive? |
| HBACTIVE_Y | 0=No<br>1=Yes<br>9=unknown | Does the woman have detectable HBV DNA and/or is HBeAg positive? |
| MODE | 2 = injecting drug user<br>5 = transfusion, non-haemophilia related<br>6 = heterosexual contact<br>7 = heterosexual contact and injecting drug user<br>8 = vertical | Mode of HIV infection |

| | | |
|---|---|---|
| | 90 = other (specify)<br>99 = unknown | |
| MODE_OTH | Character | Mode of infection – other<br>*Complete if MODE = 90* |

**Table 2 - PREG table – Information specific to each pregnancy**

Please complete one row per pregnancy per mother.

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of mother |
| PREG_ID | Character or numeric | Unique ID for this pregnancy |
| MENS | yyyy-mm-dd | Date of last menstrual period<br>If unknown, please put 1911-11-11 |
| EDD | Yyyy-mm-dd | Estimated date of delivery |
| GESTITY | Numeric | Total number of known pregnancies <u>including this current reported one</u> (including all known miscarriages, terminations, ectopic pregnancies, newborns,..etc) |
| PARITY | Numeric | Total number of prior deliveries after 22 weeks (alive or dead) - <u>excluding this reported one</u> |
| PREV_DRUG | 0=No<br>1=Yes<br>9=Unknown | Has the mother used illegal drugs before this pregnancy? |
| P_TOBACCO | 0=No<br>1=Yes<br>9=Unknown | Has the mother used tobacco during this pregnancy? |
| P_ALCOHOL | 0=No<br>1=Yes<br>9=Unknown | Has the mother used alcohol during this pregnancy? |
| P_DRUG | 0=No<br>1=Yes<br>9=Unknown | Has the mother used illegal drugs during this pregnancy? |
| CARE_D | yyyy-mm-dd | Date pre-natal care was initiated<br>*If unknown, please put 1911-11-11* |
| N_FETUS | Numeric | Number of embryos/fetuses in this pregnancy |
| OUTCOME | 1=Livebirth ≥22 weeks gestation (even if infant died after birth)<br>2= miscarriage / spontaneous abortion (<22 weeks gestation)<br>3=termination by choice<br>4=termination - ultrasound | Outcome of pregnancy / reason pregnancy was interrupted – where appropriate please put full details in DEFECTS table |

| | | |
|---|---|---|
| | abnormality<br>5=termination – other/ unknown reason<br>6=Stillbirth (≥22 gest weeks)<br>9=unknown | |
| OUTCOME_D | yyyy-mm-dd | Date of outcome |
| INT_DETAILS | Free text | Other details about miscarriage, causes of termination or intra-uterine death, if available |

**Table 3 -ART table - antiretroviral treatment data**

For this table, please only include data on drugs taken during the pregnancy. If the drug was started prior to the pregnancy and continued during the pregnancy, please include the actual start date prior to the pregnancy. Please ensure that this table is completed for **all drugs taken from the date of conception to delivery for each pregnancy** reported.

Please also include any drugs given orally during labour that were not included in antenatal regimens (e.g. single dose NVP, double dose tenofovir, raltegravir) in this table; both the start and stop date will be the date of delivery in these cases. Information on intravenous ZDV should be provided in the ZDV_INTRAPARTUM table.

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| PREG_ID | Character or numeric | Unique ID for this pregnancy |
| ART_ID | J05AX12=Dolutegravir<br>J05AR13=Triumeq (Lamivudine, abacavir and dolutegravir)<br>J05AX08=Raltegravir<br>See appendix for list of other codes - please include data on all drugs taken during the pregnancy | Code representing the antiretroviral drug |
| ART_SD | yyyy-mm-dd | Start date |
| ART_ED | yyyy-mm-dd | Stop date |
| ART_RS | Numeric with codes<br>http://hicdep.org/Wiki/v/8/pt/4/Table/47/FieldID/594 | Main reason for stopping |
| ART_RS_OTH | Character (free text) | If ART_RS=98 (other causes), please give details here |
| ART_CONC | 0=No<br>1=Yes<br>9=unknown | Was this drug being taken at the time of conception? |

**Table 4 – ZDV_INTRAPART table – intrapartum use of ZDV**
(1 row per pregnancy)

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| PREG_ID | Character or numeric | Unique ID for this pregnancy |
| ZDV_IP | 0=No<br>1=Yes<br>9=unknown | Was intrapartum ZDV given during labour? |

**Table 5 - LAB_CD4 table - CD4 data**
Please complete this table for all known CD4 measurements from conception to delivery for each pregnancy.

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| PREG_ID | Character or numeric | Unique ID for this pregnancy |
| CD4_D | yyyy-mm-dd | Date of CD4 measurement |
| CD4_V | Numeric | Value of CD4 measurement |
| CD4_U | 1=cells/mm$^3$<br>2=% | CD4 cell count or CD4 % |

**Table 6 -LAB_RNA table – HIV-1 RNA data**
Please complete this table for all known viral load measurements from conception to delivery for each pregnancy

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID |
| PREG_ID | Character or numeric | Unique ID for this pregnancy |
| RNA_D | yyyy-mm-dd | Date of HIV-1 RNA measurement |
| RNA_V | Numeric<br>For undetectable values, enter -1 in RNA_S and limit of detection in RNA_L | HIV-1 RNA measurement value (copies/ml) |
| RNA_S | -1 = less than the value in RNA_V<br>0 = exactly equal to value in RNA_V<br>1 = greater than value in RNA_V | Sign of result for RNA_V<br>Flag to indicate whether the result in RNA_V is the exact value or whether less or more |
| RNA_L | Numeric | Lower limit of HIV-1 RNA assay (leave blank if unknown) |
| RNA_U | Numeric | Upper limit of HIV-1 RNA assay (leave blank if unknown) |

**Table 7 - LINKAGE table – allows mothers, pregnancies and fetus or babies to be matched**

| Field name | Format | Description |
|---|---|---|

| | | |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| PREG_ID | Character or numeric | Unique ID for pregnancy. Twins will both have the same values of PATIENT and PREG_ID |
| FETAL_ID | Character or numeric | Unique ID for fetus delivered at <22 weeks. This will be blank for other outcomes |
| BABY_ID | Character or numeric | Unique ID for baby (delivered at ≥22 weeks). This will be blank for other outcomes |

**Table 8 - FETAL_LOSS table – data regarding reasons for pregnancy loss or interruption before 22 weeks** (1 row per fetus)
Please complete this table for pregnancies ending in miscarriage or termination before 22 weeks. For any pregnancies ending after 22 weeks, the NEWBORN table should be completed.

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| PREG_ID | Character or numeric | Unique ID for pregnancy |
| FETAL_ID | Character or numeric | Unique ID for this fetus. |
| US_ABN | 0=No<br>1=Yes<br>9=unknown | Was there an ultrasound abnormality? |
| US_ABN_TYPE | Free Text | Describe ultrasound abnormality |
| FET_LOSS_D | Free text | Describe reason for miscarriage or termination, if known. |

Table 9 - NEWBORN table – data regarding delivery and the baby if born at 22 or more weeks

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| PREG_ID | Character or numeric | Unique ID for pregnancy |
| BABY_ID | Character or numeric | Unique ID for this baby |
| DELIV_D | yyyy-mm-dd | Date of delivery (*NB This will be the same date as OUTCOME_D)* |
| GEST_AGE | Numeric<br>99=missing | Gestational age (in completed weeks) at delivery |
| DELIV_M | 1=Vaginally, spontaneous<br>2=Vaginally, forceps<br>3=Vaginally, vacuum<br>4=Vaginally, unknown if spontaneous or instrumented<br>10=Caesarean section, primary/elective (before onset of labour and rupture of membrane)<br>11=Caesarean section, secondary ("emergency caesarean")<br>12=Caesarean section, unspecified<br>99=unknown | Mode of delivery |
| GENDER | 1=male<br>2=female<br>9=unknown | Sex of baby |
| WEIGHT | Numeric<br>999 if unknown | Weight of baby at birth in g |
| HEIGHT | Numeric<br>999 if unknown | Length of baby at birth in cm |
| HEAD | Numeric<br>999 if unknown | Head circumference of baby at birth in cm |
| NEO_DEATH | 1=stillborn<br>2=neonatal death<br>3=alive<br>9=unknown | Stillbirth or neonatal death (first 4 weeks of life) |
| DEATH_D | yyyy-mm-dd | Date of baby's death |
| DEATH_CAUSE | Free text | Cause of death. If autopsy carried out, please provide results. |
| BIRTH_DEFECT | 0=No<br>1=Yes<br>9=unknown | If yes, please provide details in separate DEFECTS table |
| NEO_PROPH | 0 = None<br>1 = ZDV prophylaxis<br>2 = Combination neonatal prophylaxis with 2 drugs | What type of neonatal prophylaxis did the baby receive? |

| | | |
|---|---|---|
| | 3 = Combination neonatal prophylaxis with 3 drugs<br>4 = Prophylaxis was given but type not known<br>5=sdNVP only<br>9 = unknown | |
| NEO_DUR | Numeric<br>99=unknown | Duration of neonatal prophylaxis in weeks |
| BRFEED_Y | 0=No<br>1=Yes<br>9=unknown | Was baby ever breastfed? |
| BRFEED_DUR | Numeric | How long did breastfeeding last (in days)? |
| HIV_BABY | 0=uninfected<br>1=infected<br>2=indeterminate<br>9=unknown | Infection status of the baby at most recent follow-up |

**Table 10 - DEFECTS table – data regarding birth defects in newborn and stillborn infants** (one row per baby per birth defect)

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| PREG_ID | Character or numeric | Unique ID for pregnancy |
| BABY_ID | Character or numeric | Unique ID for this baby – will be blank if delivery < 22 gestational weeks |
| DIAG_D | yyyy-mm-dd | Date this birth defect was diagnosed |
| DIAG_DESCRIP | Free text | Details of birth defect |
| DIAG_ICD | Code with one letter and 2 to 4 digits | ICD 10 code for birth defect if known |

**Table 11 - BABY_LAB table – HIV DNA PCR and antibody test results** *(1 row per measurement)*

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| BABY_ID | Character or numeric | Unique ID for this baby |
| VS_ID | *HIVA: HIV antibodies*<br>*HIVD: HIV DNA PCR*<br>*HIVR: HIV RNA PCR* | Test identification |
| VS_R | If antibodies or HIV DNA PCR:<br>0=negative<br>1=positive<br>9=unknown/borderline<br>If HIV RNA PCR: | Test result |

| | | |
|---|---|---|
| | 0=undetectable<br>If detectable provide value (copies/ml) | |
| VS_D | yyyy-mm-dd | Test date |

**Table 12 - OVERLAP table – patients overlapping with other cohorts**

This table only needs to be completed if you think this Mother could be reported by another cohort in the merger.

| Field name | Format | Description |
|---|---|---|
| PATIENT | Character or numeric | Cohort patient ID of the mother |
| COH_OTH | Character | Other cohort who this patient is part of |
| PAT_OTH | Character | Unique patient ID in the other cohort |

**Tables 13a and 13b – CONTEXT tables – aggregated data**

Please complete the following tables for all singleton pregnancies (not just for those you are submitting data in this merger) included in your cohort/study in women diagnosed with HIV before or during pregnancy, or as a result of intrapartum HIV testing. This will include pregnancies with DTG or RAL use, those with other ART regimens and those with no ART.

Table 13a – CONTEXT_Outcomes

| | Total number delivered (livebirths/stillbirths) or with estimated date of delivery (abortions) in each calendar year | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pregnancies | Livebirths (LB) | Stillbirths (SB) | Spontaneous abortion | Induced abortion | Unknown outcome | Of LB and SB, number preterm (<37w) |
| 2015 | | | | | | | |
| 2016 | | | | | | | |
| 2017 | | | | | | | |
| 2018 | | | | | | | |

**Table 13b – CONTEXT_ART table**

Please complete the table with the total number of singleton pregnancies with ANY exposure to drugs from these classes and the number with no ART exposure at all:

| | Total number with delivery (LB/SB) or estimated date of delivery (abortions) in each calendar year | | | |
|---|---|---|---|---|
| | INSTI | PI | NNRTI | No antenatal ART |
| 2015 | | | | |
| 2016 | | | | |
| 2017 | | | | |
| 2018 | | | | |

**Appendix**

**ART_ID** http://hicdep.org/Wiki/v/8/pt/4/Table/47/FieldID/589

| Code (Extended ATC Codes) | Anti-Retroviral Drugs |
|---|---|
| J05A | ART unspecified |
| J05A-BEV | Bevirimat |
| J05A-PBT | Participant in Blinded Trial |
| J05AE | PI unspecified |
| J05AE-MOZ | Mozenavir (DMP-450) |
| J05AE01 | Saquinavir (gel, not specified) |
| J05AE01-SQH | Saquinavir hard gel (INVIRASE) |
| J05AE01-SQS | Saquinavir soft gel (FORTOVASE) |
| J05AE02 | Indinavir (CRIXIVAN) |
| J05AE03 | Ritonavir (NORVIR) |
| J05AE03-H | Ritonavir high dose (NORVIR) |
| J05AE03-L | Ritonavir low dose (NORVIR) |
| J05AE04 | Nelfinavir (VIRACEPT) |
| J05AE05 | Amprenavir (AGENERASE) |
| J05AR10 | Lopinavir/Ritonavir (Kaletra). Former code: J05AE06 |
| J05AE07 | Fos-amprenavir (Telzir, Lexiva) |
| J05AE08 | Atazanavir (Reyataz) |
| J05AE09 | Tipranavir (Aptivus) |
| J05AE10 | Darunavir (TMC-114, Prezista) |
| J05AF | NRTI unspecified |
| J05AF-ALO | Alovudine |
| J05AF-AMD | Amdoxovir (DADP) |
| J05AF-FOZ | Fozivudine tidoxi |
| J05AF-LDN | Lodenosine (trialdrug) |
| J05AF-RVT | Reverset |
| J05AF01 | Zidovudine (AZT, RETROVIR) |
| J05AF02 | Didanosine (ddI) (VIDEX) |
| J05AF03 | Zalcitabine (ddC) (HIVID) |
| J05AF04 | Stavudine (d4T) (ZERIT) |
| J05AF05 | Lamivudine (3TC, EPIVIR) |
| J05AF06 | Abacavir (1592U89) (ZIAGEN) |
| J05AF07 | Tenofovir (VIREAD) |
| J05AF09 | Emtricitabine |
| J05AG | NNRTI unspecified |
| J05AG04 | Etravirine (TMC 125) |
| J05AG05 | Rilpivirine (TMC-278) |
| J05AG-CPV | Capravirine |

| | |
|---|---|
| J05AG-DPC083 | DPC 083 |
| J05AG-DPC961 | DPC 961 |
| J05AG-EMV | Emivirine (MKC442) |
| J05AG-LOV | Loviride |
| J05AG01 | Nevirapine (VIRAMUN) |
| J05AG02 | Delavirdine (U-90152) (RESCRIPTOR) |
| J05AG03 | Efavirenz (DMP-266) (STOCRIN, SUSTIVA) |
| J05AR01 | Combivir (Zidovudine/Lamivudine) |
| J05AR02 | Kivexa (Lamivudine/Abacavir) |
| J05AR03 | Truvada (Tenofovir/Emtricabine) |
| J05AR04 | Trizivir (Zidovudine/Lamivudine/Abacavir) |
| J05AR05 | Douvir-N (Zidovudine/Lamivudine/Nevirapine) |
| J05AR06 | Atripla (Emtricitabine/Tenofovir/Efavirenz) |
| J05AR07 | Triomune (Stavudine/Lamivudine/Nevirapine) |
| J05AR08 | Eviplera/Complera (Emtricitabine/Tenofovir/Rilpivirine) |
| J05AR09 | Stribild (Emtricitabine/Tenofovir/Elvitegravir/Cobicistat) |
| J05AR10 | Kaletra/Aluvia (Lopinavir/Ritonavir) |
| J05AR11 | Lamivudine, tenofovir disoproxil and efavirenz |
| J05AR12 | Lamivudine and tenofovir disoproxil |
| J05AR13 | Triumeq (Lamivudine, abacavir and dolutegravir) |
| J05AR14 | Darunavir and cobicistat |
| J05AR15 | Atazanavir and cobicistat |
| J05AR16 | Lamivudine and raltegravir |
| J05AR17 | Emtricitabine and tenofovir alafenamide |
| J05AR18 | Emtricitabine, tenofovir alafenamide, elvitegravir and cobicistat |
| J05AR19 | Emtricitabine, tenofovir alafenamide and rilpivirine |
| J05AX-VIC | Vicriviroc (Schering) |
| J05AX07 | Enfuvirtide (Fuzeon, T-20) |
| J05AX08 | Raltegravir (Merck) |
| J05AX09 | Maraviroc (Pfizer) |
| J05AX11 | Elvitegravir |
| J05AX12 | Dolutegravir |
| J05AX-CAB | Cabotegravir (GSK-744) |
| L01XX05 | Hydroxyurea/Hydroxycarbamid (Litalir) |
| V03AX03 | Cobicistat |

## 9.3 Data extrapolated from the EMA's SmPCs and EPARs document for the examined ARVs

| Trade name | ARVs | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Pregnancy and breastfeeding. Clinical data. | Sec 5.2 PK properties in preg/B-F | Sec 5.3 Preclinical safety data | Placenta crossing | Half-life (h) |
|---|---|---|---|---|---|---|---|---|
| Atripla | EFV/FTC/ TDF | 2007 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | WCBP/contraceptive measures: preg should be avoided, preg test& barrier contraception should be always used +another method during and for 12w after discontinuation given long t ½ of EFV. Preg: EFV 904 preg with T1 exposure; 766LB, 1 case of NTD; FTC/TDF large (>1,000) data indicates no malformative or fetal/neonatal toxicity. **B-F**: all excreted in human milk. **Recommendation:** Should not be used in preg unless clinical condition of the women requires Tx. Not used during BF/Avoid BF | No data | **EFV:** no reProdTox in rats& rabbits; but yes 3/20 fetuses/newborns of cynomolgus monkey• **FTC/TDF**: no reProdTox, TDF: ↓viability index and ↓weight of pups in a postnatal toxicity study a.m.t.d | EFV®,FTC®, TDF® | EFV 52<br>FTC 10<br>TDF 12-18 |
| Combivir | 3TC/ZDV | 1998 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | Large amount of data (>3,000, T1) from AZT, and large amount (>1,000, all Trim) from 3TC no malformative or fetal/neonatal toxicity. **B-F**: all excreted in human milk. **Recommendation:** The malformative risk is unlikely in humans based on the large amount of data. Avoid BF | PK in preg similar to that of non-preg women | **3TC:** reProdTox in rabbit (↑early embryonic death)/no teratogenicity **ZDV:** reProdTox in rats a.m.t.d (↑ incidence of malformation) | 3TC®, ZDV® | 3TC 5-7<br>ZDV 1.1 |
| Descovy | FTC/TAF | 2016 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | No-adequate& well controlled studies. Limed (<300) data on TAF; Large (>1000) data on FTC indicates no malformative or fetal/neonatal toxicity. **B-F:** TAF nk, FTC excreted in human milk. **Recommendation:** Should be used in preg only if the potential B justifies the potential R to the fetus. Avoid BF | No data | **FTC:** no reProdTox in rats &rabbits. **TAF** no evidence of harmful effects on fertility, preg, foetal development | TAF® | FTC 10<br>TAF 0.51 |
| Edurant | RPV | 2011 | **Sec 4.2, 4.4** Lower exposure of RPV in preg seen in Phase III studies; VL should be monitored & consider switching to another cART. | Limited (<300) data on RPV. Lower exposure of RPV in preg; VL should be monitored closely. **B-F:** nk in human milk. **Recommendation:** as a precautionary measure, it is preferable to avoid its use during preg. **Sec 4.4**: should be used in preg only if potential B justifies potential R. Avoid BF | **RPV:** Study TMC114HIV301 on 19 preg women showed 30% AUC's ↓ in T2-T3 vs PP | **RPV**: no reProdTox nor teratogenicity | RPV® in animal | RPV 45 |
| Emtriva | FTC | 2003 | **Sec 4.4.** Mitochondrial dysfunction following *in utero* exposure to NRTIs | Moderate (300-1,000) data on FTC indicates no malformative or fetal/neonatal toxicity. **B-F:** excreted in human milk. **Recommendation:** The use of FTC may be considered during preg, if necessary. Should not be used in BF/Avoid BF | No data | **FTC:** no reProdTox | FTC® | FTC 10 |
| Epivir | 3TC | 1996 | **Sec 4.4.** Mitochondrial dysfunction following *in utero* exposure to NRTIs | Large (>1,000) data from all Trim on 3TC indicates no malformative or fetal/neonatal toxicity. **B-F:** excreted in human milk. **Recommendation:** Can be used during pregnancy if clinically needed. The malformative risk is unlikely in humans based on those data. Avoid BF | PK: Oral administration in late preg similar to non-preg woman | **3TC:** increase in early embryonic deaths in rabbits but not in rats. | 3TC | 3TC 5-7.5 |

| Trade name | ARVs | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Pregnancy and breastfeeding. Clinical data. | Sec 5.2 PK properties in preg/B-F | Sec 5.3 Preclinical safety data | Placenta crossing | Half-life (h) |
|---|---|---|---|---|---|---|---|---|
| Eviplera | FTC/RPV/ TDF | 2011 | **Sec 4.2, 4.4** Lower exposure of RPV in preg seen in Phase III studies; VL should be monitored & consider switching to another cART.<br>**Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | WCBP/contraceptive measures: must use effective contraception. No-adequate& well controlled studies. No/limited (<300) data on RPV: lower exposure of RPV in preg; VL should be monitored closely; large (>1000) data on FTC/TDF indicate no malformation or fetal/neonatal toxicity. **B-F**: FTC/TDF excreted in human milk, RPV nk. **Recommendation:** As a precautionary measure, it is preferable to avoid the use in preg. No BF under Eviplera/Avoid BF | No data for **TDF/FTC. RPV**: TMC114HIV301o n 19 preg women showed 30% AUC's ↓ in T2-T3 vs PP (6-12w) | **FTC/TDF**: no reProdTox or developmental toxicity. **RPV:** no teratogenicity. Limited power to cross placenta | RPV in animal, FTC@, TDF@ | FTC 10<br>TDF 12-18<br>RPV 45 |
| Evotaz | ATV/COBI | 2015 | **Sec 4.2, 4.4.:** COBI levels in preg ↓ with low ATV exposure; risk of virological failure &↑risk of VT. Tx should not be initiated in preg and women who become preg should be switched to alternative cART regimen. | Moderate (300-1,000) data from ATV indicate no malformative or fetal/neonatal toxicity. Additional monitoring in the prepartum period should be considered due to the possibility of ATV to exacerbate physiological hyperbilirubinemia and kernicterus in neonates. **B-F**: ATV excreted in human milk **Recommendation:** not recommended in preg nor should be initiated in preg; this is due to substantially lower exposure of COBI & consequent lower exposure to ATV. No BF under Evotaz | | **ATV, COBI:** no reProdTox, no teratogenicity, but **COBI** in rats showed ossification changes in spinal column a.m.t.d. | COBI@<br>ATV@ | ATV 12<br>COBI 3-4 |
| Genvoya | FTC/TAF/ EVG/c | 2015 | **Sec 4.2,4.4:** COBI levels in preg ↓ with low ATV exposure; risk of virological failure &↑risk of VT. Tx should not be initiated in preg and women who become preg should be switched to alternative cART regimen. **Sec 4.4 WCBP**: contraceptive requirements Mitochondrial dysfunction following *in utero* exposure to NRTIs. | WCBP/contraceptive measures: should be used an effective methods (oral contraceptives or others). Plasma concentration of drospirenone might be ↑, hence clinical monitoring due to potential for hyperkalaemia. No/limited (<300) data; no data on TAF; Large (>1,000) data on FTC indicates no malformative or fetal/neonatal toxicity. EVG/c in T2-T3 substantial ↓ in EVG exposure which may result in VL failure and ↑VT. **B-F**: EVG/c/TAF nk in human milk. FTC excreted in human milk. **Recommendation:** Should not be initiated in preg, and women who become preg during therapy should be switched to alternative regimen. Should not be used in BF//Avoid BF | No data for **TAF/FTC. EVG/c**: IMPAACTP1026s, showed lower EVG exposure, in T2-T3 vs PP (for EVG ↓ in AUC of 24% in T2 & 44% in T3; for COBI ↓ in AUC of 44% in T2 & 59% in T3) | **EVG, FTC, TAF:** no special hazard. **COBI**: no reProdTox, but ↑ post-implantation loss and ↓ foetal weights in rats associated with a significant ↓in maternal body weights at 125mg/kg/day. | TAF@, NK for FTC, EVG, COBI | FTC 10<br>TAF 0.51<br>COBI 3.5<br>EVG 12.9 |
| Isentress | RAL | 2007 | **No data** | Moderate (300-1,000 T1) data on RAL indicate no malformative nor fetal/neonatal toxicity. APR to monitor maternal-fetal outcomes in pt inadvertently administered RAL while preg. **B-F:** nk in human milk. **Recommendation:** Should be given in preg only if expected B justifies the potential R to the fetus. Should not be used in BF/Avoid BF | No data | No reProdTox nor teratogenic; slight ↑ of supernumerary ribs only in rats of dams exposed to 4.4-fold human exposure at the RHD^. | RAL in animal | RAL 9 |

| Trade name | ARVs | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Pregnancy and breastfeeding. Clinical data. | Sec 5.2 PK properties in preg/B-F | Sec 5.3 Preclinical safety data | Placenta crossing | Half-life (h) |
|---|---|---|---|---|---|---|---|---|
| Kaletra | LPV/r | 2001 | **Sec 4.3, 4.4** contraindication in preg women due to potential risk of toxicity from excipient propylene glycol | Large (>3,000) data, including >1,000 T1 exposure to LPV mostly from APR have shown no pattern of CAs & comparable prevalence of CA to the one observed in general population. On the base of PK and CT studies proposed dose in preg and PP of 400/100 mq BID. Some studies have shown ↑ risk of prematurity with all PIs (particularly LPV/r). **BF:** excreted in human milk. **Recommendation:** Based on the data the malformative risk is unlikely in humans, can be used during pregnancy if clinically needed. Avoid BF | PK on dose adjustments in pregnancy. | **LPV/r:** reProdTox only in rats (slight embryotoxicity with preg loss, decreased fetal viability & fetal body weights) | Not stated | LPV 12 |
| Kivexa | ABC/3TC | 2004 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | Discrete (>800) data from T2; moderate (>1,000) from T2-T3 on ABC and moderate (>1,000) from all Trim on 3TC indicate no malformative nor fetal/neonatal toxicity. Mitochondrial dysfunction. **B-F:** both excreted in human milk. **Recommendation**: No data on Kivexa use in preg, but the malformative risk is unlikely in humans based on those data. Avoid BF | No data | **ABC:** toxicity to developing embryo/fetus only in rats (↓ fetal body weight, fetal oedema, ↑ skeletal variation, early intrauterine deaths and stillbirth), **3TC**: no teratogenic effect, but ↑ in early embryonic deaths only in rabbits. | ABC, 3TC both in animal & human | ABC 1.5<br>3TC 5-7.5 |
| Odefsey | FTC/RPV/ TAF | 2016 | **Sec 4.2, 4.4** Lower exposure to RPV in phase III studies in preg; risk of ↑VL; consider switching to another cART regimen<br>**Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs. | WCBP/contraceptive measures: should be used a contraception. No adequate/well-controlled studies; moderate (>1,000) data on FTC indicate no malformative nor fetal/neonatal toxicity; limited (<300) data on RPV & TAF. ↓ exposure of RPV in preg; VL should be monitored closely. **B-F:** FTC excreted in human milk; nk RPV &TAF **Recommendation:** As a precautionary measure, it is preferable to avoid its use in preg. Not used in BF/Avoid BF | No data for **TAF/FTC. RPV:** TMC114HIV301on 19 preg women showed 30% AUC's ↓ in T2-T3 vs PP (6-12w) | **FTC, RPV, TAF:** no reProdTox nor fetal development toxicity. | TAF®, FTC®, RPV® | FTC 10<br>TAF 0.51<br>RPV 45 |

| Trade name | ARV | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Pregnancy and breastfeeding.<br>Clinical data. | Sec 5.2 PK properties in preg/B-F | Sec 5.3 Preclinical safety data | Placenta crossing | Half-life (h) |
|---|---|---|---|---|---|---|---|---|
| Prezista | DRV/r | 2007 | **Sec 4.2,4.4.** low exposure to DRV in T2-T3; ↑risk of viral failure & VT. Caution for preg women with concomitant medications which may decrease further DRV exposure | No adequate/well-controlled studies. In preg **B-F:** nk if excreted in human milk. **Recommendation:** DRV/r should be used during preg only if the potential B justifies the potential R. Avoid BF | TMC114HIV3015 PK, efficacy &safety data DRV/r arm Phase 3b: 6/7 women had DRV/r's AUC of 26% & 15% lower in T2-T3 vs PP (6-12w) | **DRV/r:** no reProdTox, no teratogenicity; only in rat N of corpora lutea and implantations were ↓ a.m.t.d. | Not stated | DRV 15 |
| Reyataz | ATV/r | 2004 | **sec 4.2** 300/100mg might not provide enough exposure to ATV; caution due to drug resistance & decreased exposure to ATV due to interaction with h2-receptor antagonist/not recommended in pt with use of both h2 antagonist &TDF); in PP exposure to ATV might increase, so recommended close monitor | Moderate (300-1,000) data indicate no malformative or fetal/neonatal toxicity. In CT AI424-182 ATV/r + ZDV/3TC in 41 women in T2-T3 with no cases of lactic acidosis; 6/20 with 300/100mg & 13/21 with 400/ 100mg had grade 3 to 4 hyperbilirubinemia. In PP consider monitoring as nk if preg will exacerbate physiological hyperbilirubinemia & kernicterus in neonates/infants. **B-F:** found in human milk. **Recommendation:** Use may be considered in preg only if the potential B justifies the potential R.Avoid BF | PK: ATV plasma concentration approx. 2-fold higher in PP vs those observed historically in HIV non-preg pt. | **ATV/R:** no ReProdTox, no teratogenicity | Not stated | ATV 12 |
| Rezolsta | DRV/c | 2014 | **Sec 4.2,4.4.** low exposure to DRV in T2-T3; ↑ risk of viral failure & VT. Caution for preg women with concomitant drugs that may ↓ further DRV exposure. DRV/COBI should not be initiated in preg and women who become preg during Tx should be switched to another cART; DRV/r can be an alternative. | No adequate/well-controlled studies in preg **B-F:** nk if excreted in human milk. **Recommendation:** DRV/COBI 800/150mg in preg results in low DRV exposure and ↑ risk of TX failure and ↑risk of VT, so should not be initiated in preg and women who become preg during Tx should be switched to alternative cART regimen. Avoid BF | TMC114HIV3015 PK, efficacy and safety data from DRV/c arm Phase 3b; 6/7 women completed study & showed AUC of DRV/c 56% & 50%, respectively lower in T2-T3 vs PP (6-12w) | **DRV:** N of corpora lutea & implantations ↓ a.m.t.d; no teratogenicity<br>**COBI:** no ReProdTox, no teratogenicity, but rats showed ossification changes in spinal column a.m.t.d | Not stated | DRV 15<br>COBI 3.5 |
| Stribild | EVG/c FTC/TDF | 2013 | **Sec 4.2** EVG/c in preg results in lower EVG exposure.<br>**Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs. **WCBP**: warning re contraceptive measures/risk for hyperkalaemia. **Preg:** EVG/c T2-T3 lower EVG exposure; ↓ may result in VL failure & ↑ VT infection. Should not be initiated in preg. | WCBP/contraceptive measures: must be used an effective methods (oral contraceptives or others). Plasma concentration of drospirenone might be ↑, hence clinical monitoring for potential hyperkalaemia. No/limited (<300) data; Large (>1,000) data on FTC/TDF indicates no malformative nor fetal/neonatal toxicity. EVG/c during T2-T3 shown substantial reduction in EVG exposure, which may result in VL failure and ↑VT. **B-F**: EVG/COBI nk in human milk, FTC/TDF excreted in human milk. **Recommendation:** Should not be initiated in preg, and women who become preg during therapy should be switched to alternative regimen. Should not be use in BF/Avoid BF | No data for **TDF/FTC**. **EVG/c:** IMPAACT P1026s, showed lower EVG exposure, in II-III trim vs PP for EVG/c-containing regiments. | **EVG, FTC:** no special hazard. **COBI**: No reProdTox, but ↑ post-implantation loss & ↓ fetal weights in rats.<br>**TDF:** no reProdTox, but ↓ viability index/weight of pups | Not stated | FTC 10<br>TDF 12-18<br>COBI 3.5<br>EVG 12.9 |

| Trade name | ARV | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Pregnancy and breastfeeding.<br>Clinical data. | Sec 5.2 PK properties in preg/B-F | Sec 5.3 Preclinical safety data | Placenta crossing | Half-life (h) |
|---|---|---|---|---|---|---|---|---|
| Symtuza | DRV/c FTC/ TAF | 2017 | **Sec 4.2,4.4** low exposure to DRV/COBI 800/150mh in T2-T3 (↓ of 90% in $C_{min}$ levels); ↑ risk of viral failure & VT. Should not be initiated in preg, if so, switched to alternative cART regimen.<br>**Sec 4.4.** Mitochondrial dysfunction following *in utero* exposure to NRTIs. | No adequate/well-controlled studies alone. Tx DRV/c in preg results in low DRV exposure which might be associated with ↑ risk of Tx failure and ↑ risk of VT. **B-F:** FTC excreted in human milk, nk for the other. **Recommendation:** Should not be initiated during preg, and women who become preg while on therapy should be switched to an alternative regimen. Not to be used in BF/Avoid BF | No data for **FTC/TAF.** **DRV/c:** lower DRV exposure in T2(56% ↓AUC) & in T3(50% ↓AUC) vs PP. Main cause of low exposures is marked ↓ in COBI as consequence of preg-associated enzyme induction | **DRV/FTC/TAF/ COBI**: no reProdTox, nor teratogenicity. **COBI:** in rats ossification changes in the spinal column and sternebrae of fetuses a. (significant) m.t.d | DRV[@], TAF[@] | FTC 10<br>TAF 0.51<br>DRV 5.5 |
| Sustiva/ Stoctin | EFV | 1999 | No data | WCBP/contraceptive measures: preg should be avoided, preg test& barrier contraception should be always used+ another method during and for 12w after discontinuation given long t ½ of EFV. Preg: EFV 904 preg with T1 exposure; 766LB, 1 case of NTD; FTC/TDF large (>1,000) data indicates no malformative or fetal/neonatal toxicity. **B-F**: all excreted in human milk. **Recommendation:** Should not be used in preg unless clinical condition of the women requires Tx. Not used during BF/Avoid BF | No data | **EFV:** no reProdTox, but 3/20 fetuses/newborns of cynomolgus monkey*<br>**FTC/TDF**: no reProdTox, TDF: ↓viability index and ↓weight of pups | EFV | EFV 52 |
| Tivicay | DTG | 2014 | No data | WCBP/ contraceptive measures: preg test before Tx initiation; effective contraception. Limited data from use of DTG/RPV. Preliminary data from a surveillance study suggested ↑ incidence of NTDs (0.9%) in mother exposed at conception vs non exposed to DTG (0.1%). Moderate (>1,000) data from T2-T3 indicates no malformative nor fetal/neonatal toxicity. **B-F**: nk if excreted in human milk. **Recommendation:** Should only be used during T2-T3 of preg when the expected B justifies the potential R to the fetus. Avoid BF | No data | In rats and rabbits no developmental toxicity, nor teratogenicity. | DTG in animal | DTG 14 |

| Trade name | ARV | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Pregnancy and breastfeeding.<br>Clinical data. | Sec 5.2 PK properties in preg/B-F | Sec 5.3 Preclinical safety data | Placenta crossing | Half-life (h) |
|---|---|---|---|---|---|---|---|---|
| Tybost | COBI/c | 2013 | **Sec 4.2, 4.4** low exposure to DRV in T2-T3; ↑ risk of viral failure & VT. Caution for preg women with concomitant drugs that may ↓ further DRV exposure. **Sec 4.4** Plasma concentrations of ethinyloestradiol are ↓when co-administered with drospirenone & DRV/COBI. Alternative/additional contraceptive measures are recommended. ATV/COBI co-administered with drospirenone/ ethinyloestradiol requires monitoring due to potential for hyperkalemia. | No/limited data. Tx DRV/COBI in preg results in low DRV exposure which might be associated with ↑ risk of Tx failure and ↑ risk of VT. There are limited data from its use in combination with ATV, plasma levels of COBI and consequently of ATV may ↓ significantly in preg. **B-F:** nk in human milk. **Recommendation:** DRV/COBI should not be initiated in preg and women who become preg while on TX should be switched to an alternative regimen. ATV/COBI should only be used in preg if potential B justifies potential R to fetus & mother. Should not be used in BF. **Sec 4.4:** DRV/COBI should not be initiated in preg; DRV/r can be an alternative. Avoid BF. | No data | **COBI:** in rats& rabbits no special hazard no teratogenicity, but in rats, ossification changes in the spinal column and sternebrae of fetuses a. (significant) m.t.d. | Not stated | COBI 3-4 |
| Triumeq | ABC/3TC /DTG | 2014 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | WCBP/ contraceptive measures: preg test before Tx initiation; effective contraception. Preliminary data from a surveillance study suggested ↑ incidence of NTDs (0.9%) in mother exposed at conception vs non exposed to DTG (0.1%). Moderate (>1,000) data from T2-T3 indicates no malformative nor fetal/neonatal toxicity; moderate (> 400) data for T1 use of ABC/3TC & large (>3,000) data on T1 use of 3TC & moderate (>600) data on T1 on ABC indicates no malformative toxicity. Mitochondrial dysfunction reports in HEU exposed to NRTI. **B-F:** 3TC/ ABC excreted in human milk, DTG nk. **Recommendation:** Should be used in preg only if expected B justifies the potential R to the foetus. Avoid BF. | No data | **ABC:** toxicity to the developing embryo & fetus only in rats (↓ fetal body weight, fetal oedema, ↑ skeletal variation, early intrauterine deaths and stillbirth), **3TC:** no teratogenic but ↑ in early embryonic deaths in rabbits at low systemic exposures. **DTG**: no developmental toxicity, nor teratogenicity. | DTG, 3TC, ABC in animal | DTG 14<br>ABC 1.5<br>3TC 5-7.5 |
| Trizivir | ZDV/3TC /ABC | 2000 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | Moderate (>300) data for T1 use on ABC/3TC/ZDV as individual ARVs and large (>3,000) data for T1 use of ZDV&3TC as single ARVs & (>2,000) data from ZDV/3TC use & moderate (>600) data for T1 use of ABC indicate no malformative toxicity. Mitochondrial dysfunction reports in HEU exposed to NRTI **B-F:** all excreted in human milk. **Recommendation:** The malformative risk is unlikely in humans based on the mentioned moderate amount of data. Avoid BF. | No data | **ABC/ 3TC:** as Triumeq<br>**ZDV:** no evidence of foetal abnormalities at lower doses, only a.m.t.d. increased incidence of malformations | Not stated | ABC 1.5<br>3TC 5-7.5<br>ZDV 1.1 |

| Trade name | ARV | Year of A. | Sec 4.2 Posology<br>Sec 4.3 Contraindication<br>Sec 4.4 Special Warnings | Sec 4.6 Pregnancy and breastfeeding.<br>Clinical data. | Sec 5.2 PK properties in preg/B-F | Sec 5.3 Preclinical safety data | Placenta crossing | Half-life (h) |
|---|---|---|---|---|---|---|---|---|
| Truvada | FTC/TDF | 2005 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | Large (>1,000) data on FTC/TDF indicates no malformative nor fetal/neonatal toxicity. **B-F:** both excreted in human milk. **Recommendation:** The use may be considered in pregnancy, if necessary. should not be used in BF/Avoid BF. | No data | **TDF/FTC**: no reProdTox<br>TDF: ↓viability index and ↓weight of pups in a postnatal toxicity study a.m.t.d | FTC®, TDF® | FTC TDF 12-18 |
| Viramune | NVP | 1998 | No data | WCBP/ contraceptive measures: contraceptive measures, but not just oral as NVP can lower their plasma concentration. No adequate/well-controlled studies alone. The limited data indicates no malformative nor fetal/neonatal toxicity. Not enough data on risk of hepatotoxicity for preg women with CD4 >250cells/mm3 & detectable HIV-RNA, as no preg women was included in any CT, caution in prescribing. **B-F:** excreted in human milk. **Recommendation:** Caution should exercised when prescribing NVP to pregnant women. Avoid BF | No data | **NVP:** no teratogenicity, no reProdTox; strong placenta transfer | NVP | NVP 45 |
| Viread | TDF | 2002 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | Large (>1,000) data on TDF use indicate no malformative nor fetal/neonatal toxicity **B-F:** excreted in human milk. **Recommendation:** The use of TDF may be considered during pregnancy, if necessary. Should not be used in BF/Avoid BF | No data | **TDF:** no reProdTox, but ↓viability index and ↓weight of pups in a postnatal toxicity study a.m.t.d | TDF® | TDF 12-18 |
| Ziagen | ABC | 1999 | **Sec 4.4** Mitochondrial dysfunction following *in utero* exposure to NRTIs | Moderate (>800) data for T1 use, large (>1,000) data for T2-T3 use indicate no malformative nor fetal/neonatal toxicity. Mitochondrial dysfunction reports in HEU exposed to NRTI. **B-F:** excreted in human milk. **Recommendation:** The malformative risk is unlikely in humans based on those data. No conclusion can be drawn with regard to the teratogenic potential of ABC because of the embryo-fetal toxicity from animal studies. Avoid BF. | No data | **ABC**: toxicity to the developing embryo & fetus only in rats (↓ fetal body weight, fetal oedema, ↑ skeletal variation, early intrauterine deaths and stillbirths) | ABC | ABC 1.5 |

Info taken from: EPAR® not in the SmPC; *one animal: anencephaly and unilateral anophthalmia with secondary enlargement of the tongue, one microphthalmia and one cleft palate. NTDs= neural tube defects; nk/NK= not known; preg=pregnancy; BF= breastfeeding; WCBP=women of childbearing potential; PP=post-partum; Tx= treatment; reProdTox=reproductive toxicities tests; t ½= half-life; a.m.t.d = at maternal toxic doses ^RHD= recommended human dose; $C_{min}$=minimum blood plasms concentration; AUC=Area under the curve; ↓=increase/increased; ↑=decrease/decreased

## 9.4 Bayesian information criterion

As explained in the methods (section 3.6.1) and reported in results chapter 5 and 6, I used Bayesian information criterion as criterion for the goodness-of-fit. Table 9.1 shows the covariates fitted in the model evaluating ARV by class from which we can tell **CA..EUROCAT ~ INSTI + FirstExposure2Peri + INSTI:FirstExposure2Peri + AgeAtDelivery2Cat**, was the best as it provided the most explanation with fewer df.

Table 9.2 shows the covariates fitted in the model evaluating the five ARV combinations of interest (Drugs5) in this case the best model was **Drugs5 + AgeAtDelivery2Cat,** being the one that provides the most explanation with fewer df.

These models accounted (i.e. adjusted) for maternal age at delivery, given it is an important independent risk factor for CA, particularly for chromosomic disorders. Furthermore, the NSHPC population resulted skewed towards older age, and this is why in the models maternal age at delivery was evaluated as two categories (i.e. </>35 years of age ), rather than including the results from the regression spline.

**Table 9.1 Table 9.1 Goodness-of-fit statistic for logistic regression models to assess ARVs, by class**

| Covariates | df | Bayesian information criterion |
|---|---|---|
| CA..EUROCAT ~ INSTI + PI + NNRTI + AgeAtDelivery2Cat + FirstExposure2Peri + INSTI:FirstExposure2Peri + PI:FirstExposure2Peri + NNRTI:FirstExposure2Peri | 9 | 2289.423 |
| CA..EUROCAT ~ INSTI + AgeAtDelivery2Cat + FirstExposure2Peri + INSTI:FirstExposure2Peri | 7 | 2271.855 |
| CA..EUROCAT ~ INSTI + NNRTI + AgeAtDelivery2Cat + FirstExposure2Peri + INSTI:FirstExposure2Peri + NNRTI:FirstExposure2Peri | 9 | 2267.243 |
| **CA..EUROCAT ~ INSTI + FirstExposure2Peri + INSTI:FirstExposure2Peri + AgeAtDelivery2Cat,** | **5** | **2256.001*** |

*optimal model

**Table 9.2 Goodness-of-fit statistics for logistic regression models for CA to assess ARVs, by the five combination of interest**

| Covariates | df | Bayesian information criterion |
|---|---|---|
| **Drugs5 + AgeAtDelivery2Cat** | **7** | **919.56*** |
| Drugs5 + FirstExposure2 + AgeAtDelivery2Cat | 8 | 919.68 |
| Drugs5 * FirstExposure2 | 10 | 941.72 |
| Drugs5 * FirstExposure2 + AgeAtDelivery2Cat | 12 | 943.44 |

*optimal model

## 9.5 Publications and presentations

### Papers

Rasi V, Cortina-Borja M, Peters H, Sconza R, Thorne C. Surveillance of congenital anomalies following exposure to Raltegravir or Elvitegravir during pregnancy in the UK and Ireland, 2008-2018. Journal of Acquired Immune Deficiency Syndrome. 2019 Mar 1;80(3):264-268 doi: 10.1097/QAI.0000000000001924

### Oral presentations

Rasi V, Thorne C, Peters H, Sconza R, Cortina-Borja M. Assessing the influence of BHIVA guidelines on trends in antiretroviral use in pregnancy in the UK and Ireland in 2005–2016. 4th Joint BHIVA/BASHH, 17-20 April 2018, Edinburgh. Oral abstract O21.

November 2017: invited speaker presenting “PMTCT: epidemiology and new treatment strategies in pregnancy” at “Antiviral therapy in paediatric foresight 2017-2020, November 2017, Florence, Italy

### Research poster

Rasi V, Peters H, Sconza R, et al. Real world use of newly authorized antiretrovirals in pregnancy in the UK / Ireland and available safety data. 9th International Workshop on HIV Pediatrics, 21-22 July 2017. Paris, France.

- Won best poster award.

Sconza R, Rasi V, Peters H, et al. Raltegravir in pregnancy: patterns of use and birth outcomes in the UK and Ireland. Conference on Retroviruses and Opportunistic Infections (CROI). 4-7 March 2018. Boston, Massachusetts.

Rasi V. What is a safety signal and how to assess it: the story of Dolutegravir in pregnancy. UCL GOS ICH research poster competition 2019.

Thorne C, Rasi V, Aebi-Popp K, et al. Outcomes following prenatal exposure to Dolutegravir: the DOLOMITE-EPPICC study. Conference on Retroviruses and Opportunistic Infections (CROI). 8-11 March 2020. Boston, Massachusetts.