

Patient Response Variability to Second-Generation
Integrase Strand Transfer Inhibitors:
A Pharmacokinetic/Pharmacogenetic Approach

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Stand Up for Science!

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Table of contents

Remerciements	6
List of abbreviations	12
List of the figures	16
List of the tables	17
1 Introduction	19
1.1 HIV infection	19
1.1.1 Natural history and general introduction	19
1.1.2 Historical perspective	20
1.1.3 Current HIV epidemiology	23
1.1.3.1 World	23
1.1.3.2 Belgium	24
1.1.4 Virology	25
1.1.4.1 Classification	25
1.1.4.2 Genetic variation	25
1.1.4.3 HIV structure	26
1.1.4.4 Viral replication cycle	28
1.1.5 HIV infection and immunology of the host response	34
1.1.6 Treatments of HIV	36
1.1.6.1 Drug classes and modes of action	37
1.1.6.2 Current key concepts in HIV management and ARV therapy	42
1.1.6.3 The integrase inhibitors era	48
1.2 Pharmacogenetics and pharmacokinetics	83
1.2.1 Introduction and definitions	83
1.2.2 Principles of drug disposition	86
1.2.3 Drug metabolism	88
1.2.3.1 Phase 1 metabolism	89
1.2.3.2 Phase 2 metabolism	97
1.2.4 Drug transport	106
1.2.4.1 Introduction	106
1.2.4.2 ABCB1	108
1.2.4.3 ABCG2	112
1.2.5 Xenoreceptors	115
1.2.5.1 Introduction	115
1.2.5.2 NR1I2	116
1.2.6 Principles of pharmacogenetic research	120
1.2.6.1 <i>In vitro</i> experimental approaches	121
1.2.6.2 Clinical studies	122
1.2.7 Pharmacogenetics of HIV therapy	126

1.2.7.1	NRTI agents: the cases of ABC and TFV	127
1.2.7.2	NNRTI agents: the case of EFV	128
1.2.7.3	PI agents: the case of DRV	129
1.2.7.4	First-generation INSTIs	130
1.2.7.5	Second-generation INSTIs	131
1.2.8	Pharmacogenetics in clinical practice	135
1.2.9	Therapeutic drug monitoring	138
2	<i>Aims of the thesis</i>	141
3	<i>Results</i>	143
3.1	<i>In vitro experiments</i>	143
3.1.1	Introduction	143
3.1.2	Methods	144
3.1.3	Effect of ABCB1 Most Frequent Polymorphisms on the Accumulation of Bictegravir in Recombinant HEK293 Cell Lines	147
3.2	<i>INSTI-01 study</i>	156
3.2.1	Introduction	156
3.2.2	Methods	157
3.2.3	Results	160
3.2.3.1	Study population	160
3.2.3.2	Pharmacogenetics of Dolutegravir and Bictegravir Plasma and Intracellular Pharmacokinetics	161
3.2.3.3	Dolutegravir and Risk of Neuropsychiatric Adverse Events: A Pharmacogenetic Study	203
3.2.3.4	Associations between weight gain, integrase inhibitors antiretroviral agents, and gut microbiome in people living with HIV: A cross-sectional study.	237
3.3	<i>Virological failure under long-acting cabotegravir-rilpivirine treatment: case-control and pharmacogenetic analysis</i>	270
4	<i>Conclusions and Perspectives</i>	281
5	<i>Annexes</i>	292
5.1	Annex 1	292
5.2	Annex 2	293
5.3	Annex 3	294
5.4	Annex 4	296
5.5	Annex 5	299
	<i>References</i>	300

List of abbreviations

3TC: lamivudine
ABC: abacavir
ABCB: ATP binding cassette subfamily B
ABCC: ATP-binding cassette sub-family C
ABCG: ATP binding cassette subfamily G
ADR : adverse drug reactions
AIDS: acquired immunodeficiency syndrome
ALLINI : allosteric integrase inhibitors
ARV : antiretroviral
AhR : aryl hydrocarbon receptor
ATP : adenosine triphosphate
ATV : atazanavir
AUC : area under the curve
AZT : zidovudine
BBB : blood-brain barrier
BIC : bictegravir
BICc : Bayesian Information Criterion
BMI : body mass index
CAR : Constitutive androstenedione receptor
cART : combination antiretroviral therapy
CCD : catalytic core domain
CL/F : apparent clearance
CNS : central nervous system
COBI : cobicistat
CPIC : Clinical Pharmacogenetics Implementation Consortium
CSF : cerebrospinal fluid
CTD : C-terminal domain
C_{trough} : trough concentration
CUSL : Cliniques universitaires Saint-Luc
CV : coefficients of variation
CYP : cytochrome P450
DBD : DNA binding domain
DDI : drug drug interactions

DNA : deoxyribonucleic acid
DPWG : Dutch Pharmacogenetics Working Group
DTG : dolutegravir
EC₅₀ : half-maximal effective concentration
EMA : European Medicines Agency
ETR : etravirine
EVG/c : elvitegravir/cobicistat
FTC : emtricitabine
GALT : gut associated lymphoid tissues
GFR : glomerular filtration rate
GWAS : genome wide associated studies
HAART : highly active antiretroviral therapy
HLA : Human leukocyte antigen
HPLC : high-performance liquid chromatography
HIV : Human immunodeficiency virus
HRE : hormone response elements
HSR : hypersensitivity reaction
HTLV : Human T-lymphotropic virus
IC₅₀ : half-maximal inhibitory concentration
IDV : indinavir
IIP : instantaneous inhibitory potential
IIV : interindividual variability
IM : intramuscularly
IN: HIV integrase enzyme
INSTI : integrase strand transfer inhibitor
IQ : inhibitory quotient
IQR : interquartile range
IS : internal standards
LA : long-acting
LBD : ligand binding domain
LC–MS/MS : liquid chromatography coupled to tandem mass spectrometry
LEN : lenacapavir
LLV : low-level viremia
MAF : minor allele frequency
MATE : Multidrug and toxic compound extrusion

MRP : Multidrug resistance protein
mRNA : messenger ribonucleic acid
MSM : men who have sex with men
NADPH : nicotinamide adenine dinucleotide phosphate
NGS : next-generation sequencing
NNRTI : non-nucleoside reverse transcriptase inhibitor
NPAE : neuropsychiatric adverse events
NR : Nuclear receptor
NRTI : nucleoside reverse transcriptase inhibitor
NS : not statistically different
NSV : non-suppressible viremia
NTD : N-terminal domain
OAT : Organic anion transporter
OCT : Organic cation transporter
PA-IC₉₀ : protein-adjusted 90% inhibitory concentration
PBMC : peripheral blood mononuclear cell
PCR : polymerase chain reaction
PD : pharmacodynamics
PEP : post-exposure prophylaxis
PGx : pharmacogenetics
P-gp : P-glycoprotein
PharmVar : Pharmacogene Variation Consortium
PI : protease inhibitor
PIC : pre-integration complex
PK : pharmacokinetics
PK/PD : pharmacokinetics/pharmacodynamics
PLWH : person living with HIV
POR : NADPH-P450 oxydoreductase
PPAR α : peroxisome proliferator-activated receptor α
PrEP : pre-exposure prophylaxis
PXR : Pregnane X Receptor
RAL : raltegravir
RAM : resistance associated mutation
RCT : randomised clinical trial
RPV : rilpivirine

RSE : relative standard error
RT : reverse transcriptase
RTV : ritonavir
RNA : ribonucleic acid
RV : residual viremia
RXR : Retinoid X receptor
SD : standard deviation
SHIV : Simian-human immunodeficiency virus
SIV : Simian immunodeficiency virus
SLC : Solute carrier
SNP : single nucleotide polymorphism
SNV : single nucleotide variant
STR : single tablet regimen
TA : thymine–adenine
TAF : tenofovir alafenamide
TDF : tenofovir disoproxil fumarate
TDM : therapeutic drug monitoring
TFV : tenofovir
TJ : tight junction
TLD : tenofovir-lamivudine-dolutegravir
 T_{max} : time to maximum plasma concentration
UDP : uridine diphosphate
UGT : Uridine diphosphate glucuronosyltransferase
ULLV : ultra-low-level viremia
vDNA : viral linear DNA
VF : virological failure
VKORC: Vitamin K epoxide Reductase Complex
VL : viral load
WHO : World Health Organisation
XTC : lamivudine or emtricitabine

List of the figures

Figure 1. AIDS-related deaths, 2025 target and percentage of people living with HIV receiving treatment, global, 1990–2023.	22
Figure 2. Global testing and treatment cascade among PLWH, 2019-2023.	25
Figure 3. Schematic view of the HIV-1 genome, proteins and structure.	27
Figure 4. Schematic view of the different phases of the HIV viral replication cycle.	29
Figure 5. Schematic view of the HIV proviral DNA integration process.	32
Figure 6. Number of AIDS-related deaths: current situation versus scenario without available therapy, 1990-2022.	37
Figure 7. Chemical structures of available integrase strand transfer inhibitors (INSTIs).	42
Figure 8. Use of INSTI-based cART as initial ARV treatment over time in Switzerland.	49
Figure 9. Different models proposed to explain the change in bodyweight over time in participants from the ADVANCE trial.	82
Figure 10. General catalytic cycle for CYP reactions.	91
Figure 11. Genomic organization of UGT1 loci.	100
Figure 12. Localization of drug membrane transporters in the human intestine, liver and kidney.	108
Figure 13. Schematic illustration of PXR mode of action.	118
Figure 14. Schematic view of the principle of pharmacogenetic case-control study.	125
Figure 15. Overview of the site-directed mutagenesis process.	145
Figure 16. Framework of the substudies conducted under the INSTI-01 umbrella.	157
Figure 17. Venn diagram representing the INSTI-01 study population.	160
Figure 18. Graphical abstract highlighting key findings from the INSTI-01 study.	291
Figure 19. Hypothetical dose-response curves to illustrate the impact of the curve's slope on antiretroviral efficacy.	295
Figure 20. Detail of 41 genes covered by the SOPHiA GENETICS® Pharmacogenomics Community Panel test.	299

List of the tables

Table 1. Summary of drug classes used to treat HIV.	41
Table 2. Summary of literature findings regarding association between DTG plasma exposure and the occurrence of NPAE.	75
Table 3. Description of different types of impacts of pharmacogenetic variants on drug efficacy and toxicity.	85
Table 4. Description of CYP3A4, CYP3A5 and POR most important polymorphisms.	96
Table 5. Description of UGT1A1 most important polymorphisms.	105
Table 6. Description of key ABCB1 polymorphisms.	112
Table 7. Description of key ABCG2 polymorphisms.	115
Table 8. NR1I2 most important target genes in humans regarding drug metabolism and transport.	118
Table 9. Summary of the available literature regarding the effect of PGx variants on DTG PK.	134
Table 10. List of the ARVs currently used in Belgium	292
Table 11. Main pharmacological characteristics of second-generation INSTIs	293

1 Introduction

1.1 HIV infection

1.1.1 Natural history and general introduction

The Human immunodeficiency viruses (HIV), HIV-1 and HIV-2, are highly pathogenic virus that can infect humans. HIV transmission can occur through sexual intercourse, exposure to blood or vertically from infected mothers to their infants (4). On average about 3 weeks after the primary infection, about one half to two-thirds of infected individuals will present nonspecific symptoms of mononucleosis-like illness, a syndrome called acute retroviral infection. Persons infected with HIV-1 will then enter an asymptomatic phase, but in the absence of an adequate treatment, HIV will lead the vast majority of them to gradually develop worsening clinical manifestations. After a median duration of 7 to 10 years, those symptoms will eventually evolve to acquired immunodeficiency syndrome (AIDS), the HIV final clinical stage (5, 6). Untreated patients reaching the AIDS stage have a poor prognosis, with a median survival of 12-18 months (7). Infection with HIV-2 displays a distinct natural history, with slower CD4⁺ T-cell count declines, longer asymptomatic period, and inconstant progression to AIDS (8).

Clinical manifestations of HIV infection are mainly due to two concomitant phenomena: (i) a progressive dysfunction of the immune system, driven by a depletion of CD4⁺ T-cells, a class of lymphocytes that plays a central role in the immune system and constitutes the main target of HIV (although some

other immune effectors are also displaying quantitative and qualitative defects linked to HIV infection) ; and (ii) the seemingly paradoxical chronic activation of a dysfunctional immune system, directly driven by HIV replication and the host immune response against the virus (9). A deficient intestinal mucosal barrier inducing bacterial products translocation is also thought to play an important role in the persistence of “low-grade” chronic inflammation persisting in persons living with HIV (PLWH), even after viral replication control has been reached with modern antiretroviral (ARV) treatments (10).

1.1.2 Historical perspective

The first report related to the AIDS epidemic occurred in June 1981, when a CDC report described an outbreak of *Pneumocystis jirovecii* infection among young and previously healthy homosexual men in the United States (11). Several reports followed, describing a variety of other clinical manifestations and initially identifying 4 risk groups: homosexual/bisexual men, intravenous (heroin) drug users, Haitians and haemophilic patients, leading some people to call this syndrome the “4H disease” in 1982 (12). In 1984, a Belgian case series reported similar clinical findings that had occurred between 1979 and 1983 in several previously healthy patients from African origin. At the same period, several reports identified similar cases in Central Africa. All those cases occurred in heterosexual persons not using intravenous drug (13-15). Over the following years, the number of cases rapidly increased all over the world. Despite extensive media coverage (16) and the gradual understanding

of HIV transmission modes—particularly the lack of transmission through everyday non-sexual interactions (17)—high levels of stigma and discrimination also spread throughout the general population.

It took two years from AIDS description until in 1983 a virus - initially named *Lymphadenopathy-associated virus* (LAV) or *Human T-lymphotropic virus type III* (HTLV-III), later renamed HIV-1 - was identified as its causative agent, first in France and later that year in the US (18, 19). In 1986 HIV-2 was isolated and identified as a distinct cause of AIDS in patients from West-Africa (20). Identification of those viruses as causative agents opened the way for the development of serologic tests and a better comprehension of the epidemiology of HIV infection (21).

The retrospective identification of HIV-1 in a plasma sample collected in 1959, and later from a lymph-node collected in 1960, along with the demonstration that those viral sequences were genetically diverse, lead to the estimation that they originated from a common ancestor at the beginning of the twentieth century (22-24). Further phylogenetic and epidemiologic studies of the closely related Simian immunodeficiency viruses (SIVs) demonstrated that while those viruses had undergone multiple cross-species transmissions, the HIV-1 group M (the principal cause of the AIDS pandemic, as detailed below) most certainly originated from a single transmission event from chimpanzees in southeastern Cameroon, before spreading unnoticedly in Africa and from there overseas (25).

Over the years, HIV continuous spread caused devastating effects across all continents. Scientific progresses in the comprehension of the disease, its prevention and improved management including the development of active ARV therapies were unable to curb down the epidemic. An impact became apparent only when these advances were paired with increased access to treatments—a result of years of activism and advocacy—with AIDS-related deaths peaking in 2004 (**Figure 1**). As of 2024, it is estimated that approximately 88 million people have become infected with HIV and 42 million people have died from AIDS-related illnesses since the start of the epidemic (26).

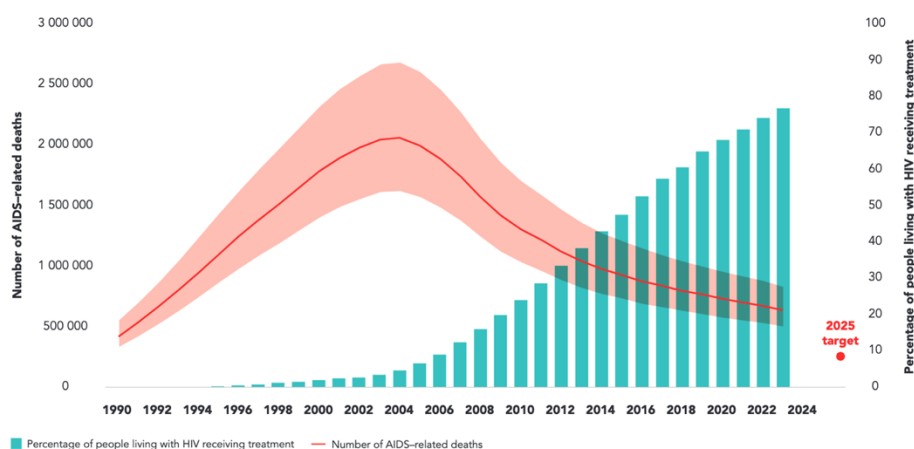


Figure 1. AIDS-related deaths, 2025 target and percentage of people living with HIV receiving treatment, global, 1990–2023.
From *Global AIDS Report 2024*, UNAIDS (27).

1.1.3 Current HIV epidemiology

1.1.3.1 World

It is estimated that 39.9 million (36.1-44.6 million) people were living with HIV worldwide at the end of 2023, including 1.4 million (1.1-1.7 million) children, and that a total of 630 000 (500 000-820 000) people died from AIDS-related illnesses in 2023. Despite being still desperately high, those global numbers represent a progress: the number of new HIV infections is estimated to be at its lowest since the late 1980s, and the number of AIDS-related deaths at its lowest level since its 2004 peak. Central to these progresses, the number of PLWH receiving ARV therapy has increased to approximately 30.7 million (27.0-31.9 million) – but this also means that about 9.3 million PLWH remain untreated. Those global progresses also mask regional disparities (e.g., sub-Saharan Africa, although still disproportionately affected, is the region making the most spectacular progresses in the reduction of new infections, while those numbers are increasing in Latin America, Eastern Europe-Central Asia, and Middle East-North Africa). Moreover, significant disparities in the access to prevention, diagnosis and treatment persist across different populations. Eventually, children still represent a disproportionately high rate of 12% of the total of AIDS-related deaths despite representing only 3% of the total PLWH (2, 27).

In line with the 2015's third Sustainable Development Goal to “*Ensure healthy lives and promote well-being for all at all ages*” (Target 3.3 : “*By 2030, end the epidemics of AIDS (...)*”), the World Health Organisation (WHO) has

updated in June 2021 its HIV testing, treatment and viral suppression targets towards a “95-95-95” target in 2025, meaning that by this date 95% of PLWH should know their HIV status, 95% of people knowing they are living with HIV should receive ARV therapy, and 95% of people receiving ARV therapy should achieve HIV viral load (VL) suppression. As depicted in **Figure 2**, despite the above-mentioned progresses, the world is not on track to reach this global target.

1.1.3.2 Belgium

It is estimated that 18 690 persons were living with HIV in Belgium by the end of 2023, with 93 % of them knowing their seropositive status, among them 95% receiving ARV treatment, and 98% of those treated being virologically suppressed. In total, this means that 87% of PLWH are virologically suppressed. Behind these seemingly positive results lie some more worrying factors, not least the +13% increase in the number of new diagnoses in 2023 after more than 10 years of continuous decrease.

In Belgium, men represented 66% of PLWH in follow-up in 2023, with 67% of them reporting homosexual contact as probable mode of transmission. Among women, 93% acquired HIV through heterosexual contacts. Intravenous drug and vertical transmission each represent 2% of mode of transmissions. Gender repartition varies according to the origin of PLWH: in 2023, 78% of PLWH from Belgian origin were men, while 66% of PLWH originating from Sub-Saharan Africa were women (28).

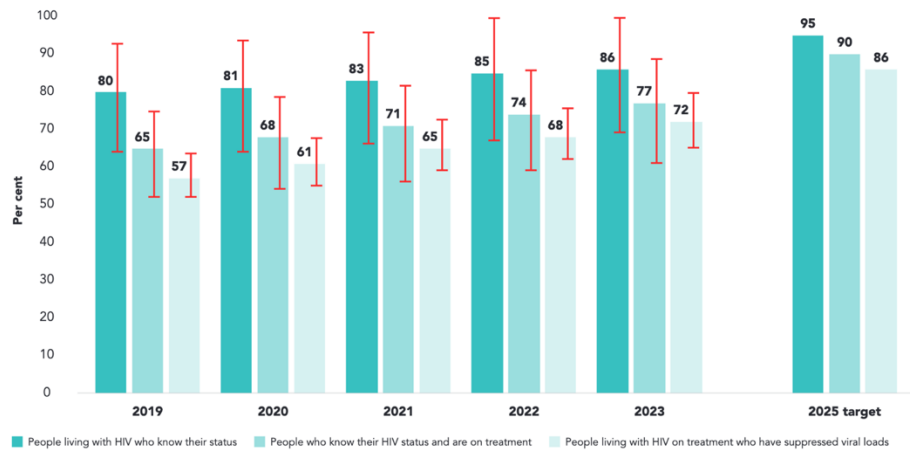


Figure 2. Global testing and treatment cascade among PLWH, 2019–2023.
From *Global AIDS Report 2024*, UNAIDS (27).

1.1.4 Virology

1.1.4.1 Classification

HIV-1 and HIV-2 are *Lentiviruses* which are ribonucleic acid (RNA) viruses, belonging to the *Orthoretrovirinae* subfamily of the *Retroviridae* family.

Other *Orthoretrovirinae* genera are named α -, β -, γ -, δ -, and ε -retroviruses. The first discovered human retroviruses, HTLV-1 and HTLV-2, are δ -retroviruses. Of note, SIV (used as model for HIV infection) is a species of *Lentiviruses* closely related to HIV (29).

1.1.4.2 Genetic variation

HIV-1 and HIV-2 evolved from different SIV strains, with HIV-1 originating from SIVs infecting chimpanzees or gorillas and HIV-2 deriving from SIV strains infecting sooty mangabey monkeys. HIV-1 strains are classified into 4 *groups*, each of them originating from a different zoonotic transmission

event: the M (“Major” or “Main”) and N (“non-M/non-O”) groups originate from chimpanzees, while the O (“Outlier”) and P groups originate from gorillas (25, 30). Group M constitutes the pandemic strain, and is further subdivided into 9 subtypes or clades (A-D, F-H, J, K), *sub-subtypes* (A1-A6, F1, F2), additional circulating recombinant forms (CRF) from different (sub-)subtypes, and further variants. It has been shown that virulence may vary from subtype to subtype, and even among subtypes, some variants have demonstrated higher virulence (31, 32). Subtype C is the most prevalent form worldwide and predominates in Africa and India, while subtype B predominates in other part of the world including Europe and North America. Other groups are much less prevalent, with Group O found almost only in West Africa, group N accounting for a few patients from Cameroon, and group P currently only identified in two individuals in Cameroon (33, 34).

1.1.4.3 HIV structure

HIV is a small virus of about 100 nm diameter. The virion is enveloped by a membrane originating from the host cell from which it derives. Glycoproteins gp41 and gp120 are noncovalently attached and form viral spikes that are inserted in the HIV-1 envelope (gp36 and gp105 are the corresponding transmembrane glycoproteins of HIV-2). The viral core is nested inside the envelope, from which it is separated by a matrix mainly constituted by the Gag protein p17. The core’s structure consists in Gag proteins p6 and p24. It contains two single-strand of RNA of about 10 kb closely associated with the Gag protein p7. It also contains the crucial viral

enzymes protease, reverse transcriptase (RT) and integrase (IN), whose role is detailed hereunder (35).

The HIV genome starts with the *gag*, *pol* and *env* genes which are found in several retroviruses and encode respectively for the core and matrix proteins, the three above mentioned enzymes, and the envelope proteins. Additionally, the HIV genome encodes genes for the accessory proteins *vif*, *vpr*, *tat*, *rev*, *vpu* (for HIV-1) or *vpx* (for HIV-2) and *nef* (**Figure 3**).

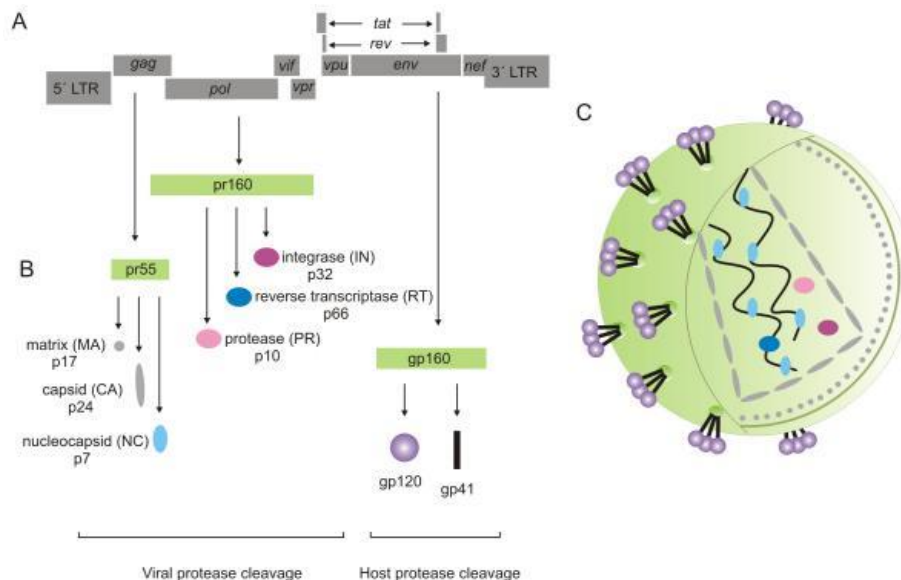


Figure 3. Schematic view of the HIV-1 genome, proteins and structure.

(A) A representation of the nine genes encoded by the HIV-1 genome. (B) The *gag*, *pol* and *env* genes encode protein precursors (pr55, pr160 and gp160) which require further processing by either viral or cellular proteases to generate structural proteins necessary for the formation of a mature virion (C). From (36)

1.1.4.4 Viral replication cycle

The HIV viral replication cycle consists in a complex succession of different phases, as depicted in **Figure 4**.

The first phase of the HIV viral cycle consists in the viral **entry** in the host cell. The **attachment** is initiated by the binding of the gp120 glycoprotein with the CD4 glycoprotein located on the host cell and serving as primary viral receptor. This binding induces a conformational change in glycoprotein gp120 that allows it to bind to its co-receptor CCR5 or CXCR4. The latter are the two alternative coreceptors and define the viral tropism: R5 viruses require CCR5, while X4 viruses require CXCR4. Of note, viral tropism can evolve over time: a single mutation in the V3 loop of the gp120 glycoprotein is sufficient to induce a tropism shift. A switch from R5 to X4 viral tropism usually occurs during HIV disease progression (37).

The liaison of gp120 to this second receptor induces a conformational change of gp41 that enables the **fusion** of the viral and host cell's membranes and the subsequent entry of the capsid core into the host cell (38). Once into the cytoplasm, the virus begins its **uncoating**, a phase that overlaps with the initiation of the **reverse transcription** during which single strand viral RNA will give rise to double strand DNA. This reverse transcription is performed by the RT, an RNA-/DNA-dependant DNA polymerase. Importantly, the HIV RT is characterized by its low fidelity rate: it is estimated that the enzyme mis-incorporates one nucleotide per

replication cycle. This high error rate paradoxically offers an advantage to HIV as it gives rise to a high HIV genetic diversity, enabling viral evolution and escape under the selective pressure induced by the host immune response or by ARVs.

During uncoating, capsid proteins play a role in trafficking the virus to the nucleus (35).

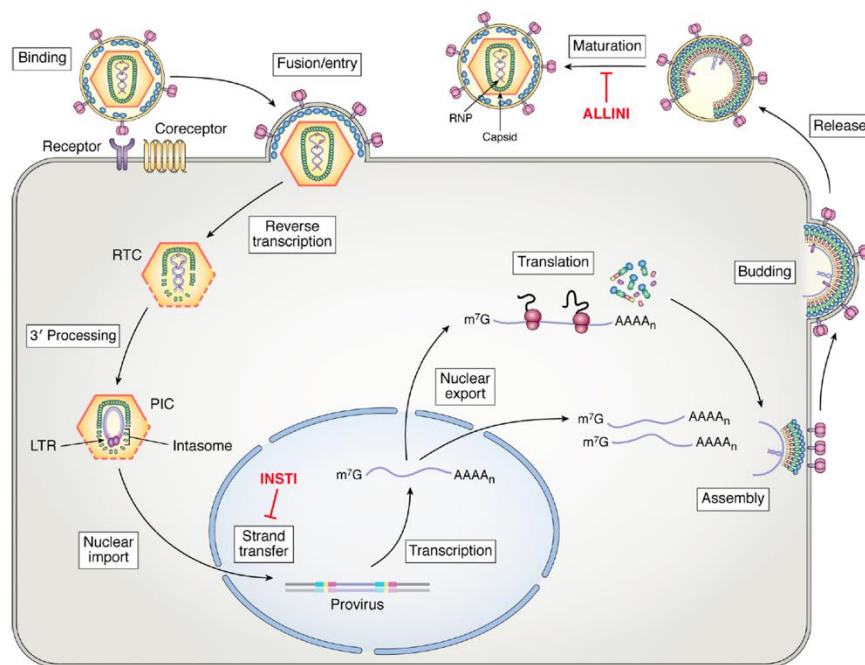


Figure 4. Schematic view of the different phases of the HIV viral replication cycle.

Abbreviations: ALLINI: allosteric integrase inhibitors; LTR: long terminal repeat; PIC: pre-integration complex; INSTI: integrase strand transfer inhibitors. From Engelmann AN (2019) (1).

The next step ends consist in the **integration** of the retro-transcribed viral linear DNA (vDNA) into the host cell chromosomal DNA, through the action of IN (further detailed in **Text Box 1**). The action of IN takes place in the pre-integration complex (PIC), which consists of a polymer of IN assembled on vDNA ends (this association is called the intasome), associated with other viral and host's proteins. Within the cytoplasm, IN catalyses the DNA strand cutting (i.e., the first stage of IN activity, also called **3' processing**) to yield the *cleaved synaptic complex* (**Figure 5**). Briefly, vDNA contains invariant CA dinucleotides at its 3' end. At each extremity, IN hydrolyses the DNA 3' end by cleaving a dinucleotide and exposing 3' hydroxyls attached to those CA dinucleotides. The PIC will eventually arrive at the nuclear pore. Interestingly, the fact that the genome will enter the nucleus through those pores is an important difference from other retroviruses, which need the nuclear membrane disruption (occurring during host cell division) to penetrate the nucleus. This feature offers the possibility for HIV to infect nondividing cells, such as macrophages.

The second stage of IN activity takes place after entering the nucleus and consists in **strand transfer**, i.e., the process of joining host and vDNAs. For this, the intasome binds to its target DNA and uses the 3' hydroxyl groups to cut chromosomal DNA, with vDNA 3' extremity directly joined to the 5' extremities of the chromosomal DNA. Cellular enzymes will end up the process by cutting the two unpaired 5' viral nucleotides and repairing the remaining gapped DNA resulting from the cut. Eventually, the integrated viral DNA is flanked by a short-duplicated target DNA sequence (1, 39, 40). This

stage is considered as a point of no return because infected cells now become permanent carrier of integrated viral DNA, which is referred to as the **provirus**. Importantly, although not site restricted, the integration appears to occur predominantly into or nearby active genes, with IN being involved in integration site selection (2, 41).

During the next phases of viral replication, proviral DNA **transcription** into mRNAs and viral genomic RNA is performed using the host cell's proteins in a highly regulated process. The Transactivator of transcription (Tat) protein activates viral transcription via interaction with the HIV long-terminal repeats RNA regulating factor (42). Moreover, the Regulator of expression of virion (Rev) protein, which is produced from initial fully spliced viral RNA transcripts, enables the cytoplasmic exportation of underspliced HIV RNAs that were initially retained into the nucleus. Those underspliced RNAs include single-spliced RNAs that encode for the late viral polyproteins such as Gag, Gag-Pol, Env and other accessory proteins, but also genomic RNAs (43).

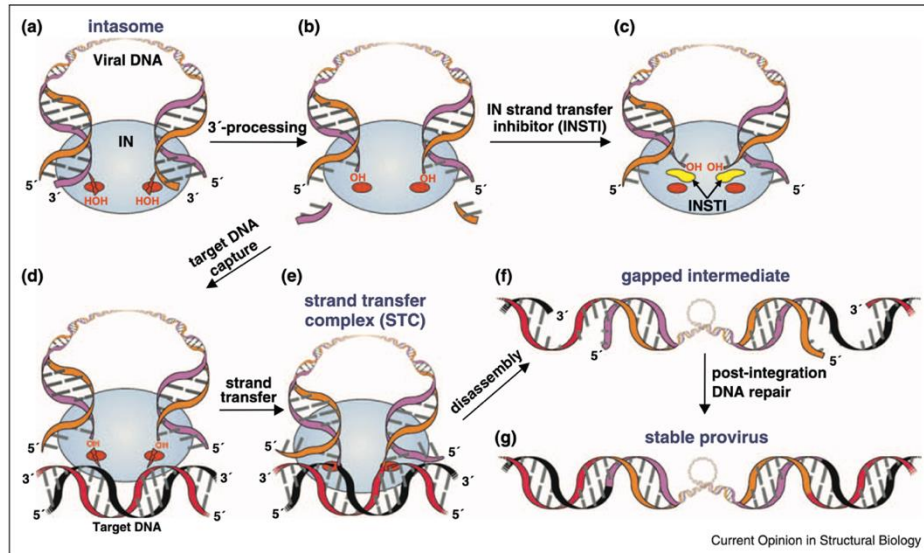


Figure 5. Schematic view of the HIV proviral DNA integration process.
The large blue oval represents a multimer of IN, while small red ovals represent IN active sites. From Cherepanov P. (2011) (39).

HIV virion formation enters its **assembly** stage, with Gag and Gag-Pol polyproteins assembling at the host cell membrane and mediating the incorporation of envelope (Env) glycoproteins gp120 and gp41. The p17 matrix proteins associate with the cytoplasmic domain of gp41. Meanwhile, genomic RNA is packaged into the nascent virion. Virions **budding** from the cell surface into the surrounding media occurs, and eventually the viral replicative process is finalized with the virus **maturation** that requires the cleavage of the Gag and Gag-Pol polyproteins by the viral protease (29, 44).

Text Box 1. HIV IN and its inhibitors

IN is of particular interest in this work as the enzyme is the target of the integrase strand transfer inhibitors (INSTIs) drug class. IN is a 32-kDa, 288-amino-acid protein, and one of the three *pol* gene products. It is synthesized from a *gag-pol* mRNA that translates in a common Gag-Pol polypeptide precursor cleaved by the viral protease.

IN is a polynucleotidyl transferase constituted of three structural domains: the N-terminal domain (NTD), the catalytic core domain (CCD), and the C-terminal domain (CTD). NTD and CTD play important roles of interaction with DNA substrates and in the arrangement of the intasome structure. The CCD contains the enzyme active site which uses catalytic divalent cations (Mg^{2+} or Mn^{2+}) that interact with its carboxylate sites in order to destabilize phosphodiester bonds and hydrolyse the 3' DNA ends, forming the *cleaved synaptic complex*. During the strand transfer phase, IN will use the 3' hydroxyl groups to cut chromosomal DNA, resulting eventually in the integration of proviral DNA as detailed hereabove. It has also been suggested that IN could play additional roles in the HIV lifecycle, such as in the process of uncoating and the trafficking of PIC to the nucleus (1).

INSTIs mode of action has been well described: those compounds engage divalent metal ions at the enzyme active site through interaction with oxygen atoms at their pharmacophore. This enables the INSTIs to occupy

the IN active site and to displace the reactive 3'-OH vDNA that normally cuts chromosomal DNA to engage the strand transfer. This results in the inactivation of the intasome (1, 2).

Another class of agents called allosteric integrase inhibitors (**ALLINI**) also inhibits the HIV-1 IN. ALLINI do not inhibit the IN active site but rather target a binding pocket by which IN interacts with the LEDGF/p75 host factor. The latter interaction is involved in preferential proviral integration in host's active genes. However, ALLINI mode of action does not primarily involve the inhibition of the integration, as ALLINI rather block the maturation of virions by inducing hypermultimerization of IN (1, 2). ALLINI agents are currently undergoing preclinical (3) and phase 1 evaluation (NCT03634085).

1.1.5 HIV infection and immunology of the host response

The most frequent mode of transmission of HIV is through sexual intercourse - accounting for approximately 97% of infections in PLWH in follow-up in Belgium (28). Cell-free or cell-associated viruses present in sexual fluids are thought to quickly cross the mucosal barrier and interact with either dendritic cells, macrophages and/or resting CD4+ T-cells. Virus captured by dendritic cells may be retained on cellular surface or into endosomes and are then transferred to draining lymphoid tissues within 3 to 4 days, where HIV is transferred to its target CD4+ T-cells. From there, the virus will quickly

replicate with formation of new virions, as described hereabove. The infection disseminates throughout the body via the blood within days to week and to lymphatic tissues, giving rise to the acute retroviral infection syndrome. From this early phase begins a continuous deleterious effect of HIV on the CD4+ T-cells, in which depletion is associated to cellular dysfunction. This takes place in peripheral blood but more crucially in lymphoid tissues: lymph nodes become hyperplastic but functionally deficient and gradually architecturally damaged; gut associated lymphoid tissues (GALT) become rapidly depleted in CD4+ memory T-cells and mucosal barrier integrity is affected (45). It has been established that as early as primary infection, the virus constitutes a reservoir with the integration of proviral DNA in the host cells. This reservoir is located in various lymphoid and non-lymphoid tissues and consists in different cellular types such as CD4+ T cells, macrophages, microglial cells (46). It is responsible for the currently impossible eradication of HIV, as ARV can only block the viral replication, and VL quickly rebounds after treatment discontinuation in almost all individuals (47).

A striking particularity of HIV infection is the inability of infected individuals to elicit an immune response able to eliminate the virus or effectively control viral replication. The exceptions are *elite controllers* and long-term non progressors, two rare categories of patients who are able to preserve normal CD4+ T-cell counts respectively with or without undetectable VLs in the absence of ARV therapy (48). Host genetics, immune related or virological factors are thought to play a role in the occurrence of this specific

phenotype, but a definitive understanding is still lacking (49, 50). For the remaining vast majority of infected individuals, no adequate effective HIV-immune response is mounted.

Although a humoral response quickly develops, it lacks a robust and durable neutralizing activity – except in very few individuals (51). This is at least partly due to the quickly evolutive nature of HIV. Indeed, the virus displays a high mutation rate during replication, thus resulting in highly diverse viral populations in infected individuals and allowing the virus to further evolve to escape when exposed to selective pressures, such as those exerted by antibodies (52). Similarly, although cytotoxic CD8+ T-cells participate into the restriction of HIV replication, these lymphocytes are generally unable to provide complete control of viral replication.

1.1.6 Treatments of HIV

The advent of effective ARV therapies has dramatically changed the prognosis of HIV infection but also the face of the pandemic. It has been estimated that HIV treatments have averted almost 21 million deaths between 1996 and 2022 (**Figure 6**).

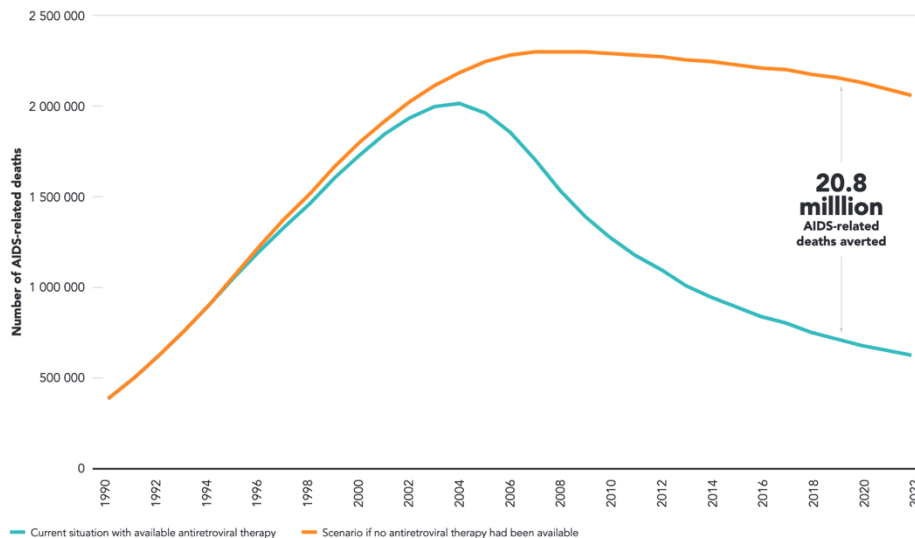


Figure 6. Number of AIDS-related deaths: current situation versus scenario without available therapy, 1990-2022.

From *Global AIDS Report 2023*, UNAIDS (53).

1.1.6.1 Drug classes and modes of action

ARV treatments are classified according to the targeted stage of the HIV replication and/or the targeted viral enzyme or component (**Table 1**). Nine therapeutic classes of ARVs have been developed against HIV-1, with some of them being also active against HIV-2. A table detailing current agents available in Belgium is provided as **Annex 1**.

Four classes of drugs inhibit the entry stage:

- CCR5 antagonists (often called *entry inhibitors*) can be used against R5 tropic viruses. Maraviroc was approved by the European Medicines Agency (EMA) in 2007. The use of this drug can be considered in case of drug resistance, but it is now rarely used.

- Fusion inhibitors target and block gp41 glycoprotein of HIV-1. Only one agent (enfuvirtide) has been fully developed and was EMA approved in 2003. The drug is not on the Belgian market anymore, and is now seldom used globally.
- Attachment inhibitors constitute a more recent drug class. Fostemsavir, currently the only available attachment inhibitor, targets gp120 (54). The drug was EMA-approved in 2021 and can be used in case of HIV-1 multiple-class resistance.
- Post-attachment inhibitors: The monoclonal antibody ibalizumab blocks the viral entry by binding to the CD4 receptor at the CD4+ T-cell membrane, preventing gp120-induced conformational changes that are required for HIV entry (55). The drug was granted EMA approval in 2019 for the treatment of multi-drug resistant HIV-1, but this approval was withdrawn in 2023 at the request of the company, for commercial reasons.

Five other drug classes act at subsequent stages of viral replication:

- Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) were the first drugs to enter clinical use (zidovudine (AZT) in 1987) (56), and currently still constitute a cornerstone of HIV treatment. After intracellular phosphorylation, NRTIs block the viral RT due to their analogies with native nucleotides, causing the termination of the vDNA elongation. Importantly, those agents can cause mitochondrial dysfunction as they may also inhibit the action of the host's cellular DNA polymerase including the mitochondrial DNA polymerase.

However, this toxicity is mainly a hallmark of some early agents (didanosine, stavudine, AZT) that have nowadays been replaced by the less toxic lamivudine (3TC), emtricitabine (FTC), abacavir (ABC) and tenofovir (TFV). The latter comes in two main forms: TFV disoproxil fumarate (TDF) or TFV alafenamide (TAF). Both are prodrugs that are converted into the active form, the TFV diphosphate metabolite. TAF has been developed as an alternative TFV prodrug to TDF, characterised by a better pharmacokinetic profile enabling increased TFV intracellular accumulation, with the aim of reducing systemic exposure and off-target bone and kidney effects compared to TDF (57).

- Non-nucleoside reverse transcriptase inhibitors (NNRTIs): those agents act as non-competitive RT inhibitors of HIV-1 by fixing to a binding site far from the enzyme's active site, reducing its activity. Nevirapine (NVP) and efavirenz (EFV) are first-generation NNRTIs that were respectively EMA approved in 1998 and 2012. EFV was recommended as first-line anchor drug by WHO and extensively used until recently, before its global replacement by DTG. Rilpivirine (RPV) remains a largely prescribed and recommended drug, including as long-acting (LA) treatment with the INSTI cabotegravir (CAB) as companion drug. Doravirine is the most recent NNRTI, EMA approved in 2018.
- The protease inhibitors (PIs) act by binding to and inhibiting the action of the viral protease that is required to cleave viral polyproteins into functional proteins, thus interfering with viral

maturation. Saquinavir, the first developed PI, was EMA approved in 1996, swiftly followed by indinavir (IDV) and several other agents, notably the widely prescribed lopinavir. Old agents were since then replaced by more recent agents. Darunavir (DRV) is currently the last PI routinely used in clinical practice. One inconvenient of those agents is the fact they require combination use with a pharmacokinetic booster such as ritonavir (RTV) or cobicistat (COBI).

- The integrase strand transfer inhibitors (INSTIs) inhibit the HIV IN and are the subject of this work. Five agents are currently available: elvitegravir (EVG), raltegravir (RAL), dolutegravir (DTG), bictegravir (BIC) and CAB (**Figure 7**). More details on this class are provided hereunder.
- Capsid inhibitors are the most recently available drug class, with lenacapavir (LEN) being EMA-approved in 2022. Two important features of this drug are that (i) by interfering with capsid proteins it targets different stages of the viral replication, especially the nuclear uptake of the PIC, and the formation of new virions, and (ii) its long terminal half-life enables its use as a LA agent (58). LEN is currently used for treatment of multidrug-resistant HIV-1 and has proven effective as HIV prophylaxis.

Table 1. Summary of drug classes used to treat HIV.

Targeted stage of viral cycle	Drug class	Molecular target	Key agents
Entry	Entry inhibitors	CCR5 (host)	Maraviroc
	Fusion inhibitors	gp41	Enfuvirtide
	Attachment inhibitors	gp120	Fostemsavir
	Post-attachment inhibitors	CD4 (host)	Ibalizumab
Reverse transcription	Nucleoside reverse transcriptase inhibitors	Reverse transcriptase	Zidovudine, didanoside, stavudine, lamivudine, emtricitabine, abacavir
	Nucleotide reverse transcriptase inhibitors	Reverse transcriptase	Tenofovir disoproxil fumarate, tenofovir alafenamide
	Non-nucleoside reverse transcriptase inhibitors	Reverse transcriptase	Etravirine, nevirapine, efavirenz, rilpivirine, doravirine
Integration (strand transfer step)	Integrase strand transfer inhibitors	Integrase	Elvitegravir, raltegravir, dolutegravir, bictegravir, cabotegravir
Maturation	Protease inhibitors	Protease	Saquinavir, indinavir, lopinavir, ritonavir, atazanavir, darunavir
PIC nuclear uptake; virion formation	Capsid inhibitors	Capsid	Lenacapavir

Regarding HIV-2, this virus is intrinsically resistant to NNRTIs, the fusion inhibitor enfuvirtide and the attachment inhibitor fostemsavir. Different PIs have variable levels of intrinsic activity. Other agents are generally active, although limited data are available regarding the most recent agents (59). Of note, INSTIs have demonstrated high efficacy against HIV-2 infection (60).

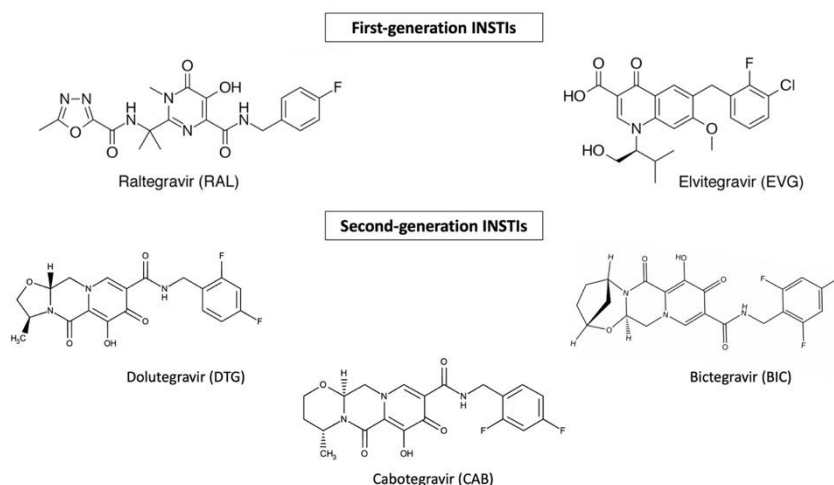


Figure 7. Chemical structures of available integrase strand transfer inhibitors (INSTIs).

1.1.6.2 Current key concepts in HIV management and ARV therapy

HIV diagnosis is now often made before the AIDS stage, and most PLWH who have access to treatment do not present in an immediately life-threatening situation. Rather, PLWH face challenges common to other chronic diseases, to which must be added numerous HIV-specific issues such as significant impacts on relational and social life, as stigma remains a deeply entrenched reality. For HIV physicians, acute care remains a reality as some patients still

present late, but care has mainly moved towards supporting long-term quality of life. At a population level, delivering not only active ARVs but also high-quality care to millions of people across the globe remains challenging, and several populations remain underserved – including in high-income countries (53). For researchers and the pharmaceutical industry, objectives have shifted from simply controlling viral replication to ensuring long-term effective and safe treatments. Eventually, the search for a curative treatment continues and its success remains uncertain despite several important progresses.

1.1.6.2.1 Use of combination ARV therapy

Although the discovery of the first ARV agents active against HIV paved the way for later successes, their initial use as monotherapy did not prove successful. At best, some agents such as AZT when used as monotherapy were shown to delay the clinical progression of HIV infection (56). In 1997, a large randomised clinical trial (RCT) demonstrated a reduction of AIDS and deaths by half among patients with advanced HIV treated with the combination IDV/AZT/3TC, as compared to those treated with AZT/3TC (61). This demonstration of the superiority of triple combination ARV therapy (cART) - formerly called highly active antiretroviral therapy (HAART) can be considered a founding event of modern era of HIV treatment. Its implementation in clinical practice has led to dramatic decreases in deaths rates among PLWH (62).

Currently recommended ARV regimens still consist of a combination of agents from at least two drug classes: one or two NRTI(s) that serve as

“backbone” drug(s), and one other drug from another class (INSTI, PI or NNRTI) serving as second/third agent, also called “anchor” drug, with INSTI being the first choice in the current recommendations (63). Of note, more complex cART regimens are recommended in case of suspected or proven viral resistance.

1.1.6.2.2 Towards safer and simplified ARV regimens

Although early cART regimens reduced HIV replication and improved patients’ prognosis, they were often complex to administer with high pill burdens, inconvenient dosing and stringent food requirements linked to unfavorable pharmacokinetic profiles – notwithstanding the high number of drug-drug interactions (DDIs). Drugs were also fraught with significant toxicities such as mitochondrial toxicity (linked to NRTIs), central nervous system (CNS) toxicity (particularly with NNRTIs), digestive disorders, or hypersensitivity (especially with NVP and ABC).

Progresses in HIV treatment thus aimed to make treatments “*as simple as possible to ensure long-term adherence, as effective as possible to ensure rapid and sustained virologic suppression, and as safe as possible to ensure long-term health*” (64).

An important milestone in the journey towards simplified regimens was the advent in 2006 of the first once daily single tablet regiment (STR), consisting in the combination TDF/FTC/EFV (Atripla®). Further progress in the

simplification of treatments involved development of more flexible therapies (with fewer restrictions regarding pill intake) and smaller tablets.

Improved drug potency and increased resistance-barrier of the more-recent agents also enabled a search for reduced cumulative drug exposure, with the aim of improving drug adherence, reducing long-term toxicities, but also reducing treatment costs. While monotherapies generally failed to prove sufficiently effective (65), dual therapies combining two drugs from different classes were demonstrated to be effective and promising options (66). The demonstration that newer cARTs offer increased forgiveness (i.e., the ability to achieve virological suppression despite uncomplete adherence) (67), also opened the way for another approach: reducing the frequency of drug intake instead of reducing the number of drugs per intake. Short-cycle intermittent triple therapy (4-consecutive-days-on and 3-days-off) has proven its effectiveness as maintenance therapy in a large RCT (68). This practice has currently not been recommended by the European AIDS Clinical Society (EACS) or the American guidelines, but is a proposed option in the French guidelines provided several conditions are fulfilled: after at least 12 months of virological suppression, for patients with a nadir CD4+ T-cell count > 250 cells/ μ L and with no previous virological failure (VF), and in the absence of documented resistance associated mutations (RAMs) to the concerned regimen (69).

Eventually, one of the most recent and promising progress in treatment simplification is the advent of LA therapies. In this regard, the demonstration

of the effectiveness as maintenance treatment of monthly (and later, every two months) injections with the combination of CAB/RPV is undoubtedly the latest milestone toward person-centered simple and effective cART (70).

1.1.6.2.3 Early treatment for all PLWH

Treatment recommendations have evolved over the years regarding treatment indications, another key aspect in the field. While in the lower- and middle-income countries, treatment indications have steadily enlarged following the increased availability of treatments, the evolution has been less linear in developed countries. The original mantra “*hit hard, hit early*” developed after the advent of effective cART quickly evolved to more restrictive indications based on the CD4+ T-cell count, as challenges linked to adherence, toxicities, and risks of emergence of resistance appeared (71). Indications broadened however after development of improved treatment options, and landmark studies demonstrated benefits of early and continuous cART treatment. In 2006, the SMART study demonstrated better outcomes with continuous treatment as compared to episodic CD4+ T-cell counts-guided cART (72). A few years later, the INSIGHT START trial definitively resolved the debate on the optimal timing for initiating cART, demonstrating a significant decreased incidence of serious AIDS-related event, serious non-AIDS-related event, or death from any cause in PLWH starting cART early (>500 CD4+ T-cell count) as compared to those deferring the start until CD4+ T-cell count fell under 350 or until the development of AIDS (73).

1.1.6.2.4 Effective treatment prevents HIV transmission

Another crucial argument for recommending early HIV treatment for all PLWH has been the transforming public health impact of this measure. Treatment of pregnant women is now a standard of care since it was recognized that effective ARV treatment prevents HIV mother to child transmission (74, 75). In other contexts, studies have demonstrated that beyond its individual health benefit, the suppression of the viral replication dramatically reduces the infectivity and eliminates the risk of sexual transmission (76, 77). The latter studies have served as foundation for two pivotal concepts: “*Undetectable = Untransmittable*” (“U=U”), and the public health strategy known as “*Treatment as Prevention*”.

1.1.6.2.5 ARV treatment as HIV prophylaxis

Besides their crucial role for the management of PLWH, ARVs have also proven effective in preventing infection by HIV. This role encompasses two different concepts: *post-exposure prophylaxis* (PEP) and *pre-exposure prophylaxis* (PrEP).

PEP consists in the early (within 24-72h) administration of cART after mucocutaneous or sexual exposure to HIV to reduce the risk of viral acquisition (78). Several cART regimens have been recommended for this purpose, including DRV/c, RAL, DTG and BIC-based regimens (63). The local protocol at Cliniques universitaires Saint-Luc (CUSL) involves the use of the EVG/c/TDF/FTC STR.

PrEP consists in the use of ARV agents to prevent HIV acquisition by sexual intercourse. Several studies have demonstrated the effectiveness of this

strategy using different agents: TDF/FTC, TAF/FTC, CAB and more recently LEN (79-82).

1.1.6.3 The integrase inhibitors era

1.1.6.3.1 Introduction

Despite early interest and the identification of *in vitro* active IN inhibitors, the development of a compound both inhibiting IN and showing antiviral activity *in vivo* presented a real challenge (1). In 2000, *Hazuda et al.* demonstrated that diketo acid compounds display an antiviral activity through the inhibition of strand transfer activity (83). Further developments led to the demonstration that one of those compounds, L-870812, was active against simian-human immunodeficiency virus (SHIV) replication *in vivo* and prevented viral-induced effects on the immune system of Rhesus-macaques, paving the way for preclinical and clinical development of the first INSTIs (84). Over the years, several INSTIs have been developed and recommended as first-line agents for both naïve and experienced PLWH. Their use has been continuously growing, and second-generation INSTIs now constitute a cornerstone in the management of HIV (**Figure 8**).

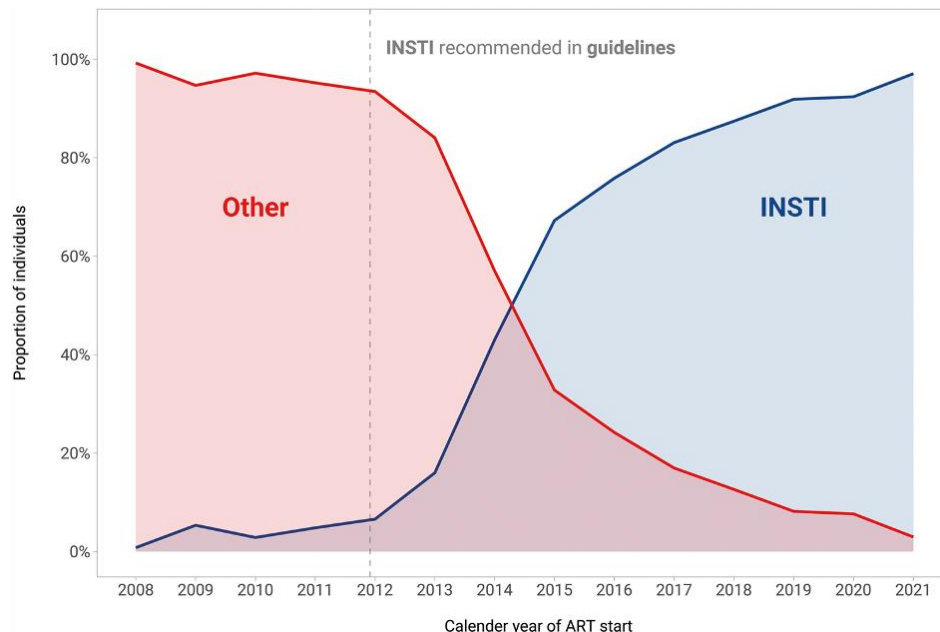


Figure 8. Use of INSTI-based cART as initial ARV treatment over time in Switzerland.

Data from the Swiss HIV cohort Study (85).

1.1.6.3.2 First generation INSTIs

RAL (MK-0518) was the first-in-class INSTI drug. It was approved by EMA in 2007 after demonstrating its efficacy and safety in naïve and experimented patients (86, 87). The medication was recommended as part of first-line drug regimens for naïve patients in 2010.

EVG (GS-9137) was developed in 2006 as a derivative from quinolone antibiotics, displaying a potent antiviral activity (88). The drug was initially tested in combination with two NRTIs (FTC and TDF) and COBI, to be used as a once-a-day regimen. COBI serves as a pharmacokinetic booster, without

any antiviral activity, based on its selective and potent inhibitory activity on cytochrome P450 (CYP) 3A4. The EVG/c/FTC/TDC combination was EMA approved in 2013 based on the demonstration of its efficacy and safety in naïve and experimented patients (89-91). A later developed single-tablet formulation replaced TDF by TAF, and was EMA-approved in 2015 (92).

Importantly, both RAL and EVG share the same drug resistance mutations profile, thus precluding the sequential use of those two drugs in case of VF.

1.1.6.3.3 Second-generation INSTIs

Second-generation INSTIs have several advantages over the first-generation agents, especially their ability to overcome some – but not all – mutations associated to resistance against RAL and EVG. Moreover, these drugs do not require multiple daily dosing or the use of a pharmacokinetic booster. A summary of the main pharmacological characteristics of the three available agents (DTG, BIC, CAB) can be found in **Annex 2**.

1.1.6.3.3.1 DTG

1.1.6.3.3.1.1 *Pharmacology*

S/GSK1349572, later named dolutegravir, was developed as the first next-generation INSTI drug. The molecule was developed on the basis of first-generation INSTIs, following findings in structure–activity relationships, and demonstrated its potential once-daily dosing use associated with superior

potency against resistant viral strains (93). The drug displays a moderate molecular weight and a lipophilic nature (94).

In vitro, the drug displayed a 50% inhibitory concentration (IC_{50} , i.e., the concentration required to inhibit 50% of the activity of the integrase strand transfer activity) of 2.7 nM and a 50% effective concentration (EC_{50} , i.e., the concentration required to inhibit 50% of the viral replication) of 0.51 nM in peripheral blood mononuclear cells (PBMCs) (95). Protein adjusted 90% inhibitory concentration ($PA-IC_{90}$) was measured at 64 ng/mL (96). The drug exhibits a higher resistance barrier than RAL and EVG (95).

Pharmacokinetic (PK) studies in healthy volunteers and PLWH demonstrated that the drug is readily absorbed with a time to maximum plasma concentration (T_{max}) of approximately 0.5-2 hours and a half-life of approximately 13-15 hours. The drug is highly protein bound (> 99%), but with a low affinity. It was shown that once daily dosing of 10 to 50 mg of DTG exceed the $PA-IC_{90}$ by 5- to 26-fold, depending of the dose used (96-98). A phase II pharmacokinetics/pharmacodynamics (PK/PD) study demonstrated the potent antiviral activity of DTG at a dose of 50 mg once daily, with 7 out of 10 previously untreated patients achieving VL <50 copies/mL after 10 days of DTG monotherapy. The study also demonstrated that trough concentration (C_{trough} , i.e., the concentration at the end of the dosing interval) was the best PK predictor of VL reduction (99). Although DTG EC_{50} , IC_{50} , and their relative protein adjusted values are respectively in the same low nanomolar and nanomolar range as first-generation INSTIs, the drug

displays a significantly higher VL reduction potency. This higher potency of DTG compared to first generation INSTIs is attributed to the increased slope of its dose-response curve (100). This means that for a similar ratio value of measured drug concentration over drug IC_{50} , the drug will have increased efficacy (101) (**Annex 3**).

Overall, DTG exhibits low to moderate intersubject PK variability, with coefficients of variation (CV) <30% for both the area under the curve (AUC) and C_{max} . DTG absorption is increased by food intake, especially fat meals, but the extent of this increase is not considered clinically significant (97). DTG population PK modelling has demonstrated several factors to be associated with higher plasma exposure: lower body weight, non-smoking status, lower age, higher total bilirubin and female gender (102, 103).

Mass balance studies have demonstrated that 64% and 32% of the DTG dose are recovered respectively in feces and urine and that the drug undergoes extensive metabolization. Glucuronidation into an inactive metabolite by uridine diphosphate (UDP) glucuronosyltransferase (UGT) 1A1 has been described as the main biotransformation pathway, with oxidation by CYP3A4 being the secondary pathway. Minor contributions come from UGT1A3, UGT1A9, and an oxidative defluorination followed by glutathione substitution (104, 105). Interestingly, two more recent publications have reported additional or distinct pathways: firstly, using an *in vitro* metabolomic approach, *Zhu et al.* reported that CYP1A1 and 1B1 contribute to the formation of DTG aldehyde metabolites (106); secondly, using *in vitro*

assays, *Liu et al.* described important contribution of extrahepatic (intestinal and renal) glucuronidation in DTG metabolism by UGT1A3, UGT1A8, UGT1A1 and UGT1A9. Moreover, their results also indicated a possible undervaluation of UGT1A9 contribution in DTG liver metabolism, as their models suggested its contribution could exceed that of UGT1A1 (107).

DTG is a substrate for ATP binding cassette subfamily B, isoform 1 (ABCB1), also called P-glycoprotein (P-gp) and to a similar extent by ATP binding cassette subfamily G, isoform 2 (ABCG2), also called Breast Cancer Resistance Protein (BCRP) both being key efflux transporters (97). DTG has been demonstrated to be a moderate activator of the Pregnane X Receptor (PXR), also called Nuclear Receptor Subfamily 1, group I, member 2 (NR1I2) (108). However, importantly, DTG does not display any significative induction or inhibition effect at clinical concentration on phase I or phase II biotransformation enzymes, neither on drug transporters, with the exception of the renal transporter Solute carrier (SLC) family 22 member 2 (SLC22A2), also called Organic cation transporter (OCT) 2 and the renal and biliary transporter SLC family 47 member 1 (SLC47A1), also called Multidrug and toxin extrusion protein (MATE) 1. Both are inhibited by DTG - but do not transport the drug (109).

The recommended DTG daily dose is generally 50 mg once daily for adults. However, for patients with integrase inhibitors RAMs, the dose of 50 mg twice daily is recommended. Increased dose has also been recommended in some cases of coadministration with UGT1A1 and/or CYP3A4 inducers.

Lower doses are recommended for children (40 mg for 14-20 kg). No dose adjustment is recommended in case of kidney or liver impairment, except for severe liver impairment (Child-Pugh class C) for which the drug use is not recommended due to lack of studies (109).

1.1.6.3.3.1.2 Efficacy

Efficacy of DTG in cART was demonstrated in several phase III RCTs.

First, in ART-naïve PLWH, the SINGLE study demonstrated the superiority of DTG/ABC/3TC over EFV/TDF/FTC in achieving VL suppression (<50 copies/mL) at 48 weeks (88% versus 81%) (110). Superiority was maintained at week 144 and mainly driven by fewer discontinuations due to adverse events in the DTG arm (111). The FLAMINGO trial demonstrated the superiority of DTG compared to DRV/r (80% versus 68%), combined with investigator-selected TDF/FTC or ABC/3TC (112), while the SPRING-2 study showed the non-inferiority of DTG to RAL with similar backbone options (81% versus 76%), both evaluating VL suppression at 96 weeks.

In non-naïve patients, the SAILING trial evaluated DTG versus RAL (both with investigator-selected background therapy) in INSTI-naïve PLWH and demonstrated the superiority of DTG, with 71% versus 64% of participants demonstrating VL suppression at week 48 (113). Eventually, a non-randomized phase III study confirmed the efficacy of DTG 50mg twice daily with optimized background therapy in highly treatment-experienced PLWH with INSTI-resistant virus and failing regimens, as 69% achieved undetectable VL at week 24 (114).

Based on these successes, DTG-based dual therapies prompted interest and the first regimen to be successfully evaluated was DTG/RPV, showing non-inferiority to continued triple cART among virologically suppressed PLWH in the SWORD trials (115). Further developments came from the association DTG/3TC, a more convenient association in terms of risk of drug interactions and administration. The dual regimen was proven efficacious in ART-naïve individuals, demonstrating non-inferiority to DTG/TDF/FTC in the GEMINI trials (116). In ART-experienced individuals, DTG/3TC demonstrated non-inferiority to TAF-based regimens when continuing a 3- or 4-drug regimen in the TANGO and SALSA trials (117, 118).

Eventually, DTG monotherapy has also been tested, but although a significant proportion treated participants maintained viral suppression (90 % at 48 weeks in the MonCAY trial), studies were discontinued as it failed to demonstrate non-inferiority to cART and some participants developed INSTI RAMs. (119, 120).

Besides RCTs, the long-term effectiveness of DTG-based regimens was confirmed in numerous clinical and real-life scenarios (naïve or experienced PLWH, triple or dual cART, wild-type HIV or virus harboring RAMs to lamivudine or minor grade INSTI resistance, HIV-1 or HIV-2 infection) (121-126). In a multicenter real-life retrospective cohort study in Belgium, 96% of 4101 DTG-treated PLWH demonstrated an HIV-1 VL below 50 copies/mL at 96 weeks (121).

1.1.6.3.3.1.3 Recommendations and use

DTG is part of some of the first-line regimens recommended in Europe and the US (63, 127). Since 2018, the drug has also been recommended in combination with TDF/3TC or TDF/FTC as first-line regimen by the WHO (128).

In Belgium, DTG is currently available as stand-alone drug (Tivicay®), or as a co-formulation (DTG/ABC/3TC, Triumeq®; DTG/RPV, Juluca®; DTG/3TC, Dovato®; all from ViiV Healthcare®). In developing countries, the drug is now largely available, usually distributed as co-formulated DTG/TDF/3TC (also named TLD, for tenofovir-lamivudine-dolutegravir) (129). The drug is produced by several generic drug companies. It has been estimated that more than 90% of PLWH in low and middle income countries were treated with DTG-based regimens by 2023, thus accounting for more than 22 millions of persons intaking the drug every day (130).

1.1.6.3.3.2 BIC

1.1.6.3.3.2.1 Pharmacology

BIC (GS-9883) was developed by performing serial structure-activity relationship analysis and modifying iteratively the structure of DTG, aiming to improve the drug potency and other PK-related characteristics. Compared to DTG, BIC displays a higher solubility, lower PXR activation, and preliminary data indicated an increased potency against some INSTI-resistant mutants (131).

In vitro, BIC displayed an IC_{50} of 7.5 nM for the inhibition of IN strand transfer activity, almost identical to the DTG IC_{50} reported by the researchers using similar methodologies, and an EC_{50} of 1.5 nM in CD4+ T-cell and 6.6 nM in macrophages, both values slightly increased as compared to DTG (132). PA- IC_{95} was measured at 162 ng/mL (133).

PK studies in healthy volunteers and PLWH demonstrated that the drug is readily absorbed, has a T_{max} of 1-2 hours, and a half-life of approximately 17 hours (133, 134). The drug is highly protein-bound (> 99%), but with a low affinity (98, 131). A phase Ib study including mostly non-naïve PLWH with uncontrolled VL showed that C_t is above the PA- IC_{95} with any of the studied doses ranging from 5 to 100 mg of BIC once daily. Moreover, all the tested doses displayed a significant antiviral activity (133).

Overall, BIC exhibits low to moderate intersubject PK variability, with CVs of 22-35% for AUC, C_{trough} and C_{max} . BIC absorption is increased by food intake, especially fat meals, but the extent of this increase is not considered clinically significant (135). Very limited data are available on cofactors that could influence plasma exposure (136).

Mass balance studies have demonstrated that 58.5% and 34% of the BIC dose are recovered respectively in feces and urine (136). The drug undergoes extensive metabolization, with minimal renal clearance: only 1% of the dose is found unchanged in the urine. BIC metabolism is predominantly and to a similar extent mediated by CYP3A4 and UGT1A1. UGT1A3, 1A8 and 1A9 are

secondary contributors to glucuronidation. CYP3A5 also metabolizes BIC but the precise extent is unknown (137).

BIC is a substrate for the ABCB1 and ABCG2 transporters, as demonstrated in transfected overexpressing cellular models (138). BIC is a modest PXR activator (40% of maximal activation effect of rifampicin), with a lower PXR activation potency than DTG (131, 137, 139). Similarly to DTG, the drug is not an important perpetrator of DDIs, as it does not display any significant induction or inhibition effect at clinical concentration on phase I or phase II biotransformation enzymes, neither on drug transporter, with the exception of OCT2 and MATE1 (138). The latter two, however, did not translate in important DDIs (140).

The standard recommended BIC dose is 50 mg once daily. For children (from the age of 2 and weighing <25 kg) a daily dose of 30 mg is recommended. No dose adjustment is recommended in case of liver impairment, except for severe liver impairment (Child-Pugh class C) for which the drug use is not recommended due to lack of studies. Despite renal elimination being negligible, BIC use avoidance is recommended in case of renal failure with estimated glomerular filtration rate <30 mL/min because the drug is only available as a co-formulated tablet with FTC and TAF, and safety has not been established in that population (135).

1.1.6.3.3.2.2 Efficacy

Efficacy of BIC in adults was demonstrated in several phase III RCTs.

In treatment-naïve PLWH, the GS-US-380-1489 trial demonstrated non inferiority of BIC/TAF/FTC to DTG/ABC/3TC for the primary endpoint of plasma HIV-1 VL less than 50 copies/mL at week 48 (92% versus 93%) (141), while the GS-US-380-1490 demonstrated non inferiority to DTG/TAF/FTC for the same endpoint at 48 weeks (89% versus 93%) (142). Both results were confirmed at 96 weeks (143, 144).

In treatment-experienced PLWH, switching to BIC/FTC/TAF was shown non-inferior to continuing DTG/ABC/3TC in the GS-US-380-1844 trial, with respectively 1% and <1% of participants displaying HIV-1 VL of ≥ 50 copies/mL at week 48 (145). Moreover, another trial found no difference for treatment experienced individuals (of which approximately one fourth harbored RAMs to NRTIs) receiving DTG/TDF/FTC or DTG/TAF/FTC and switching to either BIC/TAF/FTC or DTG/TAF/FTC (146). Besides head-to-head comparisons with DTG-based regimens, BIC was also tested against PI-based regimens (GS-US-380-1878): BIC/TAF/FTC was found non inferior to continued boosted PI therapy with 2% of participants in each group displaying HIV-1 VL of ≥ 50 copies/mL at week 48 (147). Eventually, a study carried in women originating mostly from non-high-income countries demonstrated the non-inferiority of BIC/TAF/FTC versus continued TDF or TAF-based regimen (mostly with co-formulated EVG/c/FTC), with a similar endpoint occurring in 2% of participants in each group (148).

Real-life studies have confirmed the high-efficacy of BIC/TAF/FTC. In Belgium, a large multicentric retrospective study found 93.5% of 4001 included PLWH to be virologically suppressed at 48 weeks, with similar rates across sexes,

ages, sexual orientation groups, origin, HIV treatment status (naïve versus experienced) (149).

1.1.6.3.3.2.3 Recommendations and use

BIC/TAF/FTC is currently recommended among first-line cART regimen in Europe and the USA (63, 127). BIC/TAF/FTC is only distributed as a STR, in Belgium under the name Biktarvy® (Gilead Sciences).

1.1.6.3.3.3 CAB

1.1.6.3.3.3.1 Pharmacology

CAB (GSK1265744) was discovered together with DTG, as a modified INSTI with improved PK profile and superior potency against resistant viral strains compared to first-generation agents (93).

In vitro, the drug displayed an IC₅₀ of 3 nM and an EC₅₀ of 0.22 nM in PBMCs. PA-IC₉₀ was measured at 166 ng/mL. The drug demonstrated a distinct resistance profile to RAL and EVG, with an increased resistance barrier. Preclinical studies identified that the drug displays some interesting features such as high protein binding, low aqueous solubility and low clearance in animals as compared to DTG, justifying the further development of the drug for LA therapies (150, 151).

PK studies of the oral drug in healthy volunteers and PLWH demonstrated that it is readily absorbed, has a T_{max} of 0.5-1.5 hours, and confirmed a prolonged half-life measured at approximately 31.5 hours. The drug also

showed a high protein binding (> 99%). Monotherapy with doses ranging from 5 to 30 mg daily displayed potent antiviral activity (151). PK studies of CAB nanosuspension delivered by injection confirmed that the drug was a good candidate for development as part of a LA regimen: plasma drug concentrations were found measurable up to one year after a single injection, with apparent terminal half-lives of 25-54 days. It was shown that a dose of 800 mg intramuscularly (IM) achieved mean plasma concentrations above the PA-IC₉₀ for approximately 16 weeks (152).

High levels of interindividual variability as well as intra-individual (between observation) variability have been described for CAB. Population PK modelling has identified female sex and increased weight as being respectively associated with decreased absorption rate and increased apparent clearance (153-155). Resorption from nanoparticles and absorption have been shown to be the limiting factor for CAB elimination after IM administration (154).

Mass balance studies have demonstrated that 58.5% of CAB is eliminated in the feces and approximately 27% in the urine. Due to logistical constraints linked to the drug prolonged half-life, studies were conducted after oral intake but concordant results were found when analyzing urine and plasma samples from IM administration. The drug undergoes extensive hepatic metabolization, although unchanged CAB is also excreted in the bile. Renal clearance is minimal and unchanged CAB was not detected in urine. CAB is mainly metabolized by UGT1A1 (around two thirds), and secondarily by

UGT1A9 (around one third), while oxidative metabolism through CYP enzymes play no significant role (156-158).

CAB is a known substrate for ABCB1 and ABCG2; however, the impact of those transporters on the drug absorption is expected to be limited due to the is high intrinsic membrane permeability (159).

CAB has a limited potential of causing significant DDIs. Regarding drug metabolic pathways, it has demonstrated in vitro a limited and not clinically relevant inhibitor effect on CYP3A4 and UGT1A9. Regarding transporters, the drug displays no significant effect on OCT2 and MATE-1, contrarily to DTG and BIC, but causes notable inhibition of Organic anion transporter (OAT) 1 and OAT3, basolateral membrane transporters expressed by the kidney proximal tubular cells. This could translate in DDI risks with substrate drugs such as methotrexate or TFV. Regarding TFV, however, it should be noted that this is not expected to increase risk of TFV nephrotoxicity, as CAB does not inhibit the apical efflux transporter ATP-binding cassette sub-family C member 4 (ABCC4, also called Multidrug resistance protein (MRP) 4), and as such prevents the toxic accumulation of TFV in tubular cells (159, 160).

The recommended dose of CAB in the treatment of HIV depends on the administration route. Oral route can be used for treatment initiation to confirm drug tolerance ("oral lead-in", usually for one month), or occasionally as bridge until next IM injection if an injection has to be delayed by more than 7 days. Oral recommended dose is 30 mg once daily (in combination with 25 mg RPV daily). IM administration dose is 400 mg

monthly (with 600mg RPV) or 600 mg every 2 months (with 900 mg RPV), the latter being the only dosage available in Belgium. Oral lead-in can be safely replaced by IM loading scheme consisting in one single 600 mg CAB/900 mg RPV administration one month before starting the regular 2-months interval scheme. CAB can also be used as LA monotherapy for HIV PrEP, using a similar dosing as the 600 mg treatment scheme.

1.1.6.3.3.2 Efficacy

Efficacy of LA regimens combining CAB with RPV for the treatment of non-naïve adults living with HIV was demonstrated in 3 phase III RCTs. The ATLAS trial demonstrated non inferiority of monthly LA therapy compared to continuing standard therapy in PLWH with previously undetectable VL for at least 6 months, with 1.6% versus 1% of participants being detectable (≥ 50 copies/mL) at 48 weeks (70). The FLAIR trial compared switching to CAB/RPV versus continuing unchanged oral regimen after a 20-weeks long oral induction therapy with DTG/ABC/3TC in cART naïve PLWH. Non inferiority was demonstrated, with 2.1% versus 2.8% of virologically detectable participants at 48 weeks (161). The use of a two-month interval scheme was validated in the ATLAS-2M study, demonstrating non-inferiority of LA CAB/RPV injections every 8 weeks compared to every 4 weeks at 48 and eventually at 96 weeks (161, 162). Importantly, LA-CAB/RPV every 8-weeks was demonstrated safe and not-inferior to oral treatment in a large African RCT, despite simplified monitoring and care (with no genotype available at switch and sparse VL monitoring). This suggests this treatment could fit in

care models from low- and middle-income countries following the recommended “public health approach” (163).

Eventually, LA-CAB administered every 2 months was demonstrated to be superior to daily TDF/FTC for the prevention of HIV in men who have sex with men (MSM) and transgender women (164), as well as in cisgender women in a second trial carried in Sub-Saharan Africa (165).

Although real-world studies confirmed the overall efficacy of CAB/RPV LA regimens, these studies also demonstrated that a small proportion of patients experience VF despite on-time injections, usually during the first year after treatment initiation - although late failures have also been reported. In a single center French study, 79% of 126 patients starting CAB/RPV were still receiving this treatment after a median follow-up of 9 months, and most discontinuations were not linked to treatment efficacy or tolerability but rather to patients’ convenience. However, 5 (4%) discontinued the LA therapy due to VF (166). Other studies have found a lower risk of VF around 1-1.5 % (154, 167). Importantly, studies have found a substantial risk of selecting NNRTI or INSTIs RAM in case of failure (168). A post-hoc analysis from large trials, identified 3 factors associated to VF, with the combination of two or more of those disproportionately increasing this risk: pre-existing RPV RAMs, HIV-1 subtype A6/A1, and a high BMI above 30 kg/m² (169). Recent real-life studies however found no effect of weight on risk of VF (170). Despite several reports having shown drug concentrations in the low range in LA-treated PLWH presenting VF, the link between plasma

drug exposure and VF remains poorly understood. Different target concentrations have been suggested, including maintaining CAB concentrations above 4 times de PA-IC₉₀ (i.e., above 664 ng/mL), but it was found that a substantial proportion of LA-treated PLWH display low or very low concentrations without translating in VF (154, 168, 171).

1.1.6.3.3.3 Recommendations and use

CAB is currently recommended in Europe and the US as a switch strategy for PLWH with sustained virological suppression (at least 3-6 months), as well as for PrEP (63, 127). Moreover, the American IAS-USA guidelines suggest CAB injections could be used in some selected cases for PLWH with detectable viremia and unable to take oral cART consistently despite extensive efforts and clinical support (172).

CAB is commercialised as Vocabria® for HIV treatment and Apretude® for PrEP. The latter has been EMA-approved but is currently not available in Belgium.

1.1.6.3.3.4 Low-level, ultra-low-level and residual viremia with second-generation INSTIs

Measuring HIV VL during cART is a standard practice. However, much heterogeneity exists regarding virological outcomes and their management in routine clinical practice. First, the definition of HIV VL undetectability varies according to the sensitivity of used assays, with recent assays displaying limits of detection and/or quantification generally between 20 to

50 copies/mL (173). Secondly, detecting VL in a treated PLWH is generally not sufficient to consider the treatment as failing.

Although consensus is lacking, some definitions have been proposed to describe the most important virological outcomes encountered in clinical practice (174-177):

- Virologic suppression: VL measured ≤ 50 copies/mL. Some authors do not use a defined threshold but refer to the lower limit of detection of available assays.
- Blip: VL of 51- 999 copies/mL measured at a single occasion preceded and followed by VL of ≤ 50 copies/mL. Some authors restrict the definition to VLs of 51-199 copies/mL (175).
- Low-level viremia (LLV): ≥ 2 consecutive VLs of 51–199 copies/mL occurring ≥ 30 days apart. For some authors, LLV can be considered with a single episode of VL of 51–199 copies/mL (175).
- Virological failure (VF): The inability to achieve or maintain suppression of viral replication while on ART. This can be defined by ≥ 2 consecutive VLs of ≥ 200 copies/mL or a single VL of ≥ 1000 copies/mL. Some authors use the threshold of a single VL of ≥ 200 copies/mL (175).

Of note, the EACS uses significantly different definitions, with VF defined as VL > 50 copies/mL and this outcome being further subdivided in *incomplete suppression*, defined as VL > 50 copies/mL at 6 months after starting therapy in a person not previously on ART, and *virological rebound*, defined as

confirmed VL >50 copies/mL in someone with previously undetectable VL. No definition is provided for LLV. The EACS also proposes different management strategies of VF according to the level of VL (simple follow-up if VL <200 copies/mL) (63).

Another important concept that has emerged recently in the literature is non-suppressible viremia (NSV). It refers to the situation where persistent detectable VL, generally in the LLV range, is not due to incomplete treatment adherence, detrimental pharmacological interactions nor by drug RAMs, and does not progress to VF. It has been estimated that 1 in 250 treated PLWH may experience NSV (178). This situation may or not occur after an initial period of virological suppression under ARV therapy. Available evidence suggests that NSV is due to release of viral particles (usually defective, although this is not always the case) from the clonal expansion of latently infected cells (177).

Some authors have also defined ultra-low-level viremia (ULLV, also called very-low-level viremia) as the occurrence of VL measured as detectable and/or quantifiable but ≤ 50 copies/mL (173). Eventually, residual viremia (RV) has been defined as cryptic viremia during cART that is below 10 to 20 copies/mL and unaffected by treatment intensification (173, 177).

One should remember that the best thresholds and VL targets are poorly defined beyond 200-1000 copies/mL. From a public health perspective, upper range thresholds are acceptable, as demonstrated in a recent

systematic review showing that the risk of HIV transmission was null below 600 copies/mL and very close to zero for VL below 1000 copies/mL (179). From an individual health perspective, lower range thresholds are generally regarded as better, although the precise impact of LLV remains incompletely understood - this is even more true for values under 50 copies/mL. Several studies have pointed towards higher risk of VF after LLV, especially with persistent LLV and when using the cut-off value of ≥ 200 copies/mL to define VF (174, 175, 180). This appears to hold true also for more recent agents including second-generation INSTIs (175, 181). Whether ULLV has a clinical significance or prognostic value has been debated, but those values are usually disregarded (182-185). An association between LLV and increased all-cause mortality has also been shown (186). Regarding RV, this phenomenon has been shown to be present for prolonged periods in almost all ARV-treated PLWH - with a reported median VL of 3.1 copies/mL in a long-term longitudinal study (187).

Comprehension of the origin and mechanisms of RV, ULLV and LLV remains elusive (188). The production of replicative or defective viral particles by the activation of latently infected cells is usually considered as the most likely cause. However, another nonexclusive theory links detectable viremia to active replication in sanctuary sites with limited drug exposure (189, 190). While persistent viremia was shown independent of inadequate plasma exposure (191, 192), low drug levels in anatomical sanctuary sites such as lymphatic tissues may play a role (193-195).

Limited data are available regarding LLV and ULLV with INSTIs, but published evidence have suggested that INSTI-based regimens could be associated with lower incidences of LLV, blips and/or ULLV as compared to other regimens (190, 196, 197). A large French study has reported that 11% of patients starting an INSTI-based (mostly second-generation) regimen presented at least one measure of VL between 51 and 200 copies/mL. More specifically, regarding second-generation INSTIs, a large study from Taiwan found an incidence of blips/LLV (defined as any occurrence of a VL measure between 50 and 200 copies/mL) of 3.8 per 100 person-years of follow-up for DTG- and of 6.2 per 100 person-years of follow-up for BIC-treated PLWH (198). The frequency of non-virological suppression with LA-CAB/RPV is less well-known. In phase 3 studies, 6% of participants experienced viral blips and 1% LLV (199). Recent cohort studies have reported rates of 6-13% of treated individuals presenting blips, with no data regarding LLV (154, 166).

1.1.6.3.3.5 Second-generation INSTIs' adverse events

Randomized clinical trials mentioned hereabove have all concluded that DTG, BIC and CAB were not only efficacious, but also generally safe and associated with excellent - and in some cases improved versus comparators - tolerability. Indeed, when compared to EFV, DTG appeared better tolerated with 2-3% versus 10% discontinuation due to adverse events in the SINGLE and SPRING-1 studies (110, 200). However, real world studies have quickly identified neuropsychiatric adverse events (NPAEs) - such as sleep disorders, anxiety, depression, headache - and abnormal weight-gain as two important

safety-related signals, raising the question of a possible class effect (201, 202).

1.1.6.3.3.5.1 Neuropsychiatric adverse events

The previously described better tolerability profile of DTG compared to EFV was partly driven by a reduction in NPAEs. This advantage has been outlined by several studies, including a recent prospective cohort study in an African population which confirmed that compared to EFV, DTG is associated with improved cognitive function and sleep, although it appeared associated with a possible increase in depression. This study also demonstrated that this improvement is not linked to enhanced CNS virological efficacy of DTG (203). Nonetheless, significant NPAEs were observed among DTG-treated participants in RCTs, and a meta-analysis published in 2020 showed an increased risk of depression for DTG compared to all comparators including other INSTIs, but excluding EFV (RR = 1.34, 95% IC: 1.04-1.74) (204).

The first report of unexpectedly high DTG discontinuation rates linked to NPAEs came in 2016 from the Netherlands. Among 556 PLWH starting the drug, *de Boer et al.* found a rate of 15.3% of DTG discontinuation mainly due to intolerability. ABC-containing regimens appeared disproportionately concerned by discontinuation. The three most frequent causes for drug discontinuation were insomnia/sleep disturbances (in 5.6%) and other neuropsychiatric symptoms such as anxiety, psychosis and depression (in 4.3%), along with gastrointestinal complaints (in 4.3%) (205). A German

study published at the same period found a similar rate and identified female sex and *older* age (>60 years) as risk factors additionally to ABC use (206). Other possible risk factors identified in following DTG studies included: low CD4+ counts (<350 cells/mcl), Sub-Saharan African origin, being treatment-naïve at the start of DTG, association with RPV, but also *younger* age (<50 years) in one study. Importantly, contrasting results were found regarding the association between a previous history of psychiatric conditions and the occurrence of NPAEs or NPAE-related DTG-discontinuation (121, 204, 207-212).

The overall incidence of any NPAE has been reported at variable rates in DTG cohort studies, ranging from 5 to 20.6% (213). A recent systematic review of real-world evidence reported a median rate of DTG discontinuation due to NPAE across studies of 2.8% (interquartile range (IQR): 1.4%-3.68%) (207), confirming increased rates compared to published RCTs. In the retrospective Belgian cohort study by *Nasreddine et al.*, NPAE were the most frequent cause of DTG discontinuation, with a rate of 2.4 per 100 patient-year (121). Switching from DTG to EVG or DRV has been shown as potentially beneficial in symptomatic patients in two open-label RCTs (214, 215).

Regarding BIC, the drug was first compared to DTG in RCTs, providing some reassurance regarding its safety profile. NPAEs and NPAE-related drug discontinuation occurred generally at similar rates for both drugs in those studies, although numerically decreased risks were reported in some of them (*e.g.*, 1% versus 4% for sleep disorder among BIC/TAF/FTC-treated

participants versus DTG/ABC/3TC-treated participants in the double-blind study by *Wohl et al.*) (143, 207). Moreover, a secondary analysis of patient reported symptoms and sleep quality in RCTs comparing BIC/FTC/TAF and DTG/ABC/3TC showed a favourable profile of the former regarding NPAE and sleep (216). The first information from real-world evidence came from Germany and estimated BIC/FTC/TAF discontinuation rates due to NPAEs at 3.3% after 6 months of treatment, out of a total of 5.3%, not different from discontinuation rates under DTG in the same cohort. Interestingly, the authors found a low cross intolerance between DTG and BIC (217). Later data hopefully provided more reassurance, with a synthesis of evidence describing a median rate of BIC discontinuation due to NPAEs across studies of 0.7% (IQR: 0.0%-1.7%), lower than for DTG (207). Data from the Belgian BIC cohort, not included in this literature synthesis, also found limited NPAE-discontinuations occurring in 0.4% after a median follow-up of 89 weeks (149). Moreover, regarding specifically sleep disorders, a study evaluating factors associated with poor sleep quality in PLWH identified the use of BIC as the only *protective* factor among a cohort of 132 PLWH with various cART regimens, including BIC for 28 and DTG for 19 of them (218). Eventually, a small open-label RCT found a significant decrease in sleep disorders in patients without overt neuropsychiatric symptoms randomized to shift to BIC/FTC/TAF compared to those continuing DTG/ABC/3TC, while other neuropsychiatric components evaluated through psychometric evaluations remained unchanged (219). On the contrary, a double-blind RCT showed no difference in grade 2-4 NPAEs or drug discontinuation between PLWH

presenting neuropsychiatric symptoms that were shifted either to DTG/3TC or to BIC/FTC/TAF (220).

Data from CAB RCTs found limited incidence of NPAEs (213). There is currently scarce real-world evidence regarding tolerance and drug discontinuation. In a single-center study of PLWH starting LA-CAB/RPV, 3/126 (2%) discontinued the treatment due to NPAE after a median follow-up of 9 months (166).

Of note, NPAEs have also been reported with first-generation INSTIs, albeit at a lower rate than with DTG (209, 221). Overall, while it remains hard to conclude definitively if NPAE constitute a class effect of INSTIs or second-generation INSTIs, because of shorter duration of follow-up for BIC and CAB, current data mostly point towards increased risk for DTG. In the rest of this chapter, we will only consider the case of DTG unless specifically mentioned.

Pathophysiological mechanisms underpinning these adverse events remain incompletely understood. An association between DTG plasma concentration and NPAE has initially been suggested by some authors (222). Additional arguments in favour of a direct association between NPAE and DTG plasma PK came from a small report describing improvement of sleep disorders in some patients after bringing forward pill intake from evening to morning (223). As second-generation INSTIs including DTG have been shown to diffuse readily into the cerebrospinal fluid (CSF) (224-227), this association suggested possible direct or indirect effects of CNS exposure to DTG.

However, several more recent studies have challenged this view as they found no or inconsistent associations between DTG plasma exposure and NPAEs (228-231). **Table 2** summarizes the literature findings regarding associations between DTG PK and NPAEs.

Some authors have speculated neurotoxic effects of DTG could be linked to microglial activation or neuronal apoptosis (232), but studies substantiating this affirmation could not be found. Other authors have suggested that neurotoxicity could be mediated by DTG aldehyde metabolites, but this was not supported by experimental data (106).

Table 2. Summary of literature findings regarding association between DTG plasma exposure and the occurrence of NPAE.

A summary of the study is proposed, with a (+) indicating an association between DTG PK and NPAEs.

Study	Measures (number of participants evaluated)	Results	Summary
Yagura et al. (222)	Cumulative incidence of selected NPAE (dizziness, headache, insomnia, restlessness, anxiety); DTG C _{trough} (n=107, of which 34 developed NPAE)	Higher median DTG C _{trough} in PLWH with NPAEs vs those without NPAEs Plasma DTG trough concentration ($\geq 1.06 \mu\text{g/mL}$) associated with cumulative incidence of NPAEs	+
Borghetti et al. (231)	SCL-90-R score (10 subscales + global severity index); DTG C _{trough} (n=203)	Multivariate analysis: DTG C _{trough} associated with 2 subscales (hostility, psychoticism)	? (mixed results)
Hoffmann et al. ^a (208)	DTG discontinuation due to NPAE (n=37) vs controls (n=74); sparse DTG PK	No association of NPAE-discontinuation with plasma concentrations	-
Elliot et al. (230)	6 sleep scores (PSQI, ESS, FOSQ, ISI, FSS, SDQ) and Cogstate neurocognitive test, at days 28, 90 and 180; intensive DTG PK sampling on day 28 post shift to ABC/3TC/DTG (n=38)	C _{trough} , C _{max} and AUC not correlated to day 180 sleep scores or intra-subject changes C _{trough} and AUC not correlated to day 180 neurocognitive test or intra-subject changes. Higher C _{max} associated with improvements in global cognitive function	-
Griesel et al. (229)	MMS mental health assessment and Likert scale (0-10) evaluating average quality sleep in the preceding 4 weeks, both at weeks 4, 12 and 24 post ARV initiation with DTG; intensive DTG PK (n=41) and sparse sampling (n=431)	Higher AUC associated with more improvement at week 2 and 24 from baseline in MMS Multivariable regression: increased AUC associated to decreased sleep quality, only at week 12	? (mixed results)

^aAbstract, no detailed results

Abbreviations: ESS, Epworth Sleepiness Scale; FOSQ, Functional Outcomes of Sleep Questionnaire; FSS, Fatigue Severity Scale; ISI, Insomnia Severity Index; MMS, modified mini screen; PSQI, Pittsburgh Sleep Quality Index; PK: pharmacokinetics; SDQ, Sleep Disorder Questionnaire; SCL-90-R, Symptom Checklist-90-R; vs, versus.

Another hypothesis has been raised to explain DTG-induced NPAEs is linked to the inhibitor effect of the drug on SLC22A2 (OCT2). Knowing that this transporter is expressed in the CNS and involved in the transport of several neurotransmitters, Borghetti et al. hypothesized that the SLC22A2-inhibiting effect of DTG could affect the brain function and be the origin NPAEs. In addition to testing DTG trough levels (as reported hereabove), they tested this hypothesis by evaluating the impact of the *SLC22A2* c.808C>A (rs316019) single nucleotide polymorphism (SNP) on neuropsychiatric symptoms, because this SNP has been suggested to alter the function of the transporter. The authors found inconsistent results in two separate studies (further details are provided in the pharmacogenetic section of this manuscript) (211, 231).

More recent studies, however, started to provide relevant insights into the comprehension of DTG neuro-pathophysiology. Indeed, DTG has been shown to dysregulate the blood-brain barrier (BBB). Using bovine brain microvascular cells, *Ma et al.* found that exposure to DTG induces a down-regulation of the gene expression of several tight junction (TJ) proteins (TJP1, CLDN5, JAM-2) and increased mRNA levels of the proinflammatory cytokines TNF α and IL1 β (232). In a more advanced study using different cellular and *ex vivo* mouse brain microvascular models as well as *in vivo* mice

experiments, *Huang et al.* replicated some of those results and further expanded the description of DTG effects on BBB. Not only did they describe a significant downregulation of mRNA expression of three TJ proteins (TJP1, CLDN5 and OCLN) and an upregulation of gene expression of the cytokines IL6, IL8, IL1 β and of the oxidative stress marker NOS2, but they also reported a significant downregulation of *ABCB1* and *ABCG2* mRNA expression, and a possible upregulation of *SLC2A1*. Moreover, an *in vivo* impact was demonstrated, with a 14-day DTG treatment of mice inducing a 70% increase in brain levels of Sodium fluoresceine, a marker of BBB integrity. Interestingly, the authors found that the BBB disruption induced by DTG significantly exceeded EFV and BIC effects (233). Using human and mouse BBB models *in vitro*, and mouse brain capillaries *ex vivo*, the same group subsequently confirmed the disruptive and pro-inflammatory effects of DTG, with elevated mRNA and/or protein expression of several pro-inflammatory cytokines and chemokines (Cxcl1, Cxcl2, Cxcl3, Il6, Il23, Il12), alongside downregulation of folate transporters mRNA expression. Studying the blood-CSF-barrier in the choroid plexus of DTG- and BIC-treated mice and controls, the authors found upregulation of proinflammatory cytokines and chemokine (Il6, Il1 β , Tnf α , Ccl2) and downregulation of mRNA expression of drug efflux transporters (ABCC1 (MRP1), ABCC4 (MRP4), and ABCB1) and tight junction protein (CLDN3). Overall, effects of DTG largely exceeded those of BIC (234). Eventually, the authors describe in a preliminary report that BBB disruption appears mediated by endothelial reticulum stress associated with cytosolic calcium imbalance (235).

1.1.6.3.3.5.2 Weight gain

A higher-than-expected weight gain in patients receiving second-generation INSTIs was first noticed in 2017 among a French cohort of patients starting DTG, mainly (94%) as a shift from another drug class. In their cohort, *Menard et al.* reported that 20% of patients receiving DTG gained more than 10% in weight after a mean follow-up of approximately 9 months. Interestingly, this first cohort study already identified women and patients receiving ABC/3TC (as opposed to others receiving mainly TDF/FTC) as being at particular risk (236). Several other studies reported similar trends, and confirmed that weight gain is linked to increases in fat mass, raising major concerns among HIV clinicians (237, 238). Women were confirmed at particular risk, especially in the late peri- or post-menopause period (239). Overall, a large meta-analysis concluded that weight gain is higher with DTG-based regimens compared to EFV and most other regimens, but comparable to other INSTIs except EVG/c (CAB was not studied). The same study also found that TAF leads to larger weight gains than other non XTC (3TC or FTC) NRTIs (240). In our own experience at CUSL, we observed a significant relative increase in weight of 2.80% (SEM of ± 0.23) ($P < 0.001$) during a median follow-up of 31 weeks (0.9–64.9) in PLWH starting DTG or EVG/c, with no significant effect of sex. Weight gain was higher among black compared to white people (241).

The mechanisms driving this weight gain are not fully understood but are believed to involve complex interactions with other medications including TAF, which may independently contribute to weight gain (242, 243). In addition to drug-related factors, HIV-specific considerations play a role in

weight dynamics. The '*return-to-health*' effect, a phenomenon observed after the initiation of an effective HIV treatment, leading to viral suppression and immune recovery, can result in weight gain. Several studies have underscored that advanced HIV with low CD4+ T-cell counts and/or elevated VLs are associated with increased weight gain after treatment initiation (244, 245). However, this does not fully account for the observed weight changes (246), as weight gain has also been demonstrated after treatment shifts in virologically suppressed patients (247). The introduction of cART not only reverses CD4 depletion but also diminishes HIV-related immune activation. These effects might have a stronger influence on weight with INSTIs due to the potency of these drugs (248-250). Furthermore, INSTIs have been linked to changes in adipose tissue function, evidenced by increased adipogenesis, fibrosis, mitochondrial dysfunction, and insulin resistance (242, 251-254). DTG-induced reductions in brown and beige adipocyte thermogenesis linked to mitochondrial defects have also been demonstrated (255).

Despite these possible pathophysiological phenomena, data have accumulated over the years demonstrating that weight gain is a multifactorial phenomenon that is not solely caused by INSTI drugs, as reported in a recent literature synthesis by *Wohl et al.* (256). The effects of the host's immune status, back-bone regimens, previously received treatments, as well as social factors should be taken into account. Moreover, weight gain in PLWH should always be interpreted in the context of the global rise in obesity (257).

First, it is becoming clear that when assessing the effect of INSTIs on weight gain, the comparator anchor drug should not be regarded as weight-neutral. In the ADVANCE trial, participants starting DTG/TDF/FTC gained an average of 4.3 kg compared to 2.3 kg for participants starting EFV/TDF/FTC after 96 weeks (with higher values of respectively 4.8 kg and 3.2 kg for women) (258). However, this eventually appeared to be related to EFV weight-attenuating effect. Indeed, a pharmacogenetic post-hoc analysis demonstrated important variations of levels of weight gain according to EFV-metabolizing status, as defined by *CYP2B6* genotype: the level of weight-gain was lowest in EFV intermediate- and slow-metabolizers, and was similar for EFV-extensive metabolizers and individuals receiving DTG/TDF/FTC. This supports the hypothesis of decreased weight gain with increased exposure to EFV (a drug with limited tolerability, as previously mentioned) (259). Additional evidence from other studies includes the fact that adding DTG to a stable background regimen did not increase weight gain as compared to adding a placebo (260).

Secondly, we have recently gained a better understanding of the role of backbone drugs on weight-gain. While TAF was initially thought to exert a weight-promoting effect, especially after the ADVANCE trial demonstrated a weight gain of 7.1 kg in the DTG/TAF/FTC group as compared to 4.3 kg in the DTG/TDF/FTC group, it is now thought that the most crucial impact comes from a weight-attenuating effect of TDF. Some clues came from PrEP studies, taking advantage of the absence in the PrEP context of possible interfering effects of the HIV infection, the return-to-health effect, or the combination

of TDF with INSTIs (256). A recent meta-analysis concluded that TDF has a negative effect on weight gain and may induce weight loss, as compared to the group receiving either placebo or CAB (261). TDF was also associated with decreased weight gain compared to TAF (262). Further evidence in the field of HIV treatment came from the SALSA and TANGO trials that evaluated shift to DTG/3TC, and the SOLAR trial that evaluated shift from BIC/FTC/TAF to CAB/RPV. None of these studies have demonstrated an impact of TAF removal on weight gain (117, 118, 263).

Eventually, other important findings came from studying injectable CAB monotherapy for PrEP. On one hand, weight gain appeared as similar with CAB and placebo (264). On the other hand, when compared to TDF/FTC, CAB appeared to be associated with greater weight gain in MSM and transgender women during the first year. Following this initial effect, the annual weight accrual appeared similar across groups (164).

Overall, although intrinsic effects of INSTIs especially on adipose tissue remain to be fully understood, a new model has been proposed by *Wohl et al.* to explain weight-gain associated with ARVs, which should probably be complemented by environmental and social determinants that play a role in weight-gain in the general population as well as PLWH (265, 266). **Figure 9** illustrates how this new model modifies the understanding of weight gain, using data from the ADVANCE trial.

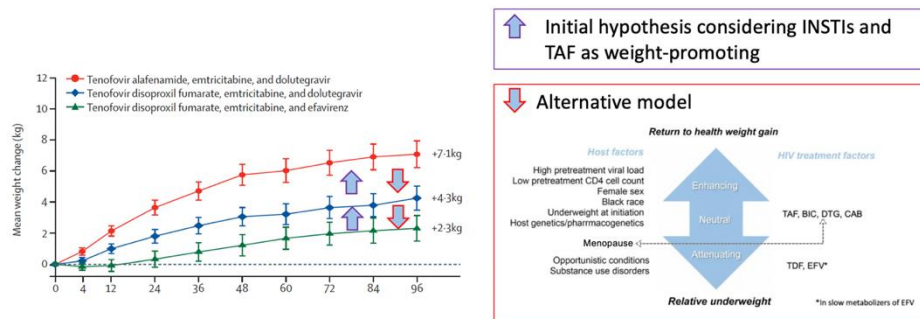


Figure 9. Different models proposed to explain the change in bodyweight over time in participants from the ADVANCE trial.
Adapted from Venter et al. and Wohl et al. (256, 258).

1.2 Pharmacogenetics and pharmacokinetics

1.2.1 Introduction and definitions

Pharmacokinetics (PK) is defined as the study of the time course of drug concentrations in the body (in a simplistic view: “*what the body does to the drug*”) and pharmacodynamics (PD) as the study of the relationship between drug dose or concentration and the intensity and time course of pharmacological, clinical, or toxicological responses (“*what the drug does to the body*”).

Apparently unpredictable interindividual variability in drug response has been a common observation in clinical practice since the advent of medicine. The first conceptualization that genetic traits could account for “*hypersusceptibility reactions and hyposusceptibility reactions*” to drugs was proposed by A.G. Motulsky in 1957. This followed the identification of inherited predispositions to adverse drug reactions (ADRs), such as primaquine-induced haemolysis or prolonged suxamethonium-induced apnea during anesthesia, respectively due to glucose-6-phosphate dehydrogenase and pseudocholinesterase deficiencies (267).

Pharmacogenetics (PGx) is the field that studies how inherited genetic factors can affect the interindividual variability in drug response, in terms of efficacy and toxicity. The first use of this term can be found in a 1959 treatise by F. Vogel entitled “*Moderne Probleme der Humangenetik*” (268). Definitions have evolved following progresses in genetics and pharmacology,

and the International Conference on Harmonisation has proposed to define *pharmacogenomics* as the study of variations of DNA and RNA characteristics as related to drug response, and *pharmacogenetics* as the study of variations in DNA sequence as related to drug response (269, 270). Some other important definitions used in the field of PGx that will be used regularly in this manuscript as well as a summary of key principles of genetic nomenclature are provided in **Annex 4**.

A wide range of unexpected responses to drugs have been described. As initially suggested by Motulsky, genetic variations can impact on both drug efficacy and drug toxicity. In a classical view, ADRs are divided in type A reactions if they are predictable based on the drug expected pharmacological effect (most often through exaggerated effects) or type B reactions (also called idiosyncratic reactions) if they are unrelated to the drug pharmacological effect. Other authors have proposed more complex classifications to take into consideration ADR mediated by treatment duration or drug withdrawal (271).

The effects of pharmacogenetic variants on drug efficacy and toxicity can be mediated by variations in drug concentrations (PK variation) or can be independent of the drug concentration (PD variation). PK variations are mediated by modifications in transport and/or metabolism (or biotransformation) of the drug or any of its pharmacologically active metabolites. PD variations are generally mediated by modifications in the immune response to a drug, or modifications in the drug target or receptor

(270, 271). Different illustrations of those categories of PGx effects are illustrated in **Table 3**.

Table 3. Description of different types of impacts of pharmacogenetic variants on drug efficacy and toxicity.

	Effect on efficacy	Effect on toxicity (ADR)	
		Type A	Type B
Effect mediated by drug concentration (PK variation)	CYP2D6 poor metabolizers have diminished analgetic effect of codeine, a prodrug that requires bioactivation by CYP2D6 in its active metabolite morphine (272)	CYP2D6 ultrarapid metabolizers exhibit an enhanced narcotic effect from codeine	<i>CYP2C9*3</i> reduces phenytoin clearance and is associated with severe cutaneous adverse reactions (Stevens-Johnson, toxic epidermal necrolysis, DRESS)
Effect independent of drug concentration (PD variation)	Loss of function variants in <i>VKORC1</i> , the target of warfarin, cause familial warfarin resistance, i.e., an absence of INR	Variants in regulatory regions of <i>VKORC1</i> associated with lower gene	<i>HLA57*01</i> polymorphism is associated with severe systemic reactions in

	increase even with high doses of warfarin	expression affect warfarin response	patients receiving abacavir

Abbreviations: CYP: cytochrome P450; DRESS: Drug reaction with eosinophilia and systemic symptom; HLA: Human leukocyte antigen; INR: International Normalized Ratio; VKORC: Vitamin K epOxide Reductase Complex (VKORC).

1.2.2 Principles of drug disposition

PK studies describe the disposition of a drug, classically divided into four components (phases): *absorption, distribution, metabolism* and *excretion* (ADME). These components involve processes that concur simultaneously and may interact with each other. All of them can be affected by genetic factors (273). Given that much of this work focuses on the interaction between PGx and PK, it is crucial to understand at which levels these interactions can occur.

The absorption of a drug refers to its passage from its administration site into the systemic circulation. Several factors impact on a drug absorption and its bioavailability (designated by F , the fraction of an administered drug dose that reaches the systemic circulation). For orally administered drugs, it can be affected by transport proteins expressed by enterocytes, with different transport proteins exerting different effects, decreasing or increasing the drug absorption. Moreover, *pre-systemic metabolism* may occur: enterocytes express enzymes that have the ability to metabolize drugs before they enter the portal circulation (*intestinal first-pass effect*), and

hepatocytes can metabolize drugs before they enter the systemic circulation (*hepatic first-pass effect*). The action of both those proteins and enzymes may vary as a consequence of genetic variability in their encoding genes. Of note, DDIs may also impact on proteins and enzymes activity. The term *phenocopy* is used when DDIs mimic the effect of a genetic variant.

The distribution of a drug refers to its reversible transfer from the systemic circulation to the various tissues and organs of the body. It is influenced by several physiochemical drug characteristics (drug lipid solubility, drug binding to plasma protein or to blood cells) and host physiological characteristics (blood flow, protein levels in the plasma or the tissues). Distribution into tissues is also influenced by the action of influx and efflux transport proteins.

The metabolism (or biotransformation) of a drug refers to the chemical processes leading to the modification of a drug into its metabolite(s), generally making it more polarized and water soluble. The liver is the main organ responsible for metabolism, but other organs (the kidney, lung, gastro-intestinal tract...) can also participate in drug metabolism. Metabolism involves enzymatic processes that are subject to important inter- or intra-individual variability associated to physiological processes (e.g., age or pregnancy), pathological processes (e.g., liver or kidney failure), DDIs, or genetic variants.

The excretion is the final step by which the drug and/or its metabolites are eliminated from the body, mainly involving the kidney and the liver. First, the unbound fraction of xenobiotics can be passively filtered through the glomerular membrane, some of those (particularly the lipid-soluble drugs) being subsequently passively reabsorbed along their concentration gradient in renal tubules, where urine is concentrated. Secondly, some molecules are eliminated at the kidney proximal tubule through an active secretion process involving transport proteins (cellular uptake and efflux transporters). Eventually, several molecules are excreted into the bile and eventually the feces, also through active transport. Some of them will be further reabsorbed, usually after deconjugation (*enterohepatic cycle*) (274, 275).

The most important biotransformation enzymes and transporters involved in INSTIs pharmacokinetics, as well as their main polymorphisms, will be presented in the next chapters.

1.2.3 Drug metabolism

Metabolism (or biotransformation) is a crucial component of drug elimination, together with drug excretion. It is especially important for lipophilic drugs that are not efficiently excreted by the kidney. Drug (and to a larger extent xenobiotic) metabolism reactions aim at enhancing their excreatability and are categorized into phase 1 and phase 2 reactions, which in several cases occur sequentially. Importantly, drug metabolism (especially

phase 1) can also in some cases form active and/or toxic compounds (a process called bioactivating effect).

1.2.3.1 Phase 1 metabolism

1.2.3.1.1 Introduction

Phase 1 reactions (also called *functionalization reactions*) are catabolic reactions that introduce or expose functional groups on the chemical structure of a compound, usually to make them more reactive and accessible for phase 2 reactions. They can consist in processes such as oxidation, hydroxylation, hydrolysis, dealkylation, deamination or reduction. Phase 1 reactions mainly involve the CYP monooxygenases, although other enzymes also play important roles (e.g., alcohol dehydrogenase, xanthine oxidase, monoamine oxidase) (274).

1.2.3.1.2 The CYP monooxygenase system

CYP constitute a large superfamily of related haem proteins that have crucial enzymatic roles in almost all forms of life. They are named as “CYP” followed by a conjunction of numbers and letters classifying them into families and subfamilies based on their genetic sequence identity: members of a CYP family share >40% amino acid identity while members of subfamilies share >55% amino acid identity. The human genome encodes 57 CYPs classified in 18 families and 43 subfamilies (276).

Overall, CYPs are mainly expressed in the liver, but they are also found in several other tissues including the intestine, the kidney or the brain. Besides

xenobiotic metabolism, CYPs hold important physiological functions that have not all been fully uncovered, and CYP enzymes not only differ according to their genetic sequence but also to their substrates and substrates classes (being steroids, xenobiotics, fatty acids, eicosanoids, vitamins or unknown for “orphan” CYPs), with some of them handling more than one category (277). CYPs are responsible for roughly 80% of small molecules metabolism and for the majority of drug metabolism, with a small set of them (CYP1A2, 2C9, 2C19, 2D6, and 3A4/3A5) catalyzing the vast majority of drug oxidation reactions (278).

CYPs are often described as *microsomal* enzymes because of their cellular localisation in the smooth endoplasmic reticulum. This localization has a crucial implication: to be metabolized by CYP, a drug must cross the cellular plasma membrane, either passively or helped by the action of a transporter (phase 0 of metabolism) if the drug has a low lipid solubility. To catalyse drug oxidation, CYP requires an electron source (nicotinamide adenine dinucleotide phosphate, NADPH) and a flavoprotein coenzyme (NADPH-P450 oxydoreductase, POR). The catalytic cycle is illustrated in **Figure 10**. Briefly, the catalytic cycle starts with the substrate binding to CYP, which allows the heme iron of CYP (in its ferric state, Fe^{3+}) to become accessible for reduction. An electron is transferred from POR that will reduce the ferric iron to ferrous iron (Fe^{2+}). The reduced CYP will react with O_2 to form a ferrous- O_2 complex, that will receive a second electron usually from POR, and undergo protonation to yield an iron hydroperoxide complex. This complex loses a water molecule to generate an unstable FeO^{3+} complex named

compound 1, that will abstract a hydrogen atom from the substrate, creating a substrate radical. Eventually the Fe-O bond of FeOH^3 will split and the hydroxyl group is transferred to the substrate, which is then released (274, 276).

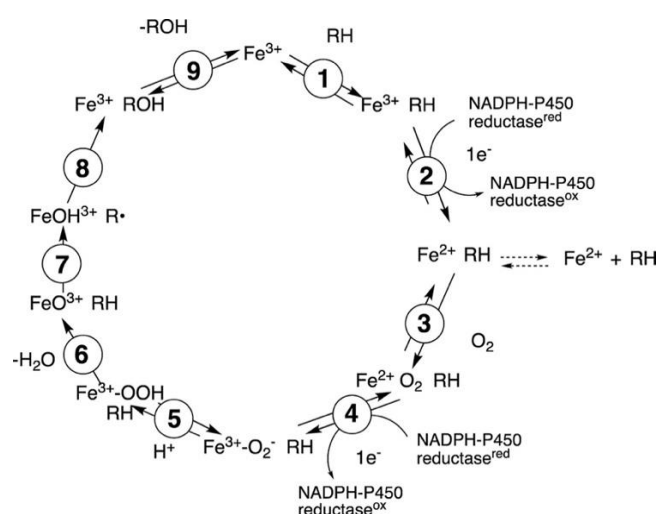


Figure 10. General catalytic cycle for CYP reactions.
From Guengerich et al (276).

1.2.3.1.3 Variability in CYP activity

CYP activity displays significant inter-individual variability. This mainly results from two mechanisms: inherited genetic variability, and the induction and inhibition phenomenon by environmental factors, herbal extracts or drugs (DDI).

1.2.3.1.3.1 Non-genetic variability

Examples of important factors affecting CYPs are the CYP3A4 inhibiting effect of furanocoumarins present in grapefruit, or the inducing effects of tobacco

and cruciferous vegetables (such as Brussels sprout) on CYP1A2 (279, 280). DDIs are numerous and frequently involve CYPs. Inhibition of CYPs can be competitive or non-competitive. Noncompetitive inhibition can be reversible (e.g., ketoconazole binds with the ferric iron of the CYP3A4 haem) or non-reversible when an oxidation product binds with the CYP and destroys it, in a mechanism called suicide inhibition. RTV and COBI are important irreversible CYP inhibitors, frequently used as PK boosters in the field of HIV. CYP induction involves ligand-activated transcription factors acting as xenobiotic receptors, such as the aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptor α (PPAR α), constitutive androstane receptor (CAR, also called NR1I3) or PXR (NR1I2) (274, 281). The latter will be detailed in a further chapter.

The use of databases and online interaction checkers is recommended in case of poly-medication. In the context of HIV, the *HIV Drug Interactions* website from University of Liverpool is a reference website (<https://www.hiv-druginteractions.org>).

1.2.3.1.3.2 Genetic variability

1.2.3.1.3.2.1 CYP3A4 and CYP3A5

CYP3A4 and its isoenzyme CYP3A5 deserve further attention in this manuscript, due to their important role in metabolism of INSTIs. Both are members of the *CYP3* family which in humans counts only two additional members: *CYP3A7*, which represents a major CYP during foetal life but is less important later, and *CYP3A43*, which appears less crucial in drug metabolism. These *CYP3A* subfamily members are located on chromosomal

band 7q21-q22.1 and each of them comprises 13 exons. *CYP3A4* and *CYP3A5* share 85% similarity in their amino-acid sequences and their substrate specificity largely overlaps. The relative contribution of *CYP3A4* and *CYP3A5* to drug metabolism varies from one substrate to the other. Crucially, it also varies from one individual to the other, depending on (i) the sex, with female displaying higher levels of *CYP3A4* proteins than male, (ii) the carriage of *CYP3A4* variants, and (iii) the *CYP3A5* genotype (**Table 4**) (282, 283).

Several *CYP3A4* variants have been described, but most of them show no or little effect on *CYP3A4* activity. In its guidelines, the Dutch Pharmacogenetics Working Group (DPWG) reports that *CYP3A4**8, *11, *12, *13, *16, *17, *18 and *22 display decreased function and *CYP3A4**6, *20 and *26 display no function, but most of the underlying evidence comes from *in vitro* data. Also, most of these variants are very rare in the population, with exception of *22, described hereunder, *20, with a reported minor allele frequency (MAF) of 0.7% in Spain, and *16 with a MAF of 2-5% in the Japanese population (284).

*CYP3A4**22 (rs35599367, c.522-191C>T) appears as the most frequent among *CYP3A4* function-impacting variants, and is almost only present in White individuals. This SNP in intron 6 has been shown to alter RNA splicing leading to partial intron 6 retention, with production of a non-functional protein in the liver, but not the intestine (285). This leads in turn to a reduced *CYP3A4* activity (282).

CYP3A5 is a polymorphic gene with five variants listed on the Pharmacogene Variation Consortium (PharmVar) database (286). The *CYP3A5* functional allele is *CYP3A5*1*, and three major non-functional variants exist (*CYP3A5*3*, *CYP3A5*6* and *CYP3A5*7*). Their main characteristics are summarized in **Table 4** (287). Carriers of at least one *CYP3A5*1* functional variant (**1/*1*, **1/*3*, **1/*6* or **1/*7*) are classified as *CYP3A5* expressors. Those individuals metabolize some *CYP3A* substrates (such as tacrolimus) more rapidly than non-expressors (any non **1* harbouring haplotype, such as e.g., **3/*3* or **3/*6*) (288, 289). Those individuals may also be prone to genotype-based DDIs: in the context of HIV, a study carried at CUSL previously demonstrated that DRV concentrations are decreased for *CYP3A5* expressors versus non-expressors when DRV is co-administered with etravirine (ETR), a *CYP3A* inducer drug (290).

*CYP3A5*3* (rs776746, g.6986A>G, c.219-237A>G) is the most frequent non-functional variant. The change from “A” to “G” creates a cryptic splice in intron 3 that alters the reading frame and generates the exon 3b. Splicing of this exon into the *CYP3A5*3* transcript introduces a stop codon, leading to a truncated protein (291).

*CYP3A5*6* (rs10264272, g.14690 G>A, c.624G>A) is another non-functional allele that is found less frequently and mainly in the populations from African origin. The change from “G” to “A” also results in an alternative splicing with deletion of exon 7, again leading to a truncated protein (291).

*CYP3A5**7 (rs41303343, g.27131-27132insT, c.1035_1036insT) is the third non-functional allele, with roughly similar allele frequency as *CYP3A5**6. This insertion polymorphism results in a shift in reading frame, introducing a stop codon, and leading to a truncated protein (289).

It has been suggested that combining the *CYP3A4* and *CYP3A5* genotypes could provide an overview of CYP3A overall activity and be used to define three CYP3A phenotypes: poor metabolizers (*CYP3A4**22 carrier and *CYP3A5**3/*3), extensive metabolizers (*CYP3A4**1/*1 and *CYP3A5**1 carrier) and intermediate metabolizer (all other combinations) (282).

Table 4. Description of CYP3A4, CYP3A5 and POR most important polymorphisms.

Allele frequencies for CYP3A4*22 and POR*28 are retrieved from the 1000 Genome Project and for CYP3A5 polymorphisms from the All of Us Research Program (292-294).

Gene and Allele	Nucleotide change	Impact	Underlying mechanism	Allele Frequency
CYP3A4				
CYP3A4*22 (rs35599367)	c.522-191C>T	Decreased activity	Alternative splicing leading to non-function protein accumulation (liver)	European 5% <1% in other ethnic groups
CYP3A5				
CYP3A5*3 (rs776746)	c.219-237A>G	Non-functional	Cryptic splicing leading to truncated protein	African 29% East Asian 72% European 93% South Asian 73%
CYP3A5*6 (rs10264272)	c.624G>A	Non-functional	Alternative splicing leading to truncated protein	African 13% <1% in other ethnic groups
CYP3A5*7 (rs41303343)	c.1035_1036insT	Non-functional	Reading frame shift leading to truncated protein	African 10% <1% in other ethnic groups
POR				
POR*28 (rs1057868)	c.1508C>T	Variable		African 17% East Asian 37% European 30% South Asian 35%

1.2.3.1.3.2.2 *P450 oxydoreductase*

POR is a 79 kDa diflavin membrane-bound protein that serves as electron donor for CYP as well as other important oxygenase enzymes. *POR* is encoded on chromosomal band 7q11.2 and contains 16 exons. The gene is highly polymorphic. Defective mutations are rare but when present in a homozygous state, *POR* deficiency results in type 2 Antley-Bixler syndrome with severe bone dysplasia and defects in steroid hormone biosynthesis. *POR*28* (rs1057868, c.1508C>T) is a much more frequent variant (**Table 4**). This SNP in exon 12 leads to an A503V substitution and has been associated with variable impacts on different CYP isoforms, either decreasing or increasing their activities (295). For CYP3A4, the nature and the magnitude of *POR*28* impact varied significantly, possibly in line with the fact that substrate may induce conformational changes to CYP3A4 that affect the efficiency of electron transfer from POR (296). Probably the most clinically relevant impact described for *POR*28* is its effect on tacrolimus PK. Carriage of *POR*28* in CYP3A5 expressors was associated with increased tacrolimus metabolism (297). A similar effect was also shown with *POR*28* homozygosity in CYP3A5 non-expressors (298).

1.2.3.2 Phase 2 metabolism

1.2.3.2.1 Introduction

Phase 2 reactions consists in anabolic reactions leading to the conjugation of a substrate, usually making it inactive and facilitating its excretion. Most prominent phase 2 reactions are glucuronidation by UGTs and this chapter

will focus on those because of their key role in INSTIs metabolism. However, other important types of conjugations exist such as methylation, acetylation, sulphation or conjugation with glutathione.

1.2.3.2.2 Glucuronidation

UDP-*glycosyltransferase* form an important superfamily of enzymes that share a common function: catalyzing the transfer of a sugar from nucleotide UDP-sugar donors to functional groups on lipophilic molecules or metabolites from phase I reactions. In humans, this mainly involves the transfer of glucuronic acid, and for this reason, the term UGT most frequently refers to “UDP-*glucuronosyltransferase*”. The transfer of this hydrophilic and negatively charged sugar onto endogenous or exogenous compounds (such as respectively hormones, bilirubin or xenobiotics) makes them more water soluble and facilitates their elimination. Because in counterpart this reduces their lipophilicity and thus their passive membrane permeability, hepatocyte further rely on drug transporters expressed at the apical/canicular membrane (such as ABCB1, ABCC2 (MRP2) and ABCG2) for conjugate biliary excretion (phase 3 of metabolism) (299). ABCC3 (MRP3) and ABCC4 (MRP4) transporters that are expressed at the basolateral sinusoidal membrane of hepatocytes exert opposite effects as they transport conjugate into the blood, from where those can be excreted into the urine (further details are provided in the *Drug transport* chapter and on Figure 12) (158, 300).

Human UGTs are divided into four families: UGT1, 2 (further subdivided in subfamilies 2A and 2B), 3 and 8. UGTs are expressed in several tissues,

including the liver, kidney, intestine, mammary gland and the brain, with variable distribution according to the isoenzyme. UGT1 and 2 are considered as important pharmacogenes because they are the main responsible for phase 2 drug metabolism, but they metabolize several other substrates such as bilirubin, bile acids, steroids, environmental toxins or carcinogens. UGT3 and UGT8 appear to conjugate mainly endogenous lipophilic substrates - although they can also be involved in some pharmacological or toxicological mechanisms. The latter also appear to preferentially or exclusively catalyze the transfer of non-glucuronic acid sugars, being involved in several physiological mechanisms, including myelin synthesis for UGT8 (301).

The UGT1 family and its unique human subfamily, UGT1A, will be the focus of this chapter, due to their crucial role in metabolism of INSTIs. *UGT1A* gene locus is located at 2q37 and counts 13 exons. It encodes for 9 different functional isoenzymes: UGT1A1 and UGT1A3 to UGT1A10. This diversity of proteins encoded in one unique gene results from the alternative splicing of a unique exon 1 out of 9 isoform-specific first exons located at the 5' extremity of the locus, combined with the 4 common exons (exons 2 to 4 and exon 5a) located at the 3' side of the locus. Moreover, the gene can further give rise to 18 additional mRNAs through alternative splicing (at the 3' end) of exon 5b instead of 5a, or the combination of exon 5a and 5b (**Figure 11**). Those additional mRNAs can give rise to non-catalytic proteins, and probably play regulatory roles (302).

Among the UGT1A subfamily, UGT1A1 isoenzyme is mainly expressed in the liver where, besides its role in xenobiotics conjugation, it is the sole enzyme responsible for bilirubin glucuronidation (303).

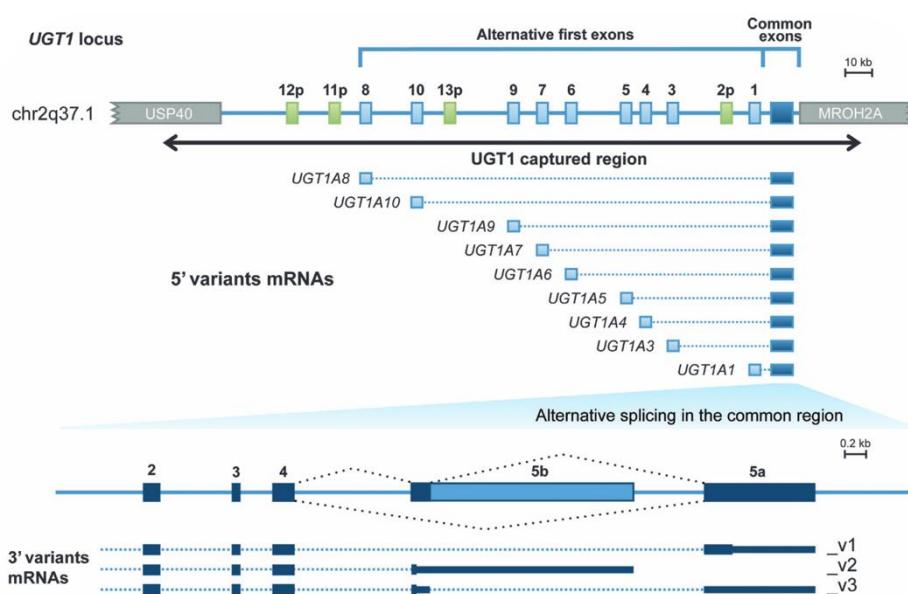


Figure 11. Genomic organization of UGT1 loci.

UGT1 locus on chromosome 2 is characterized by multiple alternative first exons (light blue boxes), three central common exons and two alternative 3' exons (dark blue boxes). Pseudo first exons are represented by green boxes. From Tourancheau et al. (302).

1.2.3.2.3 Variability in phase 2 reactions

Similar to phase 1 reactions, phase 2 reactions are subject to significant inter-individual variability resulting either from genetic variability or from the effect of exposure to drugs (DDIs), herbal extracts or environmental factors. Due to its major role in the metabolism of INSTIs, this chapter will further focus on the case of UGT1A1.

1.2.3.2.3.1 Non-genetic variability

Drug-induced variability in UGT1A enzyme activity may result directly from drug-enzyme interactions (inhibition) or be mediated by the gene regulatory mechanisms (induction) (274). Although those drugs are of less use nowadays, the case of the PI drugs IDV and atazanavir (ATV) is a classical illustration of an interaction between a drug and an endogenous substance: both drugs are competitive UGT1A1 inhibitors, and this competition has been thought to lead to reduced bilirubin conjugation and clinical hyperbilirubinemia, in an interaction further influenced by the genetical background (as detailed in a further paragraph). However, it should be mentioned that this UGT1A1-mediated interaction has been very recently questioned based on the known low-affinity and high-capacity of the UGT1A1 system: using a physiologically-based pharmacokinetic analysis, authors have suggested that ATV-induced hyperbilirubinemia might be mainly due to the ATV inhibition of OATP1B1/3, both being crucial influx transporters of conjugated and unconjugated bilirubin (304).

Phenobarbital constitutes an important *UGT1A1* inducer, and this effect can be used to enhance the bilirubin conjugation and reduce the risk of kernicterus in premature babies. Among ARVs, EFV and RTV (but not COBI) are *UGT1A1* inducers (305). UGT induction is mediated by AhR, PPAR α , NR1I2 (PXR) and NR1I3 (CAR) (274, 281, 301, 306).

1.2.3.2.3.2 Genetic variability

Numerous *UGT1A1* variants have been described, and genetic variability in *UGT1A1* locus can have important clinical consequences: the absence of *UGT1A1* function due to homozygous or compound heterozygous carriage of inactive *UGT1A1* variants leads to type I Crigler-Najjar disease, while mutations conferring a severely reduced but not abolished activity of *UGT1A1* lead to type II Crigler-Najjar disease (303).

UGT1A1 variants are named according to the UGT Nomenclature Committee, with *UGT1A1**1 referring to the wild-type allele. Several of them (rs3064744) are positioned in the thymine–adenine (TA) repeat region of *UGT1A1* promoter (TATA box), that plays an important role in the initiation of the gene transcription. The wild-type allele counts 6 TA repeats (TA₆) and it has been shown that an increase in the number of TA repeats impairs *UGT1A1* transcription. The most frequent variant is TA₇ (*UGT1A1**28). TA₅ (*UGT1A1**36) and TA₈ (*UGT1A1**37), respectively associated with an increase and decrease in *UGT1A1* expression, are more rare and almost nonexistent in people who are not of African descent. *UGT1A1**6 (rs4148323, c.211G>A) is another frequent variant that affects *UGT1A1* activity. This SNP in exon 1 leading to a glycine to arginine change at position 71 also leads to a reduced *UGT1A1* function. This variant is mainly found in the population from Asian origin. With regards to metabolizing status, it is also interesting to consider the *UGT1A1**80 variant within the promoter region (rs887829, c.-364C>T). It remains indeterminate if this variant on its

own has an impact, but its main interest is to be in very high linkage disequilibrium with *UGT1A1**28 and *37 (303, 307) allowing it to be used as a proxy for them. The most important *UGT1A1* polymorphisms are summarized in **Table 5**.

Based on those different variants' impact on UGT1A1 function, genotype-derived phenotypes can be predicted. Gilbert syndrome is mainly associated with the *UGT1A1**28/*28 genotype (308), but other combinations are possible and *UGT1A1**6 is frequently involved in Asian populations (309). It affects roughly 10% of European individuals and more in some other ethnic groups such as Africans. The syndrome is associated with an UGT1A1 activity decreased at 30%, and can lead to fluctuating unconjugated hyperbilirubinemia. Importantly, it may also impact on drug response. An important example is irinotecan, a topoisomerase I inhibitor used mainly as chemotherapy for colic cancer. Irinotecan is a prodrug of SN-38, the latter being inactivated mainly by UGT1A1. Evidence suggests that *UGT1A1**28 homozygous individuals display higher risks of severe neutropenia with the use of moderate or high doses of irinotecan (310). Similar findings were found for *UGT1A1**6 (311).

In the context of HIV, the aforementioned risk of hyperbilirubinemia with the use of ATV has been shown to be increased for individuals with decreased UGT1A1 activity, and the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends to consider an alternative ARV agent for PLWH whose genotype indicates a poor metabolizer status, as defined

according to *CPIC Diplootype-Phenotype Table*. This table (considering only the most frequent variants) proposes a useful consensus classification of phenotypes based on genotype as such: normal metabolizers (*1/*1; *1/*36; *36/*36), intermediate metabolizers (*1/*28; *1/*37; *36/*28; *36/*37; *1/*6) and poor metabolizers (*28/*28; *28/*37; *37/*37; *6/*6). The CPIC recommends that if only *UGT1A1**80 is interrogated and the patient is heterozygous or homozygous for *80, an intermediate or poor metabolizer phenotype may be inferred, respectively (307, 312).

Table 5. Description of UGT1A1 most important polymorphisms.
*Allele frequencies retrieved from the Genomes Aggregation Database v4.1.0 (313), except for UGT1A1*6 that were retrieved from the All of Us Research Program (292, 293).*

Allele	Nucleotide(s) change	Impact	Allele frequency
UGT1A1*1	Six thymine-adenine (TA) repeats within the promoter region	Wild-type allele	African 48% East Asian 87% Non-Finnish European 68% South Asian 59%
UGT1A1*6 (rs4148323)	c.211G>A missense mutation resulting in Arg71Gly	Reduced activity at 30% of normal at homozygous state	East Asian 13% South Asian 2% <1% in other ethnic groups
UGT1A1*28 (rs3064744)	Seven TA repeats within the promoter region	Decrease in transcriptional activity by approximately 70% at homozygous state	African 40% East Asian 13% Non-Finnish European 32% South Asian 41%
UGT1A1*36 (rs3064744)	Five TA repeats within the promoter region	Increased promoter activity	African 7% <1% in other ethnic groups
UGT1A1*37 (rs3064744)	Eight TA repeats within the promoter region	Reduced promoter activity to levels lower than that of promoters with the <i>UGT1A1*28</i> allele	African 5% <1% in other ethnic groups

1.2.4 Drug transport

1.2.4.1 Introduction

Drug transporters can be easily classified according to their influx (uptake into the cell) or efflux (excretion out of the cell) function (**Figure 12**).

Most human drug efflux transporters belong to the ATP binding cassette transporter superfamily (314-316). Members of this large and diverse superfamily actively transport their substrates (which can be ions, peptides, amino acids, sugars, lipids, metabolites, or other compounds including drugs) across plasma or intracellular membranes against a concentration gradient. They play key roles in several cellular and metabolic processes, including protection against xenobiotics such as drugs. The superfamily encompasses 49 members, divided into 7 subfamilies designated as ABCA to ABCG. With the exception of ABCE and ABCF subfamilies, all superfamily members share similarities in their core structure, which typically consists in two *nucleotide-binding domains* (NBD) and two *transmembrane domains* (TMD). Of note, some ATP binding cassette proteins function as monomers, while some others request homo- or hetero-dimerization to exert their activity. The NBDs bind and hydrolyze ATP to deliver the necessary energy for their substrate's active transport. The TMDs consists of twelve transmembrane helices and participates in the substrate recognition. According to the classical "alternating model", the pump activity results from the ATP-powered shift of the transporter from an inward-facing to an

outward-facing conformation, with this conformation change being associated with a reduction in substrate affinity.

Solute carrier (SLC) transporters constitute a superfamily of transporters that facilitate the passive movement of solutes across their electrochemical gradient and are mainly responsible for substrate cellular influx. This transport may either be purely passive, or be coupled to the electrochemical gradient of ions (such as Na^+) that is itself generated by ATP-dependent pumps. SLCs are divided in two families, the organic anion transporting polypeptides (OATPs) encoded by the *SLCO* family genes, and the organic anion transporters (OATs) and organic cation transporters (OCTs) encoded by the *SLC22A* family genes (317). SLCs are responsible for the transport of several endogenous compounds (such as neurotransmitters) as well as xenobiotics. They have variable tissue distributions but essentially all epithelial tissues express OCTs. With regards to drug pharmacokinetics, the most important tissues where they are expressed are the gastro-intestinal tissue, the biliary tract, the renal tubule and the blood-tissue barriers (274, 299).

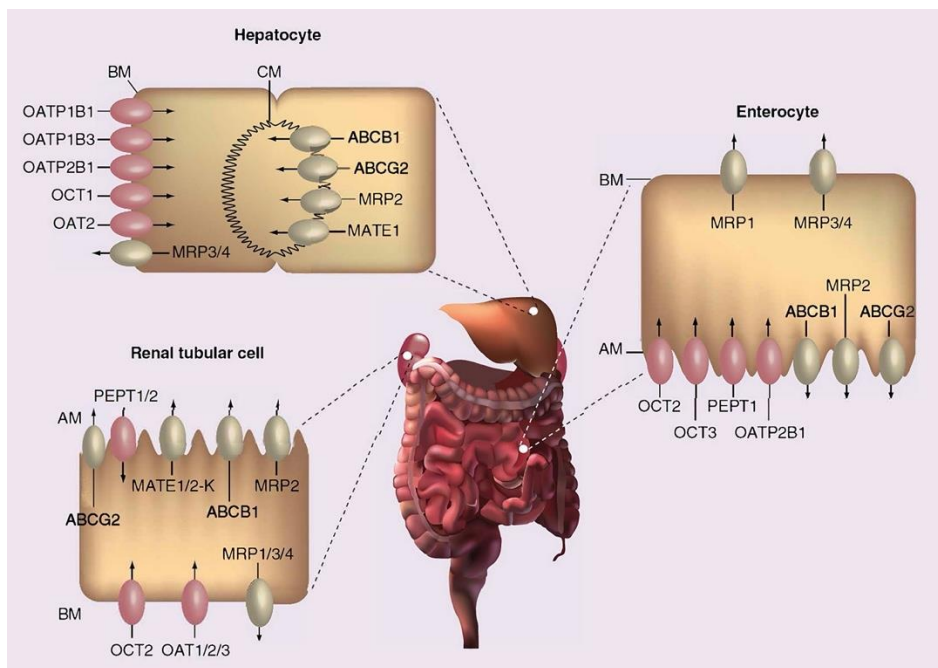


Figure 12. Localization of drug membrane transporters in the human intestine, liver and kidney.

Adapted from Zair et al. (299).

1.2.4.2 ABCB1

The existence of ABCB1 (also known as P-gp or Multidrug resistance protein 1, MDR1) was first suggested in 1970 by *Biedler et al.* who described the development of cell lines resistant to several chemotherapy agents, a characteristic that was linked to a qualitative difference in cell membrane resulting in decreased permeability (318). The *ABCB1* gene is located on chromosome 7q21.12 and consists of 28 exons. It encodes a 1280 amino acid protein that consists in two halves containing each 6 TMD and a NBD, separated by a flexible linker region (319).

ABCB1 has a broad substrate profile encompassing molecules with a large range of structural and biochemical characteristics. Numerous endogenous (peptides, steroid hormones, phospholipids, lipids, cytokines) and exogenous (xenobiotics such as drugs, dietary compounds) substrates have been described, with some of them modulating its expression (320).

Through its selective expression on the luminal side of enterocytes in the small intestine and colon, ABCB1 limits the absorption of several xenobiotics. It also exerts an excretory activity in the liver and kidneys through its expression at the canalicular pole of hepatocytes and the luminal membrane of renal tubular cells. Moreover, by being apically expressed in cells at the serosal side of blood-tissue barriers (e.g., blood-brain, blood-CSF, blood-placenta or blood-testis barriers), ABCB1 protects the so-called sanctuary organs from the accumulation of its substrates (321). Eventually, the transporter also plays an important role in the intracellular accumulation and pharmacokinetics of drugs (320). As such, ABCB1 deserves a particular attention in the field of HIV pharmacotherapy as it is expressed by PBMCs, including CD4 + T cells where the virus replicates (322, 323).

1.2.4.2.1 Variability in ABCB1 function

1.2.4.2.1.1 Non-genetic variability

Similarly to what was described earlier for phase 1 and 2 metabolism, ABCB1 function is subject to variability due to the interacting effects of drugs, herbal extracts and environmental factors. Although interactions are compound specific, the effects of different interacting agents on transporters and

biotransformation enzymes may overlap, especially for CYP3A4 and ABCB1. This can be explained by the key role of NR1I2 in *ABCB1* and *CYP3A4* gene upregulation (320, 324). RTV and COBI are both important ABCB1 inhibitors (305).

1.2.4.2.1.2 Genetic variability

Several variants have been described in *ABCB1* but investigations into their functional impact have typically centered on a select few (**Table 6**).

Despite its low frequency, the missense rs2229109 variant (c.1199G>A, p.Ser400Asn) has been subject of interest. The asparagine amino-acid substitution is located close to the ABCB1 substrate binding site. Several reports have highlighted a substrate-specific effect of this SNP, possibly related to a differential effect on ABCB1 affinity towards its substrates (325, 326). An increase of ATPase activity was also shown for 1199A as compared to 1199G (327).

The rs1128503 (c.1236C>T, p.Gly412Gly), rs2032582 (c.2677G>T, p.Ala893Ser), and rs1045642 (c.3435C>T, p.Ile1145Ile) SNPs are the three most frequent and unsurprisingly have been the most studied. Of note rs2032582 also refers to another less frequent mutation c.2677G>A (p.Ala893Thr).

- The synonymous c.1236C>T SNP has not been shown to affect protein expression, neither protein function, although a putative effect on protein folding has been speculated (319).

- The c.2677G>T SNP is non-synonymous and it has been suggested that the replacement of an alanine to a serine impacts the activity and substrate specificity of ABCB1, but not the protein expression (328).
- Despite c.3435C>T being a synonymous SNP, it has been shown to affect the structure and function of ABCB1. This SNP has been shown to alter mRNA levels, protein expression, protein folding and substrate specificity. Different underlying mechanisms have been suggested, including changes in the folding and function of ABCB1 due to ribosome stalling, as translation necessitates the use of a rarer codon (319, 329). Other authors have suggested that this SNP induces decreased mRNA stability and expression, probably related to an effect on mRNA secondary structure (330).

Those three SNPs are found in strong linkage disequilibrium, with the most frequent haplotypes in most ethnicities being the CGC and TTT combinations of variants at positions c.1236, c.2677 and c.3435, respectively (331). Functional studies have focused on the individual effects of those three individual SNPs or, more interestingly, of their haplotype combinations, and have yielded variable results (332, 333).

Table 6. Description of key ABCB1 polymorphisms.

Allele frequencies from 1000 Genome Project, phase III (293, 294).

Polymorphism	Protein change	Impact	Possible underlying mechanism	Minor Allele Frequency
c.1199G>A (rs2229109)	p.Ser400Asn (missense variant)	Substrate-dependent	Modified substrate affinity Reported increased ATPase activity for 1199A	Europeans 3% <1% in other ethnic groups
c.1236C>T (rs1128503)	p.Gly412Gly (synonymous variant)	No or limited	Unknown	African 14% East Asian 63% European 42% South Asian 59%
c.2677G>T (rs2032582)	p.Ala893Ser (missense variant)	Possible substrate dependent	Unknown	African 2% East Asian 40% European 41% South Asian 59%
c.3435C>T (rs1045642)	p.Ile1145Ile (synonymous variant)	Possible substrate dependent	Ribosome stalling Decreased mRNA stability and expression	African 15% East Asian 40% European 52% South Asian 57%

1.2.4.3 ABCG2

ABCG2 was first described in a lineage of breast cancer cells displaying resistance to chemotherapy, hence its other name Breast Cancer Resistance Protein (BCRP). The *ABCG2* gene is located on chromosome 4q22 and consists of 16 exons. Interestingly, the ABCG subfamily members share only limited amino acid sequence similarities with other ATP binding cassette

family members, with the exception of the NBD. *ABCG2* encodes for a 655 amino-acids protein, with a NBD at the N-terminus and 6 TMD. This protein is considered as a “half-transporter” as it needs to be dimerized to act as an active efflux transporter (334).

ABCG2 has a broad substrate profile, including endogenous and exogenous compounds such as uric acid, porphyrins, estrogen precursors or heterocyclic amines. Its expression profile shares some similarities with *ABCB1*, and *ABCG2* exerts an important role in drug PK: in the intestine, it limits drug absorption; at barrier sites such as the BBB or blood-placental barrier it protects sanctuary sites against drug penetration; and in the liver canalicular membranes and the kidney tubular epithelial cells it participates into drug elimination (334-336).

Several variants of *ABCG2* have been described but two of them have received the most attention (**Table 7**). The rs2231142 (c.421C>A) SNP has been the most studied, as it is both frequent and impacting. Its importance is outlined by its association with hyperuricemia and gout, with variant protein being associated with defects in *ABCG2* abundance and urate excretion especially in the intestinal tract (337). This SNP is located in exon 5, and results in a glutamine to lysine substitution at position 141 located in the NBD, probably inducing conformational changes at the TMD/NBD interface. The SNP affects *ABCG2* in two ways: (i) it impedes the transport function of *ABCG2*, probably by interfering with the ATPase activity of the protein (338); and (ii) it is associated with protein trafficking defects, with a

large proportion of ABCG2_{421A} variant proteins being degraded by proteasome or autophagy (334, 339). Rs2231142 is considered as one of the few “Highly Important Polymorphisms” by the *International Transporter Consortium* (340). It has been associated to increased systemic exposure for numerous ABCG2 substrates (332, 341, 342).

Rs2231137 (c.34G>A) is the other ABCG2 SNP that has been well-studied. It is located in exon 2 and results in a valine to methionine amino acid substitution at position 12 in the N-terminal part of the protein. Its impact on protein folding is not well understood. Some data have indicated that the SNP is associated with impaired ABCG2 membrane localization and decreased function, although other studies found no or limited effect (334, 338, 343). The SNP is generally considered to be of no or limited clinical relevance regarding drug PK (332).

Table 7. Description of key ABCG2 polymorphisms.

Allele frequencies from 1000 Genome Project, phase III (293, 294).

Polymorphism	Protein change	Impact	Possible underlying mechanism	Minor Allele Frequency
c.34G>A (rs2231137)	p.Val12Met (missense variant)	None or limited	Uncertain. Reported impaired membrane localization.	African 6% East Asian 33% European 6% South Asian 15%
c.421C>A (rs2231142)	p.Gln141Lys (missense variant)	Important	Defects in trafficking leading to reduced expression. Reduced transporter activity.	African 1% East Asian 29% European 9% South Asian 10%

1.2.5 Xenoreceptors

1.2.5.1 Introduction

Nuclear receptors (NR) constitute a large superfamily of receptors involved in transcriptional regulation of genes responsible for critical cellular functions. They play important roles in a large number of physiological functions such as development, reproduction, immunity, vascular function, xenobiotic elimination, or glucose and lipid metabolism.

NRs share a common general mode of action: the binding of ligands to a ligand-binding domain (LBD) enables the release of co-repressors and the recruitment of co-activators, and induces the transactivation of their target

gene. Some NRs are constitutively active while others require ligand binding. NRs share a common general structure, with a N-terminal ligand-independent activation function 1 (AF-1) domain, a highly conserved DNA binding domain (DBD) that targets specific DNA sequences called hormone response elements (HRE), a ligand binding domain (LBD) and finally a C-terminal second activation factor (AF-2) (344, 345).

Four classes of NRs have been described: class I consist mostly of endocrine receptors acting as homodimers, class II that act as heterodimers with the retinoid X receptor (RXR), and class III and IV that are minor groups. Another frequently used classification describes NRs as endocrine NR (such as thyroid hormone receptors, retinoic acid receptors...), orphan NR, that are reported as 'orphan' due to their unknown ligands, and 'adopted' orphan NR, for which initially unknown ligands were subsequently identified (274, 346).

In this manuscript, we will mainly focus on NR1I2 (PXR), a class II NR that acts as a critical xenobiotic receptor and is of particular interest with regards to INSTI transport and metabolism.

1.2.5.2 NR1I2

NR1I2 is best known for its role in organism detoxification. Acting as a molecule sensor, it can be activated by numerous exogenous or endogenous ligands and induce the transactivation of genes coding for proteins involved in drug transport and metabolism (347). The LBD of NR1I2 has the particularity of being able to bind a very large number of exogenous and

endogenous ligands, of various chemical structures and sizes, including steroid hormones, glucocorticoids, bile acids, drugs from several classes (including several ARV agents, antibiotics such as rifampin, statins, antitumor drugs, anticonvulsants...), environmental factors (such as bisphenol A) or synthetic chemical products (such as some pesticides) (348).

NR1I2 mode of action is close to that of NR1I3 (CAR) and can be summarized as such (**Figure 13**): the regulator is constitutively bound to co-repressors and inactive, and binding of ligands induces a conformational change that enables the release of those co-repressors and the binding of co-activators. The exact location (cytosolic or nuclear) of unactivated PXR is uncertain, but once activated, it can heterodimerize with RXR in the nucleus. NR1I2/RXR complexes will then transactivate their target gene through the DBD-HRE interaction (324, 344).

The most important targets of NR1I2 regarding drug metabolism and transport include *CYP3A4* (349), *UGT1A1* (350) and *ABCB1* (351), as summarized in **Table 8**. Some data also suggest that NR1I2 regulates the expression of *ABCG2* (352, 353).

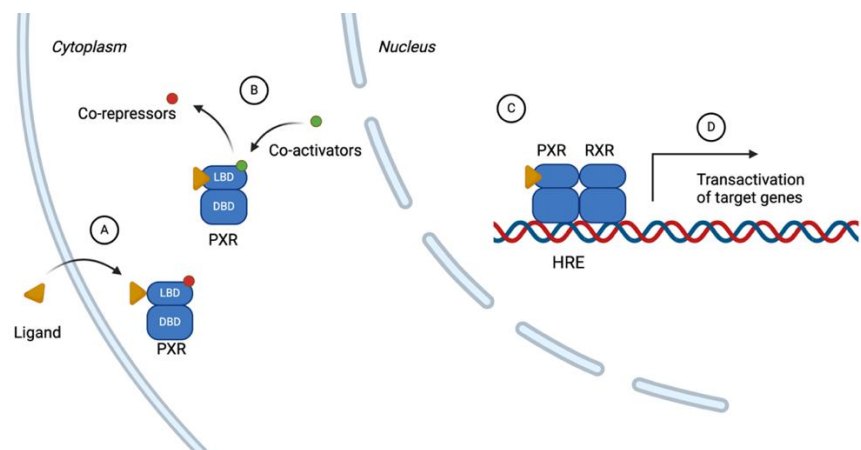


Figure 13. Schematic illustration of PXR mode of action.

(A) Ligand recognition. (B) PXR activation after the release of co-repressors and recruitment of co-activators. (C) Heterodimerization with RXR and fixation on the promoters of target genes. (D) Target gene transactivation. Adapted from Mbatchi et al. (324). Created with Biorender®.

Table 8. NR1I2 most important target genes in humans regarding drug metabolism and transport.

Based on references (347, 353-356).

Phase 1	Phase 2	Transporters
CYP2A6	UGT1A1	ABCB1
CYP2B6	UGT1A3	ABCC2
CYP2C8	UGT1A6	ABCG2 (?)
CYP2C9		
CYP2C19		
CYP3A4		
CYP3A5		
CYP3A7		
CYP7A1		

Importantly, as previously mentioned, several non-drug related functions have been described for NR1I2 and its close parent CAR (also called NR1I3), including important roles in inflammation regulation, apoptosis, energy metabolism, and more recently the brain function (357, 358). Despite its overall lower expression in brain as compared to the liver, NR1I2 was shown to be expressed in several brain regions including cerebral cortex, cerebellum or cortical capillaries. However, the role and importance of NR1I2 in the CNS remain poorly understood so far, and the expression and the response to ligand-based activation both appear highly tissue- and cell-dependent (358). At the BBB, upregulation of ABCB1 and ABCG2 expression by NR1I2 has been shown, thus possibly enhancing the BBB protective effect (353, 359, 360).

The gene that encodes NR1I2 is located in the chromosome 3q12-13.3. It contains 10 exons including two alternative exons (1A and 1B). The most frequent isoform is transcribed from exon 1A, and contains 434 amino acids (361). Numerous polymorphisms of *NR1I2* have been reported, with the possibility for some of them to modify interactions with ligands, promoters of target genes, or cofactors. Moreover, if located in non-coding region, polymorphisms could impact on *NR1I2* transcription or translation. Overall, their possible impact on NR1I2 function and subsequently on drug metabolism and transport is complex to elucidate and remains largely unclear (324).

One particular SNP that is of interest in this work is the c.-22-7659C>T (rs2472677, also referred to as 63396C>T). Located in intron 1b, this SNP (in

linkage disequilibrium with three other SNPs and a polymorphic repeat in intron 1) was originally described in 2008, with demonstration that persons homozygous for 63396C had a 3-fold lower basal CYP3A4 activity compared to subjects heterozygous or homozygous for the 63396T. However, when exposed to rifampin those 63396C homozygous showed a higher CYP3A4 fold-induction than 63396T homozygous subjects. Moreover, 63396C homozygous subjects had lower levels of *PAR2* mRNA, an alternatively spliced isoform of *NR1I2*. This SNP and the other variants found in linkage disequilibrium have been predicted to alter binding sites for the hepatic nuclear factor 3 β transcription factor (324, 362). MAF of the SNP is 39% in Africans, 59% in European and ~60% in Asians (363).

1.2.6 Principles of pharmacogenetic research

Translational research methods have proven effective in understanding how genetic variants impact on drug response (364). *In vitro* assessments as well as clinical PGx studies are often carried during the drug development process, but those approaches are frequently complemented by post-marketing studies because (i) RCTs may suffer from selection bias and frequently do not include participants from diverse ethnic and genetic backgrounds (365), and (ii), as described for INSTIs, post-marketing studies may identify safety issues that were not captured in RCTs.

1.2.6.1 *In vitro* experimental approaches

Several approaches have been used to assess experimentally the impact of a genetic variant on the function of a transporter or biotransformation enzyme. Those approaches can be used early and may guide the drug development process, or they can be used as confirmation studies for clinical findings, especially as *in vitro* studies may offer the opportunity to control environmental and other confounding factors - including unstudied genetic covariates (364, 366, 367).

Proving *in vitro* the functional impact of a genetic variant on the transport or biotransformation of a specific drug can be seen as a crucial step to accept it as a determinant of a drug PK. However, this is often insufficient, as several findings of variants demonstrating *in vitro* impacts do not translate in clinically relevant impacts, especially for drug transporters. One important reason for this inconsistency is the existence of a high degree of overlap in transporters' substrate specificity, with the possibility for one transporter to take over the activity of another deficient transporter. The high level of regulation of transporters and biotransformation enzymes also offers an explanation to discrepancies between *in vitro* models and real-life evidence (299, 332).

1.2.6.2 Clinical studies

1.2.6.2.1 Study objectives

PGx studies aim at demonstrating the association between a genetic variant (genotype) and an outcome that consists in a specific drug-response phenotype. Two types of phenotypes can be defined (270, 368):

- *Endophenotypes* are sensitive phenotypes, easier to assess than clinical phenotypes. They can be classified as PK endophenotypes, such as a measured drug concentration, or PD endophenotypes, such as the evolution of a biomarker under treatment (e.g., INR for warfarin). Endophenotypes may not always be clinically relevant.
- *Clinical phenotypes* are by definition clinically relevant, but usually more complicated to assess, especially if they are not related to a single or a few genes with large effect size (e.g., phenotypes that are not directly PK-dependent) or if they have multifactorial origins.

1.2.6.2.2 Targeted and untargeted approaches

Research methodologies to investigate the association between genetic variants and a drug response phenotype can be classified as targeted (*candidate gene or SNPs studies*) or untargeted (*genome wide associated studies, GWAS*) approaches. Both approaches are valid and should be seen as complementary, with findings of untargeted approaches usually being independently validated by targeted approaches (366, 368).

The candidate gene/SNPs studies focus on genes involved in biological mechanisms related or expected to be related to the drug target, its

metabolism, or the studied outcome. Those studies frequently focus on carefully selected SNPs in transport proteins or biotransformation enzymes. Candidate SNPs studies have classically used SNP assays to test a single SNP or a combination of selected variants known to affect the activity of transporters or enzymes. However, with the advent of pharmacogenetic panel tests, larger studies may also be carried that assess a larger number of variants in one or several pharmacogenes (369).

GWAS approaches differ from candidate gene/SNP approaches in that studied genes are not selected a priori, with a very large number of variants assessed simultaneously. GWASs offer the possibility to identify unexpected associations, in some cases enhancing the comprehension of a drug effect. Their main inconvenient is that large cohorts are required, because testing multiple variants requires statistical correction for multiple testing. Progresses in next-generation sequencing (NGS) and panel tests have also enabled the advent of more hybrid studies where candidate genes (typically coding for transporters and biotransformation enzymes) are sequenced. Whole exome or whole genome approaches are other approaches that remain limited by cost and complexity (368, 370).

Considering that drug response is generally a complex and multifactorial phenomenon involving the host genomic factors but also other host's characteristics and environmental factors, some authors have proposed to move beyond genetic studies and to assess the drug-host-disease interactions using network medicine approaches. Those approaches typically

study large amounts of phenotypes, genotypes, transcriptomic, proteomic, and/or metabolomic data to generate network models predictive of drug response (371).

1.2.6.2.3 Study designs

PGx clinical studies may be carried during clinical development or post-marketing (364). PGx may be studied in phase I studies, or panel phase I studies in which participants with genotypes of interests are preselected. Those studies are however limited by their size, and they can generally only assess endophenotypes.

Phase II and phase III studies can also encompass PGx analysis. Those studies include more participants and allow for broader analysis as compared to phase I studies. Their prospective nature makes them less prone to bias. Moreover, in the case of RCTs, the inclusion of a control arm can theoretically allow to identify genotypes predicting a specific drug effect to be differentiated from a genotype that would be a drug independent prognostic factor (e.g., a genotype specifically predicting the occurrence of DTG-induced NPAE should theoretically prove no association with NPAE in participants receiving a placebo, as compared to identifying a genotype associated with NPAE in all the participants). Interesting examples of nested pharmacogenetic analysis in the context of INSTIs include the aforementioned demonstration of the impact of EFV-slow metabolism on weight gain after switch to DTG, as well as the demonstration that *UGT1A1* and *SLC22A2* polymorphisms did not affect sleep quality nor NPAEs in an African cohort, both within the context of the ADVANCE trial (229, 259).

Several types of post-marketing (phase IV) pharmacogenetic studies exist, from the low-grade evidence case reports or case series to more advanced designs such as cross-sectional or longitudinal studies. One of the most powerful post-marketing study designs consists in case-control studies. The basic principle of those studies is to compare the genotypes of treated persons presenting a specific outcome (defined as *cases*) to treated persons who did not present that outcome (**Figure 14**). Although those studies may be prone to some bias, they are particularly relevant for studying rare outcomes. Of note, pharmacogenetic case-control studies (or case-cohort studies if cases are compared to the cohort) may also be nested phase III RCTs (372).

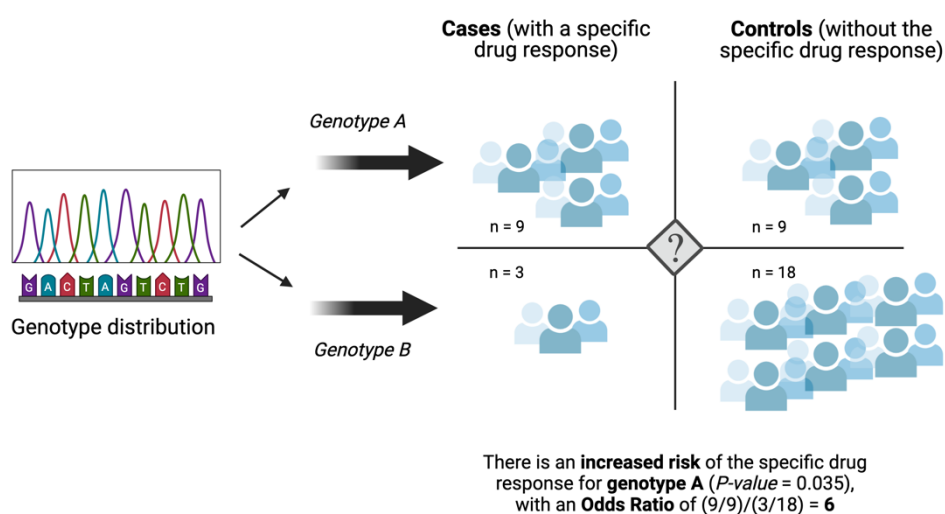


Figure 14. Schematic view of the principle of pharmacogenetic case-control study.

In the presented example, the frequency of genotypes A and B are compared among treated persons presenting or not presenting a specific drug response. Created with Biorender®.

1.2.6.2.4 Validity and utility

Overall, any finding of an association between a genetic variant and a clinical outcome will need to be replicated in independent cohorts to prove its “clinical validity”. While advancing our understanding of pathophysiological processes is often a highly valuable outcome of pharmacogenetic studies, their greatest and often elusive impact lies in identifying clinically actionable genetic tests that directly influence drug response. For this, the “clinical utility” of an association should ideally also be demonstrated by prospective clinical trials evaluating the impact of the test implementation on the drug response, although this might not always be feasible nor necessary (e.g., for rare but severe outcomes and if supporting evidence is strong) (364, 368).

1.2.7 Pharmacogenetics of HIV therapy

This chapter will review some critical findings regarding the impact of pharmacogenetics on ARV therapies. Structured as a narrative review, it selectively examines on one or two antiretroviral agents per class, emphasizing significant findings with regards to contemporary practices in ARV therapy, with the exception of second-generation INSTIs for which a state-of-the-art review is reported.

1.2.7.1 NRTI agents: the cases of ABC and TFV

Although this agent is less used nowadays, the case of *HLA-B*57:01* screening test before the prescription of ABC remains particularly relevant because it represents one of the most successful implementations of a targeted pharmacogenetic testing in clinical practice. The use of ABC was initially associated with hypersensitivity reactions (HSRs) in approximately 5-8% of users during the first weeks of treatment, ranging from mild symptoms to life-threatening reactions especially in case of prolonged therapy or drug rechallenge. The association between ABC-induced HSR and carriage of the *HLA-B*57:01* (an allele carried at 5-7% rates in European populations but much lower in other ethnic groups such as Africans) was initially demonstrated independently in a cohort study and a case-control study (373, 374). After confirmation of the association in several independent cohorts, the use of the test became widely recommended after a large double-blind RCT demonstrated that screening eliminated immunologically confirmed HSRs with a negative predictive value of 100% and a positive predictive value of almost 50% (375). Among key factors that can explain the success of this test implementation are the limited number needed to test to prevent one reaction (estimated at 13), the severity of the prevented toxic effect, and the easy interpretation of the result (either positive or negative) (270, 365).

Several studies have attempted to identify pharmacogenetic predictors of TFV kidney toxicity, one of the rare but important toxicity of current cART. Indeed, this drug is actively transported into proximal renal tubular cells by

OCT1 and OCT2 and transported out of these cells by MRP2 and MRP4, and it has been suggested that tubular toxicity could be associated with intracellular TFV concentration. However, despite initial promising findings (376), later studies have found conflicting results (377). In a recent substudy from the ADVANCE trial, investigators carried a candidate SNP approach complemented by a candidate gene approach that investigated all available polymorphisms in 14 genes relevant to TFV, TDF and TAF disposition, as well as a GWAS approach to assess genetic associations with changes in glomerular filtration rate (GFR) and tubular dysfunction markers. In the candidate SNP approach, the authors found two polymorphisms in *ABCC4* (*MRP4*) (rs1059751 and rs899494) to be associated with TDF toxicity, but this finding did not withstand correction for multiple testing. The GWAS approach also yielded an association between a polymorphism in the gene encoding collagen type XXVII alpha 1 chain and GFR, but mechanistic explanations were not found (378).

1.2.7.2 NNRTI agents: the case of EFV

Pharmacogenetics of EFV have been extensively studied. Although the use of the drug has dramatically decreased nowadays, its study remains of interest as it has been recommended as first-line regimen in many countries before the advent of DTG. Moreover, switching off EFV has been associated with weight gain in a pharmacogenetically determined fashion, as previously mentioned.

Although generally safe, EFV is associated with significant toxicities, especially neurotoxicity. The occurrence of toxicity has been associated with increased plasma drug concentrations, and therapeutic margin of 1-4 µg/mL has been proposed (379). EFV metabolism primarily involves CYP2B6 in the liver. Several studies using candidate gene approaches have identified *CYP2B6* polymorphisms associated with the drug PK. In particular, the 516G>T (rs3745274, defining the decreased function allele *CYP2B6*6*) and the 983T>C (rs28399499, defining the no-function allele *CYP2B6*18*) SNPs, both more frequent in persons from African descent, predicted increased drug exposure. Those SNP as well as 15582C>T were further found associated with EFV C_{min} using a GWAS approach, and were shown to explain approximately one third of C_{min} variance (380). Subsequent studies have demonstrated associations of *CYP2B6* genotypes with adverse reactions (381, 382). The CPIC has emitted EFV dosing recommendations based on *CYP2B6* diplotype-phenotype associations (383).

1.2.7.3 PI agents: the case of DRV

The importance of UGT1A1 metabolizing status on metabolism of ATV has already been discussed in this manuscript. The impact of PGx polymorphisms on DRV PK has been extensively studied in the doctoral work of Prof. L. Belkhir (384). As already mentioned, a genotype-based interaction between DRV and ETR was found, mediated by CYP3A5 expressing status (290). In the complete cohort (including participants not on ETR), *CYP3A5*1* genotype had no effect on DRV plasma PK. Other studied polymorphisms were *CYP3A4*22* (for plasma PK), the c.1199G>A, c.1236C>T, c.2677G>T/A and

c.3435C>T SNPs in *ABCB1*, and the rs8027174 and rs4294800 SNP in *SLCO3A1* (for plasma PK and intracellular accumulation), and no effect was found on either parameter. One possible explanation raised was that, if existing, an impact of the studied polymorphisms might have been blurred by the CYP3A and transporters inhibiting effect exerted by the co-administered RTV.

1.2.7.4 First-generation INSTIs

RAL constitutes an interesting case for PGx studies. Indeed, the drug is characterized by a high intra-individual variability associated to a high inter-individual variability (385). The drug is only metabolized by UGT1A1, and several studies have evaluated the impact of UGT1A1 metabolizing status on RAL PK. In the second part of her doctoral work, Prof. L. Belkhir showed higher RAL exposure in *UGT1A1**28 carriers compared to *UGT1A1**1/*1. Overall, those results were confirmed by some studies but could not be replicated by others (386, 387). Interestingly, one of the studies reporting negative results nevertheless reported a significantly lower incidence of VF in PLWH homozygous for *UGT1A1**28 (although significance was lost after adjustment for both baseline CD4 cell count and VL). Authors argue that the high intraindividual variability in the PK of RAL could have masked the impact of PGx polymorphisms (387). Regarding drug transporters, an association between *ABCG2* c.421C>A and *ABCB1* c.*193A>G (rs3842) variants and higher RAL C_{max} has been described (388). No impact of the c.1236C>T, c.2677G>T/A and c.3435C>T SNPs in *ABCB1* and of the *SLC22A6* c.453G>A

(rs4149170) and *SLC22A6* c.728C>T (rs11568626) were demonstrated on RAL PK (387, 388).

1.2.7.5 Second-generation INSTIs

1.2.7.5.1 DTG

Pharmacogenetic determinants of DTG plasma exposure have previously been studied and an overview of published evidence can be found in **Table 9**. Briefly, regarding *UGT1A1*, the metabolizing status linked to the decreased function variants *28, *37 (rs3064744) and *6 (rs4148323) has been associated with increases in systemic drug exposure (222, 389-392). The c.-22-7659C>T SNP in *NR1I2* (rs2472677) and the c.421C>A SNP in *ABCG2* (rs2231142) have both been significantly associated with increases in some pharmacokinetics parameters (390, 393) but those results were not replicated in other studies (391, 392). Moreover, an experimental study found no difference in systemic and tissue DTG exposure between wild-type and *ABCG2*-deficient mice (394).

Some PD studies have also investigated the impact of genetic polymorphisms on clinical response to DTG (395). First, regarding NPAE, one Japanese study found *UGT1A1* poor metabolizing status and increased DTG exposure to be associated with the occurrence of NPAEs (222), but this finding could not be replicated in a larger study carried in an African population (229). Another study has found an association of some neuropsychiatric symptoms (3 out of the 11 tested components evaluated through pharmacometric scoring, as well as moderate to severe headache) with the c.808C>A SNP in *SLC22A2*. As introduced in a previous section of this manuscript, the authors postulated

that both could possibly synergistically affect neurocognitive function, as SLC22A2 is involved in the transport of neurotransmitters and is inhibited by DTG (231). However, the same group found in another study of PLWH that, while CA/AA genotype was associated with pre-existing psychiatric comorbidities, it was not associated to DTG discontinuation due to NPAE. Studying the interaction of both factors, they found that the highest risk of DTG discontinuation due to NPAE was for PLWH with pre-existing psychiatric comorbidities and the CC genotype (211).

Secondly, regarding weight gain and DTG, besides the aforementioned influence of CYP2B6 metabolizing status, another substudy of the ADVANCE trial investigated the association between weight gain and polymorphisms in several DTG-, TDF- and TAF-related pharmacogenes, including UGT1A1 and ABCG2 but not NR1H2. No association was found with any of the polymorphisms and the extent of weight gain. When further applying a genome-wide approach, the authors found a significant association between weight gain and the rs7590091 polymorphism in *TMEM163*, a gene encoding a transmembrane zinc-binding protein that has been involved in insulin secretion and glucose homeostasis (396).

1.2.7.5.2 BIC

Intriguingly, besides our manuscript presented in the result section (397), no single *in vitro*, *in vivo* or clinical study assessing the impact of PGx polymorphisms on BIC disposition and drug response could be found at the time of writing this manuscript.

1.2.7.5.3 CAB

Little is known on the impact of pharmacogenetic polymorphisms on CAB disposition and drug response. An analysis of participants from phase 1 and 2 studies assessed the impact of selected polymorphisms in *UGT1A1* and *UGT1A9* on CAB PK and found a small but probably non-clinically relevant association between carriage of *UGT1A1**6, *28 and *37 and increased CAB exposure (398).

In a recently presented abstract, *Cusato et al.* report the findings of their study assessing the impact of polymorphisms in genes encoding enzymes and transporters involved in CAB and RPV metabolism and elimination (*CYP2C19*, *CYP3A4*, *CYP3A5*, *UGT1A1*, *ABCB1*, *ABCG2*) and plasma and intra-PBMC concentrations for those drugs. They found *CYP2C19**2/*2 and *ABCG2* c.421C>A AA genotype to be predictors of the CAB below the cut-off value of 664 ng/mL, and *CYP3A4**22 as the only predictor of RPV concentration below 50 ng/mL (399).

Table 9. Summary of the available literature regarding the effect of PGx variants on DTG PK.

	PK variable				Remarks	Ref.
	C _{max}	C _{trough}	AUC	CL/F		
UGT1A1						
UGT1A1 PM vs NM/IM	NS	+44%	+36%		Geometric mean differences	(390)
	NS	NS	NS	NS	Children (limited sample, low number PM)	(391)
UGT1A1 PM/IM vs NM	+22%		+31%	-23%	Geometric mean differences	(389)
UGT1A1 PM vs NM	+32%		+46%	-32%	Geometric mean differences	(389)
		↑			For *6/*6 (Asian population, NS for *28/*28 but limited number)	(222)
	NS	NS	NS	NS	Children, increased concentrations for PM but NS (limited sample, low number PM)	(391)
UGT1A1 IM vs NM		↑			For heterozygous *6	(222)
		↑			For heterozygous *28	(222)
	↓	↓	↓	NS	Children, limited sample	(391)
UGT1A1*80			↑		Candidate SNP used as proxy for *28 or *37, confirmed by GWAS	(392)
CYP3A4						
CYP3A4*22	NS	NS	NS		Effect studied per allele	(390)
CYP3A4*1B	NS		NS	NS	Effect studied per allele	(400)
CYP3A5						
CYP3A5*3	NS	NS	NS		Effect studied per allele	(390)
			NS		GWAS	(392)
	NS		NS	NS	Effect studied per allele	(400)
CYP3A5*6	NS		NS	NS	Effect studied per allele	(400)
CYP3A5*7	NS		NS	NS	Effect studied per allele	(400)
ABCG2						
c.421C>A	↑				Stat: AA vs CA vs CC. C _{max-AA} =1.95*C _{max-CC}	(393)
	↑	NS	NS		Effect studied per allele. C _{max-AA} =1.28*C _{max-CC}	(390)

			NS		GWAS approach	(392)
	↓	NS	NS	NS	Stat: AA/CA vs CC, children	(391)
c.34C>T	NS	NS	NS		Effect studied per allele	(390)
			NS		GWAS	(392)
ABCB1						
c.1236 C>T	NS				Stat: CC vs VT vs TT	(393)
			NS		Candidate SNP	(392)
c.2677 G>T/A	NS				Stat: GG vs GT vs TT vs TA vs GA	(393)
c.3435C>T	NS				Stat: CC vs CT vs TT	(393)
			NS		Candidate SNP	(392)
c.4036 A>G	NS				Stat: AA vs AG vs GG	(393)
			NS		Candidate SNP	(392)
NR1I2						
c.-22-7659C>T	↑	NS	↑		Effect studied per allele. $C_{\max-TT}=1.24*C_{\max-CC}$; $AUC_{TT}=1.27*AUC_{CC}$	(390)
			NS		Candidate SNP	(392)
	NS	NS	NS	NS	Stat: TT/CT vs CC, children	(391)
c.-25385C>T	NS		NS	NS	Effect studied per allele	(400)
c.7635A>G	NS		NS	NS	Effect studied per allele	(400)
c.44477A>G		NS			Candidate SNP	(392)
	NS	NS	NS		Effect studied per allele	(390)

Percentages are reported in the table only when adequate statistical analysis was performed for group comparisons and values are reported in the source. Abbreviations: NS: not statistically different; vs: versus.

1.2.8 Pharmacogenetics in clinical practice

Implementing PGx in clinical practice holds the promise to ensure maximal clinical benefit of a drug while simultaneously reducing risks but also waste (401).

Three types of techniques are available for PGx profiling in the clinical setting: polymerase chain reaction (PCR), sequencing and microarrays (402). PCRs are adapted for focused genotyping, as the technique is based on the use of specific primers. Next-generation sequencing (NGS) techniques are used much more frequently nowadays than Sanger sequencing, due to increased throughput and its capacity to assess structural variants. NGS can be defined based on the length of reads as short read or long read sequencing. While the latter enables improved PGx characterization, short read is used in clinical settings due to its lower cost and greater practicality. Microarrays are an alternative technique that enable to detect simultaneously a large number of SNVs, based on hybridization with specific DNA probes fixed on dedicated arrays.

PGx profiling can be targeted to a single or a limited number of polymorphisms in a single gene, generally using PCR techniques. A typical and classical example is the genotyping for *HLA-B*57:01* before the prescription of ABC, or genotyping for the most frequent variants associated with thiopurine-S-methyltransferase deficiency before the prescription of azathioprine (274). However, the PGx field is gradually moving from single gene characterisation to the simultaneous profiling of an individual for a large number of genes, a technique called *panel testing*. NGS and microarray techniques are typically well-suited for panel testing.

While panel tests are attractive and promising, several questions remain to be answered regarding the number and list of genes and variants to be

included and/or reported by laboratories, their exact indications and clinical utility (403), and challenges regarding practical implementation of PGx panel testing. Regarding coverage, some defined panels have been proposed for pre-emptive PGx testing, such as the PGx-passport proposed by the DPWG encompassing 58 variant alleles, located within 14 genes (*CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *F5*, *HLA-A*, *HLA-B*, *NUDT15*, *SLCO1B1*, *TPMT*, *UGT1A1*, and *VKORC1*), that are considered as “actionable variants” based on described genotype-phenotype associations for several drugs (404).

The PREPARE study is a recent open label, controlled, cluster-randomized trial that has evaluated the clinical utility of pre-emptive 12-gene pharmacogenetic testing to prevent ADRs (405). In this study, investigators included patients starting an index drug with an actionable drug-gene interaction and published recommendations by the DPWG. Patients either received PGx testing results within 7 days or participated in the control group. Clinically relevant adverse reactions were reduced by 30% in the intervention group, providing evidence that pre-emptive PGx testing is feasible and clinically useful.

In the context of HIV, *HLA-B*57:01* genotyping has been recommended since several years including by the EACS (63), and is a standard practice. On the contrary, the existence of recommendations for EFV and ATV has not translated in standard genotyping for those drug-gene pairs. A recent observational study has studied the usefulness of performing a

comprehensive 27 genes panel testing for PLWH regularly followed in a large HIV-clinic. PGx tests were integrated in the electronic medical records – a process that may constitute one of the challenges of integrating PGx testing in routine practice. Clinically relevant findings were found in all 96 participants, with major findings (i.e., associated with a CPIC guideline recommendation) found in 84% of participants. Findings mostly involved *CYP450*, with at least one major finding in 75% of participants. Based on the PGx test, 65 participants were provided with clinical recommendations regarding their ongoing medications, although most recommendations were considered as of mild/moderate significance. Regarding ongoing cART, 35 participants received recommendations, mostly regarding DTG or BIC. However, the level of evidence regarding the latter recommendations appears weak (e.g., monitoring closely for increased risk of DTG-related adverse event in participants with UGT1A1 IM or PM status, although patients were found asymptomatic). The authors also report 5 cases of DTG intolerance that “*could be attributed to*” *UGT1A1* variants. This affirmation, despite the absence of a control group or sufficient underlying evidence, serves as a clear example and reminder of the risk of *confirmation bias* in uncontrolled pharmacogenetic studies (406).

1.2.9 Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is defined as the clinical practice of measuring specific drugs concentrations in patient’s blood at designated intervals. As efficacy and toxicity are generally influenced (at least to some

extent) by drug levels, it aims at optimizing individual dosage regimens (407). It is particularly useful for drugs with a narrow therapeutic index (i.e., drugs for which small differences blood concentration may lead to significant impacts in terms of risk of failures or ADR), or for drugs with known large interindividual PK variability, provided variations within the measured concentration range impact on the drug response. TDM can also be a valuable tool to guide clinicians in case of complex DDIs. Eventually, it has also been proposed as a tool to monitor the drug compliance (408).

Regarding HIV and the efficacy of cART, although CD4 count and VL can effectively be used as markers for treatment success, the use of TDM could theoretically improve HIV care if it effectively predicts the risk of VF. The use of TDM to guide the treatment of HIV has thus been the subject of much attention, especially in the early times of ARV therapy, when treatments were mostly characterized by unfavorable pharmacokinetic profiles. The usefulness of TDM has been evaluated in several studies. While some studies demonstrated improved outcomes with TDM (e.g. an increased proportion of virological success in participants receiving IDV/AZT/3TC with TDM-adjusted drug doses versus those receiving conventional doses (409)), conflicting results were found across studies and drug regimens (410).

Overall, the use of TDM to guide ARV therapy has generally decreased over the years as new ARV agents were developed, characterized by improved efficacy and reduced toxicity. Of note, the EACS guidelines still recommend to consider the use of TDM for ARVs in case of VF and in some specific

situation such as hemodialysis, hepatic dysfunction, obesity, or for the management of complex interactions such as the use of rifampicin for the treatment of tuberculosis (63). Interestingly, TDM has recently regained interest in the context of LA-CAB/RPV treatment VF, with efforts put in the finding of optimal target drug plasma concentrations and the development consensus guidance to interpret concentrations (171).

2 Aims of the thesis

Second-generation INSTIs have become a cornerstone of HIV treatment and are recommended worldwide as key components of first-line ARV regimens

Although those agents are generally well-tolerated and highly effective, patient response variability remains a concern for DTG, BIC and CAB, especially as those agents are used by millions of PLWH worldwide, who come from diverse ancestries with differing genetic backgrounds. Importantly, this variability remains poorly understood and largely unpredictable.

Considering those knowledge gaps, the main objectives of this thesis were:

- (1) To gain knowledge on the impact of pharmacogenetic polymorphisms on second-generation INSTIs PK.
- (2) To gain knowledge on the PK and pharmacogenetic determinants of inter-individual variability in the clinical and biological response to second-generation INSTIs.

These objectives were addressed through performing *in vitro* intracellular drug accumulation experiments (focusing on BIC) and setting up a prospective clinical study (focusing on DTG and BIC). Those complementary approaches constitute the core of this thesis. Moreover, after the identification of several cases of LA-CAB/RPV failures at our HIV reference

center, we decided to launch a retrospective study to gain knowledge on possible underlying PK/PGx determinants.

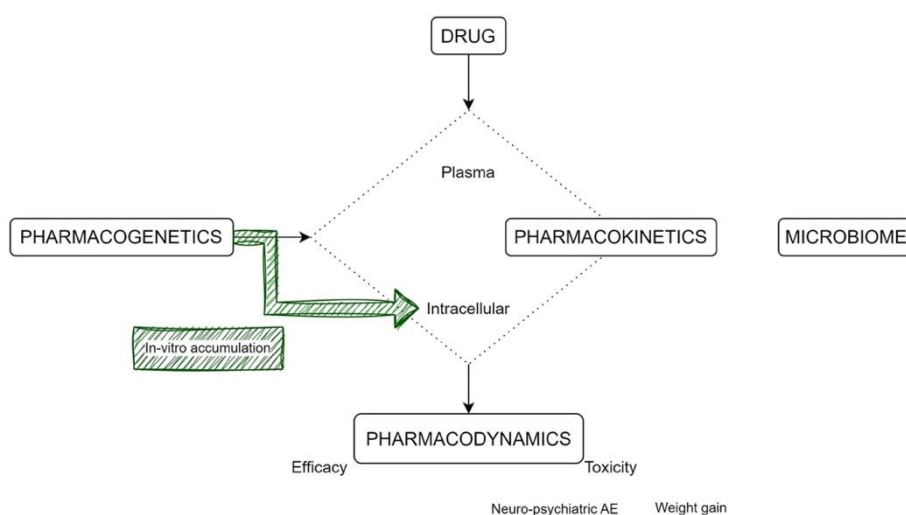
Eventually, taking advantage of the prospective clinical study launched, a secondary objective of this work was to gain knowledge on the interactions between DTG or BIC and the gut microbiome - although this objective does not directly align with the PK/PGx approach of this thesis.

3 Results

3.1 *In vitro* experiments

3.1.1 Introduction

In this work, we used a previously validated HEK293 (Human Embryonic Kidney) recombinant cell line model to investigate the effect of the four most common coding ABCB1 single nucleotide polymorphisms (i.e., c.1199G>A, c.1236C>T, c.2677G>T and c.3435C>T) on the intracellular accumulation of BIC.



Key Points

- Reduced BIC intracellular concentrations were found in cell lines overexpressing ABCB1 as compared to control cell lines.
- No significant difference was found in BIC intracellular accumulation when comparing HEK293 cells overexpressing the wild type (1236C-2677G-3435C, 1199G) or the variant (1236C-2677G-3435T, 1236T-2677T-3435T or 1199A) ABCB1 proteins.

3.1.2 Methods

The principle of these in vitro studies, as further detailed in the article section, is to compare the intracellular accumulation of BIC under standardized conditions in different cell lines overexpressing wild-type or variant ABCB1 proteins. Details on the carried experiments are reported in the article section. In this paragraph, we will briefly introduce how the recombinant cell lines used in our experiments were previously generated (326, 411).

Recombinant cell lines were generated from HEK293 cells obtained from American Type Culture Collection (ATCC). In a first step, plasmid vectors encoding mutant *ABCB1* were generated on the basis of the template pcDNA3.1 expression vector containing wild-type *ABCB1* (originally donated from Dr Rodney Ho, University of Washington) through site-directed mutagenesis (**Figure 15**): forward and reverse primers encoding the mutation of interest are designed and then used with the template wild-type DNA using the *QuickChange II XL Site-directed mutagenesis* kit (Agilent Technologies). Thermal cycling is performed to synthesize and amplify mutant plasmids. Using an endonuclease specific for methylated and hemimethylated DNA, mutant plasmids will be selected while parental DNA plasmids will be digested.

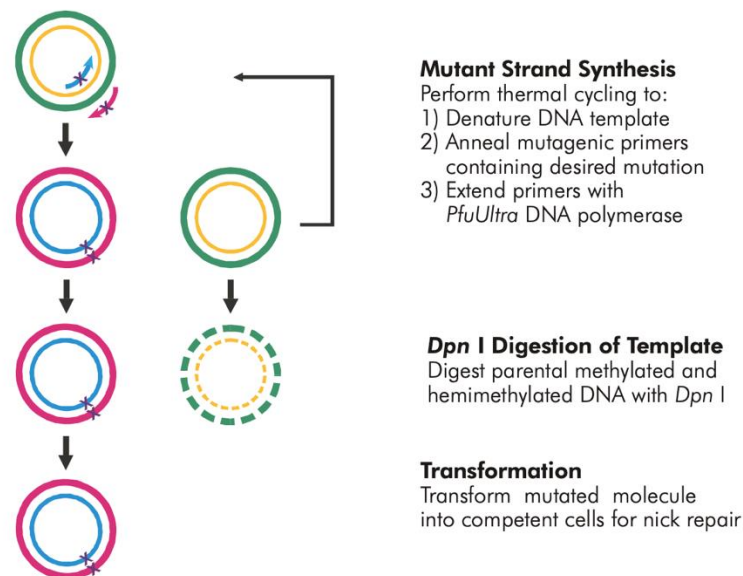


Figure 15. Overview of the site-directed mutagenesis process.
From (412).

The next step consists in the amplification of the plasmid. This is performed through genetic transformation that incorporates the plasmid in XL Gold *E. coli* cells. Transformed cells have incorporated an ampicillin resistance gene and are selectively cultured in presence of this bacterial selection antibiotic. Those cells are eventually lysed to extract DNA and the plasmid is isolated and purified using a specific anion-exchange resin. DNA sequencing is performed to confirm that the plasmid encodes the expected sequence and no additional mutation has occurred during the process.

In the last step, stable recombinant HEK293 cells are generated by lipofectamine transfection: the previously synthesized plasmids or an empty

vector plasmid not encoding *ABCB1* (serving as control) are incorporated in DNA-lipid complexes that will enable their cytoplasmic release (*lipofection*). Plasmids will migrate in the nucleus where genomic integration (required for stable expression) may occur, albeit at limited rate. Cells are cultured and those having integrated the plasmid are selected by exposition to the G418 antibiotic whose resistance is encoded in the plasmid. After culture, the expression of ABCB1 by HEK293 cells is checked by flow cytometry and cell sorting is performed as described in the article section.

3.1.3 Effect of ABCB1 Most Frequent Polymorphisms on the Accumulation of Bictegravir in Recombinant HEK293 Cell Lines

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OPEN Effect of ABCB1 most frequent polymorphisms on the accumulation of bictegravir in recombinant HEK293 cell lines

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Bictegravir, a key second-generation integrase strand transfer inhibitor in the treatment of HIV, is subject to active efflux transport mediated by ABCB1 (P-glycoprotein). Several coding variants of *ABCB1* have been described and associated with variable effects on substrate drugs pharmacokinetics. Here, we investigated the effect of the four most common coding *ABCB1* single nucleotide polymorphisms (i.e., c.1199G>A, c.1236C>T, c.2677G>T and c.3435C>T) on the intracellular accumulation of bictegravir. Using a previously validated HEK293 recombinant cell line model, we found decreased bictegravir intracellular concentrations in cell lines overexpressing ABCB1 as compared to control cell lines, in line with the known role of ABCB1 in bictegravir transport. However, we were unable to demonstrate any significant difference in bictegravir intracellular accumulation when comparing HEK293 cells overexpressing the wild type (1236C-2677G-3435C, 1199G) or the variant (1236C-2677G-3435T, 1236T-2677T-3435T or 1199A) proteins. These findings suggest that the *ABCB1* c.1199G>A and c.1236C>T-c.2677G>T-c.3435C>T variants have no or at least limited impact on the active transport of bictegravir by ABCB1.

Keywords Integrase strand transfer inhibitors, Pharmacogenetic, P-glycoprotein, P-gp, Multi-drug resistance protein 1, MDR1

Bictegravir is a second-generation integrase strand transfer inhibitor (INSTI) widely prescribed worldwide as an antiretroviral agent active against human immunodeficiency virus (HIV). The drug has been recommended as a first-line antiretroviral agent in combination with tenofovir alafenamide and emtricitabine, available as a single tablet regimen^{1,2}.

The advent of second generation INSTIs such as bictegravir, dolutegravir and cabotegravir represents a significant advancement for the management of persons living with HIV, mainly due to their overall high efficacy and favorable tolerability profile, as reported in pivotal trials³. Those drugs share a common favorable pharmacokinetic profile, although interindividual variability (IIV) has been demonstrated^{4–6}. Whether this IIV translates into significant variability in drug response (toxicity and/or efficacy) is subject of considerable interest. Indeed, since approval of second generation INSTIs, concerns have been raised regarding adverse events, including those reported with bictegravir. Abnormal weight gain is notably concerning among patients treated with dolutegravir or bictegravir^{7,8}. While initially reported for dolutegravir⁹, neuropsychiatric adverse events are a leading cause of bictegravir discontinuation¹⁰, prompting consideration of a potential class effect¹¹. Furthermore, in terms of virological response, despite the high success rate achieved with bictegravir-containing regimens, real-life studies have demonstrated that low-level viremia (LLV) defined by plasma viral load measured between 50

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and 199 copies/ml, is not an infrequent event. Chen and colleagues described an incidence of LLV as high as 6.2 per 100 person-year of follow-up¹². While a complete understanding of the mechanisms and causes of LLV is currently lacking, studies suggest that low drug tissue concentrations might be linked to residual viral transcription^{13,14}, and limited intracellular concentrations in lymphoid tissue have been shown for INSTIs^{15–17}.

Bictegravir is a known substrate of ABCB1 (also named P-glycoprotein, P-gp), an efflux transporter member of the ATP binding cassette and encoded by *ABCB1* gene^{18,19}. Through its selective expression on the luminal side of enterocytes in the small intestine and colon, ABCB1 limits the absorption of several xenobiotics. It also exerts an excretory activity in the liver and kidneys through its expression at the canalicular pole of hepatocytes and the luminal membrane of renal tubular cells. Moreover, by being apically expressed in cells at the serosal side of blood-tissue barriers (e.g., blood–brain barrier or blood–placenta barrier), ABCB1 protects the so-called sanctuary organs from the accumulation of its substrates²⁰. Eventually, the transporter plays an important role in the intracellular accumulation and pharmacokinetics of drugs. As such, ABCB1 deserves a particular attention in the field of HIV pharmacotherapy as it is expressed by peripheral blood mononuclear cells, including CD4 + T cells where the virus is known to replicate^{21,22}.

Several single nucleotide polymorphisms (SNPs) have been described in *ABCB1*, but investigations into their functional impact have typically centered on a select few. Despite its low frequency, with minor allelic frequency estimated around 3% in European populations and generally lower in other ethnic groups²³, the missense rs2229109 variant (c.1199G > A, p.Ser400Asn) has been subject of interest, as some studies have demonstrated a functional impact for the c.1199A variant^{24–26}. The rs1128503 (c.1236C > T, p.Gly412Gly), rs2032582 (c.2677G > T, p.Ala893Ser), and rs1045642 (c.3435C > T, p.Ile1145Ile) variants are the three most frequent and unsurprisingly have been the most studied. These SNPs are in strong linkage disequilibrium, with the most frequent haplotypes in most ethnicities being the CGC (thereafter named wild-type, WT) and TTT combinations of variants at positions c.1236, c.2677 and c.3435, respectively²⁷. Functional studies have focused on the effect of those individual SNPs or, more interestingly, of their haplotype combinations on drug transport, and have yielded variable results^{28,29}.

Among antiretroviral agents, protease inhibitors have been the most studied drugs for the impact of *ABCB1* variants on drug transport³⁰. A recent publication showed no effect of the four previously mentioned *ABCB1* variants on intracellular accumulation of darunavir³¹. The impact of *ABCB1* variants on bictegravir and other second generation INSTIs biodisposition remains currently unknown. Whether the most frequent *ABCB1* variants and their haplotypes significantly affect absorption, distribution and/or excretion of second generation INSTIs is however of particular interest as it could take part in the variability of their clinical response. The aim of this study is to investigate in vitro the impact of *ABCB1* c.1199G > A, c.1236C > T, c.2677G > T and c.3435C > T variants on the intracellular accumulation of bictegravir. Bictegravir was chosen for our in vitro study based on preliminary clinical pharmacokinetic assessments that indicated a higher intracellular accumulation of dolutegravir compared to bictegravir in PBMCs, suggesting that drug efflux transporters might play a more important and potentially more clinically impactful role in the transport of bictegravir.

Results

Flow cytometry

Levels of ABCB1 expression in the different cell lines assessed in our models were compared using FACS with fluorescence parameters gated on the same level of intensity. Analytical flow cytometry confirmed similar levels of ABCB1 surface expression by the different recombinant cell lines (ranging from 92.6 to 98.6% of positivity). In the HEK_{control} batch used for c.1199G > A functionality assessment, no fluorescence was detected, confirming very low levels of ABCB1 basal expression. In the HEK_{control} batch, used as a control for experiments conducted with the haplotype, a small fluorescent peak was observed with ABCB1-antibody (19.7% positivity), indicating a low but detectable ABCB1 basal expression³¹ (Fig. 1).

Functional assays

Functionality of ABCB1 expressed by the transfected cell lines in our models was confirmed using Rh123, a known fluorescent ABCB1 substrate, and comparing its intracellular accumulation in the presence or the absence of zosuquidar, a potent and specific ABCB1 inhibitor. After cell incubation with Rh123, intracellular fluorescence was significantly lower in cell lines overexpressing ABCB1 (i.e., in HEK_{WT}, HEK_{1199A}, HEK_{CGT} and HEK_{TTT}) compared to HEK_{control}, expressing low or very low ABCB1 levels. When cell lines overexpressing ABCB1 had been pre-incubated with zosuquidar, intracellular fluorescence increased to a level close to the level measured for HEK_{control} (Fig. 2). A small but significant effect of the inhibitor was observed in the HEK_{control} batch used for haplotype functional characterization, likely reflecting the higher level of ABCB1 basal expression compared to the cellular batch used for c.1199G > A experiments.

Impact of *ABCB1* c.1199G > A variant on the intracellular accumulation of bictegravir

The role of ABCB1 as a transporter for bictegravir and the impact of the *ABCB1* 1199G > A polymorphism on bictegravir transport was assessed by comparing the intracellular accumulation of bictegravir at different concentrations in the different cell lines (Fig. 3 and Supplementary Fig. 1). In accordance with what is expected for an ABCB1 substrate, bictegravir intracellular accumulations were significantly lower for HEK_{WT} and HEK_{1199A}, both cell lines overexpressing ABCB1, than for HEK_{control}. Our results did not show any significant difference of bictegravir intracellular accumulation between HEK_{WT} and HEK_{1199A}. The transport of bictegravir by ABCB1 was confirmed in a separate experiment where HEK_{1199G} cells overexpressing the wild-type ABCB1, but not HEK_{control} cells, demonstrated a significant increase in intracellular accumulation of bictegravir in the presence of zosuquidar compared to without zosuquidar (Supplementary Fig. 2).

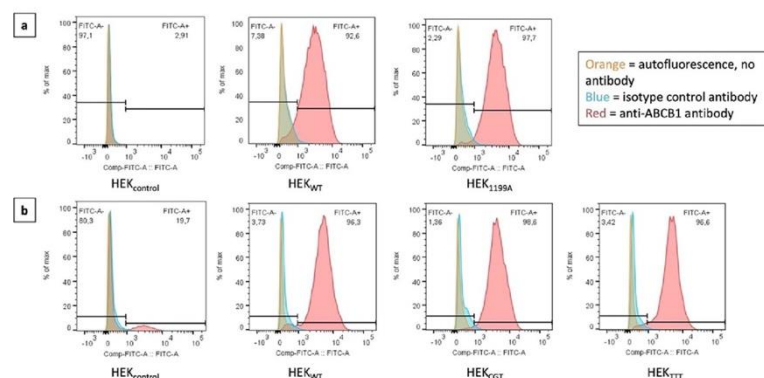


Figure 1. ABCB1 cell surface expression. Flow cytometry histograms of HEK293 cells transfected with (a) the empty pcDNA3.1 vector, *ABCB1*_{WT} or *ABCB1*_{1199A} and (b) the empty pcDNA3.1 vector, *ABCB1*_{WT}, *ABCB1*_{CGT} or *ABCB1*_{TTT}. Cells were incubated in the absence of antibody (autofluorescence, orange), with isotype control (blue) or with FITC anti-ABCB1 antibody (red).

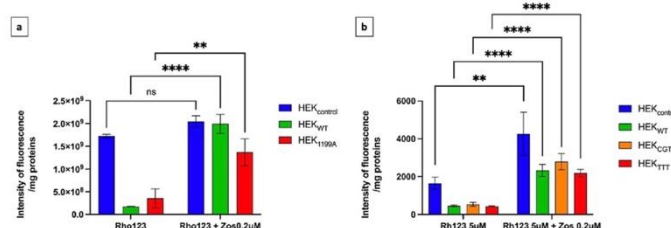


Figure 2. Model functionality assays using the ABCB1 substrate Rhodamine123 with or without the ABCB1 inhibitor Zosuquidar. Protein normalized intracellular fluorescence measured after incubation of (a) HEK_{control} and HEK_{1199A} and (b) HEK_{control}, HEK_{WT}, HEK_{CGT} and HEK_{TTT} with the fluorescent ABCB1 substrate Rhodamine123 (Rh123), in the absence (left) and in the presence (right) of Zosuquidar (Zos). Intensity of fluorescence is reported in arbitrary units. Results from a single experiment (N = 1 for (a) and N = 1 for (b)) reported as mean $\pm$ standard error (number of technical replicates performed per condition (n) = 3 for (a); n = 5 for (b)). Statistical analysis reported: ANOVA-2, after natural logarithmic transformation for (b). **p < 0.01; ****p < 0.0001.

Impact of *ABCB1* c.1236C>T-c.2677G>T-c.3435C>T variants on the intracellular accumulation of bicitegravir

Next, the impact of the most frequent haplotypes linked to the *ABCB1* 1236C>T, *ABCB1* 2677G>T and *ABCB1* 3435C>T variants on bicitegravir transport was assessed by comparing the intracellular accumulation of bicitegravir in the different cell lines (Fig. 4 and Supplementary Fig. 3). Again, as expected for a substrate drug, intracellular accumulation of bicitegravir was significantly lower for cell lines overexpressing ABCB1, namely HEK_{WT}, HEK_{CGT} and HEK_{TTT} than for HEK_{control}. Our results did not show any significant difference of bicitegravir intracellular accumulation between the cell lines overexpressing the different *ABCB1* haplotypes (HEK_{WT} vs HEK_{CGT}, HEK_{WT} vs HEK_{TTT} and HEK_{CGT} vs HEK_{TTT}).

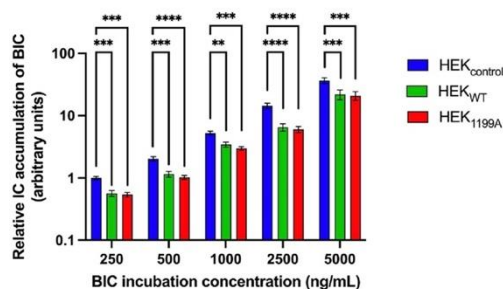


Figure 3. Intracellular protein-normalized bictegravir (BIC) concentrations in HEK_{control}, HEK_{WT} and HEK_{1199A} cells after 2 h of incubation at several dose levels. Results of three experiments were pooled (N = 3). Protein normalized BIC intracellular concentrations are reported as fold-change of the mean of the protein normalized BIC intracellular concentration observed at the lowest BIC exposition concentration for HEK_{control} (number of technical replicates performed per condition and per experiment = 3). Results reported as mean $\pm$ standard error. Statistical analysis reported: ANOVA-2 performed on pooled Ln-transformed data. **p < 0.01 ***p < 0.001 ****p < 0.0001.

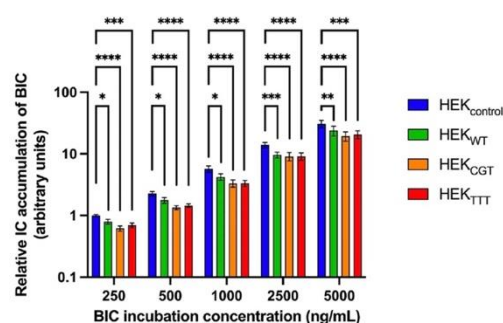


Figure 4. Intracellular protein-normalized bictegravir (BIC) concentrations in HEK_{control}, HEK_{WT}, HEK_{CGT} and HEK_{TTT} cells after 2 h of incubation at several dose levels. Results of three experiments were pooled (N = 3). Protein normalized BIC intracellular concentrations are reported as fold-change of the mean of the protein normalized BIC intracellular concentration observed at the lowest BIC exposition concentration for HEK_{control} (number of technical replicates performed per condition and per experiment = 3). Results reported as mean $\pm$ standard error. Statistical analysis reported: ANOVA-2 performed on pooled Ln-transformed data. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Discussion

We report the findings of our in vitro experiments investigating the effect of four *ABCB1* variants on the intracellular accumulation of bictegravir, a second generation INSTI. Using a previously validated HEK293 recombinant cell line model, we found that bictegravir intracellular concentrations were significantly decreased in cell lines overexpressing *ABCB1* as compared to control cell lines, in line with the known role of *ABCB1* in the transport of bictegravir. However, we were unable to demonstrate any significant difference in intracellular accumulation between HEK_{WT} and HEK_{1199A}, neither between HEK_{WT}, HEK_{CGT} and HEK_{TTT}. Those findings suggest that the *ABCB1* c.1199G > A and c.1236C > T-c.2677G > T-c.3435C > T variants have no or at least limited impact on the active transport of bictegravir by *ABCB1*.

Our results are consistent with an active transport of bicittegravir by ABCB1. This finding was recently described using ABCB1-overexpressing Madin–Darby canine kidney strain II transfected cells compared with non-transfected cells⁵. Bicittegravir is a lipophilic drug ($\text{LogD}_{7.4} = 2.1$) that belongs to the class II of the biopharmaceutical classification system (high permeability, low solubility). High transepithelial permeability has been shown for bicittegravir and its high oral bioavailability is thought to be mainly due to passive diffusion⁵. Saturability of efflux transport has been considered likely, based on in vitro human colorectal adenocarcinoma (Caco-2) cell monolayers bidirectional permeability assays at high concentrations such as those associated to the therapeutic oral dose⁵. The extent to which in vivo intestinal absorption of bicittegravir is affected by ABCB1 efflux activity remains unknown and caution should be exercised when using ABCB1 inducers or inhibitors.

On the contrary to the elevated drug diffusion from the intestinal compartment to the blood, low ratios of cerebrospinal fluid over plasma bicittegravir concentrations have been demonstrated. This finding is probably mainly driven by the high plasma protein binding^{22,35}. However, active efflux mechanisms may also play a role, and with low unbound plasma bicittegravir concentrations, saturation of efflux transporters at blood-tissues barriers cannot be assumed. While lymphoid tissues have not been studied so far, limited bicittegravir accumulation into human peripheral blood mononuclear cells has been recently shown³⁴. The precise contribution of ABCB1 and other efflux transporters remains to be determined.

We investigated the impact of four important variants and their haplotype combinations, for which a body of evidence suggests a plausible effect on the drug transport by ABCB1, despite inconclusive effects reported by several in vitro and in vivo studies. Substrate-specific effects but also variations in experimental designs and unappreciated confounding factors are thought to explain at least partially this heterogeneity^{29,38,39,55,56}.

The first variant investigated in this work is c.1199G > A (rs2229109), which leads to a serine to asparagine amino-acid substitution located close to the ABCB1 substrate binding site. Several reports have highlighted a substrate-specific effect of this SNP, possibly related to a differential effect on ABCB1 affinity towards its substrates^{38,55}. An increase of ATPase activity was also shown for 1199A as compared to 1199G⁵⁴.

The three other studied variants are in strong linkage disequilibrium, and we investigated the effect of the two most frequent haplotypes, being the wild-type CGC and the TTT combination. Studying haplotypes instead of isolated variants has been recommended as various SNPs can potentially have interacting effects³⁷. The intermediate CGT haplotype was also included in the analysis, with the aim of deciphering the relative contribution of the c.3435C > T variant. Despite the latter (rs1045642) being a synonymous SNP, it has been shown to affect the structure and function of ABCB1. Different underlying mechanisms have been suggested, including changes in the folding and function of ABCB1 due to ribosome stalling, as translation necessitates the use of a rarer codon^{38,39}. Other authors have suggested that this variant induces decreased mRNA stability and expression, probably related to an effect of the variant on mRNA secondary structure⁴⁶. However, our recombinant cellular models have been generated to express a similar level of ABCB1, making them more suitable for testing the functional impact of the haplotype rather than its effect on mRNA expression and stability. On the contrary, the synonymous c.1236C > T (rs1128503) variant has not been shown to affect protein expression, neither protein function, although a putative effect on protein folding has been speculated³⁹. The c.2677G > T variant (rs2032582) is non-synonymous and it has been suggested that the replacement of an alanine to a serine impacts the activity and substrate specificity of ABCB1, but not the protein expression⁴⁶.

To the best of our knowledge, the effect of the studied variants on bicittegravir transport has never been evaluated in vitro or in a clinical setting. In contrast, using the same cellular model and similar methods, our research group has previously demonstrated that the c.1199G > A variant has a significant impact on the intracellular accumulation of tacrolimus but not ciclosporin³⁶. The same variant was also shown to affect the transport of imatinib, nilotinib and dasatinib³⁵. Moreover, it was demonstrated that the c.1236C > T, c.2677G > T and c.3435C > T variants impact the anti-proliferative activity of tyrosine kinase inhibitors, with a more pronounced effect towards imatinib than nilotinib, dasatinib or ponatinib⁴⁰. Lastly, the effect of the four variants were studied for darunavir, with no significant impact demonstrated³⁹. Those different effects with different substrates but using similar in vitro methodologies point towards a substrate-dependent effect of the studied variants or their haplotype combinations, a hypothesis also supported by findings from other research groups using other methodologies for different substrates³⁹, including HIV protease inhibitors, or anticancer drugs^{44,45}. Importantly, as already stated, the clinical impact of those findings remains uncertain, as clinical studies have reported heterogeneous results^{28,29}.

We believe that the absence of demonstrated significant effect of the most frequent ABCB1 variants on the drug transport offers reassurance to the use of the drug in populations from different ethnic origins with different pharmacogenetic backgrounds. Although we cannot exclude that our assays were not powered to detect small differences between the different studied haplotypes, we believe those minor differences would probably not reach a level of clinical significance. One previously acknowledged limitation of our models, is that they assess the functional effect of the studied polymorphisms, and are not designed to evaluate variations in protein expression. As detailed previously, the polymorphisms that we studied have been associated with functional impacts (for c.1199G > A, c.2677G > T, c.3435C > T and possibly c.1236C > T) as well as possible expression impacts (for c.3435C > T) and as such our models are expected to be able to detect a significant impact of polymorphisms on drug transport by ABCB1. Further studies using other models assessing the effect of ABCB1 protein expression would usefully complement our approach.

In summary, we found that the second generation INSTI drug bicittegravir accumulates significantly less in HEK293 transfected cell lines overexpressing ABCB1 than in transfected cell lines not overexpressing ABCB1, a finding consistent with the active efflux of bicittegravir by ABCB1. We were unable to demonstrate any significant effect of the ABCB1 c.1236C > T, c.2677G > T, c.3435C > T and c.1199G > A variants on bicittegravir intracellular accumulation. Those findings are suggestive of an absence of functional impact, or at least a limited impact, of the aforementioned polymorphisms on bicittegravir clinical pharmacokinetics. Future studies should evaluate the effect of frequent polymorphisms in other relevant bicittegravir transporters such as ABCG2. Moreover, clinical

studies should confirm our findings in vivo, and ideally assess the impact of the main polymorphisms of those different transporters, as well as the net effect of their combinations on bicitegravir transport.

Methods

All methods were carried out in accordance with relevant guidelines and regulations; in accordance with the Belgian legislation, no approval from the institutional ethical board was sought for this study.

Chemicals and reagents

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, and enzyme-free cell dissociation buffer were purchased from Gibco (Thermo Fisher Scientific); G-418 from Roche; Poly-L-Lysine and Rhodamine (Rh123) from Sigma-Aldrich; flow cytometry antibodies (FITC mouse anti-human CD243, clone 17F9, reference 557002; and FITC mouse IgG2b κ isotype control, clone 27-35, reference 555742) from BD Biosciences; zosuquidar hydrochloride (LY335979) from Bio-connect; bicitegravir from Alsachim (Schimadzu) and deuterated bicitegravir (bicitegravir-15N, d2) from Toronto Research Chemicals. All chemicals used in drug quantification were of analytical grade.

Generation and characterization of recombinant cell lines

Generation of cell lines

The generation and characterization of recombinant cell lines have been described in previous work conducted by our lab^{36,42}. Briefly, human embryonic kidney (HEK293) cells were purchased from American Type Culture Collection and transfected with plasmids carrying either wild-type *ABCB1* 1199G-1236C-2677G-3435C (thereafter named HEK_{WT}) or several *ABCB1* variant combinations obtained using site-directed mutagenesis, 1199A-1236C-2677G-3435C (thereafter named HEK_(199A)), 1199G-1236C-2677G-3435T (thereafter named HEK₍₂₆₇₇₎) and 1199G-1236T-2677T-3435T (thereafter named HEK_(TTT)); or an empty vector (thereafter named HEK_{empty}). HEK₍₂₆₇₇₎ was selected, in addition to HEK_{WT} (carrying the CGC haplotype) and HEK_(TTT) to test the potential impact of c.3435C>T variant. HEK293 cells feature low endogenous levels of expression of *ABCB1*, ensuring that *ABCB1* expression originates almost exclusively from the transfection process³¹. This model has previously been extensively characterized using flow cytometry, Western blot, and fluorescence microscopy, and validated using reference substrates and inhibitors of *ABCB1*^{36,42}. As the same cells were used, only flow cytometry and functional tests using reference substrates and inhibitors were used to re-characterize them.

Flow cytometry

After thawing, cells were put in culture for at least seven days in the presence of the selection antibiotic G418 (1 g/l). Cells were detached from culture plates, centrifugated and 0.5×10^6 cells were counted, resuspended in 2 ml and washed twice with ice-cold fluorescence activated cell sorting (FACS) Buffer (PBS, FBS 1% (v/v), EDTA 1 mM). Cells were then re-suspended in FACS buffer supplemented with 10% anti-*ABCB1* antibody or 10% isotype control or in buffer with no antibody, and left to incubate for 45 min on ice and in the dark. After a final wash followed by resuspension in FACS buffer, cells were characterized using a BD FACSVers flow cytometer and sorted using a BD FACSAria III (BD Biosciences). Raw data were analyzed using FlowJo version 10.8.1 (Ashland).

Functional assays

0.05×10^6 cells were seeded on poly-L-lysine coated 96-well plates and incubated overnight. The next day, Rh123 and zosuquidar dilutions were prepared from a stock solution. Cells were incubated for 90 min at 37 °C in the presence of Rh123 at 5 μ M. The effect of *ABCB1* inhibition was assessed by preincubation of the cells for 15 min with zosuquidar at a concentration of 0.2 μ M. After incubation with Rh123, the supernatants were discarded. The cells were washed two times with ice-cold PBS. Sterile water was then added and detachment and lysis were performed through scratching of the well's surface followed by sonication. Finally, fluorescence of Rh123 was analyzed by an iD3 Spectramax (Molecular Devices) fluorometer (c.1199G>A model validation) or a M3 Spectramax (Molecular Devices) fluorometer (haplotype model validation); excitation wavelength was set at 485 nm and emission at 530 nm. The solution was then used for protein quantification for results normalization.

Cell culture

Transfected HEK293 cells were cultured at 37 °C and 5% CO₂ in DMEM with high glucose, L-glutamine, and sodium pyruvate supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin. For re-culturing, the cells were detached with enzyme-free cell dissociation buffer and culture medium was changed at regular intervals.

Intracellular bicitegravir accumulation experiments

0.7×10^6 cells were seeded on poly-L-lysine-coated 12-well plates and incubated overnight. The next day, bicitegravir dilutions were prepared in DMEM from a stock solution and added in each well to obtain a final concentration of 0.25, 0.5, 1, 2.5 or 5 mg/l, and a final volume of 1 ml per well. These concentrations were chosen to cover the range of total plasma concentrations found in bicitegravir-treated patients. Cells were incubated in triplicate at 37 °C in the presence of 5% CO₂ for 120 min. Subsequently, plates were then centrifuged for 3 min at 4 °C, and maintained on ice throughout the experiment to block drug efflux. Cells were washed twice with 1 ml of ice-cold PBS. Supernatant was discarded and 400 μ l of a mixture of methanol/water 60/40% (v/v) containing 20 ng/ml of bicitegravir-15N, d2 as internal standard were added for drug extraction. The well's surfaces were scratched followed by a 60 min agitation on a rocking platform at 4 °C. Cell suspensions were collected and set aside, while

the wells were rinsed with 300 µl of sterile water. Cell suspensions were centrifuged for 10 min at 10,500×g, 4 °C and 100 µl of the supernatant was transferred to a vial for bictegravir liquid chromatography-tandem mass spectrometry (LC–MS/MS) quantification. The remaining fraction was combined with the 300 µl of water used to rinse the well. This mixed solution was used for protein quantification for results normalization.

Drug quantification

Bictegravir intracellular concentrations were determined using an adapted version of a previously published LC–MS/MS method on a Xevo TQS-micro tandem quadrupole mass spectrometer (Waters)⁴⁶. Calibrators ranging from 1 to 500 ng/ml of bictegravir concentrations, containing the internal standard solution at 20 ng/ml of dolutegravir-d4 and 20 ng/ml of bictegravir-15N, d2 were prepared. Chromatographic separation was achieved on a Waters UPLC BEH C18 1.7 µm column (2.1 × 50 mm) maintained at 40 °C. The injection volume was 10 µl and the flow rate was 0.5 ml/min. The mobile phase consisted of a gradient of water/formic acid 0.1% (mobile phase A) and acetonitrile (mobile phase B), starting with 95% A and 5% B, transitioning to 80% B over 6 min, returning to 5% B at 6.1 min and maintaining this ratio until the end of the run (total run time: 8 min). The following ion transitions were monitored: 450.1 > 289.1 for bictegravir and 453.2 > 289.2 for bictegravir-15N, d2.

Protein quantification

Total proteins were quantified in pellets using the BCA kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Absolute bictegravir concentrations were normalized by total protein content.

Statistical analysis

Statistical analyses were carried out using GraphPad Prism version 9.5.1 for Mac OS X (GraphPad Software). For the functional assays, protein normalized intracellular fluorescences were compared after log-transformation using the natural logarithm (Ln), when required. Comparison was performed using a two-way ANOVA ($\alpha = 0.05$) followed by a post-hoc analysis testing the effect of the inhibitor, with Bonferroni correction for multiple testing. For the intracellular bictegravir accumulation experiments, triplicates were performed during each experiment, and each experiment was performed thrice. For each experiment, protein normalized bictegravir intracellular concentrations were reported as fold-change of the mean of the protein normalized bictegravir intracellular concentration observed at the lowest bictegravir exposition concentration for HEK_{293T} during the same experiment. Fold-change results from the three biological replicates were pooled and are shown on Figs. 3 and 4. Statistical analysis were performed on Ln transformed data that were pooled using the same principle as described here above. Fold-changes were compared using a two-way ANOVA ($\alpha = 0.05$) followed by a post-hoc analysis comparing fold-changes at each dose level, with Bonferroni correction for multiple testing.

Data availability

The data collected from this study are available from the corresponding author on reasonable request.

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Author contributions

J.D.G., V.H., L.B. and L.E. designed the study. J.D.G., N.P., M.Ak., M.An., E.H. and G.M. performed the experiments. J.D.G. and L.E. performed statistical analysis. All authors contributed to data analysis and visualization. J.D.G. wrote the first draft of the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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3.2 INSTI-01 study

3.2.1 Introduction

The *Gut microbiota, pharmacogenetics and Integrase Strand Transfer Inhibitors response study* (INSTI-01 study) was launched as part of this thesis. The clinical trial was approved by the Belgian Federal Agency for Medicines and Health Products on 15th December 2020 (Eudra-CT Number 2020-001103-17). It was further approved by the local ethical committee (*Comité d'éthique hospital-facultaire Cliniques universitaires Saint-Luc-UCLouvain*) on 11th January 2021 (2020/30NOV/593).

The specific objectives of the INSTI-01 study were:

- To study the impact of genetic polymorphisms in selected pharmacogenes (coding for biotransformation enzymes and transport proteins) on DTG and BIC PK parameters and biomarkers relevant for TDM, such as trough (C_{trough}) and intracellular concentrations.
- To determine whether genetic polymorphisms in selected pharmacogenes might affect INSTIs efficacy, as assessed by the measurement of the VL in the ranges of LLV and ULLV.
- To identify associations between genetic polymorphisms in selected pharmacogenes and the occurrence of NPAE.
- To describe how INSTIs affect the gut microbiome of treated patients, and to determine in turn how the gut microbiome might influence the clinical response to INSTIs, with a focus on weight gain.

3.2.2 Methods

Study design

Different complementary strategies were applied in this prospective phase IV study and different substudies were carried under the INSTI-01 umbrella to explore some of the potential determinants of the patient's drug response phenotypes (**Figure 16**).

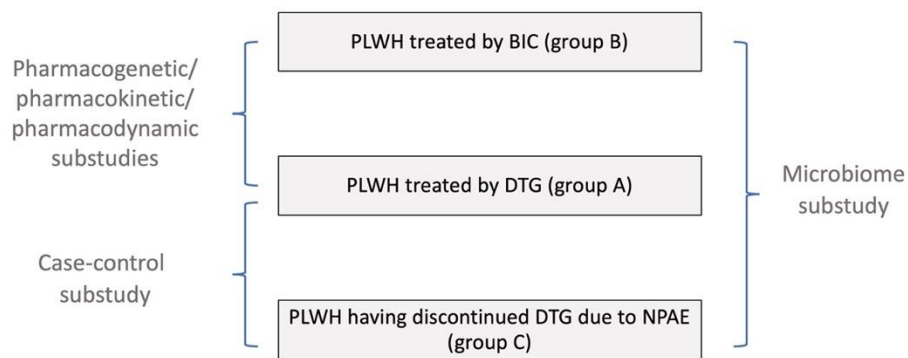


Figure 16. Framework of the substudies conducted under the INSTI-01 umbrella.

Based on recruitment possibilities and *a priori* calculation for the case-control study (detailed further), the study aimed at recruiting 200 patients treated by DTG or BIC among the cohort of PLWH followed at CUSL (N > 1400 PLWH):

- (1) 100 DTG-treated PLWH (group A).
- (2) 50 BIC-treated PLWH (group B).
- (3) 50 PLWH having previously discontinued DTG due to the occurrence of NPAE (group C). Those were identified from our pre-existing

institutional database recording the main HIV and ARV therapy-related data of patients in follow-up.

Study procedures

Participants were recruited during their regular HIV follow-up at the CUSL HIV/AIDS Reference Center. After providing informed consent, different measures were performed during follow-up, in addition to the regular clinical follow-up:

- PGx profiling (groups A, B and C):
 - High-throughput sequencing using a 44-gene PGx panel was performed. Detail of the coverage of test are provided in **Annex 5**.
- PK profiling (groups A and B):
 - C_{trough} and intracellular concentration were measured during regular visits. Patients were asked to take their medication after blood sampling. To take into account the intra-individual variability in DTG/BIC C_{trough} , we aimed at collecting a minimum of three plasma samples (different occasions) for each included patient. Due to technical constraints a single intracellular drug concentration quantification was planned.
 - *Of note, it is important to remind that due to the intrinsic design of this study, PK profile of group C patients could not be determined.*
- Ultra-low-level HIV-1 VL quantification (groups A and B):

- This analysis will be done at the AIDS reference laboratory, MBLG, UCLouvain. ULLV quantification was performed on the blood sample collected for standard VL quantification.
- Microbiota profiling (groups A, B, C):
 - A sample of stools was collected (collection at home using a kit with DNA stabilizer).
- Metabolite profiling (groups A, B, C):
 - With the aim of enabling future analysis, a plasma sample was collected when the patient provided the stool sample. It remains currently biobanked at -80°C.
- Psychometric evaluation (groups A and B):
 - Participants were requested to answer to 4 standardized questionnaires (Pittsburgh Sleep Quality Index, Pichot fatigue Questionnaire, Hospital Anxiety and Depression Scale and Symptom Checklist-90-R).
- Weight measurement (A, B and C).

3.2.3 Results

3.2.3.1 Study population

A total of 180 PLWH were recruited in the INSTI-01 study. Some participants were included in more than one group due to treatment shifts during the study follow-up. The population included in the different subgroups is described in **Figure 17**. Of note, not all participants were included in study analysis:

- 2 participants from group A and 1 participant from group B were not included in PK analysis due to irregular drug intake during days before blood sampling.
- 3 participants from group C were excluded as drug causality in NPAE could not be confirmed (further details in the results).

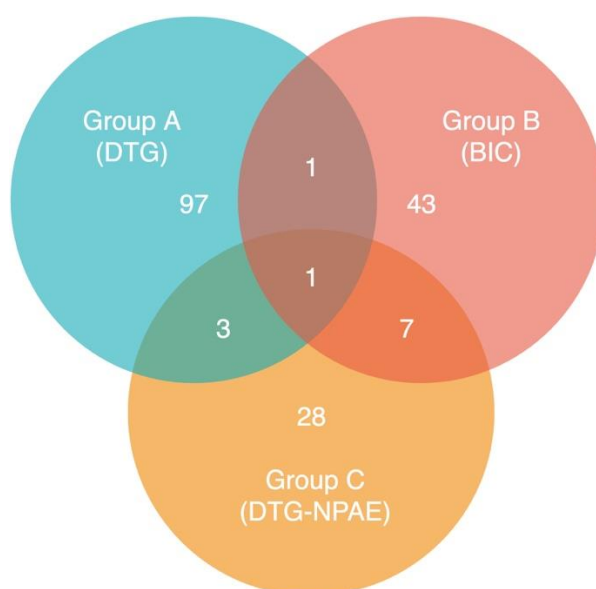
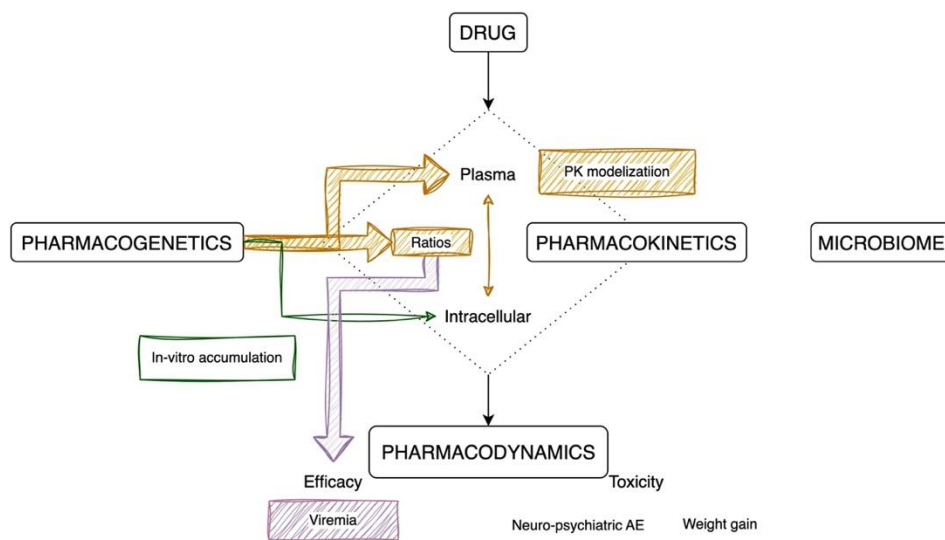


Figure 17. Venn diagram representing the INSTI-01 study population. Participants are classified according to their subgroup(s) (N = 180).

3.2.3.2 Pharmacogenetics of Dolutegravir and Bictegravir Plasma and Intracellular Pharmacokinetics



Key Points

- In this PGx study, plasma PK and intracellular drug accumulation within PBMCs were studied for DTG and BIC, and VL at low or ultra-low-level were measured.
- UGT1A1 poor metabolizing status was found associated with a decrease in apparent clearance for DTG.
- The *ABCG2* c.421C>A SNP was found to impact on intracellular accumulation of DTG and BIC.
- Intracellular drug concentrations were not associated with the occurrence of ULLV or LLV.

3.2.3.2.1 Manuscript

To be submitted

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3.2.3.2.1.1 Abstract

Introduction: Several uncertainties exist regarding the impact of pharmacogenetic polymorphisms in transport proteins and biotransformation enzymes on dolutegravir (DTG) pharmacokinetics and pharmacodynamics, and data are even more limited for bictegravir (BIC), despite the importance of both second-generation integrase strand transfer inhibitors (INSTIs) in the treatment of HIV.

Methods: This pharmacokinetic-pharmacogenetic study assessed the potential impact of relevant pharmacogenetic polymorphisms on plasma pharmacokinetics of DTG and intracellular/plasma accumulation ratios of DTG and BIC. Associations between intracellular drug accumulation and presence of (ultra-)low-level viremia (10-200 copies/mL) were also evaluated.

Results: UGT1A1 poor metabolizers showed a 32% decrease in plasma clearance of DTG. Studying intracellular drug accumulation, DTG was found to accumulate significantly more in peripheral blood mononuclear cell than BIC ($P < 0.0001$). The *ABCG2* c.421C>A SNP impacted intracellular drug accumulation, with increased accumulation for A carriers in DTG ($P = 0.01$) and BIC ($P = 0.06$). Drug intracellular/plasma ratios were not associated with detectable (ultra) low-level viremia.

Conclusions: These findings indicate that both UGT1A1 metabolizing status and the *ABCG2* c.421C>A SNP have impacts on pharmacokinetics of second generation INSTIs.

3.2.3.2.1.2 Introduction

Dolutegravir (DTG) and bictegravir (BIC) are second generation integrase strand transfer inhibitors (INSTIs). Both share low toxicity and high efficacy and are recommended as part of first line regimens against HIV (63, 176). Despite their overall favourable profile, real-life studies have raised some specific safety concerns and demonstrated that in some cases virological suppression is not reached despite potent INSTI-based regimens. Indeed, on one hand neuropsychiatric adverse event (NPAE) and abnormal weight gain have been reported (209, 240, 413), and on the other hand, detecting persistent low-level viremia (LLV, i.e., plasma HIV viral load (VL) of 50–200 copies/mL) during follow-up is not rare. The possibility of persistent viral replication under treatment has been suggested in some cases, although the origin and implications of such events remain subject of debate (414-416).

DTG is mainly metabolized by the liver, where it undergoes biotransformation by uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) and to a lower extent by cytochrome P450 3A4 (CYP3A4). Both enzymes contribute equally to BIC biotransformation. Both DTG and BIC are transported to a comparable extent by the ATP Binding Cassette (ABC) transporters ABCB1 (also called P-glycoprotein, P-gp) and ABCG2 (also called breast cancer resistance protein, BCRP) (105, 138). Those active efflux transporters limit drug absorption and participate in excretion through their expression on apical cell membrane of enterocytes, renal tubular cells or hepatocytes. Moreover, ABCB1 and ABCG2 impact drug distribution,

protecting sanctuaries through their expression at serosal pole of blood-tissue barriers. Expression by peripheral blood mononuclear cells (PBMCs) has also been demonstrated, although its importance is unclear (417-419).

Pharmacogenetic determinants of DTG plasma exposure have previously been explored. Regarding *UGT1A1*, decreased function variants such as *28, *37 (rs3064744) and *6 (rs4148323) have been associated with increases in systemic drug exposure (222, 389-392). Some studies have also shown pharmacokinetic impacts of the c.-22-7659C>T (rs2472677) single nucleotide polymorphism (SNP) in the nuclear receptor (NR) gene *NR1/2* (encoding the Pregnane X receptor, PXR), and the c.421C>A SNP (rs2231142) in *ABCG2* (respectively (390) and (390, 393)) but evidence remains more limited (395). To the best of our knowledge, no study has investigated the pharmacogenetics of intracellular accumulation of DTG. Regarding BIC, no clinical study has yet evaluated pharmacokinetic (PK)-pharmacogenetic associations, but using *in-vitro* intracellular drug accumulation assays we have previously shown that the most common coding *ABCB1* SNPs (i.e., c.1199G>A, c.1236C>T, c.2677G>T and c.3435C>T) have no significant impact on BIC transport (397).

Considering those uncertainties and knowledge gaps, the aim of this study was to investigate the impact of important polymorphisms in relevant pharmacogenes on plasma and intracellular PK of DTG and BIC, and on drug pharmacodynamics.

3.2.3.2.1.3 Material and Methods

Clinical study design and setting

This study is part of a phase IV study aiming at understanding the interindividual variability in clinical and biological response to DTG and BIC. Briefly, the parent study aimed at recruiting participants from different subgroups during their regular HIV follow-up at Cliniques universitaires Saint-Luc, an academic hospital located in Brussels, Belgium: (i) BIC-treated persons living with HIV (PLWH), (ii) DTG-treated PLWH and (iii) PLWH having previously discontinued DTG due to NPAE. The latter group will not be considered in this manuscript and was included in a separate analysis currently under review.

After providing informed consent, all participants were genotyped and different measures were performed during follow-up, including sparse sampling for DTG and BIC plasma concentrations measurements at multiple occasions during routine follow-up, and intracellular drug quantification in PBMCs at one single of the aforementioned occasions. Measuring simultaneously intracellular and plasma drug concentrations enabled the calculation of intracellular/plasma drug ratios. In order to obtain a post-intake sampling time close to the trough level, participants were personally contacted by phone a few days before each study visit to ensure not taking the medication on the day of sampling before blood was drawn. Samples for which treatment non-adherence within days before sampling was either confirmed by the patient or suspected based on uncontrolled VL were excluded from the analysis.

The study (NCT04805944) received approval by the local ethical committee (Comité d'éthique hospitalo-facultaire Saint-Luc-UCLouvain, 2020/30NOV/593) and regulatory authorities (Eudra-CT Number 2020-001103-17).

Dolutegravir and bictegravir concentrations measurements

Blood samples were obtained on heparinized tubes and immediately centrifuged at 1540xg for 5 minutes at room temperature (RT). Plasma was then collected and stored at -20°C until the day of quantification. The DTG and BIC plasma concentrations were measured according to a method described in the Supplementary appendix, by liquid chromatography with tandem mass spectrometry (LC-MC/MS).

Collection of PBMCs from patients and determination of intracellular drug concentrations

About 8 ml of blood were collected in cell preparation tube (Becton Dickinson Vacutainer). Blood samples were first centrifuged at 1790xg for 20 min at room temperature. The cell layer was collected with a Pasteur pipette and transferred to an ice-cold 15 ml size conical centrifuge tube. The volume was completed to 15 ml with ice-cold Dulbecco's phosphate buffered saline (DPBS) to block enzymatic activity and avoid drug efflux. PBMCs were then washed three times (at 1240xg for 10 min at 4°C). The cell pellet was resuspended in 1ml of DPBS. 20 µl aliquot was diluted in 180 µl of DPBS and directly used for cell counting using a Sysmex analyzer. After a final centrifugation of the remaining 980 µl at 700xg (10 min at 4°C), the

supernatant was aspirated and the cell pellet was stored immediately at -80°C until the drug extraction. This method was adapted from Elens et al. (420). The concentration of DTG and BIC in cell pellets was determined by LC-MS/MS using an adaptation of a previously validated method (421). Once the concentration in the cell pellet was determined, the absolute amount of drug (in ng) present in extracted cells was calculated. This amount was then reported on the number of cells (in million) and the DTG or BIC intracellular concentration was determined assuming that the mean volume of a PBMC is 0.4 pl (420). Proteins were quantified in the cell pellets and were shown to correlate to the PBMCs quantification ($P < 0.0001$), thus providing cross-validation of the validity of denominator used to determine PBMC intracellular concentrations (Supplementary Appendix).

VL quantification for pharmacodynamic assessment

All participants underwent routine HIV-1 VL measurement during all clinical visits (m2000 real time system (Abbott); LOQ: 40 copies/ml). Moreover, for samples drawn on the day of isolation of PBMCs and for which VL was undetectable by this technique, ultra-low-level viremia (ULLV) was quantified on the same blood sample using the QX200TM Droplet Digital PCR system (Bio-Rad; LOQ: 10 copies/ml). Samples with uncontrolled VL above 200 copies/ml were excluded from the analysis owing to uncertain drug adherence. Participants with viremia between 10 and 200 copies/ml were grouped and compared with participants with undetectable VLs.

Candidate Genes, Polymorphisms and Genotype-based predicted Phenotypes

The selection of pharmacogenetic polymorphisms considered for the DTG plasma PK analysis was based on previous associations with DTG PK (222, 389-393) or previous evidence of association with drug-phenotypes (282, 287, 298, 323, 422). Considering that both drugs share their main metabolizing enzymes and transport proteins, the same genotypes were studied for BIC: *UGT1A1**28, *36, *37 (rs3064744), *6 (rs4148323) (coded as extensive metabolizers (EM) (*1/*1; *1/*36; *36/*36), intermediate metabolizers (IM) (*1/*28; *1/*37; *36/*28; *36/*37; *1/*6) and poor metabolizers (PM) (*28/*28; *28/*37; *37/*37; *6/*6), in accordance with the Clinical Pharmacogenetics Implementation Consortium classification for *UGT1A1* genotype-based predicted phenotypic function (423)); *CYP3A4**22 (rs35599367); *CYP3A5**3 (rs776746), *6 (rs10264272), *7 (rs41303343) (coded as *CYP3A5* expressors if participants carried at least one *CYP3A5**1 allele); *ABCB1* c.1199G>A (rs2229109) and c.3435C>T (rs1045642); *ABCG2* c.421C>A (rs2231142); *NR1I2* c.-22-7659C>T (rs2472677) and *POR**28 (rs1057868).

For the intracellular/plasma drug ratio analysis, we first used a targeted approach to study the c.421C>A (rs2231142) SNP in *ABCG2*, considering its demonstrated impact on PK of the INSTI drugs raltegravir (RAL) and DTG (388, 390, 393). Secondly, in an untargeted exploratory approach, we assessed the impact of all other variants of *ABCB1* (for which full gene sequencing was performed, see further), *ABCG2* and *NR1I2* that were

identified in our cohort. *NR1I2* was studied considering its regulatory role for transporters (324, 353).

Pharmacogenomic Sample Collection, DNA Extraction and Genotyping

Whole blood was collected from participants in an EDTA tube. Samples were stored at +4°C until DNA extraction, using the QIAamp® DNA blood kit according to manufacturer's instructions. Next-generation sequencing (NGS) pharmacogenomic testing was then performed using SOPHiA GENETICS™ Community Panel for Pharmacogenomics (SOPHiA DDM® for Pharmacogenomics), on an Illumina MiSeq® platform. This test covers target coding and non-coding regions and hotspots in 41 key pharmacogenes, and includes a full gene sequencing of some genes including *ABCB1*.

For *UGT1A1*, the most frequent polymorphisms are characterised by a variation in the number of TA repetitions in the promoter region, with *UGT1A1**1, containing 6 repeats (TA₆), as the reference allele. To confirm *UGT1A1* genotyping unambiguously, all samples containing *36 (TA₅) and *37 (TA₈) alleles in addition to the common *1 or *28 (TA₇) alleles were confirmed by a validated high-resolution melting analysis method and by Sanger sequencing. Additionally, all *28 and *37 alleles were also confirmed using the *UGT1A1**80 (rs887829) proxy.

Modelling the pharmacogenetics of plasma DTG

The data was modelled using non-linear mixed effect models, where the concentration C_{ij} in subject i at time t_{ij} on occasion j was described by the following equation:

$$C_{ij} = f(t_{ij}, \psi_{ij}) + g(t_{ij}, \psi_{ij})\epsilon_{ij}$$

In this equation, f represents the structural model, g quantifies the variability of the residual error, ψ_{ij} is the vector of parameters for subject i at occasion j , and ϵ_{ij} is assumed to follow a standard normal distribution. The parameters were assumed to be log-normally distributed with interindividual variability Ω and interoccasion variability K . The data was too sparse to develop a PK model, and we used the PK model developed for DTG by Parant et al., a steady-state one-compartment model with first-order absorption with proportional residual error. f was therefore a function depending on 4 parameters: lag time (T_{lag}), absorption rate constant (k_a), apparent volume of distribution (V/F) and apparent clearance (CL/F). In the model, CL/F and V/F increased with body weight according to an exponential model, and smoking status as well as co-administration of ritonavir (RTV)-boosted darunavir were associated with higher CL (103).

We used Monolix (version 2024R1) (424) for the analyses, and R (version 4.3.2, www.r-project.org) for data management and statistical tests. In a first step, we fixed the population parameters to the values estimated by Parant et al. to estimate the individual parameters as the mean of the conditional distributions in Monolix (with no iterations in the estimation). The parameter estimates were then used to predict the residual concentrations 24 hours

after the dose for each patient (C_{trough}). We tested the effect of genotypes using univariate linear mixed effect models (425). In a second step, we used the values estimated by Parant et al. as priors and re-estimated V/F and CL/F as well as their associated variabilities, and with this setting we explored the relationships between CL/F and the genetic covariates. In Monolix, log-normal priors are applied to fixed effects and normal priors to covariate effects, and we set the mean to the estimated value and the variance to the estimated variance of estimation. Inverse Wishart priors are used for the standard deviations, with the mean again set to the estimated values and the number of degrees of freedom set to 1 (default value). No prior was set for the coefficients of the genetic covariates. We used the COSSAC algorithm (426), locking in the known covariate-parameter relationships. Other covariates were not included at this stage, as demographic covariates have already been studied in Parant et al. The covariates selected in the COSSAC procedure were then entered in a full model, and the final model was built by backward selection, eliminating relationships between parameters and genetic covariates that did not improve the corrected Bayesian Information Criterion (BICc) (427) or for which the relative standard error of estimation (RSE) was higher than 50%. The final model was assessed through graphical diagnostics and a run assessment was performed, running the model 5 times from different initial conditions and different random generator seed, to check for stability. Sensitivity analyses were also performed to check the influence of the priors, by increasing the variances by a factor 2 and the number of degrees of freedom to 25 (for an approximate CV of 30%), to decrease the informativeness of the priors. Finally, we used the resulting

model to predict the individual profiles. Uncertainty was estimated by sampling 200 individual parameters in conditional distributions estimated by Monolix (increasing the number of iterations and the size of the convergence sample), and taking the 95% interval of the C_{trough} predicted using these parameters.

Other Statistical Analysis

Continuous data are presented as means with their standard deviation or as medians with their interquartile ranges if not normally distributed. Categorical data are presented as counts and proportions. Continuous variables were compared between groups using one-way ANOVA, or Wilcoxon test for non-gaussian variables, and proportions were compared using Pearson Chi-squared or Fischer's Exact tests, as appropriate. The impact of SNPs on drug intracellular/plasma ratios was explored through univariate analysis by one-way ANOVA, and results were corrected for multiple testing, with raw and false discover rate (FDR)-corrected P values reported. To study the impact of intracellular concentrations on the occurrence of (U)LLV, we performed multiple logistic regressions taking into account the interaction between intracellular drug concentration and time between drug intake and PBMCs collection. All statistical analysis of the drug intracellular/plasma ratios and drug concentrations were performed on log-transformed data.

A bilateral P value less than .05 was considered statistically significant. The statistical analyses reported in this paragraph were carried out using JMP®

Pro 17.1.0 (JMP Statistical Discovery LLC) and figures using GraphPad Prism version 9.5.1 for Mac OS X (GraphPad Software).

3.2.3.2.1.4 Results

Study population

A total of 180 PLWH were included in the parent study, and 100 subjects receiving DTG and 51 receiving BIC were included for the plasma PK analysis (respectively 2 and 1 participants were excluded from the analysis due to consistent non-adherence to treatment) with a total of respectively 288 (with 84 (84%) participants contributing at least 3 occasions) and 138 plasma samples (with 35 (69%) participants contributing at least 3 occasions) included in the analysis. Moreover, 80 and 45 PLWH receiving respectively DTG and BIC were included for the intracellular/plasma drug concentration ratio analysis. Eventually, concomitant measures of LLV or ULLV were available for 76 and 41 PLWH receiving respectively DTG and BIC. Study population general characteristics and genotype distribution are described in Table 1. All polymorphisms were in Hardy–Weinberg equilibrium in both drug groups, including for *CYP3A5*, *UGT1A1*, and *NR1I2* after stratification for ethnic origin.

Table 1. Description of the study population.

	DTG (n=100)	BIC (n=51)	P value
Female sex, n (%)	37 (37)	20 (39)	0.79
Age, mean (SD), y	51 (12)	45 (12)	0.003
BMI ^a , median (IQR), kg/m ²	27 (24-30)	28 (24-31)	0.64
Weight ^a , mean (SD), kg	82 (15)	82 (17)	0.83
Tobacco use, n (%)	21 (21)	12 (24)	0.72
CD4+ T-cell count ^b , median (IQR)	696 (530-863)	562 (439-692)	0.0003
Lowest CD4+ T-cell count ^c , median (IQR)	234 (118-342)	199 (113-346)	0.62
Past AIDS ^d	48 (48)	29 (57)	0.33
Ethnicity			0.35
Sub-Saharan African	35 (35)	25 (49)	
White	59 (59)	23 (45)	
Hispanic	4 (4)	2 (4)	
Asian	2 (2)	1 (2)	
Number of subjects with 1/2/3/4 sampling occasions	7/9/73/11	3/13/31/4	
UGT1A1^e			
Extensive metabolizer	50 (50)	14 (27)	
Intermediate metabolizer	38 (38)	26 (51)	
Poor metabolizer	12 (12)	11 (22)	
CYP3A4*22 (rs35599367)			
-/-	89 (89)	45 (88)	
-/ *22	10 (10)	6 (12)	
*22/*22	1 (1)	0 (0)	
CYP3A5^f			
Expressor	34 (34)	25 (49)	
Non-expressor	66 (66)	26 (51)	
ABCB1 c.3435C>T (rs1045642)			
CC	49 (49)	29 (57)	
CT	39 (39)	17 (33)	

TT	12 (12)	5 (10)
ABCB1 1199G>A (rs2229109)		
GG	95 (95)	47 (92)
GA	5 (5)	4 (8)
ABCG2 c.421C>A (rs2231142)		
CC	82 (82)	42 (82)
CA	18 (18)	9 (18)
NR1I2 c.-22-7659C>T (rs2472677)		
	16 (16)	12 (24)
CC	44 (44)	17 (33)
CT	40 (40)	22 (43)
TT		
POR*28 (rs1057868)		
-/-	59 (59)	28 (51)
-*28	32 (32)	18 (35)
*28/*28	9 (9)	7 (14)

Two participants were included in both DTG and BIC groups as they were treated successively with DTG and BIC. Data are presented as number of subjects (%) unless otherwise indicated. Data are presented at study inclusion, unless otherwise indicated. Abbreviations: AIDS = acquired immunodeficiency syndrome; BMI = body mass index; IQR = interquartile range; SD = standard deviation; y = years.

^a unavailable for 1 participant in the DTG group.

^b unavailable for 1 participant in the BIC group.

^c unavailable for 3 participants in the DTG group and 1 participant in the BIC group.

^d unavailable for 1 participant in the DTG group.

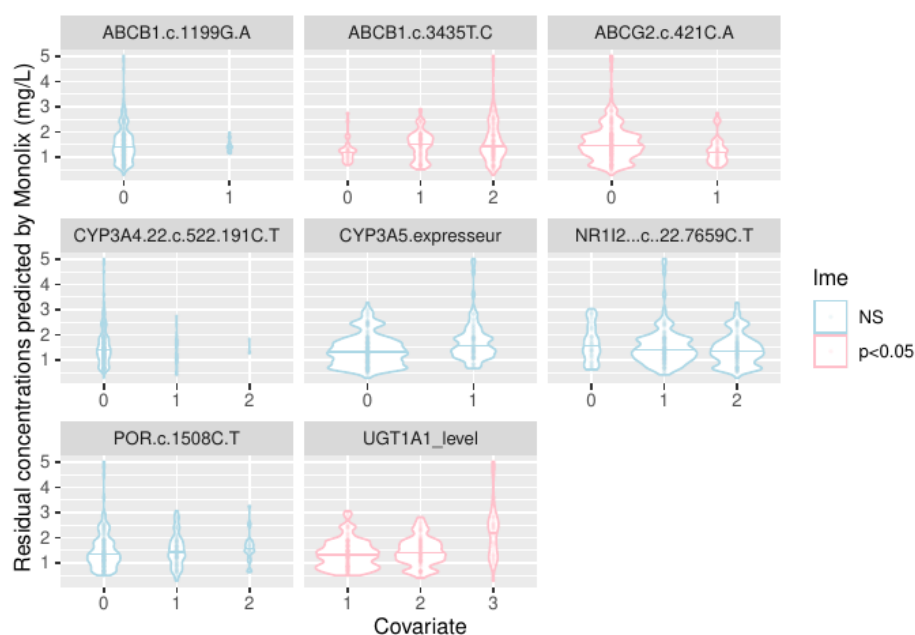
^e Classified according to Clinical Pharmacogenetics Implementation Consortium (CPIC) classification for UGT1A1 genotype-predicted phenotypic function: extensive metabolizer (*1 or *36)/(*1 or *36), intermediate metabolizer (*1 or *36)/(*28 or *37), poor metabolizer (*28 or *37)/(*28 or *37). No participant displayed the *6 allele.

^f Expressor participants display at least one copy of a normal function allele: *1/(*1 or *3 or *6 or *7); non-expressor participants have two copies of a no function allele: (*3 or *6 or *7)/(*3 or *6 or *7).

Impact of pharmacogenetic polymorphisms on DTG plasma pharmacokinetics

The Parant model demonstrated its ability to predict the observed concentrations accurately. The bias was less than 5% for the parameters estimated via their conditional modes and 11% for the estimates based on the conditional means, and the accuracy, computed by the relative root mean square error (RRMSE) was around 24% for both. Using a linear mixed effect model to take into account the repeated measurements, univariate analyses showed that *UGT1A1* PM ($P < 0.003$), *ABCG2* c.421C>A and *ABCB1* c.3435C>T ($P < 0.03$) were significantly associated with residual concentrations (Figure 1).

Figure 1. Violin plots showing the relationship between the predicted residual concentrations and the different genotypes. Blue plots indicate non-significant relationships while red plots indicate statistical significance. Uncertainty was estimated by sampling 200 individual parameters in conditional distributions estimated by Monolix (increasing the number of iterations and the size of the convergence sample), and taking the 95% interval of the C_{trough} predicted using these parameters.



To take into account uncertainty on previously estimated model parameters in a methodologically correct way, we used a Bayesian approach to integrate priors in the covariate selection algorithm. The covariates found by the COSSAC algorithm were *CYP3A5* status and *UGT1A1* metabolizing status. However, the RSE for the effect of *CYP3A5* was large ($(\beta_{CYP3A5}, CL=-0.11, RSE=58\%)$) and the model including *CYP3A5* had a higher BICc. Also, there was no difference between EM and IM for *UGT1A1*. Therefore, the final

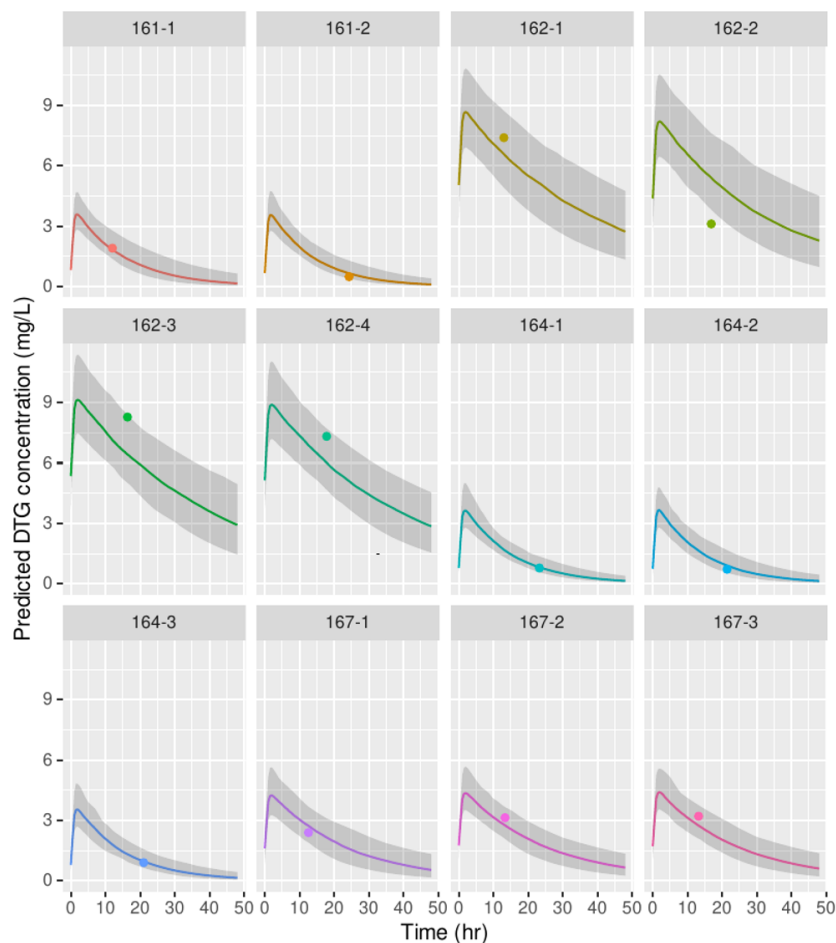
model included only *UGT1A1*, with EM and IM regrouped versus PM. The estimates for the final model remained close to the estimates found in Parant et al. (see Table 2). A sensitivity analysis was performed by increasing the variances of the priors, showing stable estimates, although the variabilities on V were more difficult to distinguish when less information was provided to the model, with higher RSE on interindividual variability. Finally, a run assessment also showed stable estimates. Note that RSE was large in all runs for smoking status and comedication with cobicistat (COBI). Figure 2 shows the prediction intervals for the 4 last subjects over their different occasions. These plots illustrate the variability both between and within subjects as well as the good fit of the model.

Table 2. Parameter estimates for the final model with genotypes. The variances of the priors on the fixed effects were set to the RSE reported in Parant et al. and the number of degrees of freedom for the variance parameters was set to 25 corresponding to a CV of 30%. As in Parant et al. Tlag and ka were fixed without IIV.

Parameter	Estimate (RSE%)	IIV (RSE%)	IOV (RSE%)
Tlag (hr)	0.263 (-)		
ka (hr ⁻¹)	2.24 (-)		
V/F (L)	15.0 (13)	0.16 (84)	0.12 (61)
b_WT, V/F (-)	0.70 (47)		
CL/F (L/h)	0.73 (6)	0.24 (16)	0.12 (25)
b_WT, CL/F (-)	0.46 (38)		
b_RTV, CL/F (-)	0.21 (91)		
b_smoke, CL/F (-)	0.12 (59)		
b_UGT1A1PM, CL/F (-)	-0.34 (27)		
s (-)	0.28 (10)		

Abbreviations: CL/F, apparent clearance; IIV: interindividual variability; IOV: interoccasion variability; ka, absorption rate; %RSE, percentage of relative standard error of the estimate; RTV: ritonavir; Tlag, lag time; UGT1A1PM : UGT1A1 poor metabolizing status; V/F, apparent volume of distribution.

Figure 2. Individual predicted profiles with their prediction intervals, for the last four subjects in the study, over their different occasions.



Intracellular accumulation of DTG and BIC

Comparing intracellular accumulation of both drugs, we found significantly higher intracellular/plasma ratios for DTG (median 0.085; IQR: 0.048-0.139) compared to BIC (median 0.039; IQR: 0.030-0.071) ($P < 0.0001$) (Figure 3-A). The mean intracellular/plasma ratio for BIC was calculated to be 46% of that of DTG.

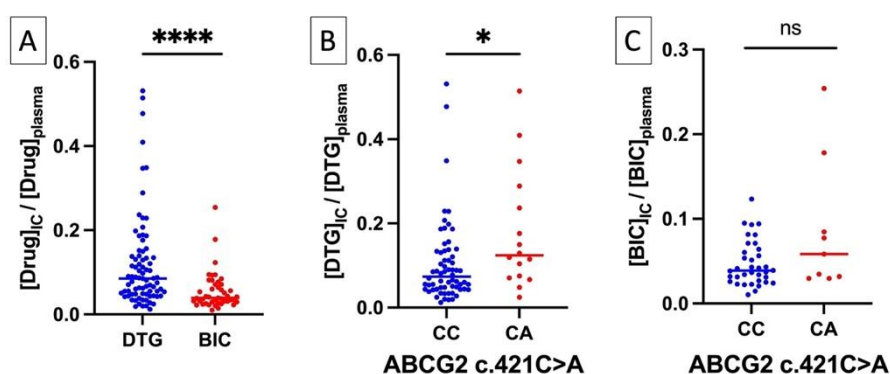
Impact of pharmacogenetic polymorphisms on DTG and BIC intracellular pharmacokinetics

We next assessed the impact of *ABCG2* c.421C>A on DTG and BIC intracellular/plasma ratios (Figure 3-B and 3-C). We found significantly higher intracellular/plasma ratios for DTG in CA genotypes (median 0.124; IQR: 0.072-0.276) compared to CC genotypes (median 0.074; IQR 0.044-0.133) ($P = 0.012$). Our results were confirmed in a sensitivity analysis excluding the 2 participants receiving COBI, a known *ABCG2* and *ABCB1* inhibitor. For BIC, we found a trend towards higher ratios for CA genotypes (median 0.058; IQR: 0.031-0.131) compared to CC genotypes (median 0.039; IQR 0.026-0.063) ($P = 0.056$).

We next assessed the impact of all other polymorphisms identified in *ABCB1*, *ABCG2* and *NR1I2* on drug intracellular/plasma ratios. *ABCG2* c.421C>A was the only polymorphism that showed an uncorrected significant effect on ratios for DTG, but the result did not withstand correction for multiple testing. We further found that *ABCB1* c.3320A>C (rs55852620) showed a near-significant effect (uncorrected $P = 0.050$). Similar results were found when excluding the 2 participants receiving COBI (uncorrected $P = 0.048$ for *ABCB1* c.3320A>C). Of note, only one patient carried the C allele (at heterozygous state). For BIC, none of the studied polymorphisms was found significantly associated with the drug intracellular/plasma ratios. Besides *ABCG2* c.421C>A, the c.*193A>G SNP in *ABCB1* (rs3842) also showed a near-significant effect on in the intracellular drug accumulation (uncorrected $P =$

0.053). Detailed results are reported in Supplementary Tables 1 and 2. Distribution of BIC intracellular/plasma ratios according to *ABCB1* c.*193A>G genotype is reported in the Supplementary figure 1.

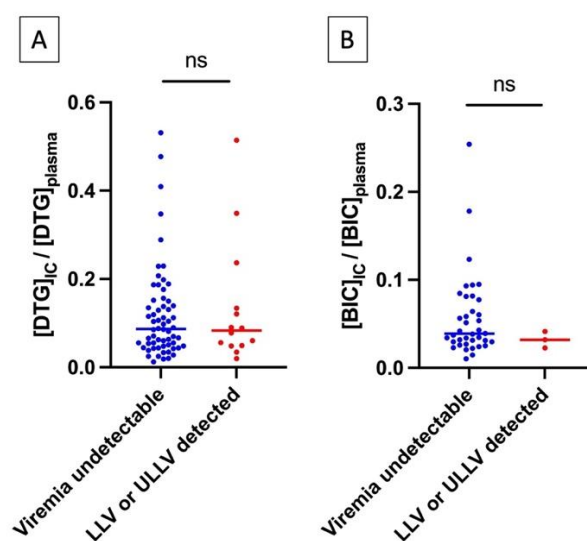
Figure 3. Drug intracellular/plasma ratios. (A) Distribution according to drug. (B) Distribution according to *ABCG2* c.421C>A genotype for DTG. (C) Distribution according to *ABCG2* c.421C>A genotype for BIC.



Pharmacodynamic evaluation

LLV or ULLV for HIV-1 was detected in 14 (18%) and 3 (7%) of participants receiving respectively DTG and BIC, with median values of 16 (range: 10-151) and 24 (range: 19-39) copies/ml. We found no difference in the distribution of intracellular/plasma drug ratios between LLV/ULLV detectable and undetectable participants ($P = 0.86$ for DTG ratios; $P = 0.36$ for BIC ratios) (Figure 4). Moreover, we found no effect of intracellular drug concentrations on the occurrence of LLV/ULLV ($P = 0.49$ and $P = 0.35$ for the models including time and respectively DTG and BIC concentrations).

Figure 4. Drug intracellular/plasma ratios according to results for VL measurement. (A) For DTG; (B) for BIC. Lower limit of quantification for VL: 10 copies/mL. VLs measured > 200 copies/mL were excluded from the analysis owing to concerns regarding regular drug adherence.



Considering that pharmacogenetic polymorphisms could possibly impact on drug concentrations in other tissues where residual viral replication possibly occurs, and to expand our pharmacodynamic (PD) analysis to all participants with LLV/ULLV measured (including those with no intracellular drug concentration available), we tested the association between detectable LLV/ULLV and the *UGT1A1* PM status, *ABCG2* c.421C>A and *NR1I2* c.-22-7659C>T genotypes for both DTG and BIC, and found no impact of either genotype (Supplementary Tables 3 and 4).

Association between ABCB1 genotype and LLV/ULLV

Eventually, considering that *ABCB1* expression has previously been linked to HIV viral replication (428, 429), we tested the association between detectable LLV/ULLV and *ABCB1* c.3435C>T or c.1199G>A genotypes, independently of treatment group, and found no impact of either genotype (respectively $P = 0.58$ and $P = 0.26$).

3.2.3.2.1.5 Discussion

We report the results of a pharmacogenetic study assessing the effect of genetic polymorphisms in selected drug transport proteins and biotransformation enzymes on plasma and intracellular PK of DTG and BIC. We identified UGT1A1 poor metabolizing status as a significant determinant of DTG plasma exposure, while *ABCG2* c.421C>A appeared to impact on DTG and BIC transport, with respectively significantly and near-significantly higher intracellular accumulation for A carriers. Detectable viremia at low- and ultra-low-level appeared independent of intracellular drug concentrations or accumulation ratios, and independent of these genotypes as well as *ABCB1* c.3435C>T and c.1999G>A.

Systemic DTG exposure

Using a Bayesian approach, we estimated genotype effects on the PK of DTG, showing that apparent clearance was decreased by 34% in UGT1A1 PM. The modelling confirmed the preliminary findings of the statistical tests between predicted C_{trough} and genotype groups, while taking into account parameter

uncertainty through Bayesian inference with non-linear mixed effect models. We used samples from the conditional distributions estimated by Monolix for each individual subject to build prediction intervals for the final model over each occasion. Another possibility would have been to pool the data from different occasions to predict the average profile for each subject.

The covariates found in the previous analysis by Parant et al. (103) were kept in the model, but the estimates had larger estimation errors, and varied more when priors were relaxed, suggesting those relationships were only weakly supported by the available data. This could be due in part to the size of the dataset (100 subjects versus 279 in the previous publications, with 20% of smokers and only 2 subjects receiving COBI), but mostly to the design, as the current dataset included routine sampling times and did not allow for a full investigation of the PK. This supports the Bayesian approach used in this study, as it helped consolidate the prior knowledge in particular on the volume of distribution, which was more sensitive to the informativeness of priors, so that we could test the effects of genotypes on clearances. In this study of plasmatic concentrations, we assumed that the effect of COBI on DTG clearance was similar to RTV effect. Although RTV and COBI have comparable inhibitory effects on CYP3A4, they differ in their effect on UGT1A1: RTV acts as an inducer, while COBI has no significant effect (305). Our assumption was based on the finding that COBI coadministration has nevertheless been shown to decrease DTG C_{trough} by 10% on average (430). In any case, the limited number of individuals receiving COBI limits the impact of this covariable on our findings.

Regarding DTG plasma PK, our modelling results confirm the previously demonstrated impact of UGT1A1 metabolizing status (395). A signal was also found for CYP3A5 expressing status during the COSSAC exploration but its effect was small and it was not retained in the final model according to BICc. This aligns with previous studies that did not find an impact of *CYP3A5* genotype on DTG plasma PK (390, 392, 400). Of note, our preliminary analysis also found the *ABCG2* c.421C>A SNP to impact on DTG estimated C_{trough} , with lower concentrations for A carriers. This result as well as the effect of *ABCB1* c.3435C>T on C_{trough} are intriguing, but should be taken with caution as they were not found by the independent modelling study which should be considered as more robust statistically, as it takes into account the uncertainty in predicted C_{trough} .

Interestingly, the effect size of UGT1A1 PM status in our study was close to what has been previously described. Indeed, Chen et al. have reported a 23% lower apparent clearance in the group of IM and PM compared to EM, and 32% lower when comparing PM to EM, while Cindi et al. reported a 26% decrease in clearance in subjects homozygous for *UGT1A1**80 (389, 392). Those limited impacts are generally thought to be non-clinically significant based on previous PK/PD analysis investigating virological and safety endpoint (102) and inconsistent associations between DTG plasma exposure and NPAE (222, 228-231). Aligning with this interpretation regarding efficacy, our analysis showed no association between UGT1A1 metabolizing status and viremia evaluated at low and ultra-low levels.

Intracellular accumulation

Besides DTG plasma PK, our study investigated the intracellular accumulation of DTG and BIC, assessed by the measure of drug intracellular/plasma ratios. One striking result of our analysis was the significant difference in intracellular accumulation between both drugs, with DTG accumulating on average two-times more intracellularly as compared to BIC. To the best of our knowledge, this difference in the characteristics of the two closely related drugs has never been reported before. We hypothesize this might be linked to differences in protein binding. Indeed, comparative studies have demonstrated higher protein binding for BIC compared to DTG (98, 132), and in two separate studies for BIC and DTG, Gelé et al. found unbound plasma fractions of respectively 0.33% and 0.66% (224, 225). Assessing intracellular/unbound plasma drug exposure could confirm this hypothesis. Whether higher NPAE rates with DTG compared to BIC could be linked to these differences is an open question, especially as other variables should be taken into account such as generally higher clinical concentration ranges for BIC compared to DTG, and intrinsic biochemical differences between both components.

Another important finding of our study was the impact of *ABCG2* c.421C>A on DTG and BIC intracellular accumulation. This SNP is considered as one of the few “Highly Important Polymorphisms” by the *International Transporter Consortium* (340). It has been associated to increased systemic exposure for

numerous ABCG2 substrates (332, 341, 342). Our findings of an effect on DTG intracellular drug accumulation but no clear impact on DTG systemic exposure are intriguing. However, this aligns with existing literature in adults that reports that this SNP impacts DTG C_{max} (390, 393) but neither C_{trough} nor AUC (390-392). It also aligns with the contrasting results reported in children, for whom a study found an association with decreased C_{max} and no impact on C_{trough} , AUC and CL/F (391). Of note, similar features were found for RAL in a study, with increases in C_{max} but not C_{trough} for A carriers (388). Interestingly, an experimental study has also demonstrated an absence of impact of ABCG2 deficiency on DTG PK and metabolism in mice (394). Some hypothesis can be proposed to reconcile a decrease in DTG efflux (as suggested by our intracellular accumulation assays) and an impact of the SNP on C_{max} (as found in the literature) but no significant effect on DTG plasma apparent clearance. First, the ABCG2 variant has the potential to affect both the drug intestinal absorption and its distribution, and opposite effects on plasma C_{max} and C_{trough} are possible. Another possibility could be to link those seemingly contradictory features to a possibly more rapid intestinal absorption with defective ABCG2, without impact on the AUC. This might be linked to the known continuous decrease in expression of ABCG2 along the intestine (431): defective ABCG2 could lead to a decrease in intestinal drug efflux in the early absorption phase (when the drug enters the small intestine), impacting C_{max} , but the decrease in ABCG2 expression in more distal intestinal segments could blur the impact of the variant subsequently, enabling compensatory absorption of residual drug. Eventually it is important to remind that a specific impact on C_{max} was not assessed in our

study as per protocol most of our samples were drawn at the end of the dosing interval. This could possibly have impaired the ability of our study to capture an impact on CL/F.

Another interesting finding of our study is the absence of demonstrated effect of the *NR1I2* c.-22-7659C>T SNP on either plasma concentrations or intracellular accumulation ratios. For DTG, this contrasts with a study by Elliot et al. which reported an association of the T allele carriage with increases in AUC and C_{max} , a finding that the authors discussed as surprising as the TT genotype is thought to increase the expression of *NR1I2*, and in turn that of *UGT1A1*, *CYP3A4* and *ABCG2*, thus theoretically leading to decreased DTG concentrations (390). Our results, however, are aligned with other studies that found no impact of this *NR1I2* SNP on different PK parameters (391, 392).

Regarding *ABCB1*, our clinical study confirmed our previous *in vitro* analysis showing that the most frequent polymorphisms of *ABCB1* do not impact on BIC intracellular accumulation (397). Although the result did not withstand correction for multiple testing, the near-significant impact of the c*193A>G SNP in *ABCB1* on in the intracellular accumulation of BIC in univariate analysis certainly deserves some further attention. Indeed, the number of included participants in this analysis was limited as compared to the number of included SNPs, and our untargeted approach was considered as exploratory, deserving to be repeated in independent cohorts. Interestingly, this SNP has already been described as impacting on drug PK, including a

study reporting increased intracellular accumulation of the antiretroviral agent efavirenz in G carriers (432) and a study showing increased RAL C_{max} in G carriers (388). The fact that the SNP showed no effect on DTG accumulation argues for caution, but substrate-specific effects are possible for polymorphisms in transporters.

Pharmacokinetic/Pharmacodynamic evaluation

Detecting viremia at ultra-low-levels, especially under 20 copies/mL, is not uncommon in treated PLWH. This situation is often referred to as cryptic viremia and has been shown unaffected by treatment intensification (173, 177). Detection of higher levels viremia despite treatment, especially in the 20-200 copies/mL range, is a more complex situation. It can in some cases be linked to poor drug adherence, treatment interactions or the presence of resistance associated mutations. In most cases, those factors are absent and the origin remains elusive. These cases can be defined as non-suppressible viremia (NSV) (177). Production of mostly defective viral particles by the activation and clonal expansion of latently HIV-infected cells has generally been considered as its most likely cause (188). Another nonexclusive theory has linked detectable viremia to active replication in sanctuary sites with limited drug exposure (190, 193, 195). One of the important sanctuary sites appears to be lymphatic tissues, including for ISNTIs (193-195, 433, 434). Although this has been previously shown for systemic exposure (191), our PK/PD analysis provides some additional evidence that ULLV and LLV may occur despite adequate treatment exposure, as we found no association

between intracellular drug accumulation ratios or intra-PBMCs drug concentrations and the presence of VL (10-200 copies/mL). However, it remains difficult to extrapolate these findings to all different types of lymphoid cells, and to lymphatic tissues where most of viral replication occurs (435). Indeed, significant differences have been found for antiretroviral concentrations between PBMCs and different compartments of lymphatic tissues (193). However, pharmacogenetic variability in drug transporters is thought to be one of the determinants of intracellular drug also in those tissues, and as such some correlations between concentrations in those compartments are probable.

Our study is not without limitations. As already acknowledged, we focused on a sampling period close to C_{trough} , thus our PK analysis is not comprehensive. Another limitation is that our study included a relatively limited number of patients in the BIC subgroup, limiting our power and affecting our ability to draw firm conclusions for this drug. Regarding BIC, systemic exposure was not assessed, as this requires to build a PK model that was non-existing at the time of this analysis. Regarding the assessment of intracellular drug concentrations and (U)LLV, although the effect of time was accounted into the logistic regression analysis, the limited sample sizes precluded the construction of a more robust model. Eventually, another important limitation regarding our PD analysis is that we only studied one drug, out of a cART regimen consisting in two or three drugs.

In conclusion, studying extensively the PK of DTG and BIC, we confirmed the predominant impact of UGT1A1 metabolizing status on DTG systemic exposure. Studying intracellular drug accumulation, we found that DTG accumulates significantly more in PBMCs than BIC. Drug accumulation was found to be associated with the *ABCG2* c.421C>A, while the SNP showed no impact on DTG systemic exposure evaluation. Eventually, ULLV and LLV appeared independent of intracellular drug accumulation and concentration.

3.2.3.2.2 Supplementary appendix

3.2.3.2.2.1 Supplementary methods

Measurement of drug concentrations

BIC, DTG and their respective deuterated internal standards (IS) BIC 15N-d2 and DTG-d4 were purchased from Toronto Research Chemicals (Ontario, Canada); cabotegravir (CAB) was purchased from Toronto too (DTG-d4 was used for IS).

Methanol and acetonitrile high-performance liquid chromatography (HPLC) grade were purchased from Baker; formic acid and zinc sulfate from Merck. HPLC grade water was produced with Milli-Q water purification system by Millipore (Darmstadt, Germany).

BIC, CAB and DTG measurements were performed by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) using a Xevo TQS-micro tandem quadrupole mass spectrometer instrument fitted with a Z-spray ion source (Waters, Milford, USA).

The instrument was directly coupled to a Waters UHPLC Acquity I-Class system, with an integrated auto sampler thermostated at 10 °C (Waters, Milford, USA). The chromatographic separation was performed on Acquity UPLC® BEC18 1.7µm 2. 1 x 50 mm (Waters) maintained at 40 °C. The 10 µL injected aliquot was eluted at a mobile phase flow rate of 0.5 mL/min.

The mobile phase consisted of water with 0.1% formic acid (eluent A) and acetonitrile (eluent B). Initial eluent composition was 95% A directly followed by a linear 6.0 min ramp to 20% A. The mobile phase returned to 95% A at 6.1 min. Equilibration time was 1.9 min.

Ionization was in the positive ion mode using the following settings: capillary voltage 0.5 kV, cone voltage 30 V, source temperature 150 °C, desolvation temperature 500 °C at a nitrogen flow of approximately 1100 L/h, and collision gas (high-purity argon) pressure $2.8 \cdot 10^{-3}$ bar.

BIC/BIC 15N-d2/CAB/DTG/DTG-d4 were monitored in multiple reaction monitoring mode (MRM) by detecting specific product ions:

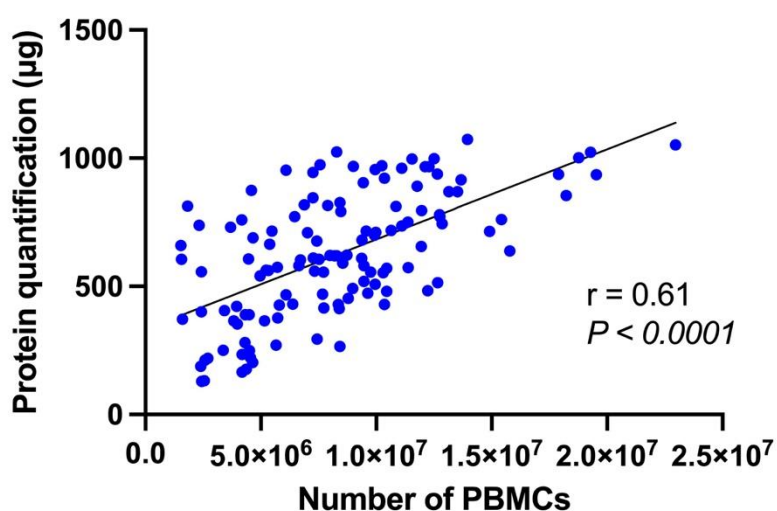
BIC m/z 450.1 > 289.1 (quant); 450.1 > 145 (qual), BIC 15N-d2 453.20 > 289.2, CABO m/z 406.20 > 127.10 (quant) ; 406.20 > 263.10 (qual) , DTG 420.10 > 126.9 (quant) , 420.10 > 277.10 (qual), DTG-d4 424.20 > 279.2. Collision energies were 30/40/35/27/25/30/25/25 for BIC, BIC 15N d2, CAB, DTG and DTG-d4 respectively.

Calibration curve was obtained using the seven calibrators and a blank control in plasma (100, 200, 400, 1000, 2500, 5000, 10000 ng/ml). 100 µL of plasma was pre-treated and vortexed with 300 µL of acetonitrile/waters (50/50) and 400 µL of acetonitrile/ZnSO₄ 0.1 M (2%) (V/V), and 10 µL internal standard (BIC 15N-d2, DTG-d4 (10 µg/L) in methanol).

Samples were vortexed for 1 min, before 5 min of centrifugation at 13500 rpm. A mix of 10 μ L of the supernatant and 90 μ L of waters was injected into the system.

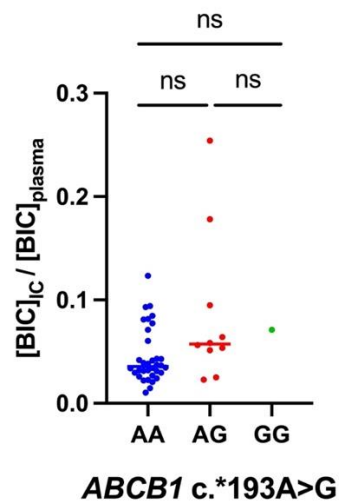
BIC retention time was 2.66 min and 2.64 min for BIC 15N-d2. CAB retention time was 2.38 min. DTG retention time was 2.57 min and 2.56 min for DTG-d4. The method is fully validated in terms of analytical performance (limit of quantification: 100 ng/ml; imprecision < 20%). The laboratory participates to the AQL EEQ Antiviraux/antiretroviraux (Asqualab, Paris, France).

Correlation between PBMCs quantification and protein concentration



3.2.3.2.2.2 Supplementary results

Supplementary figure 1. BIC intracellular/plasma ratios according to ABCB1 c.*193A>G genotype.



Statistical test: one-way ANOVA on log-transformed data with Tukey's test for multiple comparison.

P value for global comparison = 0.053.

$P = 0.055$ for AA vs AG; $P = 0.59$ for AA vs GG; $P = 0.99$ for AG vs GG.

Supplementary table 1. Univariate analysis by one-way ANOVA of the DTG intracellular/plasma ratio for ABCB1, ABCG2 and NR1I2 variants. Both raw and multiple-testing-corrected *P* values, along with effect sizes, are reported. Results are ordered according to corrected *P* values. *P* values < 0.10 are bolded.

Studied variant	<i>P</i> value	<i>P</i> value FDR	Effect size	<i>R</i> ²
ABCG2 c.421C>A	0.01163	0.395473	0.28844	0.0789
ABCB1 c.3320A>C	0.05013	0.765887	0.22575	0.0483
ABCB1 c.*89A>T	0.06758	0.765887	0.26704	0.0676
ABCB1 c.-129T>C	0.09606	0.816537	0.24957	0.0590
ABCG2 c.34G>A	0.7694	0.93053	0.08461	0.0068
ABCB1 c.-440T>C	0.59783	0.93053	0.06149	0.0036
ABCB1 c.-3_-1delinsAGG	0.86972	0.93053	0.06179	0.0036
ABCB1 c.-1A>G	0.17952	0.93053	0.21455	0.0436
ABCB1 c.61A>G	0.88329	0.93053	0.01713	0.0003
ABCB1 c.431T>C	0.76465	0.93053	0.03492	0.0012
ABCB1 c.548A>G	0.48398	0.93053	0.08153	0.0063
ABCB1 c.781A>G	0.48427	0.93053	0.08148	0.0063
ABCB1 c.2506A>G	0.39001	0.93053	0.10005	0.0095
ABCB1 c.3262G>A	0.4969	0.93053	0.07915	0.0059
ABCB1 c.3421T>A	0.87821	0.93053	0.05961	0.0034
ABCB1 c.*21T>C	0.69705	0.93053	0.09922	0.0093
ABCB1 c.*79_*82del	0.89566	0.93053	0.05491	0.0029
ABCB1 c.*146G>A	0.89566	0.93053	0.05491	0.0029
ABCB1 c.*165-*172dup	0.69705	0.93053	0.09922	0.0093
ABCB1 c.*193A>G	0.55732	0.93053	0.12609	0.0151
ABCB1 c.*252A>C	0.59391	0.93053	0.06215	0.0037
ABCB1 c.*316G>A	0.67309	0.93053	0.0492	0.0023
ABCB1 c.*355T>C	0.24882	0.93053	0.13398	0.0170
ABCB1 c.1199G>A	0.60407	0.93053	0.06045	0.0035
ABCB1 c.1236T>C	0.84806	0.93053	0.06713	0.0043
ABCB1 c.2677T>A	0.74871	0.93053	0.03736	0.0013
ABCB1 c.2677T>G	0.92905	0.93053	0.04489	0.0019
ABCB1 c.3435T>C	0.24842	0.93053	0.1936	0.0355
NR1I2 c.-1135C>T	0.93053	0.93053	0.0444	0.0019

Studied variant	<i>P</i> value	<i>P</i> value FDR	Effect size	<i>R</i> ²
NR1I2 c.-1663T>C	0.69005	0.93053	0.10059	0.0096
NR1I2 c.-22-7659C>T	0.6304	0.93053	0.11211	0.0119
NR1I2 c.-23+3234G>A	0.37485	0.93053	0.16294	0.0252
NR1I2 c.795-93G>A	0.58607	0.93053	0.12059	0.0138
NR1I2 c.938-17C>T	0.77135	0.93053	0.0842	0.0067

The response variable was log-transformed prior to analysis.

Abbreviations: FDR: false discovery rate.

Supplementary table 2. Univariate analysis by one-way ANOVA of the BIC intracellular/plasma ratio for ABCB1, ABCG2 and NR1I2 variants. Both raw and multiple-testing-corrected *P* values, along with effect sizes, are reported. Results are ordered according to corrected *P* values. *P* values < 0.10 are bolded.

Studied variant	<i>P</i> value	<i>P</i> value FDR	Effect size	<i>R</i> ²
ABCG2 c.421C>A	0.05567	0.37716	0.27774	0.0825
ABCB1 c.-1A>G	0.09639	0.37716	0.24257	0.0630
ABCB1 c.3421T>A	0.09875	0.37716	0.31234	0.1044
ABCB1 c.*21T>C	0.06219	0.37716	0.27096	0.0786
ABCB1 c.*165-*172dup	0.06219	0.37716	0.27096	0.0786
ABCB1 c.*193A>G	0.05287	0.37716	0.34941	0.1306
NR1I2 c.-22-7659C>T	0.08016	0.37716	0.32531	0.1132
NR1I2 c.795-93G>A	0.11175	0.37716	0.30432	0.0991
ABCB1 c.-129T>C	0.33625	0.70334	0.14182	0.0215
ABCB1 c.*89A>T	0.33865	0.70334	0.21672	0.0503
ABCB1 c.*252A>C	0.32002	0.70334	0.1466	0.0230
ABCB1 c.1236T>C	0.29058	0.70334	0.23111	0.0572
ABCB1 c.3435T>C	0.33251	0.70334	0.21849	0.0511
ABCB1 c.*79_*82del	0.4107	0.739256	0.19691	0.0415
ABCB1 c.1199G>A	0.39415	0.739256	0.12582	0.0169
ABCB1 c.61A>G	0.49981	0.781739	0.09979	0.0107
ABCB1 c.548A>G	0.60246	0.781739	0.07711	0.0064
ABCB1 c.3320A>C	0.69603	0.781739	0.05788	0.0036

Studied variant	P value	P value FDR	Effect size	R ²
ABCB1 c.*146G>A	0.48418	0.781739	0.17812	0.0339
ABCB1 c.*316G>A	0.68002	0.781739	0.0611	0.0040
ABCB1 c.2677T>A	0.56145	0.781739	0.08594	0.0079
ABCB1 c.2677T>G	0.72383	0.781739	0.11947	0.0153
NR1I2 c.-1663T>C	0.70701	0.781739	0.1237	0.0164
NR1I2 c.-23+3234G>A	0.63025	0.781739	0.14255	0.0217
NR1I2 c.938-17C>T	0.57073	0.781739	0.15693	0.0264
ABCG2 c.34G>A	0.89968	0.934278	0.01869	0.0004
NR1I2 c.-1135C>T	0.94184	0.941844	0.0516	0.0028

The response variable was log-transformed prior to analysis.

Supplementary Table 3. Association between viral load status and selected genotypes for DTG.

	Undetectable viremia	LLV or ULLV detected	P value
UGT1A1			
EM/IM	53 (85)	12 (86)	>0.99
PM	9 (15)	2 (14)	
ABCG2			
c.421C>A	50 (81)	11 (79)	>0.99
CC	12 (19)	3 (21)	
CA			
NR1I2			
c.-22-7659C>T	8 (13)	2 (14)	0.77
CC	30 (48)	8 (57)	
CT	24 (39)	4 (29)	
TT			

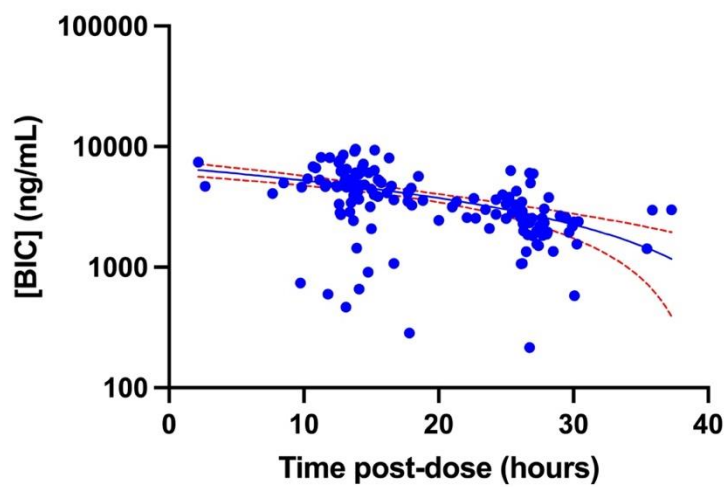
Supplementary Table 4. Association between viral load status and selected genotypes for BIC.

	Undetectable viremia	LLV or ULLV detected	P value
UGT1A1			0.17
EM/IM	29(76)	1 (33)	
PM	9 (24)	2 (67)	
ABCG2			
c.421C>A	30 (79)	2 (67)	0.54
CC	8 (21)	1 (33)	
CA			
NR1I2			
c.-22-7659C>T	8 (21)	2 (67)	0.17
CC	14 (37)	0 (0)	
CT	16 (42)	1 (33)	
TT			

3.2.3.2.3 Additional material

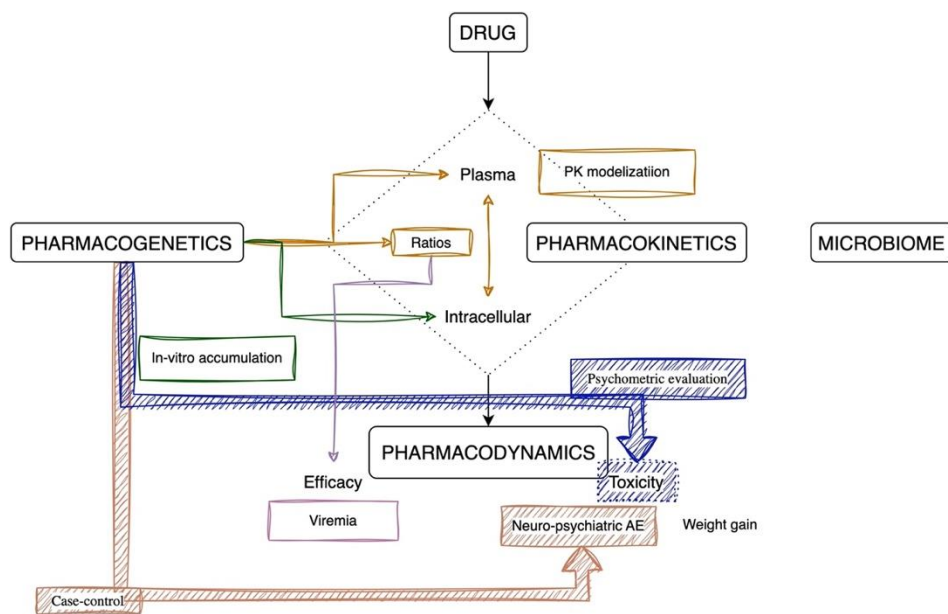
Plasma BIC PK

Additional Figure. BIC plasma concentrations measured within the INSTI-01 study, plotted over time.



Simple linear regression equation : $Y = -149,2 \cdot X + 6733$

3.2.3.3 Dolutegravir and Risk of Neuropsychiatric Adverse Events: A Pharmacogenetic Study



Key points

- Using a case-control design, we found a protective effect of the c.-22-7659C>T variant in *NR1I2* (the PXR-encoding gene) against DTG discontinuation due to NPAEs.
- Moreover, this variant was linked to decreased anxiety scores in control-group patients on DTG.

3.2.3.3.1 Article

The Journal of Infectious Diseases

BRIEF REPORT

Infectious Diseases Society of America

HIV Medicine Association

OXFORD

Dolutegravir and Risk of Neuropsychiatric Adverse Events: A Pharmacogenetic Study

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Dolutegravir (DTG) treatment can lead to neuropsychiatric adverse events (NPAEs). This study assessed the association between NPAEs and polymorphisms in DTG-related pharmacogenes, determined by next-generation sequencing panel testing. Using a case-control design, 36 patients having previously discontinued DTG due to NPAEs were compared to 98 patients tolerating DTG. In the latter group, psychometric scores were compared according to genotype, targeting polymorphisms associated with drug intolerance. *NR1I2* c.-22-7659C>T was independently associated with a reduced risk of NPAE-related DTG discontinuation (odds ratio, 0.36 [95% confidence interval, .15–.88] for T-variant allele carriage) and was linked to decreased anxiety scores in control group participants.

Keywords. HIV; pregnane X receptor; UGT1A1; SLC22A2; integrase inhibitor.

Dolutegravir (DTG) is a second-generation integrase strand transfer inhibitor (INSTI) that has become a cornerstone of human immunodeficiency virus (HIV) treatment. Several reports highlighted its propensity to cause neuropsychiatric adverse events (NPAEs), which can significantly impact quality of life

of people living with HIV and may lead to drug discontinuation. Underlying mechanisms remain poorly understood. An association with plasma concentrations remains debated [1–4], and studies investigating the impact of pharmacogenetic polymorphisms have also reported inconclusive results, especially regarding uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*), the most important DTG biotransformation enzyme [2, 3]. Besides plasma concentrations, other pharmacokinetic parameters, such as central nervous system (CNS) accumulation of DTG or its metabolites, could potentially underpin NPAEs. Surprisingly, the association between NPAEs and polymorphisms in the genes encoding ATP-binding cassette subfamily B member 1 (ABCB1) and subfamily G member 2 (ABCG2) has never been studied, despite both being DTG efflux transporters expressed at the blood-brain barrier (BBB) [5]. A study also suggested a possible implication of the inhibiting effect of DTG on SLC22A2, involved in neurotransmitter transport, and found an association between neuropsychiatric symptoms and the *SLC22A2* c.808C>A polymorphism, but this has not been confirmed [6, 7].

Considering those knowledge gaps, this study aims to determine the association between NPAE-related DTG discontinuation and selected pharmacogenetic polymorphisms.

MATERIALS AND METHODS

Study Design and Setting

This study is part of a phase 4 study aiming to understand the interindividual variability in clinical and biological responses to INSTIs. In brief, the parent study sought to recruit 200 participants from different subgroups: 50 bicitegravir-treated (group A), 100 DTG-treated (group B), and 50 patients having previously discontinued DTG due to NPAEs (group C). Details regarding sample size calculation and the study protocol are provided in the [Supplementary Appendix](#). Participants were recruited during their regular follow-up at the Saint-Luc University Hospital HIV/AIDS clinic (Brussels, Belgium), which serves a diverse patient population, predominantly White or of African descent. After providing informed consent, all participants were genotyped and measures were performed during follow-up, including psychometric evaluations for groups A and B. This manuscript reports the results of a pharmacogenetic case-control study comparing participants from groups B and C, and psychometric score distribution across genotypes for group B. The study ([ClinicalTrials.gov](#) identifier NCT04805944) received approval by the local ethics committee (2020/30NOV/593).

Inclusion Criteria

Cases were participants who previously discontinued DTG due to any NPAE, while controls were participants receiving DTG

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*V. H. and L. Be. contributed equally to this work.

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<https://doi.org/10.1093/infdis/jiaf098>

Table 1. General Characteristics of Cases and Controls

Characteristic	Cases (n = 36)	Controls (n = 98)	P Value
Age, y, mean ± SD	50.7 ± 11.1	50.5 ± 11.7	.90
Female sex	13 (36)	38 (39)	.78
BMI, kg/m ² , median (IQR)	26.5 (24.4–31.1)	26.9 (24.2–30.4)	.86
CD4 count, cells/μL, median (IQR)	763 (604–970)	693 (533–867)	.32
Nadir CD4 count ^a , cells/μL, median (IQR)	221 (143–393)	239 (124–348)	.92
Past AIDS diagnosis ^b	14 (41)	46 (47)	.53
HIV RNA <40 copies/mL	36 (100)	97 (99)	1
Ethnicity			1
White	22 (61)	56 (57)	
Sub-Saharan African	13 (36)	36 (37)	
Tobacco use ^c	8 (22)	19 (20)	.74
Use of sleep medications at DTG initiation ^{d,e}	3 (9)	14 (14)	.56
Use of antidepressants at DTG initiation ^e	2 (6)	7 (7)	1
Use of neuroleptics at DTG initiation ^e	0 (0)	2 (2)	1

Data are presented as No. (%) at study inclusion, unless otherwise indicated.

Abbreviations: BMI, body mass index; DTG, dolutegravir; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation.

^aUnavailable for 1 control.

^bUnavailable for 2 cases and 1 control.

^cUnavailable for 3 cases and 3 controls.

^dBenzodiazepines, Z-drugs, melatonin, *Valeriana officinalis*.

^eUnavailable for 1 case.

without discontinuation for NPAEs during follow-up. Cases were identified from our preexisting institutional database recording HIV and antiretroviral therapy-related data, including dates and reasons for treatment changes. Registered cases of DTG discontinuation due to NPAEs were thoroughly reviewed to assess the plausibility of drug-induced toxicity (further details in the [Supplementary Appendix](#)), considering time relationship to drug intake, existence of plausible alternative explanations, response to drug withdrawal, and effect of rechallenge (when performed). DTG-treated participants initially categorized as controls for whom DTG was discontinued due to NPAEs during the study follow-up were reclassified as cases, following the same procedure. Importantly, causality assessment was performed blinded to the pharmacogenetic results.

Pharmacogenomic Sample Collection, DNA Extraction, and Genotyping

Whole blood was collected from participants in an ethylenediaminetetraacetic acid tube and stored at 4°C until DNA extraction using the QIAamp DNA blood kit. Next-generation sequencing-based pharmacogenomic testing was performed using a 41-gene panel test (SOPHiA DDM) on an Illumina MiSeq platform. Additionally, *SLC22A2* c.808C > A (rs316019) genotyping was performed with TaqMan assay on the StepOnePlus reverse-transcription polymerase chain reaction system (Applied Biosystems). The selection process of studied candidate genes and polymorphisms is described in the [Supplementary Appendix](#). The most frequent *UGT1A1* polymorphisms are characterized by the number of thymine-adenine (TA) repetitions in the promoter region, with the reference allele *UGT1A1**1

containing 6 repeats (TA₆). All samples containing *36 (TA₅) and *37 (TA₈) alleles in addition to *1 or *28 (TA₇) alleles were confirmed by high-resolution melting analysis and Sanger sequencing. Additionally, *28 and *37 alleles were confirmed using the *UGT1A1**80 (rs887829) proxy.

Psychometric Evaluation

Four standardized questionnaires (Pittsburgh Sleep Quality Index, Pichot Fatigue Questionnaire, Hospital Anxiety and Depression Scale [HADS], and Symptom Checklist-90-R; see details in the [Supplementary Appendix](#)) were used for controls to assess if a polymorphism that would be associated with NPAE-related discontinuation of DTG could also be associated with adverse events not leading to drug discontinuation.

Statistical Analysis

Continuous variables were compared between cases and controls using Student *t* test or Wilcoxon test, and proportions were compared using χ^2 or Fisher exact test, as appropriate. Risk factors for NPAE-related DTG discontinuation were identified by stepwise multivariable logistic regression. Variables with *P* value <.10 in univariate analysis were considered for possible inclusion in the model. Odds ratios were derived using stepwise variable forward selection with backward elimination retaining only variables with *P* <.05 in the final model. For the psychometric evaluation, proportions of participants with pathological scores were compared across groups and the ranks of scores were compared across genotypes using the Kruskal-Wallis test, with Dunn test for multiple comparisons. Statistical analyses were performed using JMP Pro 17.1.0 software.

Table 2. Genotype Distribution of Cases and Controls for Selected Pharmacogenes

Genotype	All (n = 134)	Controls (n = 98)	Cases (n = 36)	P Value
<i>UGT1A1</i> ^a				.98
Extensive metabolizer	66 (49)	48 (49)	18 (50)	
Intermediate metabolizer	52 (39)	38 (39)	14 (39)	
Poor metabolizer	16 (12)	12 (12)	4 (11)	
<i>CYP3A4</i> *22 (rs35599367)				1
—/—	121 (90)	88 (90)	33 (92)	
—/*22	12 (9)	9 (9)	3 (8)	
*22/*22	1 (1)	1 (1)	0 (0)	
<i>CYP3A5</i> ^b				.88
Expressor	47 (35)	34 (35)	13 (36)	
Nonexpressor	87 (65)	64 (65)	23 (64)	
<i>ABCB1</i> c.3435C > T (rs1045642)				.96
CC	68 (51)	49 (50)	19 (53)	
CT	50 (37)	37 (38)	13 (36)	
TT	16 (12)	12 (12)	4 (11)	
<i>ABCB1</i> c.1199G > A (rs2229109)				1
GG	127 (95)	93 (95)	34 (94)	
GA	7 (5)	5 (5)	2 (6)	
<i>ABCG2</i> c.421C > A (rs2231142)				.96
CC	112 (84)	82 (84)	30 (83)	
CA	22 (16)	16 (16)	6 (17)	
<i>NR1I2</i> c.-22-7659C > T (rs2472677)				.046
CC	27 (20)	15 (15)	12 (33.5)	
CT	62 (46)	46 (47)	16 (44.5)	
TT	45 (34)	37 (38)	8 (22)	
<i>POR</i> *28 (rs1057868)				.90
—/—	83 (62)	60 (61)	23 (64)	
—/*28	40 (30)	29 (30)	11 (30.5)	
*28/*28	11 (8)	9 (9)	2 (5.5)	
<i>SLC22A2</i> c.808C > A SNP (rs316019)				.78
CC	102 (76)	74 (76)	28 (78)	
CA	32 (24)	24 (24)	8 (22)	

Data are presented as No. (%) of subjects.

Abbreviation: SNP, single-nucleotide polymorphism.

^aClassified according to Clinical Pharmacogenetics Implementation Consortium classification for *UGT1A1* genotype-predicted phenotypic function: extensive metabolizer (*1 or *36)/(+1 or +36), intermediate metabolizer (*1 or +36)/(+28 or +37), poor metabolizer (*28 or +37)/(+28 or +37). No participant displayed the *6 allele.

^bExpressor participants display at least 1 copy of a normal function allele: +1/+1 or +3 or +6 or +7; nonexpressor participants have 2 copies of a no function allele: (+3 or +6 or +7)/(+3 or +6 or +7).

RESULTS

A total of 137 participants were recruited in the study with 36 cases and 98 controls included in the case-control analysis (see flow-chart in [Supplementary Appendix](#)). Participants' characteristics are described in [Table 1](#). All participants were successfully genotyped. Genotype distribution is reported in [Table 2](#). All polymorphisms were in Hardy-Weinberg equilibrium, including for *CYP3A5* and *UGT1A1* after ethnic origin stratification.

Sleep-related disorders were the most prevalent NPAEs leading to DTG discontinuation ([Supplementary Table 1](#)). Cases had discontinued DTG due to NPAEs at a median of 16.5 (interquartile range [IQR], 7–43) months after its initiation. Median DTG treatment duration for controls, censored at the end of study follow-up, was 70 (IQR, 27.5–84) months. The most frequent DTG-containing regimen at the time of

discontinuation was DTG/abacavir/lamivudine, received by 21 cases (58%) (regimens are detailed in [Supplementary Table 2](#)).

In univariate analysis, we found a balanced distribution of all studied polymorphisms, with the exception of the *NR1I2* c.-22-7659C > T single-nucleotide polymorphism (SNP) ($P = .046$) ([Table 2](#)). The multivariable analysis, considering genetic polymorphisms, sex, ethnicity, and sleep, antidepressant, and antipsychotic medication use at DTG initiation, identified *NR1I2* c.-22-7659C > T as the sole variable independently associated with a lower risk of NPAE-related DTG discontinuation, with an odds ratio for T-allele carriers of 0.36 (95% confidence interval, .15–.88; $P = .02$).

A total of 63 (64%) controls provided valid answers to at least 1 questionnaire, and 56 (57%) to all questionnaires. A gradient was found in proportions of controls with an HADS-anxiety

score of 8 or more based on *NR1I2* genotype, with 4 of 9 (44%) for CC, 8 of 31 (26%) for CT, and 2 of 22 (9%) for TT carriers ($P = .07$; Supplementary Table 3). Furthermore, a significant difference was found in HADS-anxiety score distribution based on *NR1I2* c.-22-7659C > T ($P = .037$), with TT carriers displaying lower scores compared to CT ($P = .079$) and CC ($P = .118$) (Supplementary Figure 2). No significant difference was found for the distribution of other scores based on the *NR1I2* c.-22-7659C > T genotype (Supplementary Figures 3–15).

DISCUSSION

This pharmacogenetic case-control study investigated risk factors for NPAE-related DTG discontinuation. The *NR1I2* c.-22-7659C > T variant was independently associated with a protective effect against DTG discontinuation due to NPAEs. Notably, none of the other studied polymorphisms were associated with this outcome, despite several of them being associated with DTG plasma pharmacokinetics. Among control participants tolerating DTG, the same *NR1I2* variant was associated with reduced anxiety, as measured by the HADS-anxiety scale, further supporting the plausibility of this association.

NR1I2 is a key pharmacogene encoding the pregnane X receptor (PXR). In brief, this nuclear receptor is activated by various endogenous and exogenous ligands and regulates the expression of several genes involved in drug pharmacokinetics. Among others, PXR upregulates CYP3A4 and UGT1A1, responsible for DTG biotransformation, as well as ABCB1 and ABCG2 [5], two transporters that can limit DTG absorption through intestinal epithelium apical expression.

The precise effect of the *NR1I2* c.-22-7659C > T SNP on drug metabolism remains uncertain. The TT genotype has been associated with increased basal CYP3A4 expression, but the CC genotype has been associated with enhanced rifampin induction [8]. Regarding antiretrovirals, studies have demonstrated that TT carriers display a reduced systemic exposure to unboosted atazanavir [9], a drug primarily metabolized by CYP3A4 and transported by ABCB1. TT carriers also displayed a reduced risk of efavirenz discontinuation [10], a drug mainly metabolized by CYP2B6 and transported by ABCB1. The specific effect of this SNP on DTG pharmacokinetics remains unknown. DTG is a moderate PXR activator, and clinical studies have provided inconclusive results: an adult trial reported a modest increase in DTG exposure associated with the SNP [11], but a large study of South African adults found no effect of the SNP [12].

Our results align with several reports that challenged the idea that NPAEs are directly linked to DTG plasma concentrations. This simplified vision has been mainly supported by a study reporting higher DTG trough concentrations in patients with NPAEs compared to those without. That study also found an increased cumulative incidence of NPAEs in carriers of *UGT1A1**6, *UGT1A1**28, or both alleles [3]; however, these

results were not confirmed by other pharmacokinetic or pharmacogenetic studies [1, 2, 4, 7]. Our results further contradict this view as we found no association between DTG discontinuation due to NPAEs and genotypes linked with a decreased UGT1A1 function, despite substantial evidence of their impact on plasma exposure [7]. Moreover, we observed no effect of the *ABCG2* c.421C > A SNP, which has been previously associated with increased DTG peak concentration [7].

Other mechanisms could potentially explain the protective role of *NR1I2* c.-22-7659C > T against NPAEs. First, the SNP might reduce DTG accumulation in the CNS. Indeed, both ABCB1 and ABCG2 are expressed at the BBB, where their expression can be upregulated by PXR [5]. Thus, *NR1I2* c.-22-7659C > T could exert protective effects through PXR activation and, consequently, transporter overexpression [11]. Second, CNS toxicity might not be mediated by the parent drug, but rather by DTG metabolites. Zhu and colleagues have demonstrated that CYP1A1 and CYP1B1 play key roles in the formation of important DTG aldehyde metabolites [13]. As both CYPs are expressed in the brain and given the toxicity of aldehydes, the authors proposed that these pathways could be involved in neurotoxicity. If this hypothesis holds, *NR1I2* polymorphism could thus impact on toxicity either by an upstream influence on DTG CNS concentration, or by exerting regulatory effects on those biotransformation pathways. Finally, another hypothesis could be that *NR1I2* c.-22-7659C > T protects against neuropsychiatric manifestations independently of DTG. Indeed, PXR is expressed at the BBB as well as in specific brain regions, and its role in brain function has recently gained interest [14]. A recent study found that PXR-deficient mice exhibited increased anxiety-like behaviors and impaired recognition memory [15], an interesting finding in the light of our results, which showed lower HADS-anxiety scores in individuals homozygous for the *NR1I2* c.-22-7659C > T SNP.

Our study has several limitations. First, due to its design, we were unable to assess drug exposure in patients having discontinued DTG due to NPAEs. Second, our analysis focused on selected polymorphisms, leaving the possibility that unexplored pharmacogenetic factors may contribute to the pathogenesis of NPAEs. Third, the level of evidence from our questionnaire analysis should not be overestimated. Although the decision was made a priori to limit this analysis to SNP(s) associated with NPAE-related DTG discontinuation in the case-control analysis, the possibility of a type I error cannot be excluded. Finally, our study did not reach the initially expected sample size. Nevertheless, we believe that the homogenous distribution across cases and controls of all studied SNPs (with the notable exception of *NR1I2* c.-22-7659C > T) provides a strong indication that those are not clinically relevant risk factors for NPAEs.

In conclusion, among a selection of relevant polymorphisms in pharmacogenes influencing DTG transport and metabolism, only *NR1I2* c.-22-7659T carriage was associated with protection

against NPAA-related DTG discontinuation. Our findings confirm that DTG systemic exposure is not a key determinant of NPAEs. Further research is needed to clarify the interactions between *NR112*, DTG, and brain function.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. J. D. G., V. H., and L. Be. conceived and designed the research. P. d. T. provided assistance for designing the psychometric evaluation. J. D. G., J. C. Y., A. V., B. V., and L. Be. recruited and took care of the participants. J. D. G., L. Bo., M. P., N. P., and V. H. performed the biological sample processing and genotyping. J. D. G. and L. E. performed the statistical analysis. J. D. G. wrote the initial draft of the manuscript. V. H. and L. Be. supervised the manuscript writing. All authors critically reviewed the manuscript.

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Potential conflicts of interest. J. D. G. has received speaker's fees from ViiV Healthcare and travel support from MSD. B. V. has received speaker's fees from Pfizer, travel support from ViiV Healthcare, and advisory board fees from Pfizer. L. E. holds stocks in Pfizer. L. Be. has received consulting fees from MSD and travel support from GlaxoSmithKline and Gilead Sciences. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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3.2.3.3.2 Full manuscript

3.2.3.3.2.1 Abstract

Background: Dolutegravir (DTG) treatment in people living with HIV can lead to neuropsychiatric adverse events (NPAE), which may cause drug discontinuation. The etiology of these events remains unclear. This study aimed to assess the association between NPAE and polymorphisms in key DTG-related pharmacogenes.

Methods: In this single-centre pharmacogenetic study, patients having previously discontinued DTG due to NPAE and patients with ongoing DTG treatment were included. All participants underwent next-generation sequencing pharmacogenomic panel testing and both groups were compared using a case-control design for UGT1A1 genotype-based predicted phenotype, CYP3A5 expression, and selected genotypes for other relevant pharmacogenes (*CYP3A4*, *ABCB1*, *ABCG2*, *NR1I2*, *POR*). Four validated psychometric questionnaires were used in patients receiving DTG, and scores were compared according to genotype, targeting polymorphisms associated with NPAE-related DTG discontinuation.

Results: 36 cases and 98 controls were included. Sleep-related issues were the primary reason for DTG discontinuation. Only the c.-22-7659C>T (rs2472677) variant in *NR1I2*, encoding Pregnane X Receptor (PXR), was found to be independently associated with a reduced risk of NPAE-related DTG discontinuation, with the T-variant allele carriage associated with a reduced chance (odds ratio, 0.36 [95% confidence interval, .15–.88]).

Moreover, this variant was linked to decreased anxiety scores in control-group patients on DTG.

Conclusions: *NR1I2* c.-22-7659C>T was associated with a reduced risk of NPAE-related DTG discontinuation and lower anxiety in patients apparently tolerating DTG. As PXR is a key regulator of DTG phase I/II metabolism and transport, further research is needed to explore the interactions between PXR, DTG and brain function.

3.2.3.3.2.2 Introduction

Dolutegravir (DTG) is a second-generation HIV integrase strand transfer inhibitor (INSTI) that has become a cornerstone of HIV treatment due to its high efficacy combined to a low toxicity, as demonstrated in pivotal trials (436). However, several reports have highlighted the propensity of the drug to cause neuropsychiatric adverse effects (NPAE) such as insomnia, anxiety and depression, which can significantly impact quality of life of people living with HIV (PLWH) and hamper drug compliance (437). The median rate of DTG discontinuation due to NPAE across studies has been reported at 2.8% (1.4-3.7%) (207). Mechanisms underpinning these adverse events remain poorly understood. An association between DTG plasma concentration and NPAE was suggested by some authors (222), but has been debated (228, 229). Studies investigating the association of pharmacogenetic polymorphisms with the occurrence of NPAE have also reported inconclusive results, especially regarding the importance of polymorphisms of uridine diphosphate (UDP)-glucuronosyltransferase 1A1 (*UGT1A1*), the most

important DTG biotransformation enzyme (222, 229). Besides DTG plasma concentrations, other pharmacokinetic parameters such as central nervous system (CNS) accumulation of the drug or its metabolites could potentially impact the occurrence of NPAE. Surprisingly, the association between NPAE and polymorphisms in the genes encoding ATP-binding cassette subfamily B member 1 (ABCB1, also called P-glycoprotein) and ATP-binding cassette subfamily G member 2 (ABCG2, also called Breast Cancer Resistance Protein) has never been studied, despite both being active efflux transporters of DTG expressed at the blood-brain barrier and limiting the access of xenobiotics to the CNS (105, 353).

Considering these uncertainties, this study aims to determine the association between NPAE-related DTG discontinuation and selected pharmacogenetic polymorphisms potentially impacting DTG transport and metabolism.

3.2.3.3.2.3 Material and Methods

Study Design and Setting

This study is part of a phase IV study aiming to understand the interindividual variability in clinical and biological responses to INSTIs. Briefly, the parent study sought to recruit 200 participants from three different subgroups: (1) 50 bicitgravir (BIC)-treated patients, (2) 100 DTG-treated patients and (3) 50 patients having previously discontinued DTG due to the occurrence of NPAE (the study protocol is provided in the **Supplementary Appendix**). Participants were recruited during their regular HIV follow-up at the

HIV/AIDS Reference Center of Saint-Luc University Hospital in Brussels, Belgium, which serves a diverse patient population, predominantly of Caucasian and African descent. After providing informed consent, all participants were genotyped and different measures were performed during follow-up, including psychometric evaluations using standardized questionnaires for participants receiving DTG or BIC. This manuscript reports the results of a pharmacogenetic case-control study in which DTG-receiving participants were compared to participants having discontinued DTG due to NPAE. Psychometric scores distribution across genotypes is also reported for participants tolerating DTG. The study (NCT04805944) received approval by the local Ethical committee (2020/30NOV/593) and regulatory authorities (Eudra-CT Number 2020-001103-17).

Inclusion Criteria

Cases were defined as participants having discontinued DTG due to the occurrence of any NPAE, and controls as participants receiving DTG and for which this drug was not discontinued due to NPAE during the study follow-up. Cases were identified from our pre-existing institutional database recording the main HIV and antiretroviral therapy-related data of patients in follow-up, including dates and reasons for treatment changes, classified by systems. All registered cases of DTG discontinuation due to NPAE were thoroughly reviewed by a physician to assess the plausibility of drug-induced toxicity, considering factors such as symptoms reversibility after DTG discontinuation. Doubtful cases were independently reviewed by a second

physician. Importantly, both investigators were blinded to the pharmacogenetic results during their assessment. Participants for whom both investigators deemed the likelihood of DTG being the cause of NPAE as low were not included in the study analysis, and others were categorized as cases. DTG-treated participants were recruited consecutively during routine HIV care and were initially categorized as controls. However, if DTG was discontinued due to NPAE during the study follow-up, and the plausibility of drug toxicity was confirmed following the method described above, they were reclassified as cases.

Candidate Genes, Polymorphisms and Genotype-based predicted Phenotypes

We studied polymorphisms in the genes of the main DTG biotransformation enzymes, namely *UGT1A1*, *cytochrome P450 (CYP) 3A4*, and of transport proteins *ABCB1* and *ABCG2*. *CYP3A5* was also considered due to its potential role in DTG metabolism. Moreover, P450 oxidoreductase (POR), a critical CYP enzymes cofactor, was considered, as well as the Nuclear Receptor Subfamily 1 Group I Member 2 (NR1I2, also called Pregnane X Receptor, PXR), a key pharmacogenetic transcription factor. For these pharmacogenes, a selection of polymorphisms was analysed based on previous associations with DTG pharmacokinetics (222, 389-393), or previous strong evidence of association with drug-phenotypes: *UGT1A1**28, *36 and *37 (rs3064744); *UGT1A1**6 (rs4148323); *CYP3A4**22 (rs35599367); *CYP3A5**3 (rs776746); *CYP3A5**6 (rs10264272); *CYP3A5**7 (rs41303343); *ABCB1* c.1199G>A (rs2229109) and c.3435C>T (rs1045642); *ABCG2* c.421C>A (rs2231142); *NR1I2* c.-22-7659C>T

(rs2472677); *POR**28 (rs1057868); *SLC22A2* c.808C>A SNP (rs316019) (231, 282, 287, 298, 422). On the basis of the low expected frequencies of *UGT1A1**36 and *UGT1A1**37 and their known effects on *UGT1A1* expression, and in accordance with the Clinical Pharmacogenetics Implementation Consortium classification for *UGT1A1* genotype-based predicted phenotypic function (423), these polymorphisms were grouped with *1 and *28 alleles, respectively. This provided a 3-level ordered genotype: extensive metabolizers (*1/*1; *1/*36; *36/*36), intermediate metabolizers (*1/*28; *1/*37; *36/*28; *36/*37; *1/*6) and poor metabolizers (*28/*28; *28/*37; *37/*37; *6/*6). Regarding *CYP3A5* genotype, participants were classified as *CYP3A5* expressors if they carried at least one *CYP3A5**1 allele, and all other allele combinations as *CYP3A5* non-expressors.

Sample Size and a priori Calculation of Expected Odds Ratios

As we anticipated that maximizing the number of cases would enhance the robustness and generalizability of our findings, our aim was to include the largest possible number of potential cases identified at our centre. The inclusion of 100 controls was planned based on requirements for the pharmacokinetic-pharmacogenomic analysis of the parent study. Although a formal sample size calculation was not performed, we computed expected odds ratios (ORs) *a priori* based on anticipated recruitment numbers and a range of plausible expected genotype frequencies based on the selected candidate genetic polymorphisms and their frequencies in a diverse ethnic population. These calculations were conducted to ensure that the study

would have sufficient power to detect clinically significant associations, if present. Based on a projected number of 50 cases and 100 controls, and considering a genotype frequency among controls of 50%, the study would enable to detect true ORs for the association between genotype and risk-phenotype of 2.6 for variant genotype carriers relative to wild-type subjects with a power equal to 0.8 and type I error probability equal to 0.05. For a probability of variant genotype carriers among controls of 20%, the study would detect true ORs for the association between genotype and risk-phenotype of 3.25. Those computed ORs were considered clinically relevant, with a magnitude of effect that could justify a pre-therapeutic screening for genetic variant(s) associated with NPAEs.

Pharmacogenomic Sample Collection, DNA Extraction and Genotyping

Whole blood was collected from included participants in an EDTA tube. Samples were stored at +4°C until DNA extraction using the QIAamp® DNA blood kit according to manufacturer's instructions. Next-generation sequencing (NGS) pharmacogenomic testing was then performed using a 41-gene panel test (SOPHiA DDM® for Pharmacogenomics), on an Illumina MiSeq® platform. For *UGT1A1*, the most frequent polymorphisms are characterised by a variation in the number of TA repetitions in the promoter region, with *UGT1A1**1, containing 6 repeats (TA₆), as the reference allele. To confirm *UGT1A1* genotyping unambiguously, all samples containing *36 (TA₅) and *37 (TA₈) alleles in addition to the common *1 (TA₆) or *28 (TA₇) alleles were confirmed by a validated high-resolution melting analysis

method and by Sanger sequencing. Additionally, all *28 (TA₇) and *37 (TA₈) alleles were also confirmed using the *UGT1A1**80 (rs887829) proxy.

Psychometric Evaluation

Standardized questionnaires were used for control patients to assess if a polymorphism that would be associated with DTG discontinuation due to NPAE could also be associated with adverse events not leading to drug discontinuation. Four questionnaires were used: (1) the Pittsburgh Sleep Quality Index (PSQI) (438); (2) the Pichot fatigue Questionnaire (439); (3) the Hospital Anxiety and Depression Scale (HADS) (440); and (4) the Symptom Checklist (SCL)-90-R exploring 10 psychiatric dimensions (somatization, obsessive–compulsive disorder, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism and sleep disorders) (441). Questionnaires were delivered either electronically using the Redcap® platform (442) or on paper forms, according to participants' preference. For PSQI, Pichot and HADS, only questionnaires for which all items had been completed were included in the analysis. For SCL-90-R, according to the questionnaire manual (441), subscales were included in the analysis provided there was no more than one missing answer, and the Global severity index (GSI) was considered valid provided not more than 18 (20%) answers were missing.

Statistical analysis

Continuous data are presented as medians with their interquartile ranges and categorical data as counts and proportions. Continuous variables were

compared between cases and controls using student independent t-test for continuous gaussian variables or Wilcoxon test for non-gaussian variables, and proportions were compared using Pearson Chi-squared or Fischer's Exact tests, as appropriate. Independent risk factors for NPAE-related DTG discontinuation were identified by stepwise multivariable logistic regression. Variables with P value $< .10$ in univariate analysis were considered for possible inclusion in the multivariable model. Eventually, genotype specific ORs were derived using stepwise variable forward selection with backward elimination based on the P value and only variables with $P < .05$ were retained in the final multivariable model. For the psychometric evaluation, proportions of participants with pathological scores were compared across groups and the rank of scores were compared across genotypes using the Kruskal-Wallis test, with further testing for multiple comparisons using the Dunn test. All statistical analyses were performed using JMP® Pro 17.1.0 (JMP Statistical Discovery LLC).

3.2.3.3.2.4 *Results*

Study Cohort

A total of 137 participants were recruited: 102 receiving DTG and 35 who had previously discontinued DTG due to NPAE. During the study follow-up, 4 participants discontinued DTG because of NPAE but one of them was excluded as drug causality was not confirmed. Additionally, 2 participants who had previously discontinued DTG for NPAE were excluded for the same reason. Eventually, 36 cases and 98 controls were included in the analysis. Main participants' characteristics are described in **Table 1** and are comparable across groups.

All participants were successfully genotyped. Genotype distribution of the study population is reported in **Table 2**. All polymorphisms were in Hardy–Weinberg equilibrium, including for *CYP3A5* and *UGT1A1* after stratification for ethnic origin.

Table 1: General characteristics of cases and controls.

	Cases (n=36)	Controls (n=98)	P value
Age, mean (SD), y	50,7 (11,1)	50,5 (11,7)	0,90
Female gender	13 (36,1)	38 (38,8)	0,78
BMI, median (IQR), kg/m ²	26,5 (24,4-31,1)	26,9 (24,2-30,4)	0,86
Nadir CD4 ^a (cell/mm ³), median (IQR)	221 (143-393)	239 (124-348)	0,92
Ethnicity			1
White	22 (61,1)	56 (57,1)	
SSA	13 (36,1)	36 (36,7)	
Tobacco use ^b	8 (22,2)	19 (19,6)	0,74
Undetectable VL	36 (100)	97 (99)	1

Data are presented as number of subjects (%) unless otherwise indicated. Data are presented at study inclusion, unless otherwise indicated.

Abbreviations: BMI, body mass index; IQR, interquartile range; SSA, Sub-saharan African ; SD, standard deviation; VL, viral load; y, years.

^a Unavailable for 1 control.

^b Unavailable for 3 cases and 3 controls.

Table 2: Genotype distribution of cases and controls for selected pharmacogenes.

	All (N=134)	Controls (n=98)	Cases (n=36)	P Value
<i>UGT1A1</i> ^a				.98
Extensive metabolizer	66 (49)	48 (49)	18 (50)	
Intermediate metabolizer	52 (39)	38 (39)	14 (39)	
Poor metabolizer	16 (12)	12 (12)	4 (11)	
<i>CYP3A4</i> *22 (rs35599367)				1
-/-	121 (90)	88 (90)	33 (92)	
-/*22	12 (9)	9 (9)	3 (8)	
*22/*22	1 (1)	1 (1)	0 (0)	
<i>CYP3A5</i> ^b				.88
Expressor	47 (35)	34 (35)	13 (36)	
Non-expressor	87 (65)	64 (65)	23 (64)	
<i>ABCB1</i> c.3435C>T (rs1045642)				.96
CC	68 (51)	49 (50)	19 (53)	
CT	50 (37)	37 (38)	13 (36)	
TT	16 (12)	12 (12)	4 (11)	
<i>ABCB1</i> 1199G>A (rs2229109)				1
GG	127 (95)	93 (95)	34 (94)	
GA	7 (5)	5 (5)	2 (6)	
<i>ABCG2</i> c.421C>A (rs2231142)				.96
CC	112 (84)	82 (84)	30 (83)	
CA	22 (16)	16 (16)	6 (17)	
<i>NR1I2</i> c.-22-7659C>T (rs2472677)				.046
CC	27 (20)	15 (15)	12 (33.5)	
CT	62 (46)	46 (47)	16 (44.5)	
TT	45 (34)	37 (38)	8 (22)	
<i>POR</i> *28 (rs1057868)				.90
-/-	83 (62)	60 (61)	23 (64)	
-/*28	40 (30)	29 (30)	11 (30.5)	
*28/*28	11 (8)	9 (9)	2 (5.5)	
<i>SLC22A2</i> c.808C>A SNP (rs316019)				.78
CC	102 (76)	74 (76)	28 (78)	
CA	32 (24)	24 (24)	8 (22)	

Data are presented as number of subjects (%).

^a Classified according to Clinical Pharmacogenetics Implementation Consortium (CPIC) classification for UGT1A1 genotype-predicted phenotypic function: extensive metabolizer (*1 or *36)/(*1 or *36), intermediate metabolizer (*1 or *36)/(*28 or *37), poor metabolizer (*28 or *37)/(*28 or *37). No participant displayed the *6 allele.

^b Expressor participants display at least one copy of a normal function allele: *1/(*1 or *3 or *6 or *7); non-expressor participants have two copies of a no function allele: (*3 or *6 or *7)/(*3 or *6 or *7).

Neuropsychiatric Adverse Events

Sleep-related disorders were the most prevalent NPAEs leading to DTG discontinuation (**Supplementary Table S1**). Cases had discontinued DTG due to NPAE at a median of 15 months (IQR 7-43) after its initiation. The most frequent DTG-containing regimen at the time of drug discontinuation was the combination DTG/abacavir/lamivudine, received by 21 cases (58%) (regimen detail is provided in **Supplementary Table S2**).

Risk Factors for DTG Discontinuation due to Neuropsychiatric Adverse Events

In univariate analysis, we found a balanced distribution of all studied polymorphisms across cases and controls, with the exception of the *NR1I2* c.22-7659C>T single nucleotide polymorphism (SNP) ($P = .046$) (**Table 2**). The multivariable selection analysis found only this SNP to be independently associated with a reduced risk of DTG discontinuation due to NPAE, with an OR for T allele carriers of 0.36 (95% CI .15-.88; $P = .02$).

Psychometric Evaluation

Among 98 participants in the control group, 63 (64%) provided valid answers to at least one study questionnaire, and 56 (57%) provided valid answers to all four questionnaires. Among control patients, a gradient was found in proportions of participants with an HADS-anxiety score of 8 or more (440) based on the *NR1I2* genotype, with 4/9 (44%) for CC, 8/31 (26%) for CT and 2/22 (9%) for TT carriers ($P = .07$) (**Table 3**). Furthermore, a significant difference was found in the distribution of HADS-anxiety score based on *NR1I2* c.22-7659C>T genotype ($P = .037$), with TT genotype carriers displaying lower scores compared to CT ($P = .079$) and CC ($P = .118$) (**Supplementary Figure S1**). No significant difference was found for the distribution of other scores and proportions of elevated scores based on *NR1I2* c.22-7659C>T genotype (**Supplementary Figures S2-14**).

Table 3: Proportion of control participants with an elevated score according to *NR1I2* c.-22-7659C>T genotype.

	<i>NR1I2</i> c.-22-7659C>T			<i>P</i> value
	CC	CT	TT	
HADS anxiety score ≥ 8 (n=62)	4 (44)	8 (26)	2 (9)	.07
HADS depression score ≥ 8 (n=62)	3 (33)	8 (26)	2 (9)	.19
PSQI score ≥ 6 (n=61)	6 (67)	14 (47)	10 (45)	.57
Pichot score ≥ 23 (n=61)	0 (0)	1 (3)	2 (9)	.73
SCL-90-R GSI ≥ 1 (n=58)	1 (13)	2 (7)	2 (10)	.83

Data are presented as number of subjects (%). Proportions are calculated using as denominator the number of control participants with a valid questionnaire for each genotype.

List of abbreviations: GSI: global severity Index; HADS: Hospital Anxiety and Depression Scale; NR1I2: Nuclear Receptor Subfamily 1 Group I Member 2; PSQI: Pittsburgh Sleep Quality Index; SCL-90-R: Symptom Checklist-90 Revised.

3.2.3.3.2.5 Discussion

We report the results of a pharmacogenetic study investigating risk factors for NPAE-related DTG discontinuation. Using a case-control study design, we identified the *NR1I2* c.-22-7659C>T variant as independently associated with a protective effect against DTG discontinuation due to NPAE. Notably, none of the other studied polymorphisms were associated with this outcome, despite several of them known to be associated with DTG plasma pharmacokinetics. Among patients who did not discontinue DTG due to NPAE, we also found the same *NR1I2* variant to be linked with a decreased

anxiety, as measured by the HADS-anxiety scale, further supporting the plausibility of this association.

NR1I2 is a key pharmacogene that encodes the PXR nuclear receptor. Briefly, PXR is activated by various endogenous and exogenous ligands. Once activated, it regulates the expression of several genes involved in xenobiotics detoxification and elimination. Among others, PXR upregulates expression of CYP3A4 and UGT1A1, both of which are responsible for DTG biotransformation, as well as ABCB1 and ABCG2 (353, 443), two DTG transporters that can limit drug absorption through intestinal epithelium apical expression.

The precise effect of the *NR1I2* c.-22-7659C>T SNP on drug metabolism remains uncertain. The TT genotype has been associated with increased basal expression of CYP3A4; however, despite its lower basal expression, the CC genotype was associated with enhanced induction by rifampin (362). In the field of antiretroviral therapy, studies have demonstrated that TT carriers display a reduced systemic exposure to unboosted atazanavir (444-446), a drug primarily metabolized by CYP3A4 and transported by ABCB1. TT carriers also displayed a reduced risk of efavirenz discontinuation (447), a drug mainly metabolized by CYP2B6 and transported by ABCB1, for which CYP3A4 constitutes a minor metabolism pathway. Notably, T carriers co-treated with rifampin had significantly lower efavirenz plasma concentrations than non-carriers (448). The specific effect of this SNP on DTG pharmacokinetics remains unknown. DTG is a moderate PXR activator (108), and clinical studies

have produced inconclusive results: one adult trial reported a modest increase in DTG exposure associated with the SNP (390) and a limited-sized trial in children showed a trend towards increased exposure (391). However, the largest study carried in Southern African adults found no effect of the SNP on DTG exposure (392).

Our results align with several reports that challenged the idea that NPAE are directly linked to DTG plasma concentrations. This simplified vision has been supported by a single study carried in Japanese PLWH, which reported higher DTG trough concentrations in patients with NPAE compared to those without. That study also found an increased cumulative incidence of NPAE in carriers of *UGT1A1**6, *UGT1A1**28, or both alleles (222), however these results were not confirmed by other pharmacokinetic (102, 228, 230) or pharmacogenetic studies (229). Our results further contradict this view as we found no association between DTG discontinuation due to NPAE and genotypes linked with a decreased *UGT1A1* function, despite substantial evidence of their impact on plasma exposure (108, 222, 389, 390, 392). Moreover, we observed no effect of the *ABCG2* c.421C>A SNP which has been previously associated with increased DTG peak concentration (390, 393).

Other mechanisms not mediated by plasma exposure could potentially explain the protective role of *NR1/2* c.-22-7659C>T against NPAE. First, the SNP might reduce DTG accumulation in the CNS. Indeed, both *ABCB1* and *ABCG2* are expressed at the blood-brain barrier, where their expression can

be upregulated by PXR (353, 359). Thus, *NR1I2* c.-22-7659C>T could exert protective effects through PXR activation and, consequently, transporter overexpression (390, 422). Second, CNS toxicity might not be mediated by the parent drug, but rather by DTG metabolites. Zhu and colleagues have demonstrated that CYP1A1 and CYP1B1 play key roles in the formation of important dolutegravir aldehyde metabolites (106). As both CYPs are expressed in the brain and given the toxicity of aldehydes, the authors proposed that these pathways could be involved in neurotoxicity. If this hypothesis holds, *NR1I2* polymorphism could thus impact on toxicity either by an upstream influence on DTG CNS concentration, or by a possible regulatory effect on those biotransformation pathways (449). Finally, one hypothesis not ruled out by our study, which lacks a control group not exposed to DTG, is that *NR1I2* c.-22-7659C>T might protect against neuropsychiatric manifestations independently of DTG. Indeed, PXR is expressed at the blood-brain barrier as well as in specific brain regions, and its role in brain function has recently gained interest (358). A recent study found that PXR-deficient mice exhibited increased anxiety-like behaviors and impaired recognition memory (450), an interesting finding in the light of our results, which showed lower HADS anxiety scores in individuals homozygous for the *NR1I2* c.-22-7659C>T SNP.

Our study has several limitations. First, due to its intrinsic design, we were unable to assess drug exposure in patients having discontinued DTG due to NPAE. Secondly, our analysis focused on selected polymorphisms, leaving the possibility that unexplored pharmacogenetic factors may contribute to the

pathogenesis of NPAE. Although our NGS panel covers all major pharmacogenes and their most important polymorphisms, the *SLC22A2* 808C>A SNP (rs316019) was not included and could not be studied despite controversy regarding its role in NPAE (211, 229, 231). Finally, our study did not reach the initially expected sample size. Nevertheless, we believe that the homogenous distribution across cases and controls of all studied SNPs (with the notable exception of *NR1I2* c.-22-7659C>T) provides a strong indication that those are not clinically relevant risk factors for NPAE.

In conclusion, among a selection of the most relevant polymorphisms in pharmacogenes influencing DTG transport and metabolism, only *NR1I2* c.-22-7659T carriage was associated with protection against DTG discontinuation due to NPAE. Moreover, this SNP was associated with a decrease in anxiety in patients receiving DTG. Our findings confirm that DTG systemic exposure is not a key determinant of NPAE. Further research is needed to clarify the interactions between *NR1I2*, DTG and brain function.

3.2.3.3.3 Supplementary appendix

Supplementary Table S1: Detail of neuropsychiatric adverse events reported by cases at time of dolutegravir discontinuation.

Reported side effect	n (%)
Insomnia	22 (61)
Nightmares	7 (19)
Irritability	7 (19)
Fatigue	5 (14)
Brain fog ^a	4 (11)
Anxiety	3 (8)
Drowsiness	3 (8)
Headache	3 (8)
Dizziness	3 (8)
Depression	3 (8)
Others ^b	4 (11)

Data are presented as number of subjects (%).

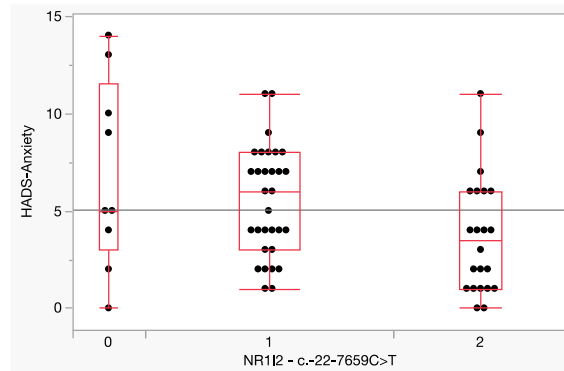
^a Defined as difficulties for concentrating or feeling mentally « cloudy ».

^b Memory loss, palpitations, behavioural disorders, « heat » in the head.

Supplementary Table S2: Distribution of antiretroviral regimens of cases at the time of DTG discontinuation.

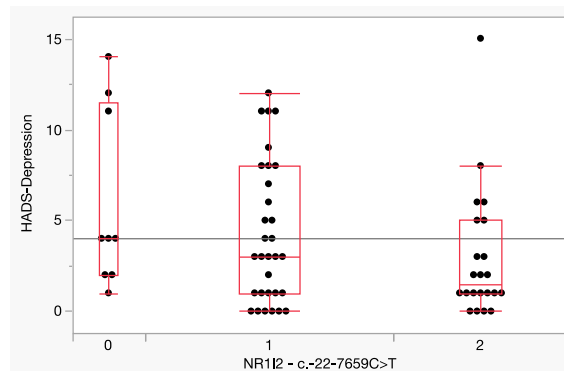
cART regimen	n (%)
DTG/ABC/FTC	21 (58,3)
DTG/TAF/FTC	7 (19,4)
DTG/3TC	4 (11,1)
DTG/TDF/FTC	3 (8,3)
DTG/3TC/RPV	1 (2,8)

Supplementary Figure S1. Distribution of Hospital Anxiety and Depression Scale - anxiety score based on *NR1I2* c.22.-7659C>T genotype among control participants.



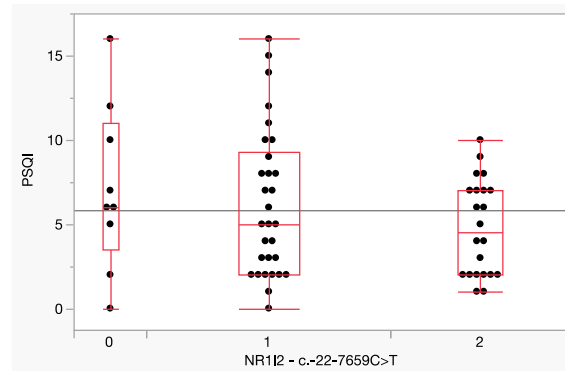
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .037 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S2. Distribution of Hospital Anxiety and Depression Scale - depression score based on *NR1I2* c.22.-7659C>T genotype among control participants.



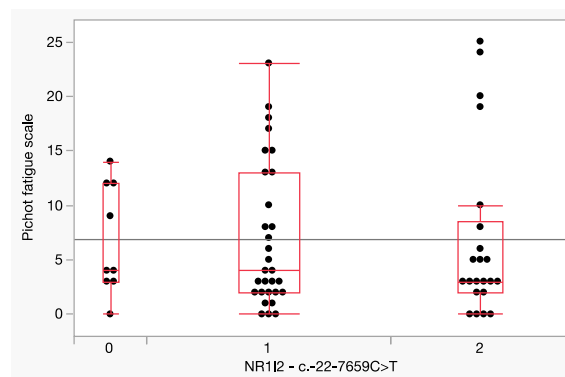
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .13 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S3. Distribution of Pittsburgh Sleep Quality Index score based on *NR1I2* c.22.-7659C>T genotype among control participants.



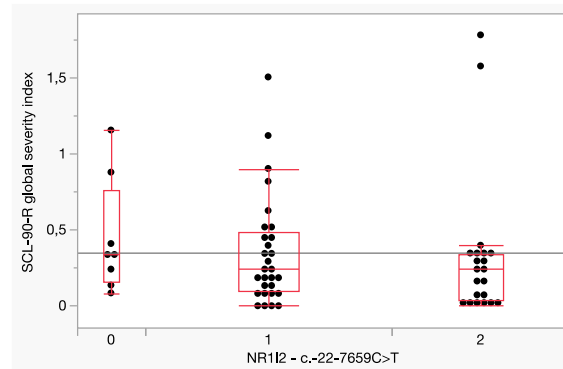
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .42 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S4. Distribution of Pichot fatigue Questionnaire score based on *NR1I2* c.22.-7659C>T genotype among control participants.



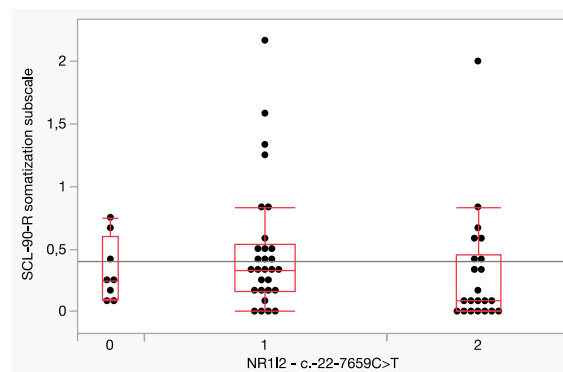
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .84 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S5. Distribution of Symptom Checklist-90-R Global Severity Index based on *NR1I2* c.-22.-7659C>T genotype among control participants.



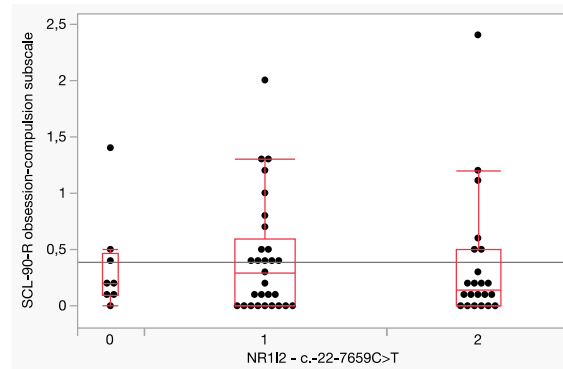
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22.-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .33 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S6. Distribution of Symptom Checklist-90-R somatization score based on *NR1I2* c.-22.-7659C>T genotype among control participants.



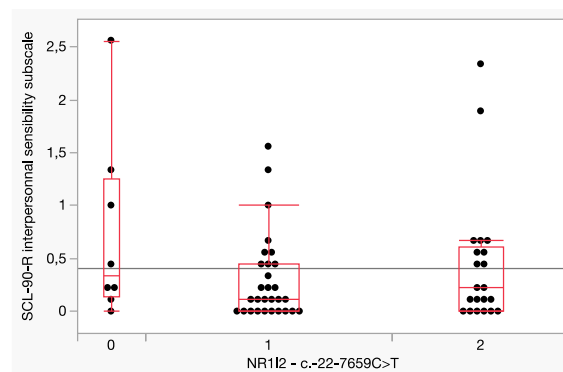
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22.-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .16 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S7. Distribution of Symptom Checklist-90-R obsession-compulsion score based on *NR1I2* c.22.-7659C>T genotype among control participants.



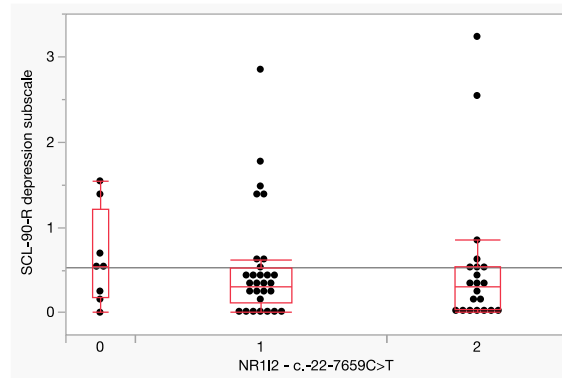
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .83 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S8. Distribution of Symptom Checklist-90-R interpersonal sensitivity score based on *NR1I2* c.22.-7659C>T genotype among control participants.



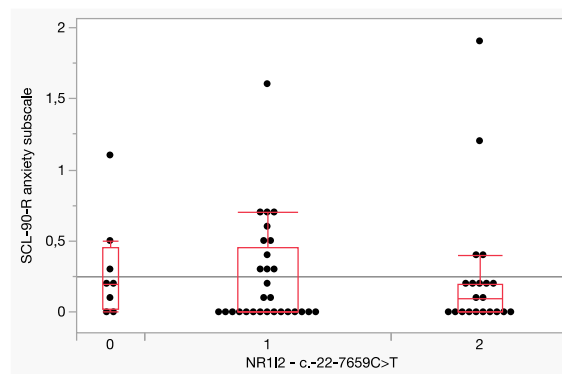
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .27 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S9. Distribution of Symptom Checklist-90-R depression score based on *NR1I2* c.22.-7659C>T genotype among control participants.



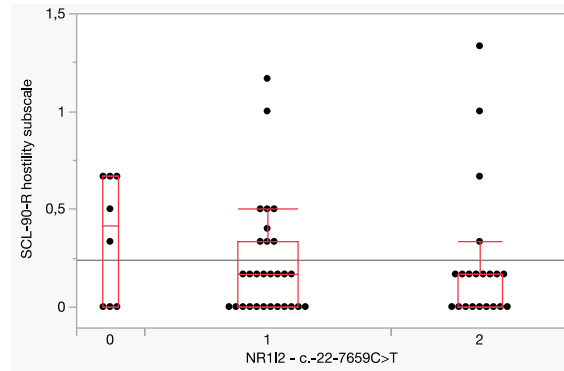
Participants are grouped according to number of T alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .50 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

Supplementary Figure S10. Distribution of Symptom Checklist-90-R anxiety score based on *NR1I2* c.22.-7659C>T genotype among control participants.

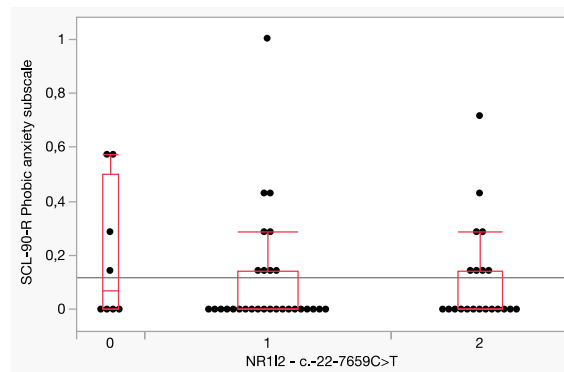


Participants are grouped according to number of T alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .60 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

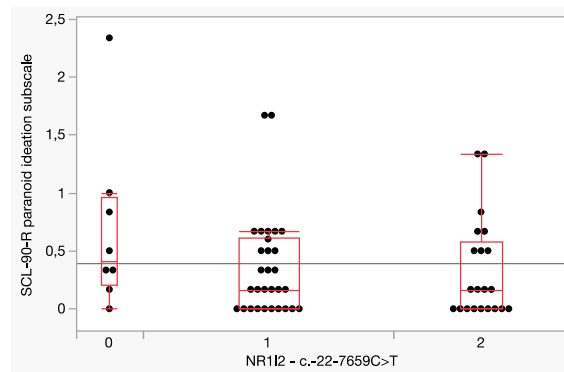
Participants are grouped according to number of T alleles carried for NR1I2 c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype. $P = .47$ for rank of scores global comparison across genotypes using the Kruskal-Wallis test.



Participants are grouped according to number of T alleles carried for NR1I2 c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype. $P = .50$ for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

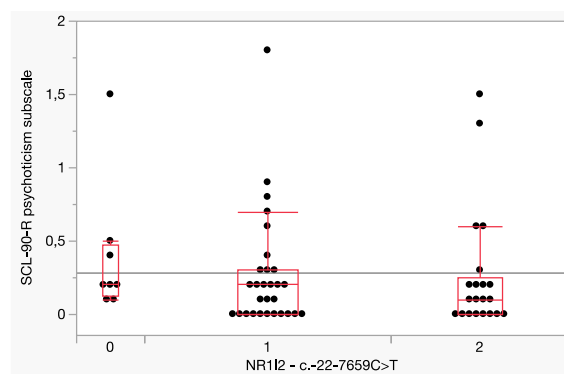


Supplementary Figure S13. Distribution of Symptom Checklist-90-R paranoid ideation score based on *NR1I2* c.22.-7659C>T genotype among control participants.



Participants are grouped according to number of *T* alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .26 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

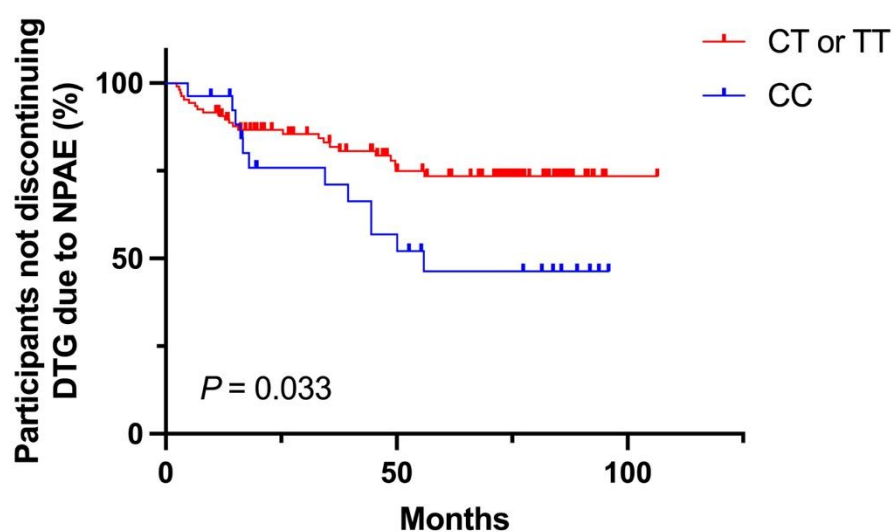
Supplementary Figure S14. Distribution of Symptom Checklist-90-R psychoticism score based on *NR1I2* c.22.-7659C>T genotype among control participants.



Participants are grouped according to number of *T* alleles carried for *NR1I2* c.-22-7659C>T (rs2472677): 0, CC genotype; 1, CT genotype; 2, TT genotype.
P = .27 for rank of scores global comparison across genotypes using the Kruskal-Wallis test.

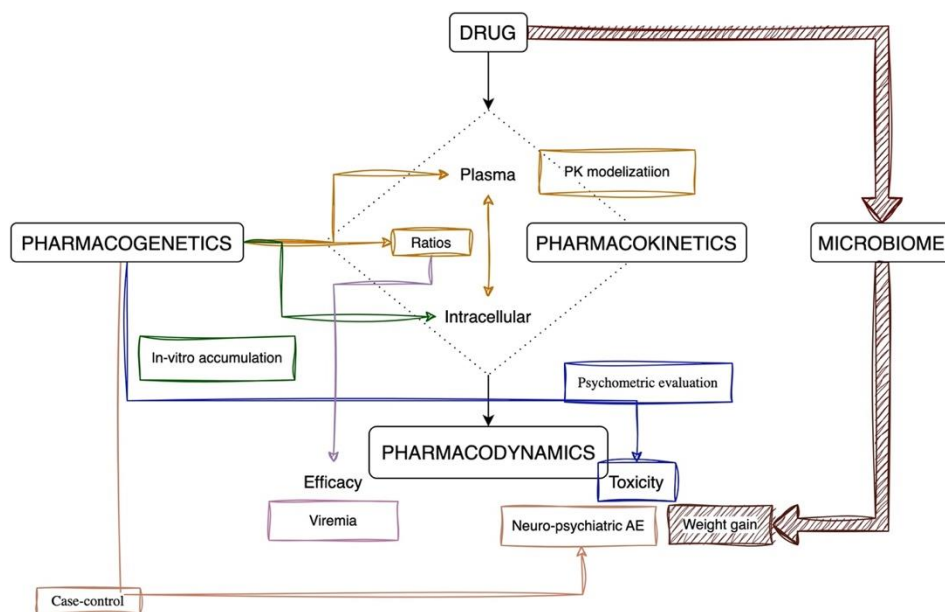
3.2.3.3.4 Additional material

Additional figure. Kaplan-Meier curves displaying percentage of participants of the case-control study not discontinuing DTG due to NPAE over time, grouped according to NR112 c.-22-7659C>T genotype.



Participants were censored at the end of the study follow-up or if they discontinued DTG for another reason. Statistical analysis reported: Mantel-Cox test.

3.2.3.4 Associations between weight gain, integrase inhibitors antiretroviral agents, and gut microbiome in people living with HIV: A cross-sectional study



Key Points

- This cross-sectional study investigated associations between microbiome and weight gain after initiation of DTG or BIC.
- Weight gain was not linked to overall bacterial diversity.
- Strong associations with specific taxa including *Haemophilus* sp. and *Dysosmobacter* sp. were demonstrated.

3.2.3.4.1 Article

Under review in Scientific Reports

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3.2.3.4.1.1 Abstract

Background: Dolutegravir and bictegravir are second-generation HIV integrase strand transfer inhibitors (INSTIs) that were previously associated with abnormal weight gain.

Methods: This monocentric cross-sectional study investigates associations between weight gain during the first year after initiation of dolutegravir, bictegravir or other anchor drugs and gut microbiome diversity as well as taxa composition.

Results: The study enrolled 79 participants receiving dolutegravir, 32 receiving bictegravir and 10 receiving non-INSTI based regimens. Most of them were treatment experienced at initiation of those anchor drugs agents. Although weight gain was not linked to overall bacterial diversity, strong associations with specific taxa were demonstrated (FDR $P < 0.01$). Using multiple linear regression, we identified 4 distinct groups of bacteria associated with either dolutegravir, bictegravir, weight loss or weight gain under treatment, allowing a machine learning model to predict 15.9% of the weight gain variability regardless of sex, age and body mass index (RMSE: 0.0126). *Dysosmobacter* sp. and *Haemophilus* sp., two bacteria previously associated with host metabolism, were among the strongest predictors.

Conclusions: Our findings link INSTIs, weight gain, and the gut microbiome. Future research should investigate the causal role of the identified taxa to

improve our understanding of microbiome-drug interactions and further support personalized antiretroviral strategies.

3.2.3.4.1.2 Background

Human immunodeficiency virus (HIV) infection has become a chronic and manageable condition with the advent of combined antiretroviral therapy (cART). cART generally comprises one or two backbone antiretroviral agents from the nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) drug class, combined with one anchor antiretroviral agent from another drug class. Integrase strand transfer inhibitors (INSTIs) are currently a key first-line anchor drug class. Among them, dolutegravir (DTG) and bictegravir (BIC) are second-generation INSTIs and both have been largely recommended as first-line agents (451, 452). Despite their good safety profile, abnormal weight gain has emerged as an important concern for both DTG and BIC (453). Exact causality and underlying mechanisms for this observed weight gain remain largely unknown. Besides INSTIs, the backbone NRTI drug tenofovir, especially when administered as tenofovir alafenamide (TAF) could play a significant role herein (240). Moreover, several factors including sex have been shown to influence the treatment-associated weight gain (454, 455).

Despite growing evidence of complex and multidirectional interactions between drugs and the gut microbiome (456, 457), the latter remains a poorly explored player in DTG- and BIC-related ADRs. However, these

interactions deserve specific attention, as on one hand, antiretroviral agents have been shown to influence patient's gut microbiome, with differential effects according to drug class (458, 459) and, on the other hand, cross talk between gut microbiome and the brain, the endocrine system and the adipose tissue has been well demonstrated (460). Moreover, gut microbiome composition has been linked to overweight, obesity and related metabolic disorders (461, 462).

We conducted a prospective phase IV study encompassing pharmacokinetic, pharmacogenetic and microbiome analysis, aiming at better understanding the interindividual variability of response to DTG and BIC at both clinical efficacy and adverse drug reaction levels. Here, we summarize the findings of a cross sectional gut microbiome analysis, deciphering complex interactions between cART, patients' characteristics and gut microbiome diversity as well as taxa composition.

3.2.3.4.1.3 *Methods*

Study design and population

The study recruited patients from three different subgroups during their regular follow-up at the HIV/AIDS Reference Center of Cliniques universitaires Saint-Luc, a tertiary academic hospital located in Brussels, Belgium: (i) BIC-treated patients, (ii) DTG-treated patients and (iii) patients having previously discontinued DTG due to the occurrence of neuropsychiatric adverse events. Two other subgroups were originally planned, consisting of patients shifting from another drug class to DTG- and

BIC-containing regimens, but were abandoned due to slow recruitment. Exclusion criteria were: (i) pregnancy at the time of inclusion or expected pregnancy within 12 months, for patients treated by DTG or BIC during the study, and (ii) liver failure (Child-Pugh A, B or C). All participants gave written informed consent before taking part in the study.

This sub-study reports the association between gut microbiome composition and different relevant parameters (other sub-studies including a pharmacogenetics and pharmacokinetics/pharmacodynamics analysis will be reported separately). All study participants were requested to bring one stool sample on a single occasion, at least 6 months after the initiation of their ongoing anchor drug (DTG, BIC or other). 180 participants were included in total, of which 131 provided a stool sample within the expected timeframe for this microbiota sub-study. Ten samples were not included as they met some predefined exclusion criteria for analysis (further details on Figure 1).

The study received approval by the local Ethical committee (2020/30NOV/593) and regulatory authorities (Eudra-CT Number 2020-001103-17; registration date 2020-12-01), and is registered with ClinicalTrials.gov (NCT04805944; registration date 2021-03-16).

Demographic and clinical characteristics

Sex, age, ethnicity, sexual orientation at HIV diagnosis, past medical history and ongoing treatments were obtained from electronic medical records. The CD4 count was measured by flow cytometry (Navios, Beckman Coulter).

Weight was prospectively measured during follow-up. Body mass index (BMI) of patients was calculated at the time of stool collection. Estimates of weight gain after anchor treatment initiation, i.e., before study inclusion was based on weight measurements which are part of standard evaluation during routine visits. When weight was not available at the date of drug shift, the weight was estimated by interpolation, using the last weight before shift, and the first weight measure after drug shift. We studied the relative weight gain normalized over time during the first year after anchor initiation, i.e., the first-year weight gain reported over the baseline weight, divided by the duration of follow-up:
$$\frac{(\Delta \text{Weight during year one after anchor start})}{(\text{Weight at anchor start})} (\%) \cdot \frac{1}{\Delta \text{Time}(\text{days})}$$
.

This enabled to compare patients taking into account (a) the uneven duration of routine follow-up from one patient to the other (as visits were not planned to occur precisely 12 months after treatment shifts), and (b) the variability in baseline weight. When not specified otherwise, the terms “weight gain” are used to describe the relative weight gain reported over time.

Fecal sample collection, DNA extraction and 16S rRNA gene sequencing

Participants received a stool collection kit with DNA stabilizer at inclusion (Invitek Diagnostics, Germany). On the day before their next appointment, they were reminded by phone to collect and bring the stool sample. Collected samples were frozen at -80°C until DNA extraction. DNA was extracted with an Invisorb Spin Universal Kit (Invitek Diagnostics, Germany), and its concentration and purity were assessed using a NanoDrop2000

(Thermo Fisher Scientific, USA). With all samples of this study included in the same run, 16 rRNA gene sequencing was performed by MrDNAlab (www.mrdnalab.com, USA) using dual-barcoded primers targeting the V4 region (515F 5'- GTGYCAGCMGCCGCGGTAA -3', and 806R 5'- GGACTACNVGGGTWTCTAAT -3').

Further information of microbiome analysis from data processing and advanced statistical methods used in this study are detailed in the supplementary method. Brief descriptions are presented as follow.

Data processing

Raw sequencing data was pre-processed using the MrDNA pipeline (www.mrdnalab.com, USA) to obtain Amplicon Sequence Variants (ASVs). Taxonomy was assigned to ASVs by importing representative sequences into Qiime2 (463), using the qiime feature-classifier (classify-sklearn), (464) against the pre-trained Silva v138 database for the V4 region (515F/806R) via RESCRIPT (465). Low abundance and extremely rare ASVs present in fewer than 3 counts in at least 10% of all samples were excluded.

Ultimately, our samples exhibited exceptionally high coverage values, exceeding 99.27%. This high quality was evident in the number of reads, with a median of 26,866 and a mean of 26,856 reads after all quality controls and filtering steps (**Table S1**). A total of 766 taxa in the form of ASVs were identified. Count data were transformed in proportion form and center-log-ratio (clr) transformed data, managed by the Phyloseq (466) and microbiome (467) packages, respectively. In metadata, BMI category was defined as

'obese' for BMIs over 30, 'normal' for BMIs 25 or below, and 'overweight' for values in between. Age categories were labelled 'old' for ages above the median and 'young' for those below. Missing values for weight gain in patients 37.MV and 69.MV were replaced with the cohort's median value.

Statistical analysis

R (version 4.2.0; www.r-project.org), Python 3.9.6 and JMP were used in statistical analysis, and data visualization operated in RStudio (version 1.4.1106) and Visual studio Code 1.84.2 on MacOS. Graphical representations were primarily created with the ggplot2 (468), ggpubr (469), Matplotlib packages (470). For comparison of demographic and clinical characteristics of the study participants, continuous variables were compared across groups using one-way ANOVA or the Kruskal-Wallis test, as appropriate. Proportions were compared using Fisher's Exact Test. Prior to analysis, all linear regression models were evaluated for linearity and independence assumptions. In microbiome-related linear models, only clr transformed data were utilized. A p-value of less than 0.05 and a false discovery rate (FDR)–adjusted p-value (q) of less than 0.05 were set as thresholds for statistical significance.

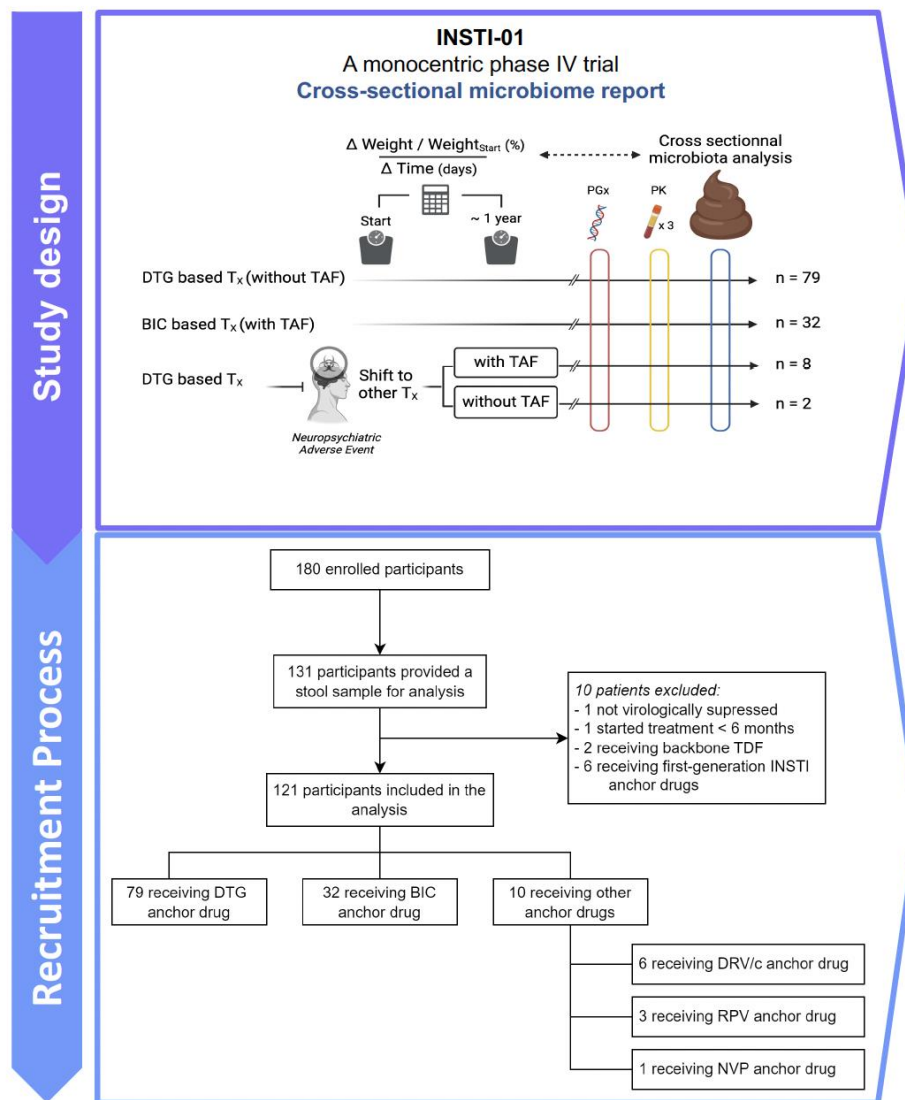
3.2.3.4.1.4 Results

Participant characteristics

We studied a cohort of 121 persons living with HIV (PLHIV), as shown in **Figure 1**. Patients were grouped according to the anchor drug in their ongoing cART: 79 received DTG, 32 received BIC and 10 received non-INSTI based regimens. Among DTG-treated patients, 69 (87%) received dual, 9 (11%) triple, and 1 (1%) quadruple cART. Patients from the two other anchor drug groups all received triple cART. None of the patients on DTG-based regimen was receiving TAF. On the contrary, all patients under BIC-based regimens received TAF. Among non-INSTI-based regimens, 8 out of 10 included TAF among backbone drugs. Further details of received treatments are provided in **Table S2**.

Main demographic and clinical characteristics were similar across the different groups, except for age, previously received cART regimen and time elapsed since the start of the current anchor drug. A vast majority of participants had received previous cART before the start of the current anchor, with only 9% in the BIC group, 13% in the DTG group and none in the non-BIC-non-DTG group being treatment-naïve.

Overall, 54% of participants experienced an increase in weight after approximately one year of treatment: 49% of DTG-treated, 74% of BIC-treated, and 30% of patients treated with non-INSTI regimens ($p = 0.015$). Further details on study participants and on weight gain after treatment initiation are provided in **Table 1**.



Abbreviations: BIC, bictegravir; DRV/c, darunavir/cobicistat; DTG, dolutegravir; INSTI, integrase strand transfer inhibitors; PGx, pharmacogenomics; PK, pharmacokinetic; RPV, rilpivirine; NVP, nevirapine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; Tx, Treatment.

Figure 1: Description of the study design and participants enrollment flowchart. This monocentric phase IV trial recruited persons living with Human

Immunodeficiency Virus grouped according to the anchor drug of their combined antiretroviral therapy. Persons living with Human immunodeficiency virus not receiving bictegavir nor dolutegravir were grouped together and were recruited among a cohort of patients that previously discontinued dolutegravir due to neuropsychiatric adverse events. Blood samples were collected for pharmacogenetic and pharmacogenomic analysis, while fecal samples were used to evaluate microbiome by 16S rRNA gene sequencing. Only the cross-sectional microbiota analysis will be reported in this paper. Fecal samples were collected, DNA extracted and sequenced before analysis to obtain raw count data of microbiome for downstream biostatistical analyses.

Table 1: Demographic and Clinical Characteristics of the study participants.

	BIC (n=32)	DTG (n=79)	Other (n=10)	P value
Female sex, n (%)	10 (31.3)	28 (35.4)	5 (50.0)	0.58
Age, mean (SD)	43.0 (9.7)	51.6 (11.6)	53.2 (7.9)	0.0007
Caucasian ethnicity, n (%)	15 (46.9)	47 (59.5)	3 (30.0)	0.15
MSM, n (%)	15 (46.9)	37 (46.8)	2 (20.0)	0.31
BMI, median (IQR)	26.2 (24.0-29.8)	26.9 (23.9-29.4)	30.0 (23.9-31.5)	0.61
Treated diabetes, n (%)	0 (0)	6 (7.6)	0 (0)	0.29
Treated dyslipidemia, n (%)	3 (9.4)	10 (12.7)	4 (40.0)	0.062
CD4+ T-cell count, median (IQR) (cells/mm³)	633 (561-834)	726 (565-877)	691 (468-851)	0.28
Time since HIV diagnosis, mean (SD) (years)	15.5 (8.8)	16.2 (7.7)	15.8 (10.8)	0.92

AIDS diagnosis history, n (%)	19 (59.4)	37 (47.4)	5 (50.0)	0.54
Nadir CD4+ T-cell count, median (IQR) (cells/mm ³)	202 (89-336)	237 (125-339)	217 (123-425)	0.87
Days since start of current anchor drug, median (IQR)	803 (721-929)	1876 (637-2239)	1118 (589-1810)	<0.0002
ART naïve at start current anchor, n (%)	3 (9.4)	10 (12.7)	0	0.72
Treatment regimen before current anchor, n (%)				
- None ¹	3 (9.1)	11 (13.9)	0	0.0004
- NNRTI-based	1 (3.0)	18 (22.8)	0	
- PI-based	1 (3.0)	17 (21.5)	3 (30.0)	
- INSTI-based	27 (84.4)	33 (41.8)	7 (70.0)	
Change in weight during first year after anchor initiation (kg) (median, IQR)	2.3 (-0.1 – 3.3)	0 (-1.7 – 2.1)	-3.4 (-5.6 – 1.3)	0.0038

All variables are reported at the time of stool collection, unless otherwise stated.

¹One non-naïve patient had interrupted treatment for 3y and 9 months before start of the DTG based regimen.

Overall association of weight gain, microbiome, treatments and host

To estimate the overall association between relative weight gain during the first year of current anchor (further referred as weight gain), microbiome, treatments, and hosts' characteristics, we first simplified the microbiome

community by calculating beta-diversity (Figure S1), and we further generated PCo1 and PCo2 which can explain about 35% of the variability of the data using principal coordinate analysis. Using four separate linear regression models, we then studied the association of sex, age category, BMI category and either the anchor drug (DTG, BIC or other) or the backbone drug (TAF or non-TAF) with weight gain, and with simplified microbiome (**Figure 2A**). We found that weight gain was different between anchor treatment groups, being highest for BIC (BIC versus DTG: $p = 0.028$ and BIC versus 'other': $p = 0.016$; **Figure 2A and 2B; Table S3**), while simplified microbiome was not. On the contrary, regardless of the effect of anchor or backbone drugs, male and female were different in the microbiome-PCo1 ($P < 1 \times 10^{-6}$) (**Figure 2B; Table S3**) but not the extent of weight gain. These differences are further illustrated in the taxonomic composition stacked bar plots (**Figure S2 and S3; Table S4**).

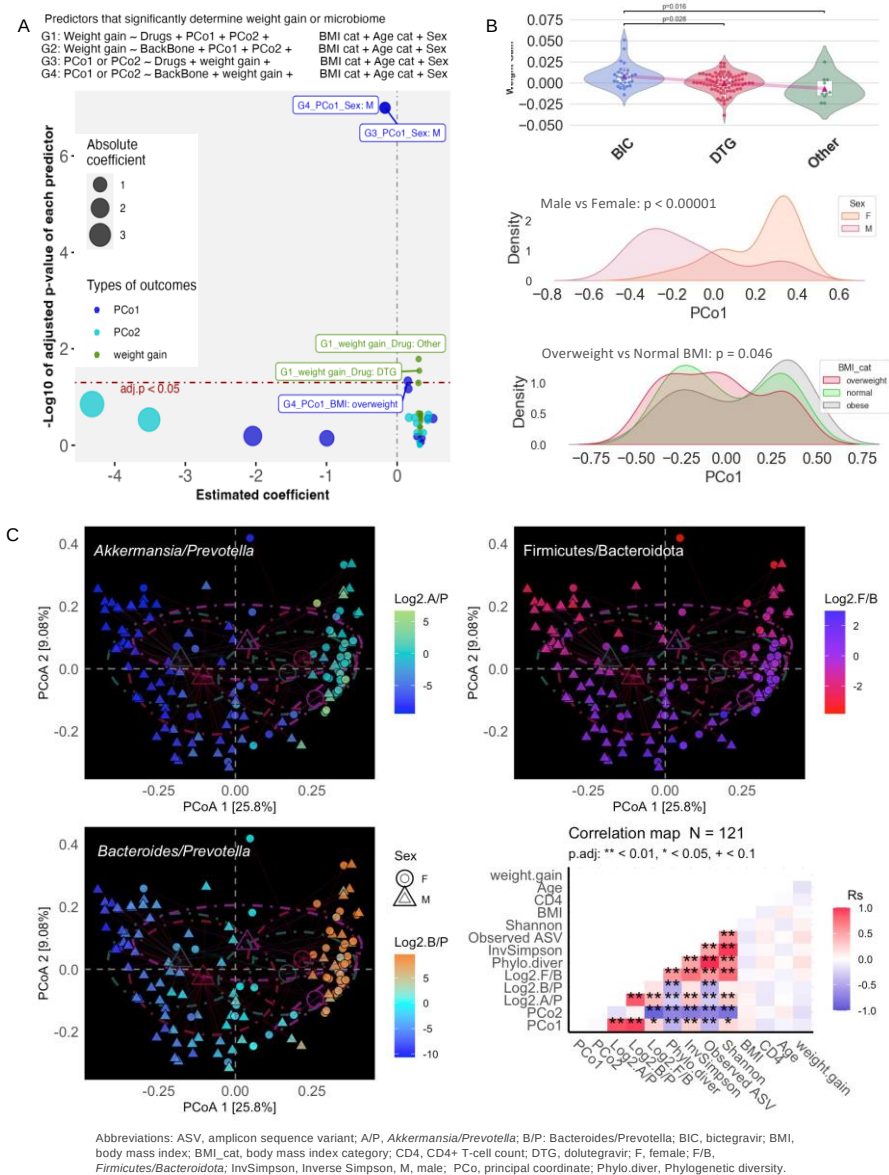


Figure 2: Exploring the relationship between weight gain, microbiome, treatments and host characteristics: Sex, Age, and BMI. A: Multiple linear regressions were performed to determine the association between weight gain, microbiome (beta-diversity: PCo1 & PCo2), drugs (DTG, BIC, Other) or backbone

(with/without-TAF), Sex, BMI category (normal, overweight, obese), Age category (old > median, young $\leq$ median; median= 51 years old). A volcano plot visualizes all predictors based on their adjusted p -value ($-\log_{10}$) and coefficients. Significant threshold is adjusted P value < 0.05. **B:** Significant results from volcano plot are further illustrated in the violin plot (weight gain in different treatments) and density plots. PCo1 & PCo2 in Bray-Curtis distance were used to represent the variability of microbiome. Difference between male & female and difference between BMI categories (overweight and normal) in only PCo1 are presented in density plots. **C:** Principal coordinate analyses of all samples ($n = 121$) based on Bray-Curtis distance metrics (PCo1 & PCo2) showing different clusters of bacteria regardless of treatment groups. Male and female samples are different in shape while color gradients represent the continuous data of $\log_2$ ratios of Akkermansia/Prevotella, Firmicutes/Bacteroidota, Bacteroides/Prevotella respectively. Spearman's correlation (R_s) heatmap between host's characteristics (BMI, CD4+ T-cell count, age, weight gain), bacterial diversities (PCo1, PCo2, alpha diversity indices: Phylo.diver, InvSimpson, Observed ASV, Shannon) and diversity associated-bacterial ratios.

Associations between weight gain, bacterial diversities, treatments and host

The overall analyses suggest a link between treatment and weight gain, as well as sex and simplified microbiome profile, but the link between weight gain, host's characteristics and microbiome diversities or bacterial clusters remains undetermined. To explore this, we initially performed Spearman correlation analysis, calculating a matrix to examine the association between weight gain, principal coordinate analysis variables (PCo1 & PCo2 calculated using Bray-Curtis dissimilarity index) of the beta diversity, various alpha diversity indices, diversity associated-bacterial ratios, and host characteristics (age, BMI and CD4+ T-cell count (CD4)), as depicted in **Figure 2C**. Surprisingly, weight gain showed no significant association with any of the variables tested.

Considering the limitations of correlation analysis, we subsequently performed multiple linear regression analyses. These allowed for simultaneous testing of associations between dependent variables (bacterial diversities, and ratios) and predictors including weight gain, treatments, and host factors. For these analyses, individuals were categorized into a “gain” group if their weight gain was greater than 0, with the remainder classified into the “lose” group (including 6 patients with unchanged weight). We hypothesize that individuals in the “gain” group might show distinct microbiome characteristics compared to those in the “lose” group.

While constructing the models with anchor and backbone treatment categories, BMI, age categories, sex, and ethnicity, we noticed a high collinearity between sex and ethnicity. This necessitated the separation of these variables into different models, which in turn allowed us to compare their respective impacts on the microbiome (**Figure S4A, Table S5**). We found no difference in microbiome diversity either between “gain” and “lose” groups or between different drugs or age groups; however, both sex and ethnicity showed significant effects (**Figure S4B, Table S3 and S5**). Notably, sex was a better predictor of outcomes than ethnicity as indicated by its inclusion in a greater number of models with superior Akaike information Criterion value (**Figure S4A, Table S5**). Further evaluation using PERMANOVA analysis on the Bray-Curtis distance of the microbiome underscored the significance of both sex and ethnicity ($p = 0.001$ and $p = 0.007$, respectively); however, sex explained the variability of microbiome 4 times more effectively than ethnicity, based on their respective R^2 value (0.0857 versus

0.02) (**Figure S1, Table S6**). Consequently, we decided to exclude ethnicity in the subsequent analyses, and conclude that weight gain did not show significant association with either bacterial community composition or key bacterial ratios.

Associations between weight gain or anchor drug and individual taxa

Although weight gain and anchor drugs did not show any association to bacterial diversities, we postulated that they might be associated with specific taxa. Therefore, we applied multiple linear regression models to determine whether some individual taxa (at the ASV and genus levels) were associated either with the use of DTG or BIC (first model), or with weight gain (second model). Due to its limited size, the group consisting of non-DTG non-BIC containing regimens was excluded from the analysis. As all BIC-treated participants received TAF and contrarily none of the DTG-treated participants received TAF, anchor drug related associations also apply to TAF.

In the first model, after correction for weight gain, sex, age and BMI categories, we found three taxa to be significantly overrepresented under specific anchor drugs. *Christensenellaceae R-7 group* (asv.199) and an uncultured bacterium (asv.925) were found to be significantly (nearly 8 to 10-fold) higher in relative abundance between DTG- and BIC-treated participants (taxa further referred as DTG-taxa). Oppositely, *Haemophilus* sp. (asv.116) was about two-fold higher in relative abundance on average (BIC versus DTG) (taxa further referred as BIC-taxa) (**Figure 3A, Figure S5A, and Table S7**).

In the second model, after correction for anchor drug (DTG or BIC), sex, age and BMI categories, we found 14 taxa to be associated either positively or negatively with weight gain, and they did not overlap with taxa identified in the first model. *Dialister* (genus.995), *UCG-002* sp. (asv.32), an uncultured organism (asv.74), *UCG-002* sp. (asv.109), metagenome (asv.186), and an uncultured bacterium (asv.397) were found to be positively associated to weight gain (taxa further referred as positive weight-gain taxa), while *incertae sedis* (genus.369), *Colidextribacter* (Genus.362), *Ruminococcus* (genus.348), *Dysosmobacter* (Genus.62), *Dysosmobacter* sp. (asv.62), *Parabacteroides* sp. (asv.510), *Ruminococcus torques group* sp. (asv.631), and gut metagenome (asv.612) were found to be negatively associated with weight gain (taxa further referred as negative weight-gain taxa) (**Figure 3B** and **Table S7**).

Considering that *Dysosmobacter* sp. (asv.62) is a likely health-beneficial bacteria which could be negatively associated with BMI (471, 472), we tested this association by linear regression. We found that *Dysosmobacter* sp. (asv. 62) is negatively associated to BMI ($P < 0.001$). Additionally, we validated our model by confirming a significant negative association between *Dysomobacter* sp. (asv.62) and BMI ($p = 0.023$, $R^2 = 0.145$) even after excluding from the analysis participants with the three highest abundances of *Dysosmobacter* sp. (asv.62), whose inclusion could have disproportionately driven the relationship (**Figure S5B**).

These preliminary findings indicate that while anchor drugs and weight gain may not significantly impact overall microbiome diversity, they could be associated with specific taxa. To delve deeper into their effects on the host microbiome, we computed Spearman's correlation inter-species matrices. These matrices assessed the relationships between DTG-taxa, BIC-taxa, weight gain, positive weight-gain taxa and negative weight-gain taxa (**Figure S5C**). DTG-taxa and BIC-taxa exhibited significant correlations with some specific taxa from both positive weight-gain taxa and negative weight-gain taxa groups. Given that weight gain was different between BIC and DTG treatment groups and drug-associated taxa formed a complex but clear pattern of association with both positive and negative weight-gain taxa, we hypothesized that these taxa together could determine the weight-gain level. To test this hypothesis, we trained linear prediction models with cross-validation on 75% of all samples and tested the performance of 2 different models: model 1 included all DTG-, BIC-, positive and negative weight-gain taxa except *asv.74* and *genus.62* because of collinearity, while model 2 included all taxa in model 1 plus sex, age, and BMI categories. Our results show that model 1 using only taxa predicted the weight gain better with a lower root mean square error (RMSE) of 0.0126 and a higher R^2 of 0.159 (**Figure 3C**). Predicted weight gain determined by the second model did not significantly correlate to the observed values ($p = 0.0502$), suggesting that sex, age and BMI categories are not very informative to predict weight gain. Individual contribution of each taxon in the model 1 was illustrated in **Figure 3D**.

In summary, our analyses have revealed contrasting interactions between four groups of bacteria, each characterized by their associations with either anchor drugs or weight gain. More importantly, our findings suggest that a set of core taxa could help to predict the magnitude of weight gain of one year taking DTG or BIC for PLHIV.

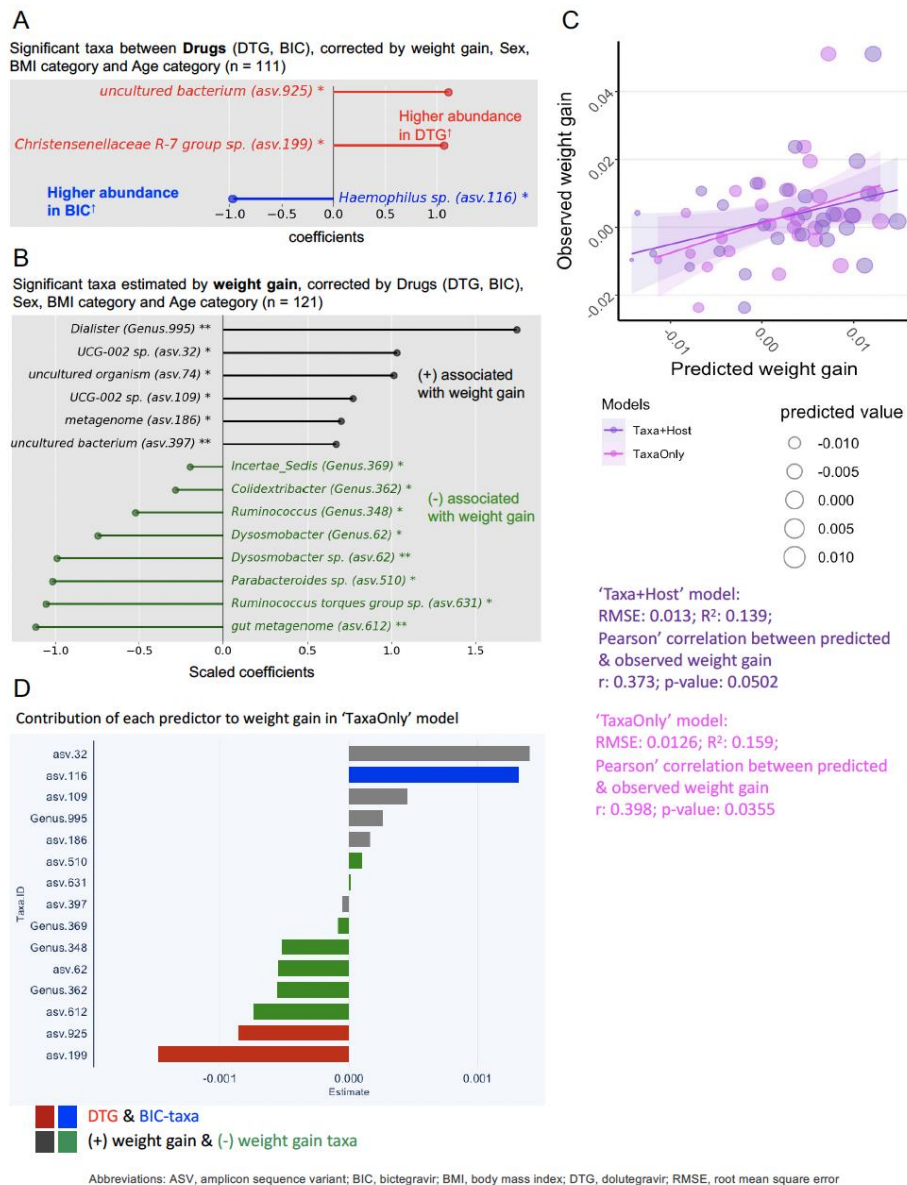


Figure 3: Interactions between Integrase Strand Transfer Inhibitors-associated weight gain and specific taxa of gut microbiome. Lollipop plots of significant taxa and their coefficient (A) or scaled coefficient (B) obtained from multiple-linear regression models in which confounders (sex, BMI and age categories) were controlled. Ten subjects taking “other” drugs than DTG or BIC were excluded in the

analysis resulting the sample size of 111 in A. **C:** a scatter plot of linear regression between predicted weight gain and its actual observed value. Purple and pink colors represent 2 different prediction linear models: (1) Taxa only model includes all taxa found in lollipop plots (A & B) excluded *asv.74* and *genus.62* because of multi-collinearity; (2) Taxa+Host model includes all taxa in the first model and host's characteristics (sex, BMI and age categories). RMSE and the R^2 estimated for each model are provided. Pearson's correlation between the observed and predicted value for each model are calculated ($N= 28$). **D:** Illustrating the contributing effect of each taxa in predicting weight gain (supporting information for Figure 2D). Estimates (effect size) obtained from trained model with 93 samples (~ 75%). Different colors present the groups of different taxa.

Identifying contributing factors to the abundance of microbial taxa associated with weight gain.

In our previous analyses, we found that while some taxa have a positive effect on weight gain, others have a negative impact, and not every individual experience weight gain. Despite this variation, our bacterial collection can successfully predict the level of weight gain. Therefore, understanding the factors that influence the abundance of these taxa is crucial. To this end, we employed LASSO regression for feature selection, aiming to identify key determinants of the abundance of positive and negative weight-gain taxa. This approach included the following predictors: weight gain, sex, age category, BMI category and DTG- and BIC-taxa. The significant predictors and their coefficients for each positively and negatively weight-gain-associated taxa are detailed in **Table S8**.

For visualization purposes, only taxa with coefficient larger than 10% of the largest absolute value of coefficient in the linear prediction model will be visualised using their coefficients (size effect) (**Figure 4**). Overall, besides

weight gain, DTG- and BIC-taxa played an important role in influencing the abundance of all dependent variables. Our model indicates a trend where all predictors are negatively associated with the abundance of negative weight-gain taxa while positively associated with the abundance of positive weight-gain taxa. It appears that DTG-taxa decrease the abundance of all negative weight-gain taxa. However, BIC-taxa (asv.116) not only contributed in a lesser proportion but also do not always follow this pattern; in some cases, it shows a positive contribution to negative weight-gain taxa such as *Ruminococcus* (genus.348) and *incertae sedis* (genus.369). Concerning *Dysosmobacter* sp. (asv.62), our analysis showed that DTG- and BIC-taxa, together with weight gain, have a negative impact on its abundance. Moreover, comparing to those with normal BMI, being overweight seem to have negative impact on the abundance of *Dysosmobacter* sp. and this finding is in line with the non-HIV population (471). These findings provide information to understand the link between host characteristic and specific bacteria which are likely to contribute to the weight gain.

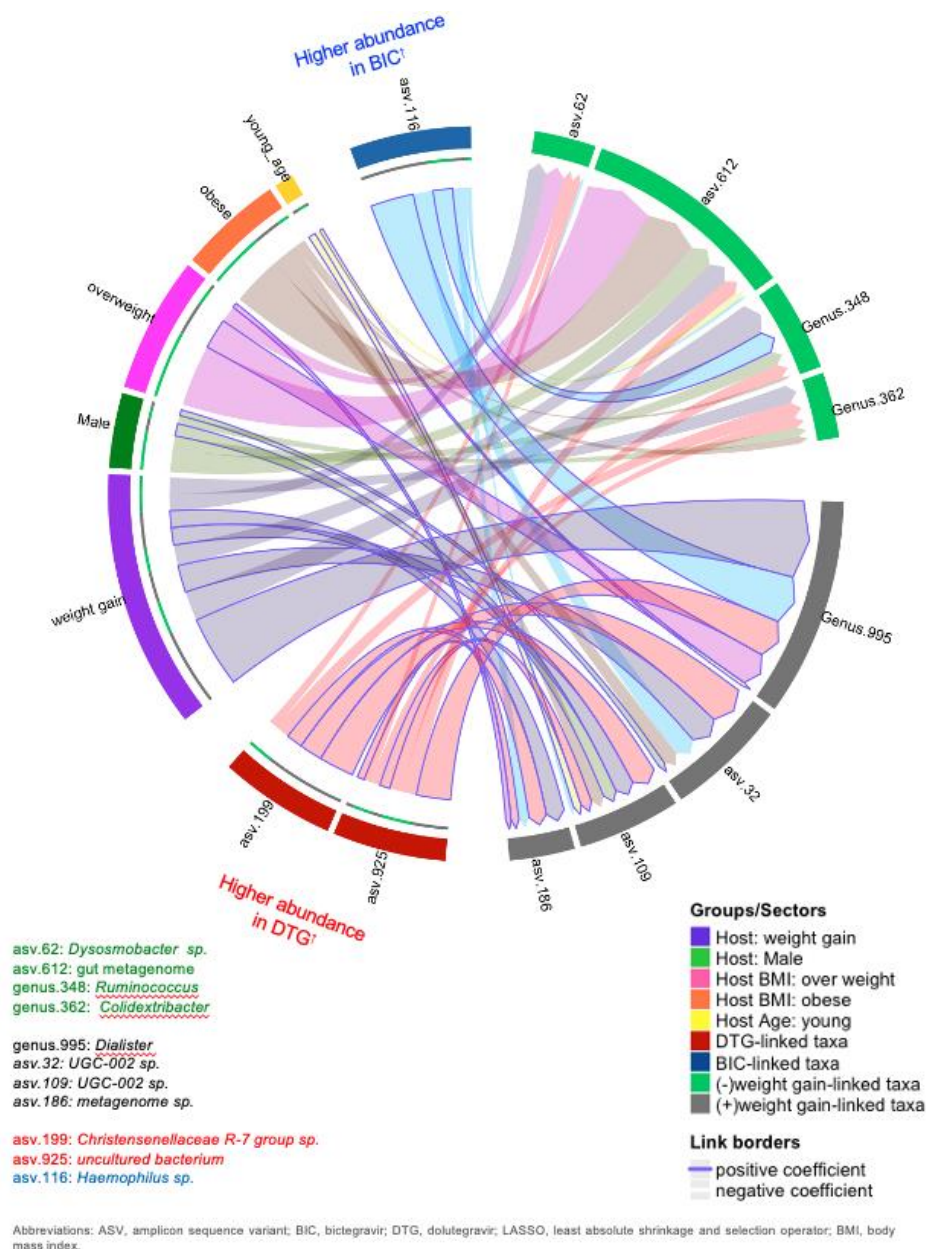


Figure 4: Chord diagram illustrating the proposed relationship between weight-gain associated taxa and each predictor by their coefficient obtained from LASSO. The arrows distinguish a dependent variable (end-point) from their predictors. Larger width of the arrow represents a larger effect of the predictor on

*the dependent variable. With or without a slate blue border distinguishes positive and negative coefficient of the predictor, respectively. One dependent variable is explained by a set of best predictors selected by LASSO. There are 3 main groups/sectors of predictors: (1) Host (weight gain, sex: Male, BMI: overweight/obese, Age: young), (2) Taxa with higher abundance in dolutegravir treatment group compared to bictegravir treatment group (Red group: asv.199; asv.925), (3) Taxa with higher abundance in bictegravir treatment group compared to dolutegravir treatment group (Blue group: asv.116). There are 2 main groups/sectors of dependent variables: (1) positively weight gain-associated taxa (palegreen group: asv.62: *Dysosmobacter* sp.; asv.612: gut metagenome; genus.362: *Colidextribacter*; genus.348: *Ruminococcus*), (2) negatively weight gain-associated taxa (black group: genus.995: *Dialister*; asv.32: UGC-002 sp.; asv.109: UGC-002 sp.; asv.186: metagenome sp.). Only taxa with coefficient larger than 10% of the largest absolute value of coefficient in the linear prediction model will be visualised.*

3.2.3.4.1.5 Discussion

Recent studies have established a link between the use of second-generation INSTIs, such as DTG and BIC, and weight gain in PLHIV (240, 473). A noteworthy percentage of individuals in the current study experienced weight gain within the first year of starting these medications. The mechanisms driving this weight gain are not fully understood but are believed to involve complex interactions with other medications, particularly TAF, which may independently contribute to weight changes (242, 243). In addition to drug-related factors, HIV-specific considerations play a role in weight dynamics. The 'return-to-health' effect, a phenomenon observed after the initiation of an effective HIV treatment, leading to viral suppression and immune recovery, can result in weight gain. This is particularly evident in individuals with advanced HIV status, where weight increase follows the

improvement of immune function. However, this does not fully account for the observed weight changes (246), and weight gain has also been demonstrated after treatment shifts in virologically suppressed (247).

The introduction of cART not only reverses CD4 depletion but also diminishes HIV-related immune activation. These effects might have a stronger influence on weight with INSTIs due to the potent action of these drugs (248, 249, 474). Furthermore, INSTIs are linked to changes in adipose tissue function, evidenced by increased adipogenesis, fibrosis, mitochondrial dysfunction, and insulin resistance (242, 251, 253). Particularly, a shift to DTG in virologically suppressed individuals has been associated with reduced levels of adiponectin, a hormone involved in regulating glucose levels and fatty acid breakdown (474). Some emerging data from large cohort studies also suggest that INSTIs could heighten the risk of developing metabolic syndrome and diabetes, underscoring the need for ongoing research into these side effects (475, 476). This complex interplay of drug effects, immune recovery, and metabolic changes highlights the importance of monitoring and managing weight gain and metabolic health in PLHIV undergoing INSTI-based therapy.

Therefore, this study aimed to elucidate the interplay between weight gain, microbiome, and antiretroviral drugs in PLHIV. We found that among a large cohort of virologically suppressed PLHIV, the received antiretroviral regimen comprising DTG or BIC did not appear to have a significant impact on gut bacterial communities.

In our study, we found no significant differences in gut microbiome diversity based on the anchor drug, although some compositional changes were noted. This observation is likely influenced by the close relationship between DTG and BIC. Unfortunately, our study mainly included INSTI-treated patients and may have been underpowered to comprehensively assess the effect of INSTIs compared to non-INSTI based regimens. However, previous cross-sectional studies showed that patients treated with INSTI exhibit gut microbiomes with bacterial diversity similar to that of uninfected controls, albeit with distinct compositional variations (477, 478). Regarding backbone drugs, no significant difference in terms of diversity was found in our cohort between participants receiving TAF and those receiving non-TAF based regimens. The effect of TAF on microbiome has not been evaluated in the context of HIV infection so far, but the drug showed no effect on gut microbiota diversity and on the relative abundance of gut microbiota at the phylum level in a study of patients receiving TAF for chronic hepatitis B (479).

Despite those drugs having seemingly a limited effect on gut microbiome diversities, receiving either DTG or BIC was associated with an overrepresentation of several specific taxa. We also identified two other groups of taxa that were negatively and positively associated with weight gain. Putting all these fifteen microbial taxa in a machine learning model, we found them capable of prognosticating weight gain, irrespective of sex, BMI, and age. The outcomes suggest that negative weight-gain taxa and those identified as DTG-taxa might negatively influence weight accretion, whereas BIC-taxa and positive weight-gain taxa could facilitate this process.

Among the taxa found to be negatively associated with the weight gain under treatment, some have previously been associated with favorable host metabolism, thus supporting the hypothesis that microbiome could play a role in the weight gain, rather than being seen as a bystander or a consequence of the weight gain. Particularly, *Dysosmobacter* sp. (asv.62) was ranked second in terms of its predictive value within the model, suggesting that an increased prevalence of this taxon is inversely related to weight gain. This phenomenon might be elucidated by the observation that the DNA sequence of this ASV exhibits a complete match (100%, spanning 253 base pairs) with the V4 region of the *Dysosmobacter welbionis* J115 16S rRNA gene, a bacterium known for butyrate production which is implicated in the attenuation of brown adipose tissue whitening and the enhancement of mitochondrial activity (480). Furthermore, this bacterium is more abundant in prebiotic responders associated with BMI reduction and better than metformin in improving glucose tolerance in mice model (472). Another significant finding concerns *Haemophilus* sp. (asv.116), which emerged as the second strongest positive predictor of weight gain (**Figure 3D**). This aligns with extant literature that delineates a positive correlation between the *Haemophilus* genus and parameters of adiposity and adult weight gain (481). Furthermore, elevated abundances of this genus have been more frequently associated with obesity compared to normal weight in a study focused on Chinese children (482). Consequently, *Dysosmobacter* sp. and *Haemophilus* sp. warrant consideration as important components in elucidating the microbiome's contributory role to weight gain in future research endeavours.

Compiling these observations, individuals in the BIC group exhibited a pronounced weight gain and a higher prevalence of *Haemophilus* sp. (asv.116—the sole significant taxon) relative to counterparts in the DTG group. Additionally, this taxon's positive association with weight gain through our prediction model enhances our comprehension of the microbiome's influence on elevated weight gain levels in subjects administered BIC over a one-year period, compared to those receiving DTG. These insights suggest potential avenues for further investigation, particularly in assessing the predictive capability of these bacterial taxa concerning weight gain in individuals prior to the administration of DTG or BIC treatments.

The link between obesity and gut microbiome has been widely accepted (483) paving a way to combat obesity by modulating gut microbiome. Regarding INSTI-associated weight gain, understanding the positive and negative weight-gain taxa is an important step in the comprehension of this adverse drug reaction that could eventually lead to interventional mitigation strategies. Therefore, we applied LASSO method to screen for significant factors that impact on the abundance of each taxon. In general, beside weight gain, DTG-taxa group is the only factor contributing to the abundance of all negative and positive weight-gain taxa. Host's characteristics (BMI, sex, age) either have small effect or contribute to a few numbers of taxa such as *Dialister*. This genus was found in a higher abundance in PLHIV than in HIV negative ones (484-486). The expansion of this genus has also been linked to adiposity in rheumatoid arthritis patients (487).

Our cohort exhibited a wide range of ethnic backgrounds, which were not evenly distributed by sex. This prompted us to investigate the effect of ethnicity on the composition of the microbiome. The influence of ethnic origin on microbiome diversity is well-documented, with environmental influences such as diet likely playing a more significant role than genetic factors (488, 489). While our analysis identified an association between ethnicity and the microbiome, ethnicity did not emerge as the primary determinant of microbiome diversity within our cohort. Instead, we observed pronounced differences in gut microbiome diversity and composition based on sex. Despite the minimal impact on weight gain-associated taxa, sex significantly influences the bacterial community composition, a finding that might be related to the fact that 69% of males included in this study were men who have sex with men. Indeed, while we decided to study sex and ethnicity based on previous findings indicating that both can affect the degree of weight gain experienced during INSTI treatment (240, 454, 455), it is important to consider that sexual orientation has been well described as an important factor influencing microbiome independently of HIV (490).

Our study has limitations, including its cross-sectional design precluding causality inference, the absence of a healthy control group, the limited size of the non-INSTI treated cohort, and a lack of dietary, fat mass and visceral fat data, all affecting the understanding of weight gain, treatments, and HIV impact on microbiome. Future longitudinal studies will be crucial to decipher HIV-related and drug class effects on the microbiome, and should take

opportunity from the use of the INSTI drug cabotegravir as monotherapy for HIV pre-exposure prophylaxis. Confounding factors, including unconsidered medications like metformin and statins, variable time elapsed between drug start and stool collection, and varied patient characteristics, might have influenced our findings. Despite these limitations, our research suggests specific taxa are linked to weight gain post-treatment. Our findings highlight the need for further research into INSTI-associated weight gain mechanisms, prediction, and management in HIV care.

3.2.3.4.1.6 *Declarations*

Availability of data and material

16S rRNA gene sequencing data have been uploaded to the NCBI's Short Read Archive and are accessible at <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1091927>. There is no new software or programming package developed as the results of this study. Supplemental and data source will be deposited to <https://zenodo.org/>.

Author contributions

Conceptualization: VH, JDG, LB and PDC. Methodology: VH, JDG, LB and PDC. Project administration: JDG, LB and VH. Funding acquisition: VH and PDC. Clinical work: JDG, LB, JCY, AV and BV. Biostatistical and Computational analysis: NKN. Management of samples and acquisition: JDG; DNA extraction: AP. Sequencing, meta, clinical data curation and data source depositing: NKN & JDG; Visualization: NKN; Writing—original draft: JDG and

NKN; Writing— review and editing: NKN, JDG, VH, and PDC. All authors read and approved the final version of the manuscript.

3.3 Virological failure under long-acting cabotegravir-rilpivirine treatment: case-control and pharmacogenetic analysis

To be submitted to AIDS

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Key Points

- This study investigated cases of VF in LA-CAB/RPV treated PLWH.
- We found a high-rate of emergence of CAB and RPV RAMs in case of VF.
- Using a case-control design, BMI and C_{trough} levels for CAB and RPV at VF appeared poorly predictive of failure.

3.3.1.1.1.1 Introduction

Long-acting (LA) cabotegravir (CAB)/rilpivirine (RPV) therapy has recently taken an important place in the antiretroviral armamentarium. LA-CAB/RPV treatments are currently recommended in Europe and the US as a switch strategy for persons living with HIV (PLWH) with sustained virological suppression (for at least 3-6 months) under stable antiretroviral regimen (63, 176). The real-world studies published so far found similar overall efficacy of LA-CAB/RPV regimens than randomized clinical trials, thus confirming that around 1 % of patients present virological failure (VF) despite on-time injections, with the exception of patients switched while viremic, who displayed VF rates of 5% in a recent meta-analysis (154, 491). VF mostly occurs during the first year after treatment initiation - although late failures are reported - and is associated with a substantial risk of selecting resistance-associated mutations (RAMs) (168, 491).

Although an association between VF and low drug exposure has been demonstrated in some cases and target concentrations have been proposed for CAB and RPV, studies have also shown that a substantial proportion of LA-treated persons living with HIV (PLWH) display low or very low concentrations without translating in VF (154, 168, 171). Other predisposing factors such as obesity, viral subtype A6/A1 and RPV RAMs at baseline have also been identified (169). Polymorphisms in genes encoding for CAB and RPV metabolizing enzymes and transport proteins could also favour VF through low systemic or intracellular drug exposure, but these have not been

assessed so far. We therefore aimed to evaluate pharmacokinetic and pharmacogenetic factors associated with VF.

3.3.1.1.1.2 Methods

Study design and participants

We launched a retrospective study describing cases of VF (defined as a viral load (VL) measured above 200 copies/mL) under LA-CAB/RPV among PLWH followed at our HIV reference center, or for which therapeutic drug monitoring (TDM) had been requested at our reference laboratory. In our center the use of LA-CAB/RPV is restricted to virologically suppressed patients, almost exclusively as a switch strategy for PLWH virologically suppressed under stable oral regimen (rarely as maintenance therapy after oral induction with another regimen). Each case was matched with two controls of the same sex, who had been on a stable LA-CAB/RPV treatment for at least as long as the case and had started CAB/RPV as close as possible to the case, with no documented VF at the time of data collection. Characteristics of cases and controls were compared at VF or matched date (calculated for a control based on the timelapse between LA-CAB/RPV initiation and VF in its matched case). Drug trough concentrations (C_{trough}) were retrospectively measured on residual plasma from the date of VF for all cases that occurred before July 2024; for those cases, controls were additionally selected based on the availability of residual plasma for C_{trough} measurement that had been drawn after a comparable interval from LA-CAB/RPV initiation. The study received approval from the local ethical

committee (Comité d'éthique hospital-facultaire Saint-Luc–UCLouvain, 2024/27JUI/323).

Clinical data, pharmacokinetic evaluation and participants genotyping

Demographic, clinical and HIV-related data were extracted from electronic medical records.

C_{trough} were measured on residual plasma from VL monitoring, by liquid chromatography with tandem mass spectrometry (LC-MC/MS) using Xevo TQS-micro tandem quadrupole mass spectrometer (Waters) coupled to an ultra-high pressure liquid chromatography system (Waters), according to a method previously described.

When available, results from next-generation sequencing (NGS) pharmacogenomic testing were reviewed in-depth for genes involved in CAB/RPV metabolism. NGS was performed on an Illumina MiSeq® platform, using a 41-gene panel testing (SOPHiA DDM® community Pharmacogenomics Solution) including *UGT1A1*, *UGT1A4*, *CYP3A4*, *CYP3A5*, *CYP2C19*, *ABCB1*, *ABCG2*, *NR1I2*. This test is routinely offered at our hospital in cases of unexpected drug reactions (regarding efficacy and/or toxicity), and is performed after obtaining the patient's informed consent.

Statistical analysis

Continuous data are presented as means with their standard deviations (SD) or medians with their interquartile ranges (IQR) and categorical data as counts and proportions. For cases-controls comparisons, continuous

variables were compared across groups using T-tests or Mann-Whitney, and proportions were compared using chi-squared or Fischer's Exact tests, as appropriate. Statistical analysis was performed using GraphPad Prism v10.

3.3.1.1.1.3 Results

Six cases of VF were identified as of 1st December 2024 among 130 patients having received at least one dose of LA-CAB/RPV and followed at our center (4.6 %), corresponding to an incidence of 2.41 per 100 persons-year of LA-CAB/RPV treatment. One additional case followed in another center and for which TDM had been requested was included. Failures were detected at a mean of 413 ± 237 days after CAB/RPV initiation, with 4 cases detected after more than 1 year. At failure, viral genotypes were available for 6, with 5 (83%) and 6 (100%) patients presenting NNRTI and INSTI RAMs, respectively. All patients were switched to darunavir/cobicistat/emtricitabine/tenofovir alafenamide with favorable virological response for 6 (1 patient awaiting follow-up VL). Characteristics of cases and controls are reported in Table 1, with no significant difference across groups. Notably, only one case displayed an A1 viral subtype and one case was obese (with a body mass index of 30.1 kg/m^2). Previous HIV genotypic data were available in 6 cases (86%), showing no RPV RAMs, although one participant displayed NNRTI-class associated RAMs (103N). Genotypic information regarding INSTIs susceptibility were available in 3 cases and displayed no INSTI-class associated RAMs.

Pharmacokinetic evaluation

C_{trough} were available for 4 cases and their 8 matched controls. We found no difference between cases and controls at the time of failure or matched timepoint, with mean C_{trough} of respectively 1682 ± 683 vs 1823 ± 564 ng/mL for CAB ($P = 0.71$) and 46.5 ± 35.0 vs 42.8 ± 32.1 for RPV ($P = 0.71$) (Figure 1). Interestingly, none of the participants displayed CAB concentrations below the suggested threshold of 664 ng/mL (i.e., 4 x the protein adjusted 90% inhibitory concentration, PA-IC₉₀). Low RPV concentrations under the suggested threshold of 32 ng/mL (i.e., the first C_{trough} quartile from phase III trials) were found in one case and four controls with the latter case displaying very low concentration of 10 ng/mL, thus below the PA-IC₉₀. All but 3 participants (1 case and 2 controls) displayed plasma concentrations below the alternatively proposed threshold of 40 ng/mL (i.e., 4 x PA-IC₉₀).

Pharmacogenetic evaluation

In depth analysis of pharmacogenetic results was available from 6 cases. Although no comparative group was available, our results are consistent with the expected distribution for a population from mixed European and African ascent (Table 2).

Table 1. Clinical and Biological Characteristics of Cases and Controls.

	Cases (n=7)	Controls (n=14)	P value
Age, years, mean (SD)	45 (14)	46 (10)	0.83
Female sex, n (%)	3 (43)	6 (43)	NA ^a
BMI, kg/m², median (SD)	27 (5)	27 (5)	0.99
eGFR^b, mL/min/1.73m², mean (SD)	87 (18)	86 (18)	0.97
HIV RNA at HIV diagnosis^c, log₁₀ copies/mL, median (IQR)	4.8 (4.4-6.4)	4.5 (3.9-5.0)	0.24
CD4+ T-cell count at LA-CAB/RPV start, mean (SD)	739 (401)	798 (297)	0.71
Time since HIV diagnosis, months, mean (SD)	142 (83)	172 (73)	0.40
Past NNRTI use, n (%)	4 (57)	5 (36)	0.40
Past RPV use, n (%)	3 (43)	4 (29)	0.64
Past INSTI use, n (%)	6 (86)	12 (86)	1
Past 2nd generation INSTI use, n (%)	5 (71)	12 (86)	0.57
Any detectable viral load within 2 years prior starting CAB/RPV^c	1 (14)	1 (7)	1
Duration of undetectability until VF/matched date^d, months, mean (SD)	75 (60)	109 (58)	0.52
Ethnicity, n (%)			0.67
- Black	4 (57)	5 (36)	
- White	3 (43)	7 (50)	
- Hispanic	0 (0)	2 (14)	

Characteristics of cases and controls are reported at VF or matched date, unless otherwise specified.

^aCases and controls were matched for sex

^beGFR estimated by CKD-EPI

^cBoth the case and the control presented with high level viral load (respectively 611 copies/mL and 1758 copies/mL) after treatment interruption. CAB/RPV was started after bictegravir/emtricitabine/tenofovir alafenamide reinduction.

^dUnavailable for 1 case and 1 control.

Table 2. Genotype distribution of cases (N=6).

Genotype	n (%)
UGT1A1	
*1/*1	1 (17)
*1/*28	4 (67)
*28/*36	1 (17)
ABCB1 c.1199G>A	
GG	6 (100)
ABCB1 c.3435C>T	
CC	2 (33)
CT	3 (40)
TT	1 (17)
CYP3A4*22	
-/-	6 (100)
CYP3A4*20	
-/-	6 (100)
CYP3A5	
Expressor ¹	3 (50)
NR1I2 - c.-22-7659C>T	
CC	3 (50)
CT	2 (33)
TT	1 (17)
ABCG2 c.421 C>A	
CC	6 (100)
CYP2C19	
*1/*1	4 (67)
*1/*2	1 (17)
*2/*2	1 (17)

¹CYP3A5 detail: n=1 for *1/*1 ; n=1 for *1/*3 ; n=1 for *1/*6 ; n=2 for *3/*3; n=1 for *3/*7.

3.3.1.1.1.4 Discussion

We report an in-depth analysis of 7 cases of VF in PLWH treated with LA-CAB/RPV, including a pharmacokinetic and pharmacogenetic study, with the

aim of evaluating possible associated risk factors. We found a significant rate of VF among CA/RPV-LA treated PLWH followed at our center, higher than the generally reported rate of 1 %. Importantly, we found several late-occurring cases, while the median time to VF in participants from FLAIR, ATLAS and ATLAS-2M studies was 24.9 weeks. Our findings confirm the risk of emergence of RAMs in case of VF, as previously reported in real-world studies and RCTs (491, 492).

Regarding VF risk factors, a post-hoc analysis from large trials identified 3 predictive factors, with the combination of two or more of those disproportionately increasing this risk: pre-existing RPV RAMs, HIV-1 subtype A6/A1, and a high BMI above 30 kg/m² (169). Another study recently showed that non-sustained viral suppression was linked to preexisting virological factors (such as VL at diagnosis or previously non-undetectable VL) (493). Moreover, some studies have found associations CAB/RPV concentrations in the low and detectable VL or VF, although the causal link remains poorly understood (154, 493). Interestingly, none of the predicted factor was found associated with VF in our analysis. Although TDM holds the potential of improving follow-up and enabling treatment individualization, its practicalities remain to be determined for LA-CAB/RPV therapy. In our analysis, we found the proposed C_{trough} targets to be poorly associated to VF and our results emphasize the need for additional data as on one hand failures occurred despite elevated CAB and/or RPV levels, and on the other hand several controls displayed low RPV levels. Of note, very low levels of RPV appeared to be associated with failure. Repeating measurements at

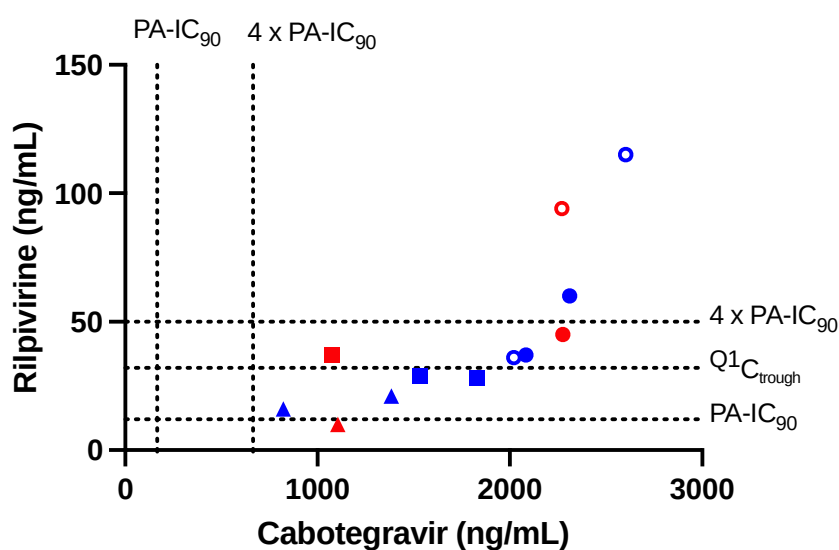
regular intervals could enhance sensitivity of TDM, but further data are needed to guide clinical practice. Moreover, the association between intracellular drug concentrations and the risk of VF certainly deserves to be studied.

This study also included a description of the pharmacogenetic profile of patients presenting VF. Although our limited data preclude any strong conclusions, it should be noted that we found no single participant with a genotype associated with an increased function for UGT1A1, which could have impacted on RPV and CAB concentrations, or for CYP2C19, which could have impacted on RPV concentrations. Moreover, we found a balanced distribution of *CYP3A5* genotypes, as a half were expressors of this enzyme that could increase the metabolism of RPV.

Our study has some limitations. First, its limited size precludes generalization, and the high rate of VF in our cohort could not be representative of treatment outcomes in other real-life cohorts. Secondly, more data would be useful regarding CAB and RPV pharmacokinetics in case of failure, especially TDM before the occurrence of VF. Lastly, larger datasets would be of interest regarding pharmacogenetic data. This would require including more cases of VF but also controls and assessing other pharmacogenes not included in our analysis despite the breadth of the 41-gene panel test performed.

In conclusion, we describe a series of 7 cases of VF not associated with most previously described risk factors. Alarming, this high rate appeared associated with high frequency of emergence of RAMs. C_{trough} levels appeared poorly associated with VF. Eventually, we were unable to find specific pharmacogenetic polymorphisms associated with VF.

Figure 1. CAB and RBV trough concentrations at time of virological failure (cases) or matched timepoint (controls). Cases are reported in red and controls in blue. Case-control pair are identified by identical symbols. Dotted lines represent drug level targets that have been proposed for CAB and RPV. Abbreviations: $PA-IC_{90}$: protein adjusted 90% inhibitory concentration; $Q1_{C_{\text{trough}}}$: first trough concentration quartile from phase III trials.



4 Conclusions and Perspectives

In this thesis, we have used a PK/PGx approach to investigate the determinants of response variability to the second-generation INSTIs (DTG, BIC and CAB). Due to their overall high efficacy and low-toxicity profiles, these agents have become a cornerstone of the treatment of HIV. This is especially the case for DTG which is now available worldwide through generic licensing agreements, and has been recommended by the WHO as first-line anchor agent within the TLD cART regimen.

Despite this broad use, several questions regarding the pharmacology of those agents remain unanswered, and the clinical experience has highlighted some crucial issues associated with their use:

- (1) **The occurrence of NPAEs**, that appears especially linked to the use of DTG.
- (2) **An increased weight gain compared to classical cART regimens**, which was particularly described for DTG and BIC when used in combination with TAF. However, it remains unclear if this is really linked to INSTIs or if it could be the flip side of the coin of the improved tolerance of modern regimens.
- (3) The ongoing **clinical challenge of (U)LLV**, which has not been solved by the use of second-generation INSTIs despite their very high antiviral potency.
- (4) The emerging concern regarding **VF associated with the use of LA-CAB/RPV** treatments which - although relatively uncommon - could

threaten long-term clinical success of cART in some PLWH due to the associated emergence of viral resistance.

These 4 challenges were addressed in this doctoral research, and we have fundamentally grounded our work in a better understanding of PGx/PK/PD interactions.

Regarding DTG, our work adds on extant literature and confirms the preponderant impact of UGT1A1 metabolizing status on systemic exposure.

On the contrary, it did not show a significant impact of several other relevant PGx polymorphisms on the drug plasma PK. Regarding *CYP3A*, although this could have been expected based on the secondary role of phase 1 enzymes in DTG metabolism, our results provide important reassurance about the broad use of the drug, especially considering the frequency of *CYP3A5* expression in persons from African ascent. Although significant, the impact of UGT1A1 metabolizing status is probably of limited clinical relevance. First, regarding efficacy, consistently with clinical practice and with published evidence, DTG levels encountered in our study were all beyond the PA-IC₉₀ - and all but two samples were beyond 4 x the PA-IC₉₀. Secondly, regarding toxicity, the evidence published so far suggesting a direct causal link between DTG systemic exposure and the occurrence of NPAE remains, at best, very tenuous (222, 228-231). Indirectly, our case control study provides some further evidence that systemic exposure is not a primary determinant of DTG

tolerability. Indeed, it is worth highlighting that we found a perfectly balanced distribution of UGT1A1 metabolizing status across cases and controls. Although some contrasting results have been reported (222), this aligns with the largest body of evidence available suggesting no clear association between *UGT1A1* genotypes and NPAE (229).

In this work, we further found an absence of impact of the *NR1I2* c.-22-7659C>T SNP on DTG systemic exposure. This is consistent with most of the available literature (391, 392), but one study also reported the finding of an association between T allele carriage and increased exposure (390). Putting these results in perspective with the contrasting results of our case-control study that found a neuropsychiatric protective effect of the T allele also argues against a direct concentration-dependent effect of DTG plasma concentrations on NPAE.

Taken together, our understanding of those different lines of evidence is that DTG systemic exposure is not a key determinant of the occurrence of NPAE.

In terms of perspectives, further PK analyses are planned based on the data generated during the INSTI-01 study. First, we want to study the association between DTG PK and participants gut microbiota diversity and composition. Recent studies have indeed shown that microbiota interferes with the regulation of transporters and biotransformation enzymes and could be an important contributor to PK variability (494-496).

Secondly, we plan to study the pharmacogenetic determinants of the systemic PK of BIC. Contrasting with pre-existing models for DTG, this PGx/PK study necessitates the building of a population PK model for BIC, a process that has already started in collaboration with the team of Prof L. Elens (LDRI/PMGK). It will be interesting to compare the results for DTG and BIC, especially given the more prominent role of CYP3A4 in BIC biotransformation. Here also microbiota analysis could be included in the analysis, in a second phase.

Another important finding of this doctoral work concerns the impact of pharmacogenetic polymorphisms on DTG and BIC intracellular accumulation.

The c.421C>A SNP in *ABCG2* was found to impact on DTG and BIC intracellular accumulation.

While the result was only near-significant for BIC, the consistent direction of results for both drugs (increased intracellular accumulation with the variant protein) combined with the existing literature indicating the importance of this SNP (340) supports the likelihood of a true effect. Importantly, whether and how the effect of this SNP on DTG and BIC transport translates on the drug systemic PK and PD remains unclear. The absence of effect of the SNP on DTG systemic exposure (CL/F) has been previously discussed, and this remains to be assessed for BIC. The possible differential impacts of the SNP

on various PK parameters ($C_{\max}$, C_{trough} and CL/F) for DTG is intriguing and will require to be explored in future clinical studies encompassing sampling times around the $T_{\max}$.

Although the study of other SNPs assessing DTG and BIC transport was exploratory, it provides some interesting insights that will deserve future exploration in independent cohorts, or using different approaches. Regarding specifically *ABCB1* and BIC, our consistent findings coming from both *in vitro* models and clinical study provide greater clarity.

We found substantiated evidence against a significant impact of the most frequent polymorphisms of *ABCB1* (c.1199G>A, c.1236C>T, c.2677G>T and c.3435C>T) on the transport of BIC.

From a broader perspective, our work highlights the relevance of applying a translational approach to those PK questions. Similar *in vitro* models are currently being applied to confirm our findings regarding *ABCG2* c.421C>A for BIC, and could also be applied to study the c.*193A>G SNP in *ABCB1*, after the generation of dedicated mutant recombinant cell lines.

Using a case-control study design, we have found a protective effect of the *NR1I2* c.-22-7659C>T SNP on DTG discontinuation due to NPAE.

With respect to DTG neurotoxicity, it is also worth mentioning that the *ABCG2* c.421C>A SNP was not associated with increased risk of DTG-discontinuation due to NPAE, despite an expected impact on drug transport at the BBB.

But if not directly DTG-concentration dependent, what causes those adverse events, and how could the NR1I2 c.-22-7659C>T SNP be involved?

Recent reports have shown that DTG disrupts the BBB affecting the expression of TJ proteins and efflux transporters - including a demonstrated significant downregulation of *ABCB1* and *ABCG2* mRNA expression (233, 234). Putting those findings in perspective with the possible opposite effects of the *NR1I2* c.-22-7659C>T SNP on drug transporters expression at the BBB, we may formulate the hypothesis that carriage of the T allele could possibly protect against NPAE by counterbalancing the local downregulation of these transporters. Another hypothesis could be that the SNP impacts on the expression of TJ proteins at the BBB in a different direction compared to DTG. Indeed, some limited data are available suggesting a role of *NR1I2* in the regulation of some TJ proteins (358, 450).

Confirming the *clinical validity* of a protective effect of *NR1I2* c.-22-7659C>T SNP against DTG-associated NPAE will undoubtedly require the replication of our findings in one or more independent clinical cohorts. Knowing the relatively low rate of significant NPAE with DTG and the variant frequency of the SNP, it may however seem improbable that this SNP could currently

demonstrate *clinical utility* and qualify for routine pre-emptive testing with avoidance of the use of DTG in the presence of the risk-associated genotype. Indeed, based on the described frequency (54%) of the T allele in the global population (363), we can calculate that 21% of the population will have the at risk CC genotype (not carrying the protective T allele). Considering a rate of DTG discontinuation due to NPAE at ~2.8% (207), we can conclude that a vast majority of PLWH identified as genetically at risk would actually not have to discontinue the drug due to NPAE¹. Excluding all those PLWH from the first line agent DTG based on a limited risk for an outcome that can be managed clinically (by discontinuing the drug) appears unjustified. Of note, genotyping

¹ Considering the aforementioned rate of drug discontinuation due to NPAE and genotype distributions found in the literature, and considering an odds ratio of 3 for the risk of NPAE-associated DTG discontinuation, we can estimate the following distribution of a population of 100 PLWH treated by DTG in the absence of preemptive pharmacogenetic testing (see table). If preemptive testing was used and DTG avoided in case of CC genotype, 16.8 persons should avoid the drug to prevent one case of drug discontinuation due to NPAE. The rate of DTG discontinuation due to NPAE would remain as high as 1.96% in the DTG-treated population.

	Will have to stop DTG due to NPAE	Will tolerate DTG
NR1I2 c.-22-7659 CC	1.25	19.75
NR1I2 c.-22-7659T carriers	1.55	77.45

might however prove useful if additional independent risk factors are clearly identified that heighten the absolute risk associated with the genotype.

Eventually, this doctoral work also included PD analysis regarding the efficacy of second-generation INSTIs. While mainly focusing on DTG and BIC, we also included a separate analysis on the relationship between CAB (and RPV) PK and drug efficacy.

Our analysis did not demonstrate an association between DTG and BIC intracellular concentrations or accumulation and the occurrence of HIV viremia at low- and ultra-low-levels.

Although the possibility of persistent HIV replication in sanctuary sites has not been rejected, it is admitted that clonal expansion of latently infected host cells is a cause of the circulation of HIV virions and nucleic acids. This phenomenon, detected as viremia during monitoring, generally occurs at (ultra-)low-levels (177, 497). Both mechanisms could coexist, with respective contributions being unknown. Our results are not surprising if (U)LLV is independent of viral replication, and could be seen as reinforcing this hypothesis. However, our findings do not exclude low concentrations in sanctuary sites such as lymphatic tissues where replication is the most prominent (435). Tests assessing the clonality of HIV RNA sequences and their genetic evolution over time could theoretically be used to confirm that a LLV (or ULLV) episode belongs to the NSV category. However, although their

use has been suggested to guide clinical practice, they remain currently complex and restricted to the research setting (178). If those tests were available, measuring plasma and intracellular ARV concentrations in PBMCs (as a proxy of measures in other lymphoid tissues because lymphatic tissues cannot be easily accessed) could prove useful in replicative cases, and the association of drug concentrations and subsequent VF should be further evaluated.

Knowing the role of GALT as a major HIV reservoir (498), the crosstalk between gut microbiota and GALT (499), the limited drug penetration in GALT (193), and the aforementioned impact of gut microbiota on regulation of intestinal transporters (494), the impacts of gut microbiota diversity and composition on LLV and ULLV deserve further attention. Building on our PK/PGx/PD and microbiota data, we could explore hypothesis such as (i) an impact of gut microbiota on drug transporters resulting in viremia linked to variations in drug levels in sanctuaries, or (ii) an impact of gut microbiota on clonal expansion of lymphoid cells into the GALT leading to clonal expansion of provirus-containing cells – the latter being or not independent of drug levels.

Eventually, this work has studied the associations between PK of CAB and RPV and VF.

Our preliminary analysis found that the association between TDM levels for both CAB and RPV and occurrence of VF is unclear.

The PK profiles of both drugs differ significantly from those of DTG and BIC due to their intrinsic characteristics and the LA suspension formulation. How this interferes with the risk of VF and emergence of RAMs remains poorly understood. In particular, the impact of varying drug concentrations over time, characterized by high IIQ at $C_{\max}$ compared to stable concentrations, warrants further investigation into their effects on viral dynamics.

Regarding PK and PGx, our preliminary study is mainly limited by the number of included participants and the single PK assessment for each patient. We believe this analysis deserves to be expanded, ideally as a multicentric case-control study to offer sufficient power to perform a rigorous assessment of the role of PK and PGx polymorphisms.

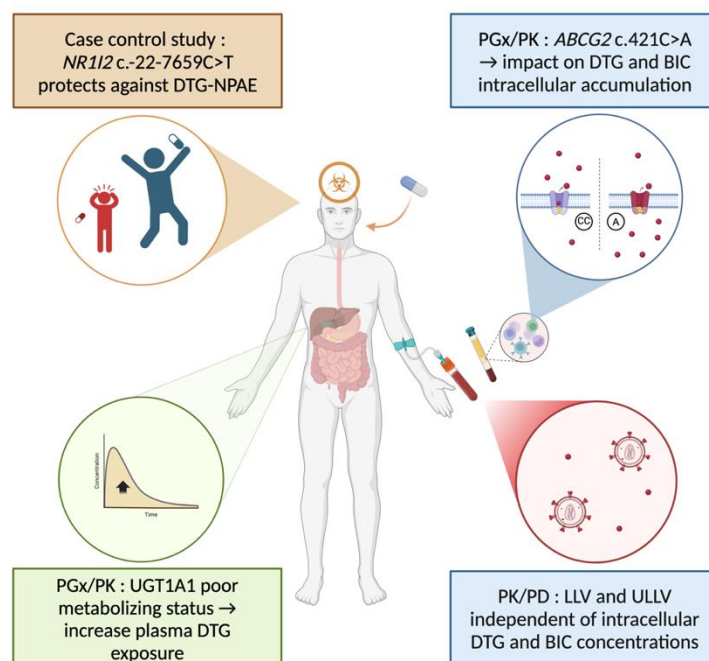


Figure 18. Graphical abstract highlighting key findings from the INSTI-01 study.

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5 Annexes

5.1 Annex 1

Table 10. List of the ARVs currently used in Belgium

Drug class	Full name	Abbreviation
NRTIs	Abacavir	ABC
	Emtricitabine	FTC
	Lamivudine	3TC
	Tenofovir disoproxil fumarate	TDF
	Tenofovir alafenamide	TAF
	Zidovudine	AZT or ZDV
NNRTIs	Doravirine	DOR
	Efavirenz	EFV
	Etravirine	ETR
	Nevirapine	NVP
	Rilpivirine	RPV
PIs	Atazanavir	ATV
	Darunavir	DRV
	Ritonavir	RTV
INSTIs (1st generation)	Elvitegravir	EVG
	Raltegravir	RAL
INSTIs (2nd generation)	Bictegravir	BIC
	Cabotegravir	CAB
	Dolutegravir	DTG
Entry inhibitors	Maraviroc	MVC
Attachment inhibitors	Fostemsavir	FTR
Capsid inhibitors	Lenacapavir	LEN

5.2 Annex 2

Table 11. Main pharmacological characteristics of second-generation INSTIs

	DTG	BIC	CAB
Other names	S/GSK1349572	GS-9883	S/GSK1265744
Usual dosage	50mg QD (PO)	50mg QD (PO)	30mg QD (PO) 600mg every 2 months (IM)
T_{max}	0.5-2h	1-2h	PO: 0.5-1.5h
Half-life	13-15h	17h	PO: 31.5h
Protein binding	>99%	>99%	>99%
Metabolism	UGT1A1 CYP3A4 UGT1A3 UGT1A8 UGT1A9	UGT1A1 CYP3A4 UGT1A3 UGT1A8 UGT1A9	UGT1A1 UGT1A9
Transport	ABCB1 ABCG2	ABCB1 ABCG2	ABCB1 ABCG2

5.3 Annex 3

Dose response curves of DTG and other INSTIs.

ARV efficacy can be described by a drug's **dose-response curve** (101, 500). **IC₅₀** is an important characteristic of a drug: it is the drug concentration that causes 50% of the maximum inhibitory effect. The **inhibitory quotient** value (IQ, the ratio of plasma drug concentrations to IC₅₀) gives important information about the drug potency, especially when expressed at certain concentrations such as C_{max} or C_{min}. The **slope (m)** of this curve is another crucial drug characteristic: for 2 hypothetical drugs with $m_A > m_B$, drug A will have increased efficacy (generally estimated *in vitro* through the measure of viral infectivity) for a similar IQ. Indeed, in the clinical concentration range (that generally lies at 10-100-fold the IC₅₀) its **instantaneous inhibitory potential** (IIP, equivalent to the number of logs by which a single round of infection is reduced in the presence of the drug at a relevant concentration) will be higher than that of drug B (**Figure 19 A-C** and **Figure 19 E**)

Among INSTIs, DTG demonstrates higher antiviral efficacy than first-generation INSTIs, with DTG dose-response curve slope of 1.3, significantly higher than RAL's slope of 1 ($P = 0.0003$) (100). Of note, INSTIs as a class do not display specifically high slopes (**Figure 19 D**).

It is important to consider that pharmacodynamic properties should ideally assessed in combination with other drugs, as cART comprises combination therapies and drug can present synergistic activities.

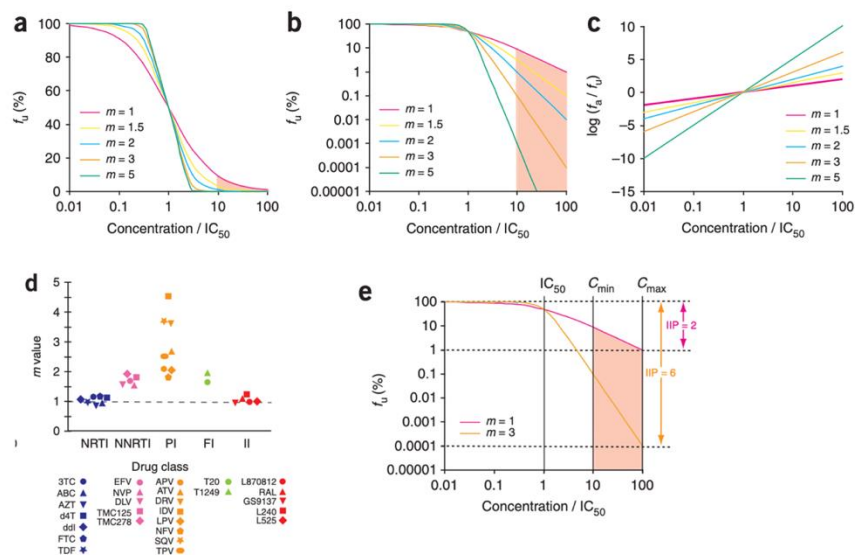


Figure 19. Hypothetical dose-response curves to illustrate the impact of the curve's slope on antiretroviral efficacy.

(a) Hypothetical linear-log plot of the fraction of viruses unaffected by a drug (f_u) versus drug concentration, on the basis of the median-effect model for drugs with different m values. Concentrations are normalized by IC_{50} . A typical clinical concentration range 10–100-fold above the IC_{50} is shaded. (b) A log-log plot of the same curves emphasizing the strong impact of slope on the suppression of infectivity. (c) A median effect plot [$\log(f_a / f_u)$ versus $\log(\text{drug concentration})$] of the same curves. The m value is the slope of the line. (d) Each class of anti-HIV-1 drugs and each agent has a characteristic slope. (e) Comparison of the antiviral activity of two hypothetical drugs with different m values. IIP is the number of logs of suppression of single-round infectivity at a clinically relevant drug concentration. At C_{max} , the IIP of a drug with $m = 3$ is 10,000-fold greater than that of a drug with $m = 1$. From (101).

Abbreviations: PI, protease inhibitors; FI, fusion inhibitors; II, integrase inhibitors; 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine, FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; EFV, efavirenz; fu, unbound fraction; NVP, nevirapine; DLV, delavirdine; APV, amprenavir; ATV, atazanavir; DRV, darunavir; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; SQV, saquinavir; TPV, tipranavir; T20, enfuvirtide; RAL, raltegravir.

5.4 Annex 4

Key definitions (368, 501, 502)

Allele: One of two or more versions of a genetic sequence at a particular region (*locus*) on a chromosome. An allele can be defined as wild-type when it represents the most common sequence of a gene in the general population. The two alleles carried by an individual define its *genotype*.

Variant: Any change in the most common DNA nucleotide sequence. The term variant can be used to describe an alteration that may be benign, pathogenic, or of unknown significance.

Polymorphism: A common variant in a specific sequence of DNA. “Common” is typically defined as an allele frequency of at least 1% in the general population.

Single nucleotide polymorphism (SNP): A DNA sequence variation that occurs when a single nucleotide (adenine, thymine, cytosine, or guanine) is different from the reference sequence. SNPs are usually present in at least 1% of the population and are the most common type of genetic variation among people, to be distinguished from *single nucleotide variants* (SNV) that can be indistinctly rare or frequent. SNVs are nucleotide substitutions, but there are several other types of simple variants, such as for instance *insertions* or *deletions* (when one or more nucleotides are respectively inserted or deleted compared to the reference sequence), or more complex variants.

Haplotype: The combination of several variants on a chromosome that are inherited in blocks, as a result of their proximity. Variants that are not independently inherited are said to be in *linkage disequilibrium*.

Different **nomenclatures** coexist to refer to genetic variants (503, 504):

- Standard notation for SNVs includes a letter prefix to indicate the type of reference sequence used (such as "c." for a coding DNA reference sequence, or "g." for a linear genomic reference sequence), followed by a number for the position of the nucleotide, followed by a one-letter abbreviation for the nucleotide (A, C, G, T or U), followed by a greater than sign (">") to indicate substitution, followed by the abbreviation of the nucleotide which replaces the former.
 - Example: c.1199G>A in *ABCB1*. The consequence at the protein level is also referred to in a standard way; in this case a Serin-to-Asparagine at position 400 change is referred as Ser400Asn.
- rs nomenclature: single base nucleotide substitutions and short deletion and insertion polymorphisms that are cataloged in the dbSNP database (<https://www.ncbi.nlm.nih.gov/snp/>) can be designated using a unique identifier that consists in "rs" (for Reference SNP cluster ID) followed by a number.
 - Example: rs2229109 holds for c.1199G>A in *ABCB1*.
- Star (*) nomenclature: This nomenclature is based on an official repository of allelic variation hosted by PharmVar (286) for some important pharmacogenes. Different haplotypes and subvariants (suballeles) are grouped using a star designation that is based on the presence of amino acid changes and/or functional non-coding sequence variations. Subvariants are identified by a numerical string

after the star designation (e.g., *CYP3A4**1.001). Strict criteria have been established for star allele and subvariant designation (505). Wild-type alleles are labelled *1.

- Example: *CYP3A4**22 designs the c.522-191C>T variant (rs35599367) that encodes a splice defect in CYP3A4.
- A standardized terminology can be used to describe the drug metabolizing phenotype for a specific genotype. This enables the classification of a subject based on the activity of a metabolizing enzyme using a predefined terminology (poor, intermediate, normal, rapid, or ultrarapid metabolizer) that is usually defined by the CPIC.

5.5 Annex 5

	△ Full gene	□ Gene hotspots	○ CNVs	☆ Star alleles	◇ Promoters/UTRs
ABCB1	△	□	○		◇
ABCC2		□	○		
ABCC4		□	○		
ABCG2		□	○		◇
ACE		□	○		
ADRB1		□	○		
ADRB2		□	○		
BCHE	△		○		
CDA		□	○		
COMT		□	○		
CYP1A2		□	○		
CYP2B6		□	○		
CYP2C19		□	○		
CYP2C8		□	○		
CYP2C9		□	○		
CYP2D6	△	○		☆	◇
CYP3A4		□	○		
CYP3A5		□	○		
CYP4F2		□	○		
DPYD	△		○		
DRD2		□	○		
G6PD		□	○		
HTR2A		□	○		
ITPA		□	○		
MTHFR		□	○		
NAT2	△		○		
NR1I2		□	○		
NR1I3		□	○		
NUDT15	△		○		
OPRM1		□	○		
P2RY12		□	○		
POR		□	○		
RYR1		□	○		
SLC22A1		□	○		
SLCO1B1		□	○		
TPMT	△		○		
TYMS		□	○		
UGT1A1		□	○		◇
UGT1A4		□	○		◇
UGT2B7		□	○		
VKORC1	△		○		◇

Figure 20. Detail of 41 genes covered by the SOPHiA GENETICS® Pharmacogenomics Community Panel test.
From (506).

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