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Courlet Perrine

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UNIL | Université de Lausanne
Faculté de biologie et de médecine

Service de pharmacologie clinique
Département de Médecine de Laboratoire et Pathologie

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par

Perrine COURLET

Pharmacienne diplômée de l'Université de Besançon, France

Jury

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Abstract

Highly active antiretroviral treatments have transformed HIV infection from a deadly disease into a chronic condition. People living with HIV (PLWH) are ageing and experience age-related comorbidities such as cardiovascular diseases. Consequently, PLWH are often polymedicated, increasing the risk of drug-drug interactions. Antiretroviral drugs are among the therapeutic agents with the highest potential for drug-drug interactions, acting essentially as perpetrators. Drug-drug interactions can affect comedications plasma exposure, increasing or decreasing drug concentrations, with a risk of adverse events or therapeutic failure, respectively. In addition, age-related physiological changes (such as renal or hepatic impairment) can affect the magnitude of the interaction.

This thesis aimed at improving knowledge about drug-drug interactions frequently encountered in clinical practice, in order to propose drug dosage adjustments and therefore optimize patient's care.

A multicentric study within the Swiss HIV Cohort Study was initiated in order to review the current state of drug prescriptions in PLWH. Cardiovascular drugs use and drug-drug interactions with this drug class were more common in elderly whilst central nervous system drugs were more prescribed and mainly involved in drug-drug interactions in younger PLWH. In order to improve knowledge about these frequent drug-drug interactions, a pharmacokinetic study with rich sampling was conducted in consenting patients receiving comedications of interest.

In addition to two bioassays for the quantification of antiretroviral drugs in human plasma, a third method was developed and validated for the determination of plasma concentrations of the most prescribed cardiovascular drugs and those with the higher risk for drug-drug interactions.

Quantitative results of these analysis allowed the development of population pharmacokinetic models for amlodipine, atorvastatin (and its major active metabolite), and rosuvastatin. These models showed that the magnitude of drug-drug interactions with antiretroviral drugs varied from weak to strong, thus potentially requiring drug dosage adjustment. In addition, a fourth model was developed for the evaluation of the pharmacokinetics of the most prescribed CNS drug escitalopram, for which no interaction with antiretroviral treatments was found, but which reveals that a significant proportion of patients were under-exposed. Finally, age was not retained as a significant covariate in any of the models, suggesting that aging has a marginal impact on the pharmacokinetics of evaluated drugs and thus, that dose adjustment based on age would *a priori* not warranted.

In conclusion, results of these studies provide overall reinsuring information on the drug exposure in PLWH, yet may be of help in clinical practice to guide the choice of comedication drugs and their dosage, taking into account patients' characteristics, specific clinical situation and the associated

antiretroviral treatment. They provide recommendations to improve to patients' care of an aging HIV-infected population, to optimize treatments to maintain clinical efficacy while insuring optimal safety.

Résumé

La mise sur le marché de puissants médicaments antirétroviraux a considérablement amélioré le pronostic de l'infection par le VIH, qui est désormais considérée comme une maladie chronique. Les personnes vivant avec le VIH deviennent ainsi de plus en plus âgées et sont touchées par diverses comorbidités habituellement liées à l'âge (maladies cardiovasculaires notamment) dont le traitement implique des associations médicamenteuses multiples et complexes, à fort risque d'interactions médicamenteuses avec les agents antirétroviraux. Ceci peut conduire à une augmentation ou une diminution des concentrations plasmatiques des comédications, avec des risques d'effets indésirables ou d'échecs thérapeutiques, respectivement. De plus, les changements physiologiques liés à l'âge (comme l'insuffisance rénale ou hépatique) sont susceptibles d'influencer l'intensité de telles interactions pharmacocinétiques.

L'objectif de cette thèse est d'améliorer nos connaissances sur les interactions médicamenteuses fréquemment rencontrées en pratique clinique, qui pourraient nécessiter d'adapter la posologie le cas échéant, dans le but d'optimiser la prise en charge thérapeutique des personnes vivant avec le VIH.

Une étude observationnelle multicentrique a été initiée sous les auspices de la *Swiss HIV Cohort Study* (SHCS) afin de dresser un état des lieux du type de prescriptions chez les personnes vivant avec le VIH, et de la prévalence des interactions médicamenteuses potentielles. Ce projet impliquait la saisie prospective détaillée de tous les médicaments pris par environ 1000 patients de la SHCS, ainsi qu'une prise de sang à l'occasion de la visite de cohorte habituelle, en même temps que les informations nécessaires à l'interprétation des taux sanguins des médicaments. Les médicaments du système cardiovasculaire et du système nerveux central étaient les plus prescrits et les plus à risque d'interactions chez les personnes âgées et les plus jeunes, respectivement. Afin d'évaluer l'importance de ces interactions dans la vie réelle, une étude de pharmacocinétique avec prélèvements riches (*e.g.* tout au long d'un intervalle de dosage de 24h) a été menée chez des patients consentants recevant les médicaments d'intérêt.

Ce projet a nécessité le développement et la validation de deux méthodes analytiques par spectrométrie de masse pour le dosage de plusieurs antirétroviraux dans le plasma, ainsi qu'une troisième méthode pour mesurer les concentrations plasmatiques des médicaments du système cardiovasculaire les plus prescrits, et les plus à risque d'interactions médicamenteuses.

Les résultats de ces dosages ont permis le développement de modèles pharmacocinétiques de population pour l'amlodipine, l'atorvastatine (et son métabolite actif majoritaire) et la rosuvastatine. Ces modèles ont montré la présence d'interactions de magnitudes faible à élevée avec les

antirétroviraux, nécessitant parfois une adaptation posologique. Enfin, un 4ème modèle a été développé pour l'analyse pharmacocinétique de l'escitalopram, pour lequel aucune interaction avec les antirétroviraux n'a été démontrée. L'âge des patients ne semble pas jouer un rôle significatif dans aucun des modèles pharmacocinétiques investigués.

En conclusion, les résultats de ces études peuvent être utiles en pratique clinique pour guider le choix de certains médicaments et de leur posologie, en prenant en compte les caractéristiques individuelles de chaque patient, leur état clinique et le traitement antirétroviral associé. Ce travail apporte de nouvelles connaissances sur les interactions médicamenteuses observées en pratique clinique. Il propose des axes d'amélioration de la prise en charge d'une population vivant avec le VIH qui vieillit, afin d'optimiser les traitements pour en optimiser l'efficacité tout en diminuant les effets indésirables.

Résumé large public

La mise sur le marché des médicaments antirétroviraux a considérablement amélioré le pronostic de l'infection par VIH, désormais considérée comme une maladie chronique. Les personnes vivant avec le VIH deviennent ainsi de plus en plus âgées, et souffrent en conséquence des comorbidités habituellement liées à l'âge pour lesquelles ces patients sont également traités. Cette situation conduit à des associations médicamenteuses multiples et complexes, à fort risque d'interactions médicamenteuses, mais dont nous ne connaissons pas l'importance réelle dans la vie de tous les jours. Les antirétroviraux sont devenus très efficaces et sont globalement bien tolérés, mais ils sont parmi les médicaments à plus haut potentiel d'interactions médicamenteuses, qui peuvent conduire à une augmentation ou une diminution des concentrations sanguines des comédications, avec un risque d'effet indésirable ou d'échec thérapeutique, respectivement. De plus, les changements physiologiques liés à l'âge (comme l'insuffisance rénale ou hépatique) peuvent modifier l'importance de l'interaction médicamenteuse. Une meilleure connaissance de ces interactions permettrait l'adaptation des posologies de médicaments le cas échéant, avec comme conséquence une amélioration de la prise en charge des patients.

Les objectifs de cette thèse étaient d'abord de dresser un état des lieux des prescriptions chez les personnes vivant avec le VIH, afin de connaître les médicaments les plus fréquemment prescrits et ceux les plus à risques d'interactions médicamenteuses. Les médicaments du système cardiovasculaire et du système nerveux central étaient les plus prescrits chez les patients âgés et plus jeunes, respectivement. Nous avons développé des méthodes analytiques notamment pour les médicaments du système cardiovasculaire qui nous ont permis de mesurer les concentrations de ces médicaments dans le sang des patients et d'étudier en détail l'importance de ces interactions médicamenteuses. Ces analyses ont finalement permis d'étudier l'évolution des concentrations sanguines de certains médicaments (amlodipine, atorvastatine et rosuvastatine) en fonction du temps, grâce à des modèles pharmacocinétiques. L'influence des caractéristiques démographiques et cliniques des patients sur les concentrations de ces médicaments, ainsi que l'importance des interactions entre les antirétroviraux et les comédications ont pu être quantifiées.

Les résultats de ces études peuvent s'avérer utiles en pratique clinique pour guider le choix de certains médicaments et de leur posologie, en prenant en compte les caractéristiques individuelles de chaque patient, leur état clinique et le traitement antirétroviral associé. En conclusion, ce travail propose des axes d'amélioration de la prise en charge d'une population vivant avec le VIH qui vieillit, afin d'optimiser les traitements pour en maximiser l'efficacité tout en diminuant les effets indésirables.

Scientific communications

Publications in peer-reviewed journal

Perrine Courlet, Monia Guidi, Susana Alves Saldanha, Matthias Cavassini, Manuel Battegay, Marcel Stoeckle, Thierry Buclin, Catia Marzolini, Laurent Decosterd, Chantal Csajka and the Swiss HIV Cohort Study. Influence of drug-drug interactions on the pharmacokinetics of atorvastatin and its active metabolite *ortho*-OH-atorvastatin in aging people living with HIV. **Clinical Pharmacokinetics. 2020.** [epub ahead of print]

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Poster presentations

Perrine Courlet, Françoise Livio, Susana Alves Saldanha, Alexandra U. Scherrer, Manuel Battegay, Matthias Cavassini, Marcel Stoeckle, Laurent Decosterd, Catia Marzolini, and the Swiss HIV Cohort Study. Real-life management of drug-drug interactions between antiretrovirals and statins. **CROI**, Boston, Massachusetts, 8-11 March **2020**.

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atorvastatin and its active metabolite *ortho*-OH-atorvastatin in people living with HIV. **PAGE meeting**, Stockholm, 11-14 June **2019**.

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Table of contents

Abbreviations

3TC	Lamivudine
ABC	Abacavir
aCSF	Artificial Cerebrospinal Fluid
ACN	Acetonitrile
ADME	Absorption, Distribution, Metabolism, Elimination
ALT	Alanine Aminotransferase
ARS	Anticholinergic Risk Score
ART	Antiretroviral Treatment
ARV	Antiretroviral
AST	Aspartate Aminotransferase
ATC	Anatomical, Therapeutic, Chemical
ATV	Atazanavir
AUC	Area Under the Curve
AUC_r	Area Under the Curve ratio
BCRP	Breast Cancer Resistance Protein
BID	Twice daily
BQL	Below the limit of quantification
BSV	Between-subject Variability
Cl	Clearance
C_{max}	Maximum plasma concentrations
C_{min}	Minimum plasma concentrations
CNS	Central Nervous System
CPE	CNS Penetration Effectiveness
CSF	Cerebrospinal Fluid
CYP	Cytochrome P450
LLOQ	Lower Limit of Quantification
ME	Matrix Effect
MeOH	Methanol
MPE	Mean Prediction Error
NCA	Non-compartmental analyses
NNRTI	Non Nucleoside Reverse Transcriptase Inhibitors
NONMEM	Nonlinear Mixed Effects Modelling
NRTI	Nucleoside Reverse Transcriptase Inhibitors
OATP1B1	Organic Anion Transporting Polypeptide
OFV	Objective function value
PBPK	Physiologically-Based Pharmacokinetic
pcVPC	prediction corrected Visual Predictive Checks
PD	Pharmacodynamic
PDDI	Potential Drug-drug Interaction
PE	Process Efficiency
PI	Protease Inhibitor
PK	Pharmacokinetic
PLWH	Person Living With HIV
popPK	Population Pharmacokinetics
PrEP	Pre-exposure Prophylaxis
pvcVPC	Prediction and variance corrected Visual Predictive Checks
Q	Inter-compartmental clearance
QC	Quality Control
QD	Once daily
QID	Four times a day

D1	Zero order constant of absorption
DDI	Drug-drug Interaction
DMSO	Dimethylsulfoxide
DRV	Darunavir
DTG	Dolutegravir
EMEA	European Medicine Agency
ER	Extraction Recovery
ESI	Electrospray Ionization
ETV	Etravirine
FA	Formic acid
FDA	Food and Drug Administration
FOCE	First Order Conditional Estimation with Interaction
$FR_{ator\text{-}oOH}$	Proportional coefficient between total atorvastatin and *o*-OH-atorvastatin absorption rate constants
FTC	Emtricitabine
Gln-ZDV	Zidovudine glucuronide
GOF	Goodness-of-fit
HAART	Highly Active Antiretroviral Therapy
HDL	High Density Lipoprotein
HIV	Human immunodeficiency virus
INSTI	Integrase strand transfer inhibitors
IPRED	Individual Predicted Value
IS	Internal Standard
IS-nER	Internal Standard normalized Extraction Recovery
IS-nME	Internal Standard normalized Matrix Effect
IS-nPE	Internal Standard normalized Process Efficiency
rCSF	real Cerobrospinal Fluid
RMSE	Root Mean Squared Error
RSE	Relative Standard Error
RT	Room Temperature
RTV	Ritonavir
SD	Standard Deviation
SE	Standard Error
SFSTP	Société Française des Sciences et Techniques Pharmaceutiques
SHCS	Swiss HIV Cohort Study
$t_{1/2}$	Half-life of elimination
TAF	Tenofovir Alafenamide
TAT	Turn–Around Time
TC	Total Cholesterol
TDF	Tenofovir Disoproxil Fumarate
TFV	Tenofovir
TG	Triglycerides
TID	Three times a day
TDM	Therapeutic Drug Monitoring
T_{max}	Time to C_{max}
UGT	UDP-glucuronyltransferase
ULOQ	Upper Limit of Quantification
UHPLC-MS/MS	Ultra-High-Pressure Liquid Chromatography coupled to tandem mass spectrometry
V_d	Volume of distribution
WS	Working Solution
ZDV	Zidovudine

IS-WS	Internal Standard Working Solution	θ	Theta: fixed effects parameters generated by NONMEM
ka	First order constant of absorption	η	Eta: inter-individual, random error in NONMEM models
LAI	Long-acting Injectable	ε	Epsilon: residual error of an observation y, also called random effect
LC-MS/MS	Liquid Chromatography coupled to tandem mass spectrometry	ω	Omega: standard deviation of parameters θ generated by NONMEM
LDL	Low Density Lipoprotein	σ	Sigma: standard deviation of residual error ε

CHAPTER I: GENERAL INTRODUCTION

More than 30 years after HIV started to spread, and almost 25 years after combination antiretroviral therapy (ART) became available, the epidemic profile of HIV epidemic in Europe has changed [1]. Control the viral infection has been the subject of many studies since the first reported cases of AIDS in 1981 and the isolation of the human immunodeficiency virus (HIV) in 1983 [2, 3]. The history of ART began with the first clinical trial of zidovudine in 1986 [4]. A better understanding of the HIV biology and pathogenesis allows the development of highly active antiretroviral therapy (HAART), with the advent of non-nucleoside reverse transcriptase inhibitors (NNRTIs) and Protease Inhibitors (PIs) in the late 1990's and 2000's and then CCR5 antagonists and HIV integrase strand transfer inhibitors (INSTIs). These days, ART regimens are currently potent, convenient (possible once daily pill combining three drugs) and allow the reduction of HIV blood concentration to undetectable values within a few weeks after treatment initiation. ARTs can control the infection and help to prevent transmission, as demonstrated by the recent decrease in the worldwide number of new HIV infections and HIV-related deaths, by 16% and 33% respectively, between 2010 and 2016 [5].

Despite the success of ART, HIV continues to be a major global public health issue. According to the 2018 World Health Organisation (WHO) report, 770 000 people all over the world died from HIV-related causes, 37.9 million were living with HIV and another 1.7 million became newly infected [5]. Since 1988, an observational and longitudinal cohort in Switzerland has been successfully enrolling people living with HIV (PLWH). The Swiss HIV Cohort Study (SHCS; www.shcs.ch) is a collaboration of all Swiss University hospitals, two large cantonal hospitals, with affiliated laboratories, smaller hospitals and private physicians. The major goal of SHCS is to provide optimal patient care, to reduce HIV transmission and to conduct research on several HIV dimensions. Approximately four hundred new individuals are recruited in SHCS every year, and the number of participants under active follow-up reached 9486 in 2018.

High levels of patient engagement in the HIV care continuum are key to the control of the global HIV epidemic. Joint United Nations Programme on HIV/AIDS (UNAIDS) has set the target of ending AIDS by 2020. They estimate that this can be achieved if 90% of people living with HIV are diagnosed; if 90% of diagnosed people are taking ART; and if 90% of people taking ART are virally suppressed – the so-called 90-90-90 target (Figure 1). Countries in Western and Southern Europe are well on their way to meeting the UNAIDS 90-90-90 targets [6].

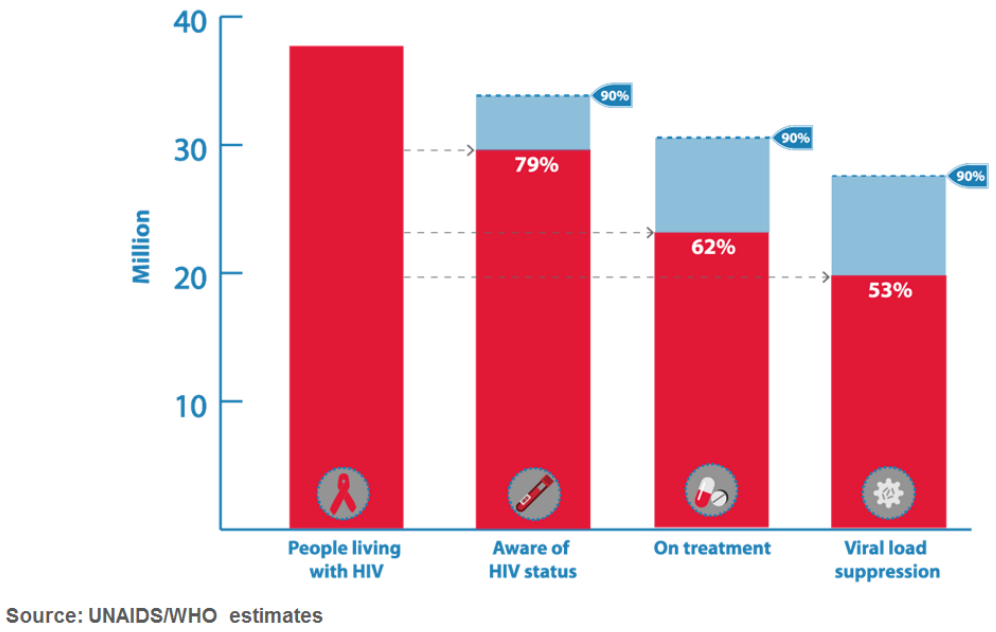

Figure 1: HIV testing and care continuum (2018) [5]

The development of ARTs is a major turning point for the HIV epidemic over the past thirty years, transforming HIV infection from a deadly disease into a chronic condition, and therefore improving quality of life of PLWH [7-9]. However, such chronic treatments raise challenging issues regarding the maintenance of both therapeutic effectiveness and long-term tolerability. Indeed, ARTs do not fully restore health, as latent T-cell infection and residual replication in sanctuary compartments such as CNS do persist.

I.1. Antiretroviral drugs

Nowadays, more than 25 drugs have been approved for the treatment of HIV infection. Antiretroviral drugs can be classified according to the five following classes based on the step by which they inhibit the viral replication and their mechanism of action (Figure 2):

1. **Entry inhibitors**, preventing viral entry into the host cell while blocking binding proteins on HIV (*e.g* fusion inhibitor enfuvirtide) or CD4 cells surface (*e.g* coreceptor CCR5 inhibitor, or humanized mAb to CD4 receptor) [10, 11].
2. **Nucleoside/nucleotide reverse transcriptase inhibitors** (NRTIs), which resemble endogenous deoxyribonucleotides and have a high affinity for the viral reverse transcriptase but their incorporation results in termination of transcription (*e.g.* abacavir, lamivudine, emtricitabine, tenofovir and zidovudine) [12].

3. **Non–nucleoside reverse transcriptase inhibitors** (NNRTIs), that also prevent viral DNA synthesis, although by allosteric inhibition (*e.g.* efavirenz, nevirapine, rilpivirine, etravirine, doravirine) [13].
4. **Integrase strand transfer inhibitors** (INSTIs), which bind the viral integrase cofactors blocking the insertion of proviral DNA into the host genome (*e.g.* raltegravir, elvitegravir, dolutegravir, cabotegravir and bictegravir) [14].
5. **Protease inhibitors** (PIs), which the viral protease active site as target, preventing cleavage of viral polypeptides and maturation of the virion (*e.g.* ritonavir, lopinavir, darunavir and atazanavir) [15].

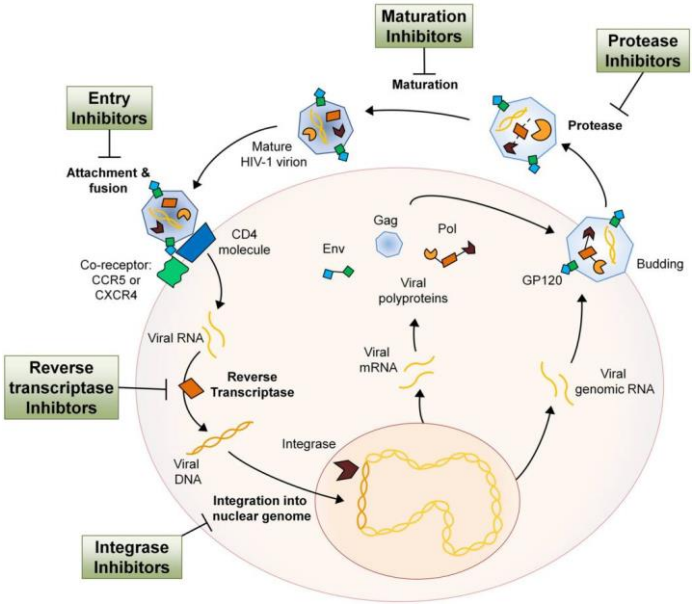

Figure 2: The HIV-1 life cycle and the antiretroviral drug class intervention points [16]

Current HIV guidelines recommend initiating ART in all PLWH, regardless of CD4 T lymphocyte cell count, to reduce the morbidity and mortality associated with HIV infection [17]. INSTI-based regimens (on top of two NRTIs, either abacavir/lamivudine or tenofovir/emtricitabine) are recommended as initial therapy for most PLWH. An alternative regimen based on NNRTIs or protease inhibitors may be preferred in certain clinical situations [18]. Globally, the regimen's efficacy, genetic barrier to resistance, adverse effects profile, drug-drug interactions potential, and patient's convenience are the factors which will guide the choice between the mechanistic classes available.

Besides therapeutic effectiveness, HIV cure is currently not available and drug tolerability and long-term health impact become key issues for treatments that need to be taken indefinitely. Treatment intolerance is one of the most important causes of treatment interruption or modification. Seven years ago, treatment intolerance was reported in approximately one third of HIV-infected patients receiving first-line antiretroviral drugs [19], leading to treatment modification or interruption. The new

generation of ARTs seems to have improved safety and tolerance profiles. Indeed, HIV drug development is currently focused on increased safety (*e.g.* tenofovir alafenamide, doravirine, bictegravir) of existing drug classes, combination therapies to facilitate adherence (*e.g.* single-tablet regimens), treatment simplification (*e.g.* long-acting injectables (LAI), cabotegravir and rilpivirine) and novel mechanisms of action (*e.g.* attachment inhibitors, maturation inhibitors, broadly neutralizing antibodies). Additionally, research and development efforts are centred on targeting persistent HIV reservoirs [20].

Currently, the first two-drug LAI formulation of cabotegravir plus rilpivirne have just reached the final phase of its clinical development. This is a promising approach to overcome adherence issues by administering long-acting drug formulations less frequently than the so far standard daily administration [21, 22]. Transition from daily oral drug intake to monthly or every two month injections can also improve treatment convenience, affect patients' privacy and reduce social stigmas associated with the treatment. Much hope has also been placed on LAI-ART for PrEP, as highly variable adherence to daily oral regimens profoundly impacts the prophylactic effect. However, clinical trials already showed an important pharmacokinetic variability of cabotegravir and rilpivirine injected intramuscularly as LAI. This variability may further increase in underweight or obese patients, in case of alteration of hepatic or renal functions, with drug-dug interactions (DDIs) involving treatments for chronic conditions (hypertension, cardiovascular problems, diabetes…) or coincident morbidities (tuberculosis, HCV infection, epilepsy, cancer...) and finally, should it occur, during pregnancy. In such instances, information of the PK profile of those novel LAI-ART are at present lacking.

I.2. Therapeutic drug monitoring of ARTs

During the past decades, blood concentrations measurements have been increasingly used to adjust the concentration exposure and optimize the therapeutic use of drugs administered to patients with serious clinical conditions (patients with life-threatening infections, intensive care unit or oncology settings, or patients with severe conditions characterized by fluctuating patho-physiological state) wherein tolerance and therapeutic efficacy are critical. This approach is based on the assumption that the actual drug concentration measured in plasma is a better predictor of the concentration at the target site than the dose administered to patient.

Therapeutic drug monitoring (TDM) is routinely used to guide dosing of certain drugs, which typically should fulfil the following criteria [23]:

- Sensitive and specific analytical method available
- Broad knowledge of their pharmacokinetic (PK) processes

- Important between-subject PK variability
- Good correlation between drug concentrations and pharmacological effects, such as established PD properties and defined therapeutic range of concentrations
- Narrow therapeutic window
- Lack of precocious clinical markers of therapeutic efficacy or toxicity

Several antiretroviral drugs meet most of the TDM criteria mentioned above [24, 25]: elvitegravir plasma concentrations showed great intra- and inter-patient variability [26]; and therapeutic range of efavirenz was based on a good correlation between plasma concentrations and efficacy and toxicity [27, 28]. Moreover, a recent study suggested a relationship between plasma dolutegravir trough concentrations and neuropsychiatric adverse events [29]. TDM also improved the virological response and/or decreased the incidence of concentration-related drug toxicities of nevirapine in pregnant women [30, 31]. Finally, TDM may also be used as a -poor- indicator of short-term adherence [32].

Generally, maintaining circulating drug concentrations over a given threshold is crucial to ensure optimal antiviral action. Suboptimal drug levels allow low-level viral replication during therapy, which substantially increases the risk of viral resistance and virological failure. On the other hand, avoiding unnecessarily high concentrations may limit the likelihood of adverse effects.

The last generation of anti-HIV agents have more favourable tolerance profiles than the earlier ones. However, FDA drug prescribing information, supported by numerous observations also made within the frame of our TDM service, indicates that heterogeneity in drug response (in both efficacy and safety) still remains of concern also for last generation drugs. To that endeavour, the availability of concentration exposure monitoring for these new drugs is certainly an important component of patients' follow-up, particularly for patients with multi-resistant HIV receiving complex regimens, for which clinicians have limited experience. Therefore, current HIV guidelines recommend performing TDM only in certain scenarios [18]:

- Suspected drug-drug interactions that might result in clinically altered efficacy or toxicity
- Changes in patho-physiological state that may impair gastrointestinal, hepatic or renal function
- Special populations, including pregnant and pediatrics patients
- Treatment-experienced patients who may have viral isolates with reduced susceptibility to ART
- Use of alternate dosing regimen whose safety and efficacy have not been established in clinical trials
- Concentration dependent toxicities

- Lack of expected virological response
- Monitoring short term adherence

In all the above-mentioned cases, it is important to highlight the role of clinical pharmacologists and clinical pharmacists for the interpretation of the concentration results, and for the dose adjustment recommendations, if necessary. It must be acknowledged however, that comprehensive studies formally assessing in the real life setting whether patient's tailored antiretroviral regimens would allow to achieve a better management of HIV-infected patients still needs to be performed.

Finally, the monitoring of antiretroviral drugs in plasma may provide useful information in the setting of pre-exposure prophylaxis (PrEP) of uninfected individuals. Indeed, antiretroviral drugs can also be taken by HIV-negative individuals at high risk for HIV exposure. Such proactive prevention strategy implies the administration of anti-HIV drugs prior to potential HIV exposure. Several clinical trials conducted from 2010 to 2011 have established the proof of concept for PrEP [33-35], demonstrating efficacy for reducing rates of HIV transmission. Then, the World Health Organization recommended PrEP as a prevention choice for people at substantial risk of HIV infection (gay community, sex workers) in combination with usual prevention approaches. The use of Truvada® (emtricitabine/tenofovir disoproxil fumarate) as PreP has been therefore approved by the FDA in July 2012. Plasma concentrations of tenofovir have been shown to be highly predictive of protection from HIV acquisition but these PrEP studies identified drug adherence as a primary obstacle to efficacy [36]. In this context, TDM can be used as short-term adherence measure for PrEP.

I.3. The importance of LC-MS/MS for TDM

Over the last 25 years, the major advances in the field of bioanalysis (involving the continuous improvement of acquisition speed and sensitivity) led to considerable progress in clinical pharmacokinetics and TDM. The LC–MS/MS technology is an important tool in TDM as it offers impressive analytical performances and improved turn–around time (TAT) as compared to the other available bioanalytical methods. Liquid chromatography coupled to MS/MS play a major role for the current deployment of TDM in clinical laboratories. Numerous LC-MS procedures dedicated to TDM have been reviewed in the last few years [37-40].

Several strength of LC-MS/MS should be acknowledged. First, the selectivity provided by tandem mass spectrometry allows to simultaneously analyse several chemically-unrelated drugs and metabolites at the same time (*i.e.* so called "multiplex") and this, within short analytical times (typically 5–7 min, including rinsing and re-equilibration steps). Therefore, numerous multiplex assays for the

quantification of drugs from the same therapeutic class in a single chromatographic run have been developed, and especially for ARTs (in plasma and cells) [41-43].

Overall, the main advantages of this approach are:

- a unique sample preparation and short analytical run
- a reduction of the required blood sample volume by simultaneously analysing commonly co-prescribed drugs
- time saving through the establishment of simultaneous calibration curves
- simplified analyses of blood samples from patients receiving either single drug, or combination regimens. This latter feature is particularly relevant for the analysis of triple therapies in HIV-infected patients [44]
- a rapid access to the analytical results on a daily basis, allowing the provision of TDM dosage adjustment advice within short time intervals
- the capacity to multiplex LC-MS assays allows the measurement of several drugs and metabolites in one run, which may also help to identify errors in drug prescription or administration.

More generally, thanks to the improved analytical performances with the achievement of a high sensitivity, the detection of extremely low drug levels in biological matrices is now possible. For drugs with a high clearance (slowly eliminated from the human body), such an increased sensitivity has allowed to extend the time during which drugs can be detected in biological fluids, leading to frequently update the half-life values of many therapeutic molecules. This is another example of the reciprocal interaction intrinsically linking clinical pharmacokinetics and bioanalytical methodologies.

A large number of factors may influence the MS response, leading to significant day-to-day variations. Therefore, internal standards (IS) are usually required to achieve reliable and accurate quantitative results. Stable isotopically-labelled IS (deuterium, ^{13}C or ^{15}N) constitute the gold standard IS for quantification and have greatly facilitated the development of LC-MS/MS methodologies. It compensates for the variability in sample preparation, recovery, injection, possible compound degradation, and especially can effectively circumvent the deleterious matrix effect variably of biological samples, which may otherwise compromise the accuracy of the analytical method. These IS are also important for the accurate quantification of analytes that are quantified simultaneously without fully optimized chromatographic separation. For the assay of drugs analysed in alternate biological matrices (such as cerebrospinal fluid, dried blood spots, cellular or tissue extracts…), the use

of isotopically-labelled IS insures that unidentified, possibly variable, matrix effect are compensated for.

This illustrates the importance of LC-MS/MS methodologies in clinical settings, enabling the development of TDM applications, with promising perspectives for TDM services in the growing context of personalized medicine. Considering the fact that “every patient is different”, such optimization should allow the administration of “the right drug at the right dosage to the right patient” in the routine setting.

I.4. Drug-drug interactions with antiretroviral treatments

The reduction in mortality due to ARTs has transformed HIV infection into a long-term chronic illness for many patients, and the growing aging HIV-infected population is increasingly affected by age-related diseases. Aging PLWH encounter chronic conditions such as hypertension, cardiovascular problems, diabetes, neurocognitive impairment and malignancies [9], which lead to increasingly complex drugs associations with a high potential of DDIs [45, 46]. Moreover, renal and hepatic functions become altered in a significant percentage of patients, necessitating appropriate treatment adjustments.

Antiretroviral drugs are among the therapeutic agents with the highest potential for DDIs. PIs , and NNRTIs are extensively metabolized by CYPs, and can inhibit and/or induce different CYP isoforms [47]. DDIs with ARTs can also occur at the level of drug transporters [48] and especially for INSTIs, at glucuronidation enzymes [49], complexation with divalent cations [50] and pH-dependent drug absorption [51]. The prevalence of DDIs with HIV therapy has been assessed in several studies performed in USA, the Netherlands, United Kingdom, Switzerland, and in resource-limited countries [46, 52-57]. These studies indicate that DDIs with HIV therapy affect 19-41% of prescriptions, while clinically significant interactions are found in one third of patients receiving antiretroviral therapy. The issue of DDIs is likely to worsen with the aging HIV population encountering age-related morbidities such as hypertension, diabetes, cardiovascular disease, osteoporosis, cancers etc. [58, 59] susceptible to increase the burden of polymedication [45]. DDIs lead to substantial risks of either toxicity or decreased efficacy and subsequent emergence of drug resistance and ultimately, potential HIV transmission to seronegative partners [60]. For these reasons, the prevention, identification, and management of DDIs are crucial for patient care. A publicly available website (www.hiv-druginteractions.org) under the auspices of the University of Liverpool, is a valuable resource about DDIs. However, 90% of the DDIs mentioned are predicted only based on mechanistic knowledge of

their metabolic pathways identified *in vitro.* This is usually sufficient to warn about a potential for drug interactions, but this neither brings quantitative information nor accounts for the complexity of multiple and contradictory effects. Indeed, there is currently a major lack of real-life clinical data on the extension and management of DDIs, especially in the context of polymorbid, polymedicated patients poorly accounted for in treatment guidelines largely elaborated for single diseases [61].
In the growing movement of personalized medicine, research efforts must therefore be pursued to improve the prescription of ART not only with regard to antiretroviral efficacy but also according to tolerability, long-term safety and potential DDIs, possibly modulated by patients' pharmacogenetics traits. These issues will be best addressed by an integrated strategy of drug selection and dosage individualization based on relevant demographic/clinical factors, genetic markers (precision medicine) and drug concentrations measured in plasma.

I.5. Population pharmacokinetic modeling

It has been recognised that for most systemic therapies, circulating drug concentration exposure is a leading factor associated with both efficacy and toxicity. Nevertheless, drugs are often prescribed at standard dosage regimens, which does not account for between-subject variability (BSV) in drug levels. However, BSV can be remarkably large for some drugs (including ART), impacting therapeutic response. Multiple sources of variability have been identified such as demographic, environmental, clinical and genetic factors. Due to the complexity of real-life data, which confound multiple pharmacokinetic, host-related, viral, environmental, genetic factors and drug-drug interactions into a resulting phenotype, it has become advisable to develop models that can incorporate all relevant determinants of treatment response. Population-based approaches represent the best way to characterize the pharmacokinetic profile of drugs in a cohort of patients, and to capture the contribution of multiple genetic and non-genetic influences affecting drug levels [62, 63]. The basic concept of population modelling is to include patient data obtained from observational studies into non-linear mixed effects regression models. One of the advantages of the population approach is the possibility to analyse sparse datasets, with often only one concentration measurement per individual. Compartmental methods are applied in PK modelling and consider the body as a system of related compartments where the drug is absorbed and distributed before being eliminated (Figure 3). PK parameters such as clearance, volume of distribution and absorption rate constant can be calculated with this approach.

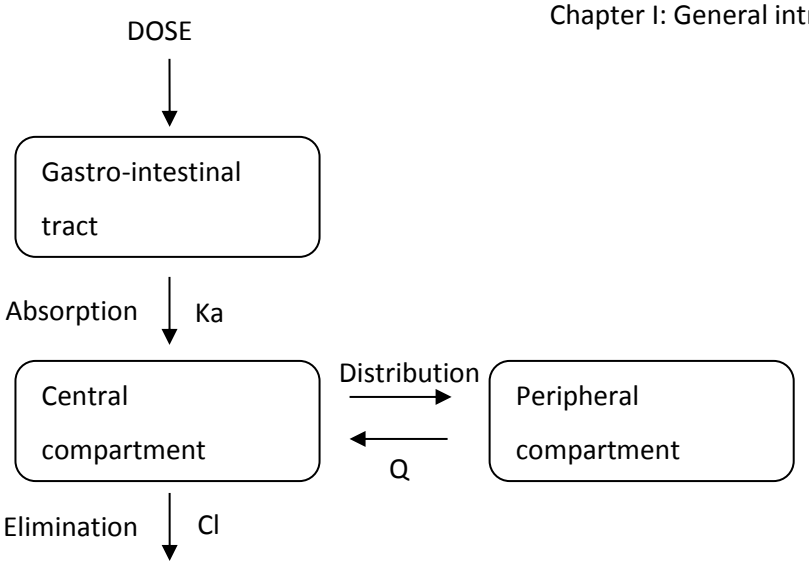

Figure 3: Representation of a two-compartments pharmacokinetic model for an orally administered drug. Ka, absorption rate; Q, intercompartmental clearance; Cl, total clearance.

In population pharmacokinetics (population PK), mathematical and statistical models are applied to obtain a typical value of PK parameters in a given population of individuals, along with between-subject variability. Then, factors that might influence these parameters will be identified to explain BSV.

Fixed effects include mean parameters (θ) that represent the population average PK parameters (*e.g.* clearance, volume of distribution…). These are susceptible to fixed effect covariates (z_i), which are individual characteristics (*e.g.* body weight, age or renal function) that induce PK variability across the population.

Two sources of variability are responsible for random effects. First, differences among two individuals defines between-subject variability. For a given individual i, $\theta_i = \theta + \eta_i$, where η_i is the individual BSV and is modelled in terms of η, which is normally distributed with a mean of 0 and variance of ω^2. Secondly, the within-subject variability, also named residual error, is the variability observed in the different measurements of the same individual and is expressed as variance σ^2. For a given individual i, and observation j, the observation is predicted by $y_{ij} = \hat{y} + \varepsilon_{ij}$, where ε_{ij} follows a normal distribution with mean of 0 and variance of σ^2.

Therefore, the general mixed effects model is represented by: $y_{ij} = f(x_{ij}, \Phi_i) + \varepsilon_{ij}$

where y_{ij} is the j^{th} observation of the i^{th} individual; f defines the PK model (one, two or three compartments, linear or nonlinear kinetics); x_{ij} is a known predictor (time and dose); Φ_i is the PK parameter vector for the i^{th} individual and ε_{ij} is the additive residual error, which could also be modelled as exponential or as combination of both.

The parameter model is then represented by: $\Phi_i = g(z_i, \theta) + \eta_i$

where g is the structural model which is function of fixed effects covariates (z_i) and PK parameters (θ). Based on a maximum likelihood approach, the software NONMEM® [64] estimates the best possible values of θ, ω^2 and σ^2. The objective function value (approximately equal to minus twice the logarithm of the maximum likelihood) allows the comparison between two hierarchical models with the likelihood ratio test. In addition, diagnostic plots, changes in coefficients of variation of PK parameters, and precision of parameter estimates are also used to determine the model that best describes the data.

Today, population PK is consistently used in the drug development process, being recommended by regulatory agencies to detect sources of variability in drug response or toxicity among individuals [65, 66]. Population pharmacokinetic analyses of the NNRTIs (efavirenz [67-69], nevirapine [70], etravirine [71]), the PIs lopinavir ([72-74], atazanavir [75], indinavir [76], darunavir [77]) and other more recent drugs (raltegravir [78], maraviroc [79], tenofovir [80], elvitegravir [26] and dolutegravir [81] have been able to quantify variability and to assess relevant sources of variations in drug levels, including DDIs. Another advantage of models is that they can be used for comprehensive simulations, in particular to test alternative dosage regimens. Noteworthy, the clinical usefulness of such approaches has been acknowledged in medicine, allowing for Bayesian feed-back adjustment of dosage regimens, considered the best strategy for TDM [82].

I.6. Hypothesis and aim of the thesis

This present research addresses a key issue in current therapeutics of PLWH, which is the complex nature of therapeutic antiviral and non-antiviral regimens resulting in an elevated risk of DDIs.

It aims at identifying the most important drug-drug interactions in SHCS patients under real-life conditions, followed by model-based approaches possibly considering further patients' demographic, pathophysiologic and possibly pharmacogenetic characteristics, and formal clinical confirmation through targeted pharmacokinetic trials. Ultimately, these investigations would allow to personalize drug treatments while addressing the problem of interactions with comedications, for providing the most efficient and the safest possible patient's care. More generally, this proposal aims to bridge a large translational research gap starting from mechanistic pharmacokinetics, going through population modeling, and ending up at the level of pharmaco-epidemiology, in an endeavor to bring significant contributions to current knowledge in the field of DDIs.

Building up on our mass spectrometry and population pharmacokinetics modelling expertise and established frameworks, we intend to launch a systematic analysis of complex DDIs relevant to

antiviral treatments and comedication in PLWH. Specifically, the following specific objectives have been undertaken:

1. Large-scale cross-sectional capture, at the occasion of the bi–annual cohort visit of SHCS patients, of TDM blood samples along with relevant therapeutic information (date/time of last drug dose and blood sampling), not only for antiretroviral treatments but also for all other drugs part of their co-medication (*i.e.* statins, cardiovascular and psychotropic drugs, etc.)
2. Multiplex mass spectrometry analyses of antiretroviral drugs and relevant comedications in plasma samples.
3. Population pharmacokinetic modeling of the exposure to antiretroviral drugs and to selected comedications in SHCS patients
4. Analyses of DDIs observed in SHCS individuals in real-life conditions, followed by model-based simulations and formal small-scale validation pharmacokinetic studies of problematic drug interactions.

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CHAPTER II: POLYPHARMACY AND DRUG-DRUG INTERACTIONS IN PEOPLE LIVING WITH HIV

Chapter II in the thesis context

Highly active antiretroviral treatments (ARTs) have transformed HIV infection from a deadly disease into a manageable chronic condition. As a consequence, PLWH live longer and become older. Moreover, ageing patients encounter chronic conditions such as hypertension, cardiovascular problems, diabetes, neurocognitive impairment and malignancies, which lead to increasingly complex drugs associations with a high potential of drug-drug interactions (DDIs) making the management of HIV-infection therapy more challenging. In addition, information about the influence of ageing on ARV pharmacokinetics is limited since PK studies are generally conducted in healthy young male volunteers which therefore do not reflect the real-life situation of elderly PLWH. Also, prescribing issues such as DDIs or inappropriate medication use can occur in elderly. Last studies that have evaluated medication use and DDIs in the SHCS date back to 2010 and 2011. However, prescribing patterns have changed with the arrival of new therapeutic classes such as strand transfer HIV integrase inhibitors (INSTIs). INSTIs are increasingly prescribed because of their safety profile, their high barrier to resistance and their lower potential from DDIs than protease inhibitors. Therefore, information about prevalence of DDIs in the SHCS needed to be updated. In addition, most studies evaluating the prevalence of polypharmacy and DDIs are based on data retrieved from patient's records completed by the physician. However, such databases are biased and error-prone since patient may not remember during the medical visit all his medications and drug dosages. To circumvent this limitations, the SHCS constitutes indeed an ideal framework since SHCS patients are keen to participate in research projects and the capture of detailed treatment information (both viral and non-virals drugs) at the usual cohort visit is facilitated by a dedicated form purposely prepared for drugs and dosage records (see Appendice VIII.2).

The first part of this chapter describes the prospective multicentre study conducted within SHCS during my thesis. This study constituted the fundamental basis of my entire thesis since it has allowed to generate fruitful qualitative and quantitative data to be analysed. This paper is a descriptive review of prescribing issues that occurred in the ageing HIV-infected population such as polypharmacy, DDIs and inappropriate medications.

The second section focuses on statins, frequently prescribed lipid-lowering agents with a known risk for DDIs. This study gives details about the management of drug-drug interactions in a real life setting, along with its pharmacokinetics and pharmacodynamics consequences.

Own contribution: Logistical organisation of the bi-centric (Lausanne and Basel) observational clinical study SHCS #815. Patients' recruitment and data collection. Writing of the research protocol and submission to the ethics committee for the pharmacokinetic study involving rich sampling

(NCT03515772) in patients consenting to participate to the 24h- pharmacokinetic study. Logistical organisation, data management, analyses and interpretation of the data. Writing of the manuscript.

II.1. Polypharmacy, drug-drug interactions and inappropriate drugs: new challenges in the aging population living with HIV.

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MAJOR ARTICLE

Polypharmacy, Drug–Drug Interactions, and Inappropriate Drugs: New Challenges in the Aging Population With HIV

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Background. Antiretroviral therapy has transformed HIV infection from a deadly into a chronic condition. Aging people with HIV (PWH) are at higher risk of polypharmacy, potential drug–drug interactions (DDIs), and potentially inappropriate medications (PIMs). This study aims to compare prescribed drugs, polypharmacy, and potential DDIs between young (<65 years old) and elderly (≥65 years old) PWH. The prevalence of PIMs was assessed in elderly.

Methods. PWH from 2 centers within the Swiss HIV Cohort Study were asked to fill in a form with all their current medications. Polypharmacy was defined as being on ≥5 non-HIV drugs. PIMs were evaluated using Beers criteria. Potential DDIs for the most prescribed therapeutic classes were screened with the Liverpool interaction database.

Results. Among the 996 PWH included, 122 were ≥65 years old. Polypharmacy was more frequent in the elderly group (44% vs 12%). Medications and potential DDIs differed according to the age group: cardiovascular drugs and related potential DDIs were more common in the elderly group (73% of forms included ≥1 cardiovascular drug; 11% of cardiovascular drugs involved potential DDIs), whereas central nervous system drugs were more prescribed and involved in potential DDIs in younger PWH (26%, 11%). Potential DDIs were mostly managed through dosage adjustments. PIMs were found in 31% of the elderly group.

Conclusions. Potential DDIs remain common, and PIMs constitute an additional burden for the elderly. It is important that prescribers develop and maintain a proactive approach for the recognition and management of DDIs and other prescribing issues frequently encountered in geriatric medicine.

Keywords. aging; drug–drug interactions; elderly; HIV; inappropriate drugs; polypharmacy.

Antiretroviral treatments (ARTs) have transformed HIV infection from a deadly disease into a chronic condition. As a consequence, people with HIV (PWH) are getting older, living long enough to develop age-related chronic conditions and consequently to receive significant polymedication in addition to their ARTs [1–4]. Furthermore, aging is characterized by physiological changes known to affect the exposure or response to drugs [5]. Thus, all together, elderly PWH are at increased risk of having polypharmacy, drug–drug interactions, and potentially inappropriate medications (PIMs) [6–10].

ARTs are among the therapeutic agents with the highest potential for DDIs, either as perpetrators (ART impacting a non-ART drug) or victims (ART being impacted by a non-ART drug). Pharmacokinetic interactions can occur at the level of absorption (eg, complexation with divalent cations, pH modification) [11, 12], distribution, metabolism, or elimination (induction/inhibition of cytochrome [CYP] isoforms, glucuronidation enzymes or transporters) [13–15]. Interactions have the potential to lead to substantial risks of either toxicity or decreased therapeutic efficacy for either ARTs or non-ARTs. For these reasons, the prevention, identification, and management of DDIs are crucial in PWH.

Little is known about PIMs in older PWH, which may harm this vulnerable population. To the best of our knowledge, only 3 studies have raised this issue, showing that 52% to 66% of older PWH had at least 1 medication-related problem [7, 16, 17].

The aim of this study was to compare prescribed medications, polypharmacy, and potential DDIs between young and elderly PWH included in 2 centers of the Swiss HIV Cohort Study (SHCS). The prevalence of PIMs was assessed in the elderly group. In addition, dosage adjustment was evaluated for

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comedications for which official dosing recommendations are available in order to assess the management of DDIs in real life.

METHODS

Study Design

The SHCS, a multicenter prospective cohort study, has been continuously enrolling PWH since its establishment in 1988 [18]. Approximately 75% of PWH receiving ART in Switzerland agree to be followed within the SHCS network. Within the framework of SHCS project 815, we have launched a comprehensive analysis of relevant DDIs between ARTs and commonly prescribed comedications from January 2017 to December 2018 in the HIV clinics at the University Hospitals of Lausanne and Basel. PWH were contacted by post 1 week before their biannual SHCS appointment and invited to report all their current medications, the respective dosage, and date/time of last drug intake before SHCS visit in a dedicated form, which they would bring back during their routine SHCS visit. Clinical nurses were responsible for gathering and checking the completeness of the medication forms. PWH were classified as "elderly" if they were ≥65 years old. This age cutoff was adopted as it represents the definition of elderly age in most developed world countries.

Description of Medications

The drugs reported in the forms included ARTs, prescription medications, and over-the-counter remedies. Comedications were classified according to the anatomical therapeutic chemical classification (ATC), as recommended by the World Health Organization [19], taking into account up to 3 digits. If a medication contained 2 or more pharmacologically active agents, each substance was counted individually in the analysis. Polypharmacy was defined as the concurrent administration of 5 or more comedications in addition to ART, which represents a rather conservative criterion, as the overall number of 5 drugs is commonly used to define polypharmacy [20]. As ARTs or comedications can be modified during the follow-up visit, all medication forms collected during the study period were considered for this analysis. Number and type of comedications were visually compared in multiple age groups.

PIMs were assessed using the most recent version of the classical Beers criteria and included, for instance, drugs with anticholinergic properties or benzodiazepines, which can impair cognition and consequently increase the risk of falls in elderly persons [3, 21]. Proton pump inhibitors were not considered a PIM, as our study did not capture treatment duration and only proton pump inhibitor treatment for longer than 8 weeks is considered inappropriate according to Beers criteria. Dosage of comedications was not taken into account in the analysis of PIMs. Anticholinergic burden was measured by means of the validated Anticholinergic Risk Scale, assigning drug points from 0 to 3, the latest corresponding to higher anticholinergic potential [22, 23]. If an elderly PWH received several drugs with anticholinergic properties, the total anticholinergic score was calculated by summing up each individual medication score.

Identification of Potential DDIs

We focused on 2 therapeutic classes, that is, cardiovascular and central nervous system (CNS), due to the fact that these therapeutic classes are largely utilized in PWH, as indicated by a previous analysis of the SHCS [24], and due to their potential for clinically relevant DDIs with ARTs. All medication forms containing at least 1 cardiovascular or CNS drug were included in the analysis. Potential DDIs between ARTs and these comedications were screened using the University of Liverpool HIV drug interaction checker [25]. These charts rank the clinical significance of an interaction from "no interaction" (green flag interaction) to "interaction of weak intensity not requiring additional action" (yellow flag interaction), "potentially clinically relevant DDI requiring either dose adaptation or close clinical monitoring" (amber flag interaction), or "contraindicated" (red flag interaction). Interactions within ARTs or within non-HIV medications were excluded from this analysis. Potential DDIs involving comedications not listed in the Liverpool drug interaction database were checked using Up-to-Date (https://www.uptodate.com/drug-interactions/#di-druglist). When a comedication was involved in several potential DDIs as a victim, the most severe potential DDI was retained.

Dosage adjustments of comedications were evaluated to assess how DDIs are managed in real life. This evaluation was performed only for comedications whose label provides dosing recommendations to overcome given DDIs. Both European and American dosing guidelines were considered [26–28].

Statistical Analyses

Statistical and graphical analyses were performed in R, using the packages tableone and ggplot2 [29]. In the descriptive analysis, continuous variables were described by their medians and interquartile ranges (IQRs) and compared between groups using the Mann-Whitney *U* or Wilcoxon tests. Categorical variables were described by proportions and compared with the χ^2 test. Repeated-measures analyses were not performed considering the time interval between 2 follow-up visits, during which both ARTs and comedications could have been changed. Medication forms fulfilled more than once by a patient were therefore considered independent measures. In addition, the proportion of patients reporting multiple medication forms was a priori expected to be similar in younger and elderly PWH, as all patients had medical appointments on a biannual basis.

RESULTS

Study Population and Medication Use

In total, 996 PWH, mostly male (69%), were included in the study. Of those, 874 (88%) were <65 years old (median [IQR], 49 [40–55] years), and 122 (12%) were ≥65 years old (median [IQR], 71 [67–74] years). Elderly PWH tended to have longer duration of HIV infection and thereby HIV treatment. Furthermore, elderly individuals tended to have more complex ARTs and more comedications. The demographic and clinical characteristics of the study population at their first recorded cohort visit, stratified by age, are summarized in Table 1. Medication forms were completed 1, 2, or 3 times by 41% (n = 403), 57% (n = 570), and 2% (n = 23) of participants, respectively.

Taking into account all the 1610 collected forms, integrase strand transfer inhibitor (INSTI)–containing regimens were the most prescribed, accounting for ~50% of overall ARTs in both age groups (Figure 1). Of interest, combined ARTs (ie, boosted protease inhibitor [PI] + INSTI or boosted PI + non-nucleoside reverse transcriptase inhibitor [NNRTI] or boosted PI + INSTI + NNRTI), representing complex ARTs characterized by a higher potential to cause DDIs, were used more in elderly PWH (21% vs 14%). The most frequently administered boosted PI was ritonavir-boosted darunavir (68% of all boosted PIs), whereas efavirenz was the most prescribed NNRTI (38% of all NNRTIs).

As expected, the number of prescribed comedications increased with age (Figure 2). Considering all the 1610

Table 1. Characteristics of the 996 PWH at Their First Visit With Fulfilled Medication Form, by Age Group

Characteristics	<65 Years Old (n = 874)	≥65 Years Old (n = 122)
Age, median [IQR], y	48.8 [40.4–55.5]	71.0 [67.3–74.0]
Male sex, No. (%)	580 (66.4)	105 (86.8)
Weight, median [IQR], kg	73.0 [64.0–83.0]	73.0 [67.0–85.0]
Ethnicity, No. (%)		
White	611 (70.1)	114 (94.2)
Black	199 (22.8)	3 (2.5)
Hispano-American	30 (3.4)	2 (1.7)
Asian	31 (3.6)	2 (1.7)
CD4, median [IQR], cells/mm^3	691.5 [527.0–919.0]	616.0 [413.0–821.0]
HIV RNA <50 copies/mL, No. (%)	845 (97.6)	114 (94.2)
Date of HIV diagnosis, No. (%)		
<1990	99 (13)	17 (17)
1990–1999	156 (20)	35 (34)
2000–2009	299 (38)	34 (33)
≥2010	228 (29)	17 (17)
ART start date, No. (%)		
<1990	1 (0.1)	0
1990–1999	235 (27)	55 (46)
2000–2009	338 (39)	45 (37)
≥2010	298 (34)	21 (17)
Non-NRTI ARTs, No. (%)		
Integrase inhibitor	413 (47.3)	57 (46.7)
Combined regimen	136 (15.6)	29 (23.8)
NNRTI	238 (27.2)	23 (18.9)
Protease inhibitor	85 (9.7)	13 (10.7)
NRTIs (backbone), No. (%)		
ABC/3TC	317 (36.3)	57 (46.7)
TDF/FTC	330 (37.8)	20 (16.4)
TAF/FTC	151 (17.3)	18 (14.8)
Others	45 (5.1)	17 (13.9)
No backbone	31 (3.5)	10 (8.2)
Number of comedications, No. (%)		
0	382 (43.7)	14 (11.5)
1	121 (13.8)	8 (6.6)
2	118 (13.5)	10 (8.2)
3	86 (9.8)	20 (16.4)
4	61 (7)	18 (14.8)
≥5	106 (12.1)	52 (42.6)

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; FTC, emtricitabine; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; PWH, people with HIV; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

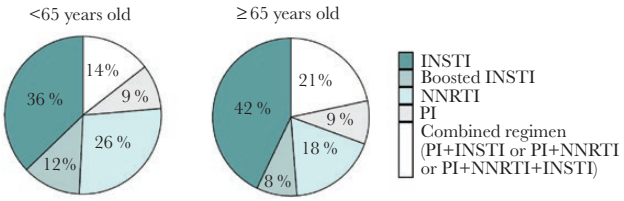

Figure 1. Distribution of the most prescribed antiretroviral regimens for the entire study period, stratified by age group. Abbreviations: INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

collected forms, elderly PWH tended to use a higher number of comedications (median [range], 4 [0–22]) compared with younger PWH (median [range], 1 [0–14]; $P < .001$). Ninety percent (n = 188) of the medication forms completed by elderly PWH included at least 1 comedication. Polypharmacy was more prevalent in PWH ≥65 years (44%) compared with the younger group (12%). As indicated in Figure 3, drugs belonging to the cardiovascular class were the most utilized in the elderly group (73% of medication forms of elderly PWH included at least 1 cardiovascular drug), whereas CNS drugs were most commonly prescribed in younger PWH (26% of forms of younger PWH included at least 1 CNS medication). Calcium/vitamin D3 and acetylsalicylic acid (prescribed as an antithrombotic agent) were the most prescribed medications in their respective therapeutic classes (33% and 52%, respectively). With the exception of CNS drugs, the use of the most prescribed therapeutic classes (ie, cardiovascular, alimentary tract and metabolism, blood and blood-forming organs) increased in an exponential way with increasing age.

Thirty-eight elderly patients (31%) had a least 1 PIM, mostly benzodiazepines and hypnotics (n = 19, 27% of PIM). Three PWH received drugs characterized by a high anticholinergic burden (anticholinergic risk scale = 3), that is, dimenhydrinate, quetiapine, and trimipramine.

Characteristics and Effect of the Identified Potential Drug–Drug Interactions With Cardiovascular and CNS Drugs

A total of 767 medications forms comprising at least 1 cardiovascular or CNS drug were collected in 500 PWH and were included in the analysis of potential DDIs. Of those, 417 prescriptions (54%) did not contain any potential DDIs. For the remainder of prescriptions, 23% (n = 178), 28% (n = 215), and 2% (n = 17) had at least 1 yellow, amber, and red flag potential DDI, respectively. These proportions were not statistically different between the 2 age groups ($P = 1$, .22, and .50 for yellow, amber, and red flag potential DDIs, respectively). However, most potential DDIs in elderly PWH were between ARTs and cardiovascular drugs, whereas in younger PWH, potential DDIs were mainly with CNS drugs. The frequency of potential DDIs with cardiovascular and CNS drugs, stratified by age, are depicted in Figure 4. Amber flag potential DDIs involved mainly zolpidem (n = 36, 12%) and rosuvastatin (n = 32, 11%), whereas red flag interactions involved predominantly the coadministration of quetiapine with boosted PIs (n = 12, 71%). Ritonavir-boosted darunavir was the most common ART involved in amber flag (n = 93, 32%) and red flag potential DDIs (n = 7, 41%). Potential pharmacodynamic DDIs resulting in potentially additive adverse effects (mostly QT prolongation interval or additive risk of nephrotoxicity) were found in 5% (n = 43) of the prescriptions.

Of note, apart from cardiovascular and CNS drugs, 5 patients (4 young and 1 elderly PWH) were treated with boosted PIs and clopidogrel, resulting in a red flag DDI.

Management of Potential DDIs With Cardiovascular and CNS Drugs

The maximum daily dose of atorvastatin recommended in the presence of boosted darunavir is 20 mg (US product label) and

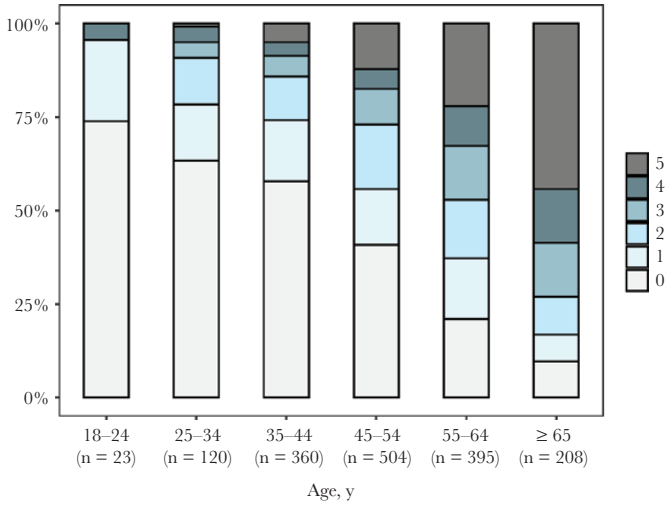

Figure 2. Overall distribution of the number of prescribed comedications for the entire study period, stratified by age group.

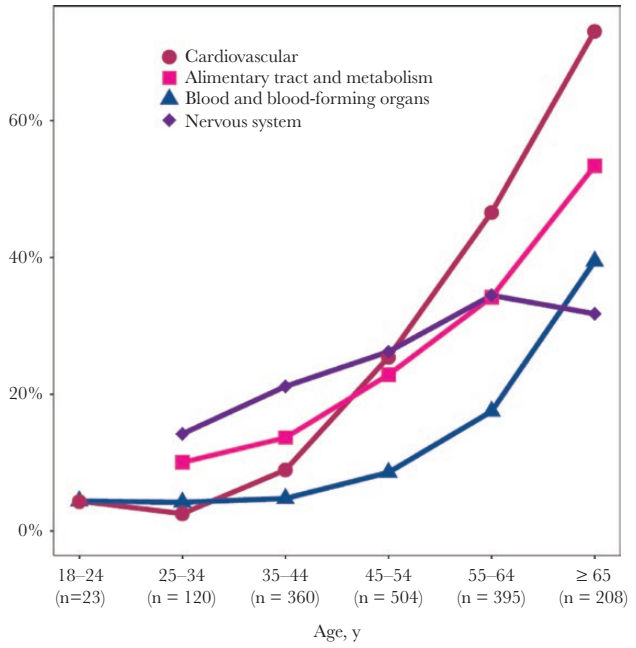

Figure 3. Percentage of people with HIV treated with at least 1 comedication of the 4 most prescribed therapeutic classes for the entire study period, stratified by age group.

40 mg (European product label). These dosing recommendations were respected in all prescriptions. Although coadministration of ritonavir-boosted atazanavir and atorvastatin is not recommended by both US and European guidelines, 1 patient (2 cohort visits) was concomitantly receiving both drugs. However, the atorvastatin dosage was limited to 10 mg once daily, which is in line with the recommendations of the University of Liverpool database [25].

Concerning rosuvastatin, the maximum daily dose is 20 mg in the presence of boosted darunavir (US product label). This recommendation was applied in all prescriptions. The Food and Drug Administration (FDA) recommends that the dosage of rosuvastatin should not exceed 10 mg when coadministered with boosted atazanavir [28], whereas the European AIDS Clinical Society suggests that rosuvastatin use is generally safe if started at a low dose, not exceeding 20 mg daily [30]. In our study, 1 patient received 20 mg of rosuvastatin daily, exceeding the maximum dose recommended by the FDA.

Finally, there are clear dosage recommendations for quetiapine when used together with boosted PIs. US prescribing information recommends that the dosage of quetiapine be reduced to one-sixth of the original dose [31]. In our study, this recommendation was followed for 11 out of the 14 patients, whereas the other 3 received quetiapine extended-release at a dosage ranging from 50 mg to 200 mg once daily.

DISCUSSION

Our findings provide evidence that a high proportion of aging PWH are polymedicated and that the overall burden of medications has shifted from ARTs to treatments for other comorbidities. Our result demonstrating that 44% of elderly

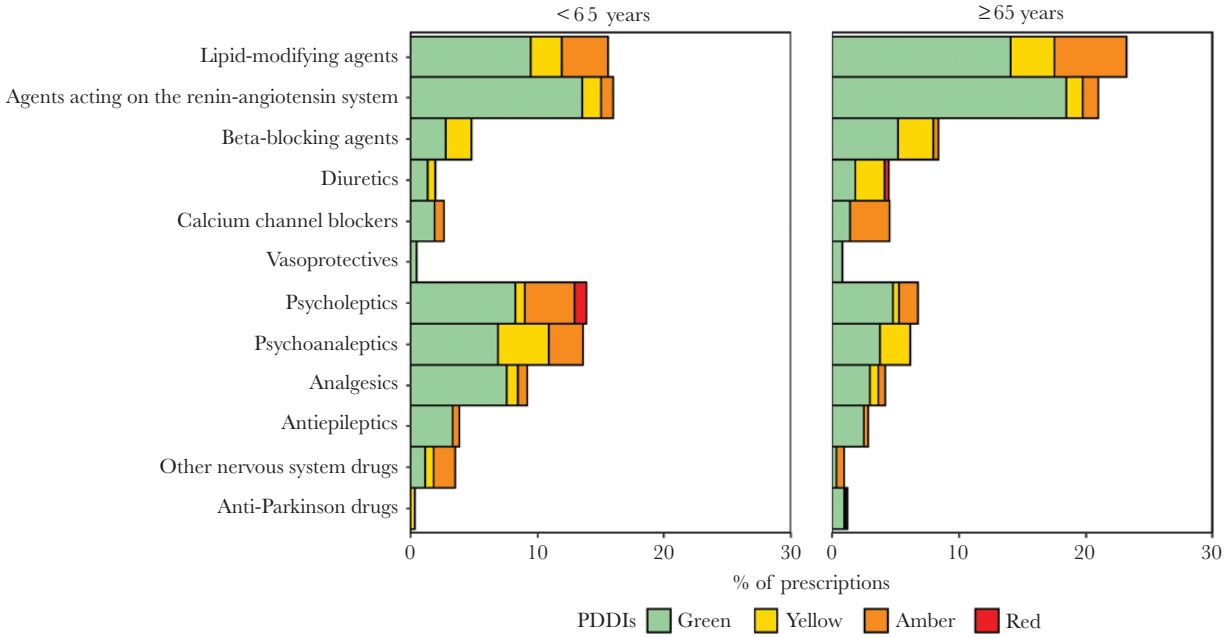

Figure 4. Percentage of prescriptions (n = 767) with at least 1 drug of the corresponding therapeutic class for the entire study period. Potential DDIs between ART and non-ART drugs are represented with different shades according to the severity of potential DDIs: red flag (deleterious), amber flag (potential clinical relevance, manageable by performing dosage adjustment or close clinical monitoring), and yellow flag (weak clinical relevance). The green flag corresponds to the absence of potential DDIs. Abbreviations: ART, antiretroviral therapy; DDI, drug–drug interaction; PDDI, potential drug–drug interaction.

PWH are polymedicated is in line with recent studies reporting a rate of 37% [32, 33]. Cardiovascular and CNS drugs were the most represented therapeutic classes in older and younger PWH, respectively. This is in agreement with a previous [24] and a more recent analysis of the SHCS [34], showing that cardiovascular disease is the first cause of comedication prescription, followed by depression. In addition to a higher number of comedications, elderly PWH received more complex ART regimens, characterized by a higher propensity to cause DDIs, than younger patients, thus further complicating their treatment. These associations of multiple ART drugs are likely to result from of a longer history of HIV infection, with the acquisition of drug resistance leading to the need for more complex ARTs. Indeed, most of the younger PWH (39%) started their ARTs between 2000 and 2009, whereas half of the elderly PWH received their first ARTs between 1990 and 1999.

Complex ARTs' associations with comedications would be expected to lead to an increased risk for DDIs in elderly PWH. Remarkably, our results did not demonstrate a higher frequency of potential DDIs in elderly PWH compared with younger patients. This observation could be explained by the fact that HIV clinicians of SHCS are well aware of the DDI potential of ART and therefore prescribe comedications devoid of interaction potential, particularly in the elderly. Of interest, the rate of red flag potential DDIs was 3%, similar to the previous value of 2% reported in an analysis of the SHCS performed in 2010 [24], whereas the rate of amber flag potential DDIs was significantly lower, likely due to a larger proportion of patients shifted to unboosted INSTIs, now recommended as firstline therapy and characterized by more favorable DDI profiles than boosted PIs or NNRTIs [35, 36]. Nevertheless, red flag potential DDIs remain clinically significant, particularly in cases involving boosted PIs coadministered with clopidogrel. It has been demonstrated that clopidogrel's active metabolite exposure was significantly reduced in PWH receiving boosted regimens, leading to insufficient inhibition of platelet aggregation in 44% of the patients [37]. Although prasugrel's active metabolite exposure was decreased to a similar extent by boosted regimens, this has no negative effect on prasugrel's pharmacodynamics, likely explained by its higher potency. Thus, prasugrel should be preferred in the presence of boosted regimens unless its use is contraindicated, in which case an alternative antiplatelet agent or ART should be considered.

Although the prevalence of potential DDIs remains important in PWH, our results demonstrated that potential DDIs notably with statins were generally managed correctly in real life through dosage adjustments, thereby reducing the probability of adverse events such as myalgia or even rhabdomyolysis. Due to our study design, it was not possible to assess the management of potential DDIs in an exhaustive manner, as for several drugs, like CNS drugs, a large range of drug doses is authorized, and dosage is adjusted mainly based on the clinical response and side effects. Finally, the prevalence of pharmacodynamic interactions was particularly low in our study due to the increasing use of tenofovir alafenamide, characterized by a lower nephrotoxicity compared with tenofovir disoproxil fumarate (TDF) [38].

PIMs were found in 31% of elderly PWH. This rate is lower than the rates reported in other studies, varying from 52% to 66% [7, 16, 17]. This difference may be explained by the fact that our study could not include all criteria defining inappropriate prescribing, for example, drugs prescribed without clinical indication, drugs administered beyond the recommended treatment duration, drugs not adjusted to the renal function of the patient, or prescribing omission. In addition, the prevalence of drugs with anticholinergic risk scale ≥3 was very low compared with the value of 17% reported by Greene et al. in PWH, even lower than the value of 4% that was reported in HIV-negative individuals in the same study [7]. This could possibly be explained by the lower number of prescribed comedications in our study (median, 4) compared with the publication of Green et al. (median, 6). In addition, differences in prescribing patterns between the United States and Europe could also explain this difference. In our study, inappropriate prescribing mainly resulted from benzodiazepines and hypnotics, which are associated with an increased risk of falls, impaired cognition, loss of independence, and hospitalization in the elderly [39]. Although clinicians might be aware of the risks associated with benzodiazepines or hypnotics in the elderly, they might not be able to stop such treatments, as patients become dependent.

Some limitations of our study should be acknowledged. First, although we focused on 2 therapeutic classes of interest, potential DDIs may also have occurred with other drug classes. Moreover, we did not assess the interactions between non-HIV comedications, resulting in an underestimation of the actual number of potential DDIs. Another limitation, common to all studies of this type, relies upon the fact that potential DDIs are assessed only between 2 compounds, which poorly accounts for the complexity of multiple and mutual DDIs encountered in polymedicated patients, not to mention pharmacogenetic issues. Finally, the lack of data about plasma drug concentrations and clinical outcomes arising from these potential DDIs prevented us from adequately evaluating their management. This was especially true for CNS drugs with a wide range of possible dosages and dosage adjustments mainly based on clinical situation.

Some strengths of our study should be emphasized nevertheless. The multicenter and prospective design provides valuable data about potential DDIs, as it reflects the general prescribing patterns and documents at best an individual's complete drug regimen. Our large sample of PWH gives to our observations a fair degree of representativeness. To our knowledge, this is the first study prospectively analyzing prescriptions filled out by PWH.

In conclusion, high rates of polypharmacy and the consequent DDI potential suggest that particular attention is needed when prescribing treatments to elderly PWH. Although the use of unboosted INSTIs is growing, one-fourth of elderly PWH had complex ARTs acting as perpetrator of DDIs. The acknowledgment that some medications may be inappropriate for aged patients constitutes an additional burden in health care provision to elderly PWH. Thus with the aging HIV population, education on geriatric medicine principles and periodic review of medicines is warranted to limit the risk of inappropriate prescribing in this vulnerable population. Clinicians should maintain a proactive approach for the recognition and management of potential DDIs, as well as for other prescribing issues traditionally encountered in geriatric medicine.

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Author contributions. Study design: P.C., C.M., L.A.D. Recruitment of participants: M.B., M.C., M.S. Recording in a database: P.C., S.A.S. Analysis and interpretation of data: P.C., C.M., F.L., M.G., C.C. Manuscript draft: P.C., C.M., F.L. Critical review and approval of the manuscript: all authors.

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II.2. Real-life management of drug-drug interactions between antiretrovirals and statins.

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Real-life management of drug–drug interactions between antiretrovirals and statins

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Background: PIs cause drug–drug interactions (DDIs) with most statins due to inhibition of drug-metabolizing enzymes and/or the hepatic uptake transporter OATP1B1, which may alter the pharmacodynamic (PD) effect of statins.

Objectives: To assess the management of DDIs between antiretrovirals (ARVs) and statins in people living with HIV (PLWH) considering statin plasma concentrations, compliance with dosing recommendations and achievement of lipid targets.

Methods: PLWH of the Swiss HIV Cohort Study were eligible if they received a statin concomitantly with ARVs. HDL, total cholesterol (TC) and statin plasma concentration were measured during follow-up visits. Individual non-HDL and TC target values were set using the Framingham score and the 2018 European AIDS Clinical Society recommendations.

Results: Data were analysed for rosuvastatin ($n = 99$), atorvastatin ($n = 92$), pravastatin ($n = 46$) and pitavastatin ($n = 21$). Rosuvastatin and atorvastatin underdosing frequently led to suboptimal PD response. Insufficient lipid control was observed with PIs despite high atorvastatin concentrations, likely explained by inhibition of OATP1B1 resulting in less statin uptake in the liver. Target lipid values were more often achieved with unboosted integrase inhibitors due to both their favourable DDI profiles and neutral effect on lipids. Insufficient lipid control was common with pravastatin and pitavastatin regardless of co-administered ARVs and despite using maximal recommended statin doses. The latter suggests lower efficacy compared with rosuvastatin or atorvastatin.

Conclusions: Suboptimal management of DDIs with statin underdosing was observed in 29% of prescriptions. Integrase inhibitor-based regimens and/or treatment with rosuvastatin or atorvastatin should be favoured in patients with refractory dyslipidaemia.

Introduction

Cardiovascular morbidities are highly prevalent in ageing people living with HIV (PLWH) as a result of traditional risk factors, HIV infection and side effects of antiretroviral drugs (ARVs).[1,2] The management of comorbidities like dyslipidaemia presents several challenges as some ARVs, such as PIs and efavirenz, can cause dyslipidaemia.[3] Furthermore, statins, the first-line drugs for the treatment of dyslipidaemia, are subject to drug–drug interactions (DDIs) with ARVs. Statins enter the liver, the site of action and subsequent metabolic elimination, via active transport by OATP1B1.[4] PIs inhibit this hepatic uptake transporter,[5] resulting in higher systemic exposure of statins and related increased risk of adverse drug reactions,[6,7] whereas their pharmacodynamic (PD) effect can be potentially impaired by them not entering the liver (Figure 1). PIs also inhibit the intestinal transporter BCRP, resulting in higher absorption of certain statins.[8] Finally, PIs are strong inhibitors of CYP3A4 and therefore may further increase the exposure of statins

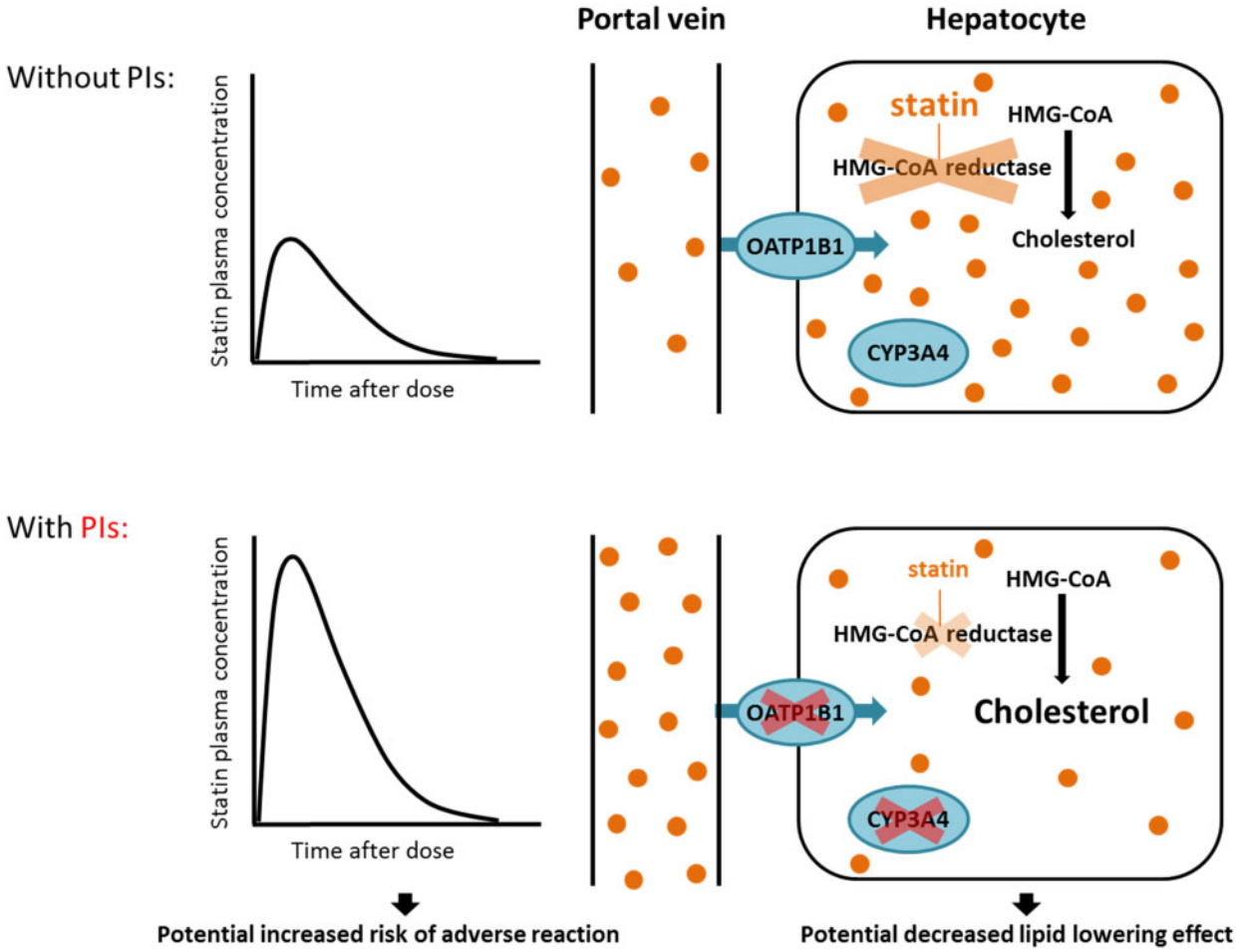

Figure 1. Mechanism of DDIs between PIs and statins, and pharmacokinetic/PD consequences. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

like atorvastatin undergoing CYP3A4-mediated metabolism due to both inhibition of transporters and CYP3A4. This concept is indeed illustrated by the observation that darunavir/cobicistat increases atorvastatin and rosuvastatin (no CYP3A4 metabolism) exposures by 290% and 93%, respectively.[9] Profound DDIs are expected with simvastatin or lovastatin as these statins are exclusively metabolized by CYP3A4[10] so their co-administration with PIs is contraindicated. Importantly, the magnitude of DDIs depends also on OATP1B1 inhibition by PIs, which is strongest for atazanavir followed by lopinavir and darunavir. Rosuvastatin exposure was shown to be increased by 213%, 107% and 48% when co-administered with atazanavir/ritonavir,[11] lopinavir/ritonavir[12] and darunavir/ritonavir,[13] respectively. Differences in the magnitude of DDIs have led to specific dosing recommendations for statins based on the co-administered PI. For instance, the European label of atorvastatin recommends not exceeding a daily dose of 10 mg with atazanavir or atazanavir/ritonavir; 20 mg with lopinavir/ritonavir or 40 mg with darunavir/ritonavir.[14] Conversely, the NNRTIs efavirenz, nevirapine and etravirine can decrease atorvastatin exposure due to CYP3A4 induction, requiring a potential increase in dosage. Finally, no DDIs are expected between statins and unboosted integrase inhibitors (INIs) like dolutegravir, raltegravir, bictegravir or the NNRTI rilpivirine as these ARVs have no inhibitory or inducing effects on CYP3A4 or drug transporters.

It is currently unknown how DDIs between ARVs and statins are systematically apprehended and managed in clinical practice. A previous study showed that a large proportion of PLWH on PI- or NNRTI-based regimens failed to reach adequate lipid targets while on statin treatment.[15] Statin levels were not measured, therefore it is unclear whether the observed suboptimal response was due to DDIs with ARVs or to poor adherence. It is unknown whether better management of dyslipidaemia can be achieved when using non-interacting ARVs (i.e. unboosted INIs) or when using tenofovir disoproxil fumarate instead of tenofovir alafenamide in the backbone. Studies have demonstrated that switching from tenofovir disoproxil fumarate to tenofovir alafenamide was associated with the development of less-favourable lipid profiles regardless of the third agent (PI, NNRTI or INI).[16]

This work aimed to assess the management of DDIs between ARVs and statins in PLWH of the Swiss HIV Cohort Study (SHCS) considering statin plasma concentrations, compliance with dosing recommendations and achievement of lipid targets.

Methods

Study design

PLWH enrolled in the SHCS and followed up in the centres of Lausanne and Basel were eligible if they received one commonly prescribed statin (i.e. rosuvastatin, atorvastatin, pravastatin or pitavastatin) concomitantly with ARVs. On the day of the follow-up medical visit, blood was collected for the measurement of lipid values and for the measurement of the statin plasma concentration. The timing of blood sampling and statin intake were documented in order to be able to interpret the statin concentration. The complete list of medications with their dosage was also documented during the medical visit. PLWH participated at most three times in this study.

Calculation of individual non-HDL and total cholesterol (TC) targets

Demographic and clinical parameters as well as lipid values measured during the medical follow-up visit were extracted from the SHCS database. Documented lipid laboratory parameters include TC, HDL cholesterol and triglycerides (TG). PLWH often have higher than normal TG values, therefore LDL values cannot be reliably derived using the Friedewald formula.[17] Thus, non-HDL values were considered instead and calculated as follows: non-HDL = TC − HDL.

Individual LDL targets were calculated using the Framingham score[18] considering the following patient parameters: age, gender, blood pressure, smoking (number of cigarettes/day), family history of diabetes and personal history of myocardial infarction. Individual non-HDL cholesterol targets were subsequently derived by adding 0.8 mmol/L to individual LDL targets.[17] The added value corresponds to the cholesterol fraction contained in 1.7 mmol/L of TG (i.e. upper accepted limit for TG).

Individual TC targets were established based on the presence or history of cardiovascular diseases (i.e. myocardial infarction, coronary angioplasty/stenting, coronary artery bypass grafting, cerebral haemorrhage or cerebral infarction) and/or diabetes mellitus. TC threshold was fixed at $\leq$5 (in the absence of cardiovascular risks) or $\leq$4 mmol/L (in the presence of cardiovascular risks) in agreement with the 2018 European AIDS Clinical Society guidelines.[19]

Plasma concentrations of statins

Blood samples collected during the SHCS follow-up visit were centrifuged and the isolated plasma was stored at −80°C until analysis. Drug concentrations were quantified for rosuvastatin, atorvastatin and pravastatin using a validated LC coupled with tandem MS analysis[20] with lower limits of quantification (LLOQs) of 0.5 ng/mL for rosuvastatin, 0.3 ng/mL for atorvastatin and 0.4 ng/mL for pravastatin. The measured drug concentrations were compared with published reference pharmacokinetic profiles of rosuvastatin,[21] atorvastatin[22–24] and pravastatin[25–27] and categorized as low, normal or high with respect to the corresponding reference profile. Recent non-adherence was strongly suspected in the case of statin concentrations below the LLOQ.

Statin dosing recommendations in the presence of ARVs

Statins are impacted differently by ARVs, leading to specific dosing recommendations based on the co-administered ARV, as indicated in the Liverpool drug-interaction database[28] and the European AIDS Clinical Society guidelines.[19] Dosing recommendations used for the evaluation of the management of DDIs between statins and ARVs are presented in Table 1. If PLWH were treated with combined ARVs (i.e. PI + NNRTI or PI + unboosted INI), statin dosing recommendations for the ARV with the strongest DDI potential (i.e. PI in this example) were applied.

Table 1. Statin maximal daily doses according to co-administered ARV[28]

	Rosuvastatin	Atorvastatin	Pravastatin	Pitavastatin
Boosted[a] atazanavir	10 mg	10 mg	40 mg	4 mg
Boosted[a] darunavir	20 mg	40 mg	40 mg	4 mg
Boosted elvitegravir	20 mg	40 mg	40 mg	4 mg
Non-interacting ARVs	20 mg	80 mg	40 mg	4 mg

[a]Boosted either with ritonavir or cobicistat.

Atorvastatin dosage above the maximal daily dose was allowed in the presence of ARVs with CYP3A4-inducing properties (i.e. efavirenz, etravirine or nevirapine) to compensate for the interaction. Since ARVs have a modest effect on pravastatin and pitavastatin exposures, maximal daily doses of pravastatin and pitavastatin were equal to the recommended daily maximal doses in the absence of interacting medications.

Evaluation of the management of DDIs between ARVs and statins

The management of DDIs between ARVs and statins was considered to be correct when both lipid values (i.e. non-HDL and TC) were within the target values for a given patient. When one or both lipid target values were not achieved, three scenarios were considered:

(i) Statin dosage agreed with dosing recommendations; however, the measured level was <LLOQ, suggesting a problem of non-adherence. In this situation, the management of DDI was considered as *a priori* correct.
(ii) Statin dosage agreed with dosing recommendations. The lipid response was suboptimal despite detectable statin levels and despite using the highest recommended dosage, suggesting a problem of statin efficacy. The management of DDI was considered as *a priori* correct.
(iii) Statin dosage did not agree with dosing recommendations (i.e. dosage outside the recommended dosage range). The management of DDI was considered as incorrect.

Results

Study population

Data were collected for 99 rosuvastatin (66 PLWH), 92 atorvastatin (61 PLWH), 46 pravastatin (34 PLWH) and 21 pitavastatin (14 PLWH) prescriptions. The demographic, clinical and ARV treatment characteristics of the study population are presented in Table 2. The median age of participants did not significantly differ between statin treatment groups. Rosuvastatin tended to be prescribed more often in PLWH with a history of a cardiovascular event and/or diabetes. Median non-HDL, TC and TG values were comparable in the rosuvastatin and atorvastatin treatment groups whereas all lipid values and TC tended to be higher in the pravastatin and pitavastatin treatment groups, respectively. Unboosted INIs (i.e. raltegravir or dolutegravir) represented the most common ARV-based regimens in the study population.

Table 2. Characteristics of the study population, stratified by statin treatment

	Rosuvastatin	Atorvastatin	Pravastatin	Pitavastatin
Number of statin prescriptions	99	92	46	21
Number of PLWH	66	61	34	14
Age, years[a]	60 (55–67)	62 (58–71)	60 (53–65)	65 (55–72)
Women[a]	8 (12)	14 (23)	8 (24)	2 (14)
Cardiovascular event and/or diabetes[b]	46 (47)	23 (25)	12 (26)	6 (29)
Non-HDL, mmol/L[b]	3.2 (2.6–3.8)	3.1 (2.6–3.7)	3.7 (2.9–4.3)	3.2 (2.6–4.1)
TC, mmol/L[b]	4.5 (3.9–5.0)	4.5 (3.8–5.1)	4.9 (4.3–5.4)	4.8 (4.3–5.4)
TG, mmol/L[b]	1.9 (1.3–2.7)	1.7 (1.3–2.3)	2.2 (1.4–2.6)	1.5 (1.1–4.2)
ARV treatment[b]				
boosted PI	8 (8)	7 (8)	4 (9)	3 (14)
boosted INI	5 (5)	3 (3)	5 (11)	0
unboosted INI	23 (23)	36 (39)	13 (28)	12 (57)
NNRTI	26 (26)	20 (22)	13 (28)	4 (19)
combined regimen[c]	37 (38)	26 (28)	11 (24)	2 (10)
Backbone[b]				
TDF-based regimen	24 (24)	20 (22)	14 (30)	2 (10)
TAF-based regimen	28 (28)	12 (13)	6 (13)	1 (5)

Values are presented as *n* (%) or median (IQR).
TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide.
[a]Results are presented considering the number of PLWH in each statin treatment group.
[b]Results are presented considering the number of prescriptions in each statin treatment group.
[c]PI + INI or PI + NNRTI or NNRTI + INI or PI + NNRTI + INI.

Data were collected once in 82 PLWH (49%), twice in 82 PLWH (49%) and three times in 4 PLWH (2%).

Overall response to statin treatment and management of DDIs

Rosuvastatin

Considering rosuvastatin dosing recommendations in the presence of ARVs, underdosing with non-achievement of lipid targets represented approximately one-third of rosuvastatin prescriptions whereas overdosage was uncommon (Figure 2). Suboptimal PD response was observed for 12% of rosuvastatin prescriptions despite using the maximal recommended dose and despite rosuvastatin levels mostly being within the normal range.

Taking into account patient non-adherence and issues of lower statin efficacy, the management of DDIs between rosuvastatin and ARVs was correct for 69% of rosuvastatin prescriptions.

Atorvastatin

Underdosing with uncontrolled lipids represented one-third of atorvastatin prescriptions (Figure 2). Of interest, suboptimal lipid control was common with PIs despite high atorvastatin concentrations (Figure 3). This observation is suggestive of a DDI impairing the entry of statin into the liver, the site of action. Thus, increasing atorvastatin dosage might not be helpful.

The management of DDIs was correct for 65% of atorvastatin prescriptions.

Pravastatin

Insufficient lipid control was common despite using the maximal pravastatin dose and despite pravastatin levels mostly being within the normal range. This observation is suggestive of an efficacy problem. Underdosing with uncontrolled lipids represented 17% of pravastatin prescriptions (Figure 2).

The management of DDIs was correct for 83% of pravastatin prescriptions.

Pitavastatin

Pitavastatin was mostly used at the highest recommended dose of 4 mg once daily. This statin demonstrated the lowest efficacy since lipid control was not achieved in 38% of pitavastatin prescriptions despite using the maximal recommended dose and despite mostly co-administering ARVs devoid of DDIs or with a neutral effect on lipids (Figure 2). Underdosing represented almost 24% of pitavastatin prescriptions.

The management of DDIs was correct for 76% of pitavastatin prescriptions.

Statin treatment and response in the presence of PIs versus non-interacting and lipid-neutral ARV drugs

Since PIs have a higher DDI potential and are less lipid-friendly, statin treatment and response were compared in the presence of PIs versus non-interacting and lipid-neutral ARVs (i.e. dolutegravir, raltegravir or rilpivirine) for each statin. This evaluation could not be performed for pitavastatin as it was mostly not prescribed with PIs and plasma levels were not quantified.

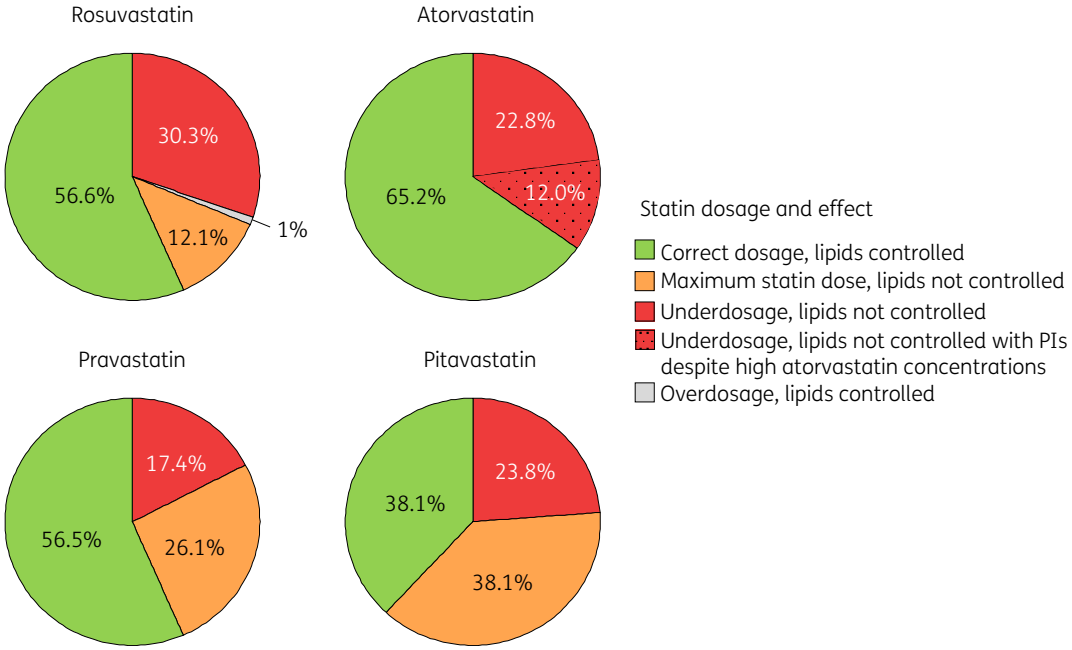

Figure 2. Achievement of lipid targets based on statin dosing recommendations considering the co-administered ARV (see Table 1 for statin dosage recommendations). Statin dosage was considered as correct if dosage was within the recommended range and the lipids were controlled. Statin dosage was considered as underdosed if the lipids were not controlled and the dosage of the statin could have been increased. Statin dosage was considered as overdosed if the dosage of the statin was above the upper limit of the recommended dosage. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Rosuvastatin

Rosuvastatin was generally prescribed at 10 mg once daily. High doses were used regardless of the presence of PIs, likely due to the fact that darunavir/ritonavir, the most prescribed PI, has only a weak effect on rosuvastatin exposure. This statement is indeed consistent with the low and comparable proportion of high rosuvastatin concentrations in the presence of PIs versus non-interacting ARVs. Non-HDL targets tended to be less often achieved with concomitant use of PIs (Figure 3).

Atorvastatin

Atorvastatin was mostly prescribed at 10 mg once daily. Lower doses were generally used in the presence of PIs whereas higher doses were more common when associated with non-interacting ARVs (Figure 3). PIs cause large DDIs with atorvastatin, as indicated by the fact that all atorvastatin concentrations were high in the presence of PIs. Conversely, low atorvastatin concentrations were observed in 42% of PLWH receiving an ARV with inducing properties (i.e. efavirenz or etravirine). Despite high concentrations, non-HDL targets were less often achieved in the presence of PIs, likely due to both their inhibitory effect on OATP1B1 and their effect on lipids.

Pravastatin

Pravastatin was mostly prescribed at the highest recommended dose of 40 mg once daily. Similar to rosuvastatin, a high dose of pravastatin was used regardless of the presence of a PI, likely owing to the known weak DDI with pravastatin. There was a tendency for less achievement of the non-HDL target with concomitant use of PIs (Figure 3).

Achievement of lipid targets in the presence of tenofovir disoproxil fumarate-containing versus tenofovir alafenamide-containing backbone

Overall, 60 and 47 prescriptions had a tenofovir disoproxil fumarate-containing or tenofovir alafenamide-containing backbone, respectively. Lipid targets tended to be more often achieved in the presence of tenofovir disoproxil fumarate-containing (88%) versus tenofovir alafenamide-containing (72%) regimens regardless of the third agent (PI, NNRTI or INI).

Discussion

Our study provides evidence of suboptimal management of dyslipidaemia in PLWH due to statin underdosing or the use of low-intensity statins, leading overall to 41% of statin prescriptions with insufficient response. Our observations are in line with previous studies reporting uncontrolled dyslipidaemia in 14% to 50% of PLWH.[15,29,30] Uncontrolled dyslipidaemia is also common in HIV-uninfected persons with a prevalence of 24% to 49%.[31–33]

Multiple factors contribute to the complexity of the management of dyslipidaemia. First, the choice of statin dosage may be guided by the magnitude of the interaction, which is high for atorvastatin (AUC increased by 200%–300%)[34] and weak for pravastatin (AUC increased by 81%)[35] and rosuvastatin (AUC increased by 48%).[13] In our study, pravastatin and rosuvastatin dosages were similar regardless of co-administered ARV, while atorvastatin was

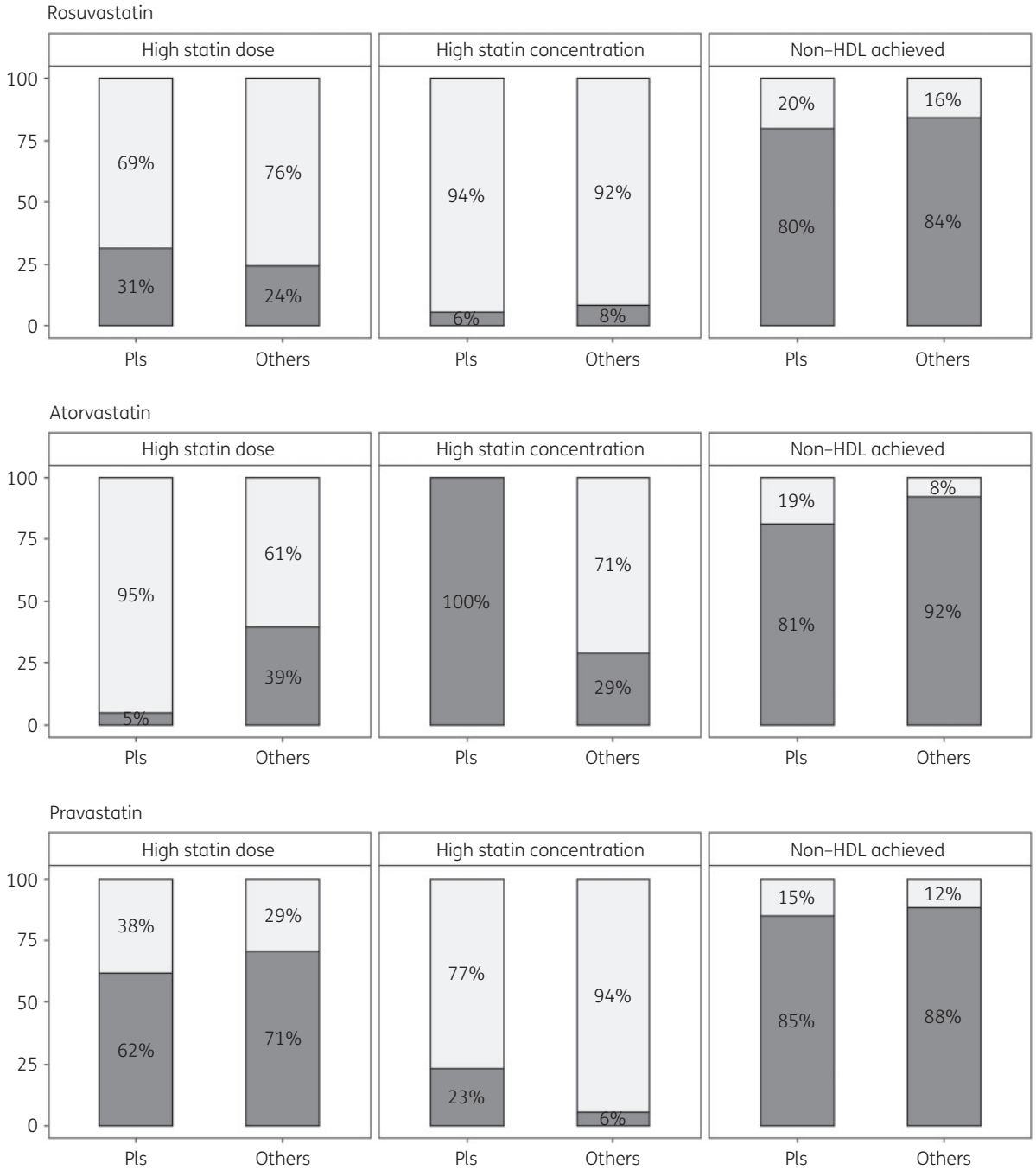

Figure 3. Percentage of statin prescriptions with high statin dose (i.e. 20 mg for rosuvastatin and 40 mg for both atorvastatin and pravastatin) (dark grey bars), high statin plasma concentrations (dark grey bars) and achievement of non-HDL cholesterol targets (dark grey bars) in the presence of PIs versus other non-interacting ARVs with neutral effects on lipids (Others: dolutegravir, raltegravir or rilpivirine).

systematically used at a lower dose in the presence of PIs, indicating that clinicians are aware of differences in the DDI profiles of ARVs. Since most statins have demonstrated a dose–response relationship in regard to the reduction of LDL cholesterol,[36–40] it is important to follow the recommendations for maximal daily doses of statins,[41] particularly in PLWH with suboptimal lipid responses. Nevertheless, our study shows that statin underdosing is one of the main factors in insufficient response to statin treatment, consistent with a previous study.[15] The deliberate low dosage of statins could possibly be explained by the overestimation of the risk of DDIs, as exemplified by a study reporting a larger proportion of PLWH with subtherapeutic levels of antidepressants compared with uninfected individuals.[42] However, the reluctance to use higher doses of statins is not limited to PLWH but has also been reported in HIV-uninfected persons regardless of the presence of interacting drugs.[31,43] This observation seems to relate to the fear

of dose-dependent adverse reactions and to the fact that clinicians do not consider lipid targets as strict criteria, above which they will not necessarily increase statin dose.

In the case of insufficient response, an increase in the statin dose should be systematically considered in the presence of ARVs with inducing metabolic properties, such as efavirenz. Of interest, we frequently observed low statin concentrations in the presence of efavirenz whereas concentrations were mostly in the reference range with nevirapine. For ARVs with no interacting properties, the dose can be increased until the statin maximal recommended daily dose is reached. However, for ARVs with inhibitory effects on OATP1B1, like PIs, an increase in the statin dose might not necessarily improve the lipid response. As depicted in Figure 1, the inhibition of OATP1B1 by PIs prevents the entry of statins into the liver, resulting in less inhibition of HMG-CoA-reductase. Inhibition of hepatic uptake will also result in less statin being metabolized and eliminated thereby leading to increased systemic concentrations and a related increased risk of adverse drug reactions. This concept is supported in our study by the observation that lipid targets were less often achieved in the presence of PIs despite high statin concentrations, particularly for atorvastatin. Studies have indeed demonstrated reduced lipid responses in individuals carrying a genetic variation in the gene encoding OATP1B1, resulting in reduced statin uptake in the liver.[44] Whether attributed to genetic variations or to DDIs, a decrease in OATP1B1 activity may alter the PD response to statins. Importantly, alteration in OATP1B1 function was shown to impact atorvastatin exposure more profoundly compared with that of rosuvastatin or pravastatin.[45]

Another factor modulating the response to statins is the effect of ARVs *per se* on lipids. Dolutegravir, raltegravir and rilpivirine have been shown to have a neutral effect on lipids[3,46] whereas PIs and efavirenz can cause dyslipidaemia.[3] Our study indeed shows that lipids are less often controlled in the presence of PIs. Thus, altogether, dyslipidaemia is more difficult to manage in PLWH on PIs as these agents have unfavourable DDIs and lipid effects. When possible, unboosted INIs should be preferred in PLWH with refractory dyslipidaemia. The choice of the backbone should also be considered since evidence suggests that tenofovir alafenamide has a less favourable lipid profile compared with tenofovir disoproxil fumarate.[16]

Finally, the efficacy of the statin is another factor to consider. Of interest, uncontrolled lipids were more commonly observed with pravastatin and pitavastatin despite using maximal recommended statin doses. This is due to their lower efficacy compared with rosuvastatin and atorvastatin, as indicated by the University of Michigan statin dose intensity and equivalence chart.[47] Our observation is consistent with multiple studies demonstrating a higher efficacy of rosuvastatin and atorvastatin compared with lower intensity statins both in PLWH[48] and uninfected individuals.[49,50]

Optimal management of dyslipidaemia is particularly critical in PLWH as they are at high risk for cardiovascular events.[51] Although large randomized controlled trials with clinical endpoints have not been completed in PLWH, a cohort study suggested that statin therapy was associated with a decrease in all-cause mortality.[52] Therefore, optimal statin therapy may reduce morbidity and mortality in PLWH similarly to in the general population.[53]

Some limitations of our study should be emphasized. Tolerability data regarding the statin treatment were not documented, thus dose escalation might have been prevented in some cases due to the presence of adverse drug reactions. This could result in a potential overestimation of cases that were not managed correctly in our study. In addition, the small number of PLWH concomitantly receiving pitavastatin with PIs and the absence of plasma levels for this statin made it difficult to draw conclusions regarding the pharmacokinetics and clinical consequences of DDIs with pitavastatin. In addition, long-term adherence to statin treatment was not measured, thus it cannot be excluded that the apparent lack of efficacy might relate to adherence issues. Nevertheless, recent non-adherence could be detected with certitude in 19 prescriptions (7.4%) as statin concentrations were below the LLOQ. This result highlights the importance of monitoring adherence to comedications, particularly because lower levels of adherence have been reported for comedications compared with ARVs in an SHCS study.[54] Finally, the study was conducted before the introduction of bictegravir so data are lacking for this novel INI. Nevertheless, bictegravir is not expected to differ from other unboosted INIs due to its low potential for DDIs and its neutral effect on lipids and therefore would also constitute a preferable option.[28,41]

Several strengths of our study should be acknowledged. This is the first study providing a systematic analysis of the management of dyslipidaemia in PLWH taking into account statin concentrations, dosing recommendations and clinical responses (non-HDL and TC levels). This comprehensive analysis allows us to better understand factors modulating the response to statins when treating PLWH.

In conclusion, suboptimal management of dyslipidaemia is common in PLWH due to statin underdosing or the use of low-intensity statins leading overall to 41% of statin prescriptions with suboptimal response. Management of dyslipidaemia in patients on PIs is challenging due to DDIs and their negative impact on lipid profile, potentially impairing the therapeutic effect of statins. Unboosted INI or rilpivirine-based regimens and/or treatment with rosuvastatin or atorvastatin should be favoured in patients with refractory dyslipidaemia.

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CHAPTER III: LC-MS/MS ANALYSES

Chapter III in the thesis context

Accurate determination of drug levels in biological fluids is a prerequisite for clinical pharmacokinetics/pharmacodynamics investigations. This requires the availability of validated LC-MS/MS methodologies for the precise and accurate measurements of drug concentration in plasma. Accuracy is a key characteristic of such bioassays since analytical results can impact TDM interpretation and clinical implications. In this thesis, the currently most prescribed antiretroviral plasma concentrations have been determined with previously developed LC-MS/MS methodologies used for the routine TDM service . However, three new analytical methods have been also developed and validated for additional drugs which were necessary for the research project

The first assay allows the determination of NRTIs in plasma and cerebrospinal fluid. This methodology is now being routinely used in our TDM service and is also applied for several projects within the frame of the SHCS, including the "Pharmacokinetic profiles of boosted darunavir, dolutegravir and lamivudine in aging patients enrolled in the Swiss HIV Cohort Study" (chapter V) and the "Emtricitabine and lamivudine concentrations in saliva: a simple suitable test for treatment adherence" (chapter VI). Results of ARV measurements in paired plasma and CSF samples of PLWH have also been the subject of a poster presented at the European AIDS conference 2019 that showed the marked differences in penetration of various ARV in CNS sanctuary compartment (Poster in Appendice VIII.5).

The second part is a LC-MS/MS method for the measurement of the highly prescribed comedications in PLWH (along with two active metabolites) with a known risk of DDIs. In the perspective to study the magnitude of DDIs between ARVs and comedications, we have developed and validated the first multiplex analytical assay quantifying simultaneously amlodipine, metoprolol, atorvastatin (and its two active metabolites *o*-OH and p-OH atorvastatin), pravastatin, and rosuvastatin. The challenge of this bioassay consisted in the accurate measurement of plasma concentrations below the nanogram per millilitre.

The third method was developed to quantify the new generation of ARV drugs. Newly approved ARV drugs, such as bictegravir and doravirine are expected to be increasingly prescribed because of their potency and the convenience once daily administration. Yet, DDIs are likely to occur also with these last generation drugs in the real life, which necessitates assays to monitor their levels in plasma. Moreover, an important pharmacokinetic variability has been reported in clinical trials of the first wave of Long-Acting Injectable (LAI) regimen cabotegravir and rilpivirine, injected intramuscularly. This variability may further increase in underweight or obese patients, in case of alteration of hepatic or renal functions, with DDIs or during pregnancy, should it occurs. In such instances, information on whether patients are exposed to appropriate plasma drug concentrations is necessary, and a bioassay is thus needed to provide such irrevocable information.

Own contribution: development and validation of analytical assays, literature review and drafting of the article.

III.1. Determination of nucleosidic/tidic reverse transcriptase inhibitors in plasma and cerebrospinal fluid by ultra-high-pressure liquid chromatography coupled with tandem mass spectrometry.

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Determination of nucleosidic/tidic reverse transcriptase inhibitors in plasma and cerebrospinal fluid by ultra-high-pressure liquid chromatography coupled with tandem mass spectrometry

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ABSTRACT

Nucleoside reverse transcriptase inhibitors (NRTIs) have been the first class of antiretroviral drugs used against HIV infection. Despite NRTI-free regimens have been eagerly sought over the years in an effort for treatment simplification, NRTIs remain in most antiretroviral combination treatment. There has been generally a limited interest for their therapeutic drug monitoring, arguably because NRTIs levels measured in plasma poorly predict the concentration of pharmacologically active metabolites in cells. Plasma concentrations do impact cellular levels, while large differences between NRTIs have been found with regard to their ability to distribute into the cerebrospinal fluid (CSF) compartment. The renewed interest for the measurements of NRTIs concentrations in plasma and CSF was raised by ongoing efforts to understand some instances of toxicity or for determining their actual implication in the development of HIV-associated neurological disorders. In this context, a 5-min multiplex ultra-high-pressure chromatography tandem mass spectrometry (UHPLC-MS/MS) analysis in human plasma and CSF was developed for NRTIs used in clinical practice: abacavir, emtricitabine, lamivudine, tenofovir and zidovudine along with zidovudine glucuronide (Gln-ZDV). The 200-fold dilution of blank human plasma was shown to be a reliable surrogate matrix for quantification of NRTIs and Gln-ZDV in CSF. Both methodologies were fully validated over the clinically relevant concentrations, and satisfactorily fulfilled all parameters for bioanalytical methods validation. This sensitive, rapid, and robust UHPLC-MS/MS assay offers a methodology for increasing our understanding of the ability of NRTIs to cross the blood-brain barrier and their potential implication in neuropsychological disorders observed in HIV-infected patients.

1. Introduction

These days, combined antiretroviral therapy (cART) constitutes the cornerstone treatment to ensure suppression of human immunodeficiency virus (HIV) infection in patients to so-called “undetectable levels” (i.e., not eradicated but below the detection limit of our currently used assays). This regimen specifies the use of three or more antiretroviral drugs, comprising two nucleoside/tide reverse transcriptase inhibitors (NRTIs), as the

Abbreviations: 3TC, lamivudine; ABC, abacavir; ACN, acetonitrile; aCSF, artificial cerebrospinal fluid; CNS, central nervous system; CSF, cerebrospinal fluid; ER, extraction recovery; ESI, electrospray ionization; FA, formic acid; FTC, emtricitabine; FWHM, full width at half maximum; Gln-ZDV, zidovudine glucuronide; IS, internal standard; IS-nER, internal standard normalized extraction recovery; IS-nME, internal standard normalized matrix effect; IS-nPE, internal standard normalized process efficiency; IS-WS, internal standard working solution; LC–MS, liquid chromatography coupled with mass spectrometry; LLOQ, lower limit of quantification; ME, matrix effect; MeOH, methanol; NRTI, nucleoside reverse transcriptase inhibitor; PE, process efficiency; PreP, Pre-Exposure Prophylaxis; QC, quality control; rCSF, real cerebrospinal fluid; RSD, relative standard deviation; RT, retention time; sCSF, surrogate cerebrospinal fluid; SRM, selected reaction monitoring; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; UHPLC–MS/MS, ultra-high-pressure liquid chromatography coupled with tandem mass spectrometry; ULOQ, upper limit of quantification; WS, working solution; ZDV, zidovudine

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backbone regimen, combined with one non-nucleoside reverse transcriptase inhibitor (NNRTI) or one protease inhibitor (PI) or one integrase strand transfer inhibitor (INSTI). At present, in the context of NRTI associations, tenofovir (TFV) and abacavir (ABC) appear among the most commonly used NRTIs, used in combination with emtricitabine (FTC) or lamivudine (3TC) [1].

NRTIs are prodrugs that require intracellular metabolic phosphorylation to be converted into the pharmacologically active phosphorylated species. Arguably, determination of the intracellular levels of NRTIs would probably be more confidently related to clinical response and toxicity than concentrations measured in plasma [2]. However, the monitoring of the very low intracellular concentrations of the active phosphate metabolites still remains an analytical challenge [3], and its actual clinical relevance remains to be formally demonstrated. Most Therapeutic Drug Monitoring (TDM) studies on antiretrovirals have been based on plasma concentrations, but there is also an interest in measuring drug levels in sanctuary compartments, such as the central nervous system (CNS), where low-level viral replication persists. NRTI concentrations in plasma and cerebrospinal fluid (CSF) have been related to some instances of toxicity: higher plasma TFV trough concentrations have been associated with renal dysfunctions [4–7], whereas high concentrations of NRTIs in CSF are reportedly associated with neurocognitive disorders [8,9].

The numerous analytical methods for NRTIs using radioimmunoassay, capillary electrophoresis and liquid chromatography coupled with UV detection or tandem–mass spectrometry (LC–MS/MS) have been previously reviewed [10]. In 2000, Kenney et al. developed the first reversed-phase LC–MS/MS method for the selective and sensitive determination of 3TC and zidovudine (ZDV) in human serum ultrafiltrate using stable-isotopically-labeled internal standards [11]. During the last decade, several LC–MS/MS methods have been developed for NRTIs analysis in various biological matrices, such as plasma [12–17], amniotic fluid [18] and peripheral blood mononuclear cells [19–23]. Regarding CSF, Best et al. and Lahiri et al. recently reported NRTIs penetration into the central nervous system only for TFV and FTC by using adaptations of previously published bioanalytical methods [24,25]. In his seminal work, Letendre et al. has proposed a CNS Penetration Effectiveness (CPE) ranking of antiretrovirals, based on their chemical properties, concentrations in CSF, and/or their antiviral effectiveness in the CNS [26]. However, the predictive role of CPE on neurocognitive and clinical outcome has been conflicting [27]. In fact, few real-life clinical data are currently available on the ability of NRTIs to cross the blood brain barrier, which could, however, be determined by measuring NRTI levels in plasma and CSF, both collected simultaneously at unselected times over the dosing interval.

The aim of this project was to develop and validate a simplified, fast and robust approach using a unique multiplex UHPLC–MS/MS assay for the simultaneous quantification of five NRTIs (i.e., abacavir, emtricitabine, lamivudine, tenofovir and zidovudine) and zidovudine glucuronide (Gln-ZDV) in plasma and CSF.

2. Materials and methods

2.1. Chemicals, reagents and biological matrices

ABC sulfate, FTC, 3TC, TFV, ZDV and Gln-ZDV were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). Their stable isotopically labeled internal standards (i.e., [$^{2}H_5$]-ABC, [$^{2}H_3$, ^{15}N]-FTC, [^{13}C, $^{2}H_2$, $^{15}N_2$]-3TC, [$^{2}H_6$]-TFV, [^{13}C, $^{2}H_3$]-ZDV) were obtained from Alsachim (Strasbourg, France). The chemical structures of the respective molecules are shown in Fig. 1.

Methanol (MeOH), acetonitrile (ACN) and formic acid (98–100%, FA) were of analytical grade (Merck, Darmstadt, Germany). Daily fresh ultrapure water was obtained from a Milli-Q® UF-Plus apparatus (Millipore Corp., Burlington, MA, USA).

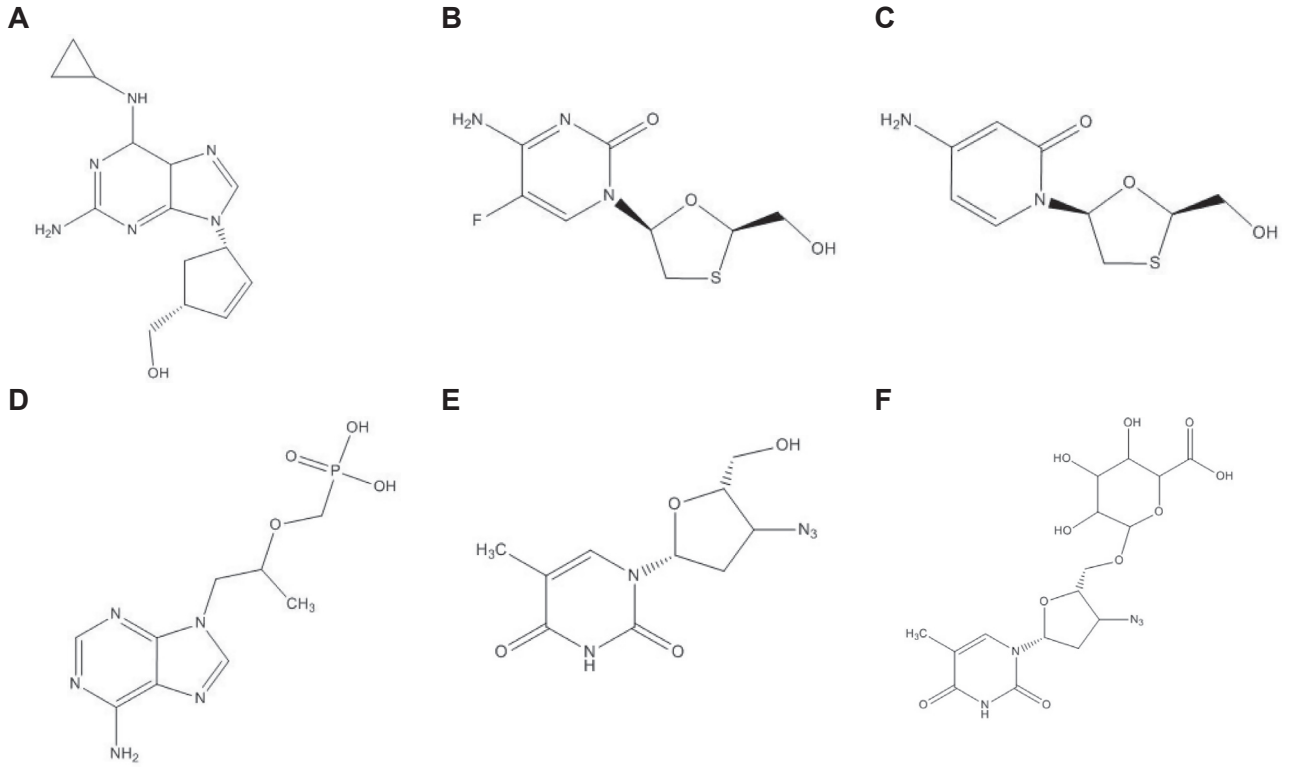

Fig. 1. Chemical structures of NRTIs. (A) Abacavir (logP = 0.39). (B) Emtricitabine (logP = −0.90). (C) Lamivudine (logP = −1.10). (D) Tenofovir (logP = −3.43). (E) Zidovudine (logP = −0.30). (F) Zidovudine glucuronide (logP = −1.3). Chemicalize was used for compound properties, 2017, https://chemicalize.com/ developed by ChemAxon (http://www.chemaxon.com).

Human blank plasma samples used during method validation experiments and for the preparation of calibration, validation and quality control (QC) samples were obtained from citrated blood (1970 g (3000 rpm)), 10 min, +4 °C, Hettich model Rotanta 460RF centrifuge) withdrawn from patients with Vaquez disease at occasion of their regular phlebotomy (CHUV, Lausanne, Switzerland). Surrogate CSF (sCSF) was obtained by diluting blank citrated human plasma 200-fold with daily fresh ultrapure water [28]. Pooled real CSF (rCSF) was obtained from the CHUV Clinical Chemistry Laboratory. Artificial CSF (aCSF) was prepared with an in-house formula: i.e., 0.2 g/L human serum albumin (Irvine Scientific, Santa Ana, USA), 7.3 g/L NaCl, 0.8 g/L glucose and 1.9 g/L $NaHCO_3$ (adjusted to pH 7.5 with phosphoric acid 85%) [29]. Reagents for aCSF preparation were obtained from Merck (Darmstadt, Germany).

2.2. NRTI and internal standard solutions

Each NRTI and Gln-ZDV were independently weighed and dissolved in the required volume of ultrapure water to obtain stock solutions at a final concentration of 1 mg/mL. The stock solutions used for ABC sulfate and Gln-ZDV sodium salt were corrected for the salt form of the analyte by applying the weighing factor. These solutions were diluted and combined to obtain a single working solution (WS) at a final concentration of 120 µg/mL for plasma and 60 µg/mL for CSF. The internal standards (IS) working solution (IS-WS), used for protein precipitation of plasma samples, was obtained by diluting the IS stock solutions in MeOH (1 mg/mL in MeOH) to obtain the following concentrations: 80 ng/mL for [$^2H_5$]-ABC, 50 ng/mL for [$^2H_3$, ^{15}N]-FTC, 100 ng/mL for [^{13}C, $^2H_2$, $^{15}N_2$]-3TC and [$^2H_6$]-TFV, and 600 ng/mL for [^{13}C, $^2H_3$]-ZDV.

For artificial, real and surrogate CSF sample preparation, IS concentrations in the WS used for sample dilution were adapted and prepared in a MeOH:H_2O 2:1 mixture to obtain the same percentage of MeOH and same IS concentrations in the final vial after plasma protein precipitation.

All solutions were prepared in 5-mL polypropylene tubes and stored at −20 °C.

2.3. Calibration/validation standards and quality controls (QCs) solutions

The WS, that includes the 6 compounds, was sequentially diluted and pooled with either: citrated blank plasma to obtain the following 9 concentration levels (k) in triplicate (n = 3): 6000, 3000, 1500, 750, 30, 10, 5, 2 and 1 ng/mL; or sCSF to obtain the following 9 concentration levels (k) in triplicate (n = 3): 3000, 1500, 750, 30, 10, 5, 2, 1 and 0.5 ng/mL. The following calibration ranges were used for quantification in plasma: 1–6000 ng/mL (k = 9) for FTC, 2–6000 ng/mL for ABC (k = 8), 5–6000 ng/mL for 3TC (k = 7), TFV and ZDV, 10–6000 ng/mL (k = 6) for Gln-ZDV. For quantification in CSF, calibration ranges in sCSF were determined as follows: 0.5–3000 ng/mL for FTC (k = 9), 1–3000 ng/mL (k = 8) for ABC, 3TC and TFV, 2–3000 ng/mL (k = 7) for ZDV, 5–3000 ng/mL (k = 6) for Gln-ZDV. Two separated series of 8 dilutions (k) of WS were prepared in plasma (i.e., 1, 2, 5, 10, 30, 600, 2400, 4800 ng/mL) and in sCSF (i.e., 0.5, 1, 2, 5, 10, 30, 600, 2400 ng/mL) for validation standard samples preparation. Determination of validation standard levels in plasma was carried out as follows: 1–4800 ng/mL (k = 8) for FTC, 2–4800 ng/mL (k = 7) for ABC, 5–4800 ng/mL (k = 6) for 3TC, TFV and ZDV, 10–4800 ng/mL for Gln-ZDV. In sCSF, validation standards were quantified as follows: 0.5–2400 ng/mL (k = 8) for FTC, 1–2400 ng/mL (k = 7) for ABC, 3TC and TFV, 2–2400 ng/mL (k = 6) for ZDV, 5–2400 ng/mL (k = 5) for Gln-ZDV. For accurately estimating the LLOQ values, each analyte had at least two low validation sample concentrations (i.e., one at the estimated LLOQ and one at 2-3x LLOQ). Calibrators, validation standards and QC samples (at different concentrations) were all prepared according to the recommendations for bioanalytical method validation, which state that the total added volume must be < 10% of the biological sample volume. The concentration ranges were chosen to encompass the expected patients' plasma and CSF levels in clinical samples [24,30–33].

2.4. Plasma and CSF sample pre-treatment

A 100 µL aliquot of spiked plasma was carefully mixed with 300 µL of methanolic IS-WS, and the mixture was centrifuged at +4 °C for 10 min at 20000*g* (14000 rpm) with a benchtop centrifuge (Benchtop Mikro 220R centrifuge, Hettich). A 150 µL aliquot of the clear supernatant was diluted 1:1 with ultrapure water directly in the glass HPLC vial.

A 100 µL aliquot of CSF was diluted with 100 µL of IS-WS in MeOH/H_2O 2:1 and the mixture underwent the same centrifugation conditions as used above for plasma. The clear solution was transferred into a glass HPLC vial for analysis.

For each matrix, the final glass HPLC vials were securely closed with crimp seals and then vortexed.

2.5. Evaluation of CSF surrogate matrices

Given the scarcity of blank human CSF, it is certainly difficult to justify its use solely for the purpose of calibration samples preparation. Thus, we have examined whether other potential matrix surrogates could confidently be used instead of CSF for the preparation of calibrator, validation and QC samples. For this endeavor, calibration samples were prepared in two separate matrices: one in 1:200 diluted plasma (sCSF) and one in artificial CSF (aCSF). Concentrations of QC samples prepared in real CSF were determined using the two types of calibrations, and trueness (%), as well as precision (RSD), were calculated. Additionally, QC results obtained with the two approaches were reported on a plot and compared using linear regression.

2.6. LC–MS/MS equipment

Chromatographic analyses were carried out using a Dionex Ultimate 3000 ultra-high-pressure liquid chromatography (UHPLC) RSLC system (Thermo Fisher Scientific, Reinach, Switzerland) integrating a binary pump and a Flow-Through-Needle (FTN) injector. The Acquity UPLC HSS T3 (Waters, Millford, USA) analytical column (2.1 × 50 mm, 1.8 µm) was kept in a thermostated oven set at +10 °C (using Peltier elements). The UHPLC system was coupled to a triple-stage quadrupole TSQ Quantiva™ mass spectrometer equipped with an Ion Max NG™ electrospray ionization (ESI) source (Thermo Fisher Scientific, Reinach, Switzerland). Data acquisition, peak integration, and quantification were performed using Xcalibur software version 1.1 (Thermo Fisher Scientific, Reinach, Switzerland).

2.7. LC–MS/MS conditions

For chromatographic separations, the mobile phase A was ultrapure water with 0.1% FA and mobile phase B was composed of MeOH with 0.1% FA. The mobile phase was delivered at a flow rate of 0.5 mL/min using the following 2-step gradient elution program: linear gradient 0% to 10% B in 1 min; up to 30% B in 0.2 min, linear gradient to 35% B until 3 min. Then, B was increased up to 90% in 0.1 min for intensive rinsing during 1 min, followed by a re-equilibration step to the initial conditions from 4.1 to 5 min (total analysis time). The column oven was set at +10 °C, whereas samples were stored at +5 °C in the autosampler. The injection volume was 5 µL for both plasma and CSF methodologies.

The ESI source parameters were set as follows: the ion transfer tube and vaporizer temperatures were set at 250 and 400 °C, respectively; ESI spray voltage was set at 3500 and 3000 V for positive mode and negative mode, respectively; sheath and auxiliary gas (nitrogen) flow rates at 30 and 15 (arbitrary units), respectively.

The first (Q1) and third (Q3) quadrupoles operated with a mass resolution of 0.7 Da (i.e., *m/z* 0.7 FWHM). The cycle time was 0.2 s. The Q2 collision gas (argon) pressure was 2 mTorr.

2.8. Analytical method validation

Validation of the analytical procedure was assessed according to current recommendations from the Food and Drug Administration (FDA) [34], European Medicine Agency (EMEA) [35], and the Société Française des Sciences et Techniques Pharmaceutiques [36,37], as well as by reference published papers for bioanalytical method validation [38–42].

2.8.1. Selectivity and carry-over

The selectivity of the developed method for potential interferences emanating from biological matrices was assessed by analyzing blank human plasma extracts from ten different sources. Analyses of blank pooled rCSF and aCSF, as well as surrogate matrices, were carried out to evaluate the presence of potential endogenous and/or exogenous compounds that could interfere with the developed assay.

The MS-based cross-talk interferences between co-eluting analytes and corresponding IS were checked by individually injecting a high concentration calibration sample of the analytes processed with pure MeOH (no IS), and blank plasma processed with the methanolic IS-WS.

Carryover was assessed by analyzing one or more blank samples immediately after the injection of the highest concentration calibrator.

2.8.2. Matrix effect, extraction recovery, process efficiency

2.8.2.1. Qualitative evaluation of matrix effect. Matrix effects (MEs) were initially examined qualitatively by the continuous post-HPLC column infusion of a standard solution of analytes, and their corresponding ISs, into the mass spectrometer during the simultaneous UHPLC–MS/MS analysis of a blank plasma extract [43]. The analyte standard mixture, at 500 ng/mL in MeOH, was infused at a flow rate of 10 µL/min and blank plasma extracts from six different sources were analyzed. A significant alteration (i.e., drift or shift) of the LC–MS signals of each selected MS/MS transition at the analytes' retention time was considered to be a matrix effect [44].

2.8.2.2. Quantitative assessment of matrix effect, extraction recovery and process efficiency. Matrix effects (MEs), extraction recoveries (ER) and process efficiencies (PE) for selected NRTIs and Gln-ZDV were quantitatively estimated at low (L, 10 ng/mL), medium (M, 300 ng/mL) and high (H, 3000 ng/mL) QC concentrations in seven different sources of human plasma [45]. Due to the difficulties in accessing a sufficient volume of real CSF from multiple donors, these parameters were assessed in surrogate CSF (sCSF, i.e., 1:200 diluted human plasma) at 10 ng/mL (LQC), 100 ng/mL (MQC) and 3000 ng/mL (HQC). For this purpose, three sets of solutions were prepared as follows: (A) three matrix-free samples (i.e., H_2O:MeOH mixture) spiked with analytes and IS, (B) seven blank human plasma or sCSF samples spiked in duplicate with both analytes and corresponding IS after extraction, and (C) seven blank human plasma or sCSF spiked in duplicate with analytes and corresponding IS before extraction. Matrix effects (i.e., B/A in %), extraction recovery (i.e., C/B in %) and process efficiency (i.e., C/A in %) for NRTIs and Gln-ZDV were calculated by taking into account the mean peak areas obtained for experiments A, B and C at the three QC levels. Variability of the parameters between the seven human plasma or sCSF sources was expressed as relative standard deviation (RSD). The IS-normalized parameters (i.e., IS-nME, IS-nER and IS-nPE) were also calculated by replacing the analyte peak area by the analyte/IS peak area ratio, with results expressed as bias [46].

For MEs, the standard line slopes approach suggested by Matuszewski was also evaluated [41]. Briefly, the RSD of slopes from linear regressions estimated through L, M, H concentrations for each plasma or sCSF source of set B (n = 7) was calculated. This value should not exceed 3–4% to consider the developed method devoid of significant relative matrix effects [41].

2.8.3. Trueness, precision, accuracy profiles and limits of quantification

Three series of validation standard samples were analyzed in triplicate over three different days for method precision and trueness determination. Concentrations of the validation standards were back-calculated by using the daily calibration curves constructed with different mathematical regression models in order to determine the best quantitative relationship between response function and concentration (i.e., lowest bias over concentration range). The trueness was determined as the percentage deviation between nominal and measured concentrations, while precision parameters (i.e., repeatability and intermediate precision) were both calculated taking into account their variances as described in the SFSTP reports [36,47]. According to recommendations, repeatability and intermediate precision were reported as relative standard deviation, based on the reference nominal value at each concentration level [40].

The accuracy profiles (i.e., total error approach), based on β-expectation tolerance intervals, were obtained by combining trueness and precision results, as previously proposed by several authors [48,49]. This approach estimates the area where β% of the future results are expected to lie [40,50] and allows confident prediction, based on results obtained during the validation phase, of the results that will be obtained during routine application of the method.

The accuracy profiles reporting absolute concentration, instead of accuracy (%), were used to precisely estimate the lower and upper limit of quantification (LLOQ and ULOQ) for NRTIs and Gln-ZDV, as described by Feinberg et al. [51]. Briefly, LLOQ as well as ULOQ were extrapolated graphically as the concentration where the β-expectation tolerance intervals cross the acceptance limits (± 30%). Indeed, total error should not exceed 30%, as stated in FDA and EMEA official documents [34,35], as well as in reference publication [52].

Linearity of the trueness is required and defines the ability of the developed assay to generate quantitative results directly proportional to known nominal analyte concentrations within the validated range. Linearity of trueness was assessed by linear square regression of the experimental concentrations plotted as function of nominal concentrations, during the three days of validation.

A dilution integrity experiment was performed with QC samples at concentrations exceeding the highest calibrator and diluted 10-fold in blank plasma or sCSF to obtain the final test concentrations within the validated range of 800 and 300 ng/mL for plasma and sCSF, respectively. Trueness and precision should be within ± 15%.

2.8.4. Short and medium stability studies

Bench-experiment sample stability, as well as medium-long stability studies for NRTIs and Gln-ZDV in human plasma and pooled rCSF were carried out as follows:

a) Stability in matrices kept up to 48 h at room temperature and at +4 °C;
b) Stability in matrices after three freeze/thaw cycles. Frozen samples were thawed at room temperature for 1 h and subsequently refrozen for 2 h;
c) Stability in matrices frozen at −20 °C and −80 °C over 6 weeks;
d) Stability in human plasma subjected to thermal viro-inactivation (i.e., 60 min at +60 °C in a water-bath) performed in our laboratory, prior to analysis. This treatment was shown to effectively inactivate HIV particles present in the samples [53–55].

Stability studies were performed in duplicate at low (i.e., 30 ng/mL for plasma and 10 ng/mL for CSF), medium (i.e., 300 ng/mL for plasma and 100 ng/mL for CSF) and high (i.e., 3000 ng/mL for both plasma and CSF) QC levels. The mean concentration of the studied samples was

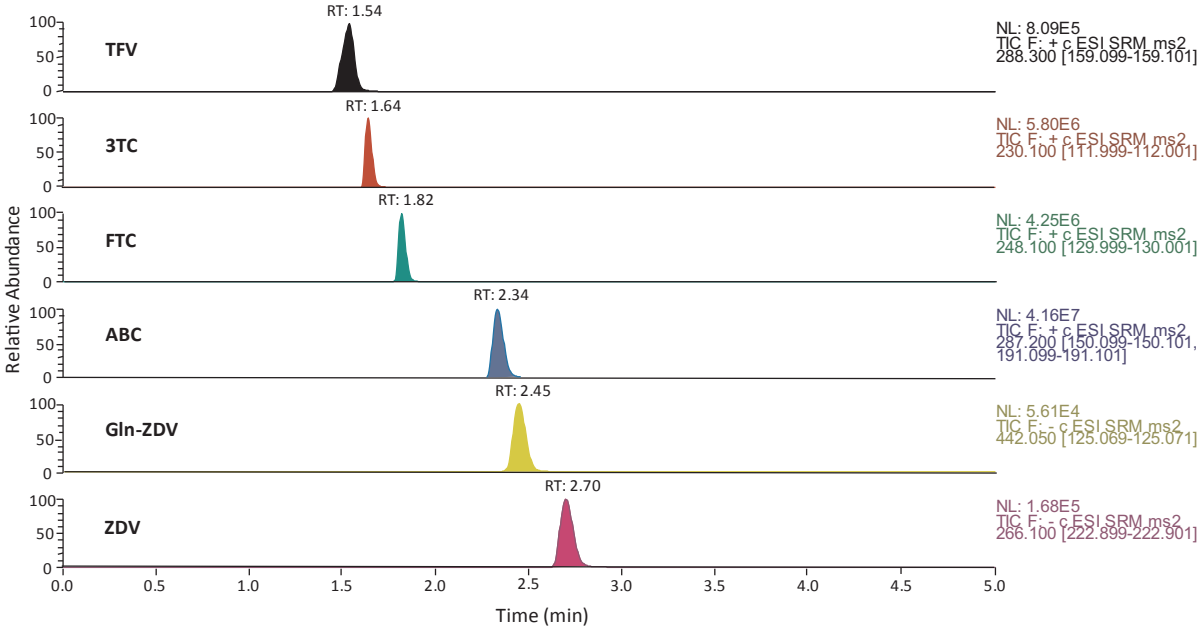

Fig. 2. UHPLC–MS/MS separation of five NRTIs and zidovudine glucuronide in plasma (calibration sample at 1500 ng/mL). Identical chromatographic separation of these analytes is obtained during CSF samples analysis.

expressed as a percentage of the initial concentration measured at the beginning (t_0) of the stability study or in absence of the parameter potentially affecting compound stability (e.g., freeze/thaw cycle or thermization process).

3. Results and discussion

3.1. Analytical method development

The selected NRTI drugs (i.e., abacavir (ABC), emtricitabine (FTC), lamivudine (3TC), tenofovir (TFV) and zidovudine (ZDV)) and ZDV-glucuronide (Gln-ZDV), despite having relatively high polarities (partition coefficient logP ranging from −3.43 to 0.39; Fig. 1), have been reported to be amenable to reverse-phase liquid chromatography [56,57]. Chromatographic separation of the six analytes was carried out on Acquity UPLC HSS T3 C18 column. This silica-based stationary phase was found compatible with the 100% aqueous mobile phase composition required at the start of the gradient program for the efficient chromatographic retention of these polar compounds. Optimization of the chromatographic conditions included: (i) evaluation of the organic solvent modifier in the mobile phase (ACN and MeOH), (ii) variation of the column temperature (+10 °C, +25 °C and +40 °C), and (iii) variation of the gradient program (one- and multi-step separation). The influence of these changes on chromatographic pattern, MS response, analyte retention time, peak selectivity and shape, and sensitivity, were thoroughly evaluated. Using MeOH as the mobile phase, with a lower eluting power than ACN, resulted in a reasonable chromatographic retention of the least retained compound, tenofovir (apparent retention factor k_e of 2.9). Moreover, sensitivity (signal/noise ratio) and peak shape were improved using MeOH in the mobile phase and also as plasma protein precipitating agent. A two-step gradient program was necessary to obtain a satisfactory chromatographic separation of analytes in terms of selectivity and analytical time. Temperature played a key role in the chromatographic resolution of the most critical pair represented by ABC and Gln-ZDV. Indeed, by decreasing the column temperature to +10 °C, a baseline separation of all compounds in less than 3 min was obtained (Fig. 2).

At this reduced column temperature, column efficiency (as evaluated by peak width) was not affected, yet an important drawback was the marked increase in column back-pressure (up to 800 bar) due to the more viscous mobile phase. The peak shape of tenofovir was found to deteriorate in the presence of the high percentage of MeOH present in the supernatant obtained after plasma protein precipitation. This could be improved by the dilution of the supernatant (1:1 with pure H_2O) and an injection volume of 5 µL which provided the best compromise in term of acceptable peak shape for tenofovir and overall sensitivity.

Table 1
MS/MS parameters and typical retention times for the analysis of five antiretroviral drugs and zidovudine glucuronide and their respective stable-isotope-labeled ISs.

Compound	ESI polarity (+/−)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision Energy (eV)	Tube lens voltage (V)	Typical retention time (min)
ABC	+	287.2	150.1	32	67	2.3
			191.1	19		
[$^2H_5$]-ABC	+	292.1	196.0	20	70	2.3
FTC	+	248.1	130.0	12	35	1.8
[$^2H_3$, ^{15}N]-FTC	+	252.1	132.1	16	35	1.8
3TC	+	230.1	112.0	13	32	1.6
[^{13}C, $^2H_2$, $^{15}N_2$]-3TC	+	235.2	115.1	13	37	1.6
TFV	+	288.3	159.1	31	89	1.5
[$^2H_6$]-TFV	+	294.2	182.0	27	85	1.5
ZDV	−	266.1	222.9	10	57	2.7
[^{13}C, $^2H_3$]-ZDV	−	270.0	227.4	10	59	2.7
Gln-ZDV	−	442.1	125.1	24	84	2.4

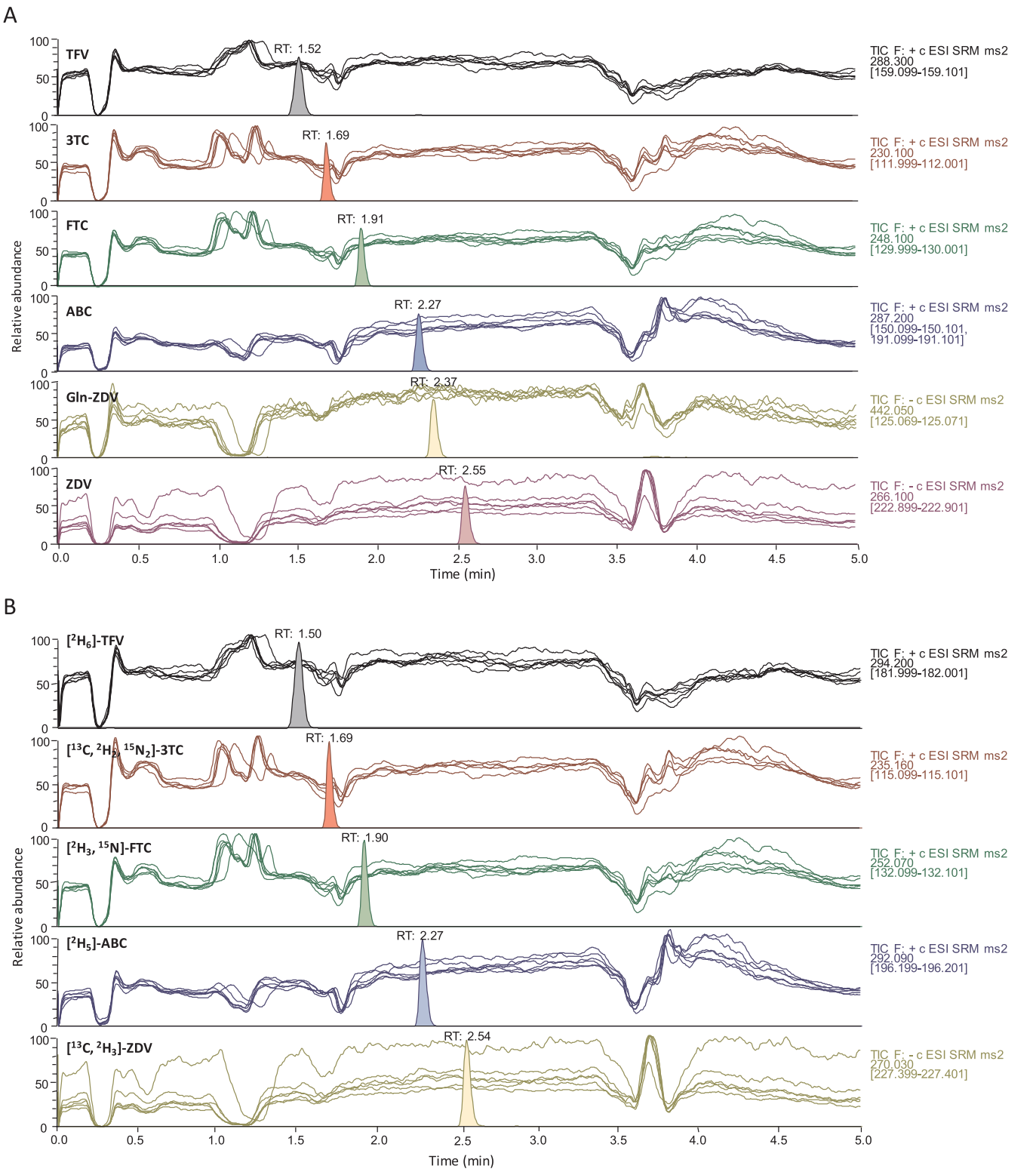

Fig. 3. Qualitative assessment of matrix effect in human plasma. Overlaid UHPLC–MS/MS traces obtained for the analysis of six blank plasma extracts during post-column infusion of a standard solution containing NRTIs, Gln-ZDV (A) and ISs (B), at 500 ng/mL. Retention times and chromatographic peaks for NRTIs, Gln-ZDV and ISs obtained during experiment were superimposed for interpretation.

Sensitivity and selectivity of MS/MS detection was determined by direct infusion of a solution of analytes (1 µg/mL in MeOH) onto the MS detector while optimizing settings and MS/MS transitions (Table 1). The most intense and selective product ion was selected for each analyte by analyzing spiked plasma samples in real LC–MS/MS conditions and was confirmed to be consistent with the molecular structure and previous reports [12–16]. For ABC, two product ions with similar signal intensities were selected to ensure sufficient sensitivity. For TFV, our preliminary selectivity experiments revealed that the TFV product ion (i.e., *m/z* 176), though chosen for SRM detection in previous studies

Table 2
Internal Standard normalized Matrix Effect (IS-nME), extraction recovery (IS-nER) and process efficiency (IS-nPE) in plasma and surrogate CSF (sCSF) for NRTIs and Gln-ZDV.

Compound	QC level[a]	IS-nME % (RSD)		IS-nER % (RSD)		IS-nPE % (RSD)	
		Plasma	sCSF	Plasma	sCSF	Plasma	sCSF
ABC/[$^{2}H_5$]-ABC	Low	−3 (5)	7 (2)	5 (7)	−4 (2)	2 (7)	3 (3)
	Middle	−4 (3)	7 (4)	−2 (6)	−7 (5)	−5 (8)	0 (3)
	High	8 (5)	9 (2)	−7 (8)	−6 (4)	0 (6)	3 (3)
FTC/[$^{2}H_3$, ^{15}N]-FTC	Low	−6 (5)	7 (3)	5 (8)	−2 (2)	0 (7)	5 (3)
	Middle	−4 (3)	6 (3)	−3 (6)	−7 (5)	−6 (8)	−1 (3)
	High	8 (5)	5 (2)	−7 (8)	−5 (4)	1 (6)	1 (2)
3TC/[^{13}C, $^{2}H_2$, $^{15}N_2$]-3TC	Low	−2 (5)	8 (3)	5 (9)	0 (3)	3 (8)	8 (3)
	Middle	−3 (4)	7 (4)	−3 (6)	−7 (5)	−6 (8)	−1 (2)
	High	9 (4)	6 (2)	−7 (7)	−5 (4)	2 (5)	0 (2)
TFV/[$^{2}H_6$]-TFV	Low	−9 (6)	2 (4)	2 (9)	10 (9)	−6 (8)	12 (10)
	Middle	−2 (4)	5 (4)	−2 (5)	−6 (6)	−4 (7)	−1 (3)
	High	6 (5)	6 (3)	−10 (8)	−6 (5)	−4 (6)	0 (3)
ZDV/[^{13}C, $^{2}H_3$]-ZDV	Low	0 (5)	2 (16)	17 (8)	13 (10)	16 (6)	15 (9)
	Middle	−10 (7)	−12 (3)	−19 (6)	12 (3)	−27 (10)	−2 (3)
	High	8 (5)	−4 (2)	−2 (6)	13 (3)	6 (4)	9 (2)
Gln-ZDV/[^{13}C, $^{2}H_3$]-ZDV	Low	−7 (5)	−15 (10)	17 (14)	12 (15)	9 (13)	−4 (8)
	Middle	−10 (8)	−13 (5)	−21 (6)	12 (5)	−29 (9)	−3 (3)
	High	8 (6)	−1 (3)	−1 (6)	14 (4)	7 (4)	13 (3)

[a] Low concentration is defined as 10 ng/mL, middle concentrations as 300 ng/mL for plasma and 100 ng/mL for sCSF, and high concentrations are defined as 3000 ng/mL.

[58–60], co-eluted in several blank plasmas with a major matrix interference; thus, the product ion *m/z* 159 was monitored instead, leading to excellent selectivity and an insignificant decrease in sensitivity. Final optimization of ESI source parameters for each analyte (reported in Section 2.7) in real LC–MS/MS conditions enabled the best compromise for this multiplex analysis, in terms of signal intensity and reduction of background noise.

Unlike the majority of previously published bioanalytical method for NRTIs, stable-isotope-labeled internal standards have been systematically used in this study to confidently manage potential MEs from complex biological matrices, such as plasma and CSF [41,42,61]. Product ions of ISs and their concentrations were selected and optimized to both reduce cross-talk interference with target analytes and improve the accuracy, precision and quantification range of the method. As no deuterated IS was commercially available for Gln-ZDV, [^{13}C, $^{2}H_3$]-ZDV was successfully used for both ZDV and Gln-ZDV. Besides the use of stable-isotope labeled IS, matrix-matched calibration is a complementary approach that can be used to compensate for any existing MEs. While access to blank human plasma for calibration sample preparation is possible, the collection of sufficient blank CSF remains difficult. To address this, Hooshfar et al. suggests that 1:200 diluted plasma constitutes a reliable surrogate matrix to replace rCSF in the preparation of calibrators and QCs [28]. In their report, the MS response of lamivudine in 1:200 diluted plasma (sCSF) closely correlated to that measured in rCSF, but less so in other surrogate matrices (i.e., aCSF or alternate plasma dilutions). In our study, (Table S1 in Supplementary Files), mean trueness values for NRTIs and Gln-ZDV were within 90 and 110%, whereas RSDs were lower than 15% for both aCSF and sCSF (i.e., diluted plasma), except for FTC (where RSD was 17%). Both calibrations, therefore, either in aCSF or sCSF provide accurate concentrations of NRTIs and Gln-ZDV in rCSF. General agreement between these two matrices was confirmed after plotting QC results obtained with aCSF versus sCSF calibration for all analytes. Determination coefficients (R^2) were all higher than 0.999, slopes ranged from 0.998 to 1.027 and intercepts varied from −2.347 to 2.919. A slope close to unity emphasizes the excellent correspondence of results obtained for the two calibration matrices. For practical reasons, the 1:200 diluted human plasma was chosen as a suitable sCSF matrix for quantifying NRTIs and Gln-ZDV in rCSF, and was used thereafter for all method validation.

3.2. Method validation

3.2.1. Selectivity and carry-over

No major interferences were observed at the retention times of the compounds in their corresponding MS/MS transition when human blank plasma samples were analyzed, confirming satisfactory selectivity of the UHPLC–MS/MS method. Selectivity was also confirmed for rCSF, aCSF and sCSF (i.e., no interfering signals from endogenous or exogenous compounds were present).

Cross-talk interferences coming from MS detection were observed between FTC and its IS, but without any consequence on quantitative data (i.e., < 10% at the LLOQ).

Carryover did not exceed 11% for any of the analytes when blank matrix samples were analyzed directly after the injection of the highest calibrator, which was in agreement with official recommendations (i.e., < 20% of the lower limit of quantification) [35].

3.2.2. Matrix effect, extraction recovery and process efficiency

The MEs in plasma were first examined qualitatively as shown in Fig. 3. Any diminutions, suppressions or enhancements of NRTI, Gln-ZDV or IS MS signals due to endogenous matrix components from extracted blank plasma samples were only observed outside the elution time of the analytes (from 1.5 to 2.6 min), emphasizing the suitability of the developed LC program. For CSF, we quantitatively determined the extraction recovery (ER) and process efficiency (PE) of the sCSF.

Quantitative determination of the internal standard normalized matrix effect (IS-nME), extraction recovery (IS-nER) and process efficiency (IS-nPE) parameters of all analytes in the two matrices (i.e., plasma and sCSF) are reported in Table 2. A value above or below 100% for the IS-nME indicates an ionization enhancement or suppression, respectively. As depicted by the results, co-eluting plasma matrix components did not appear to significantly affect the ionization process of the analytes. In plasma, IS-normalization decreases the variability for these parameters down to RSD ≤ 8% for all compounds, contrary to the variability

Table 3
Trueness, repeatability and intermediate precision in human plasma and sCSF over the validated range.

Compound	Conc. (ng/mL)	Plasma			Conc. (ng/mL)	sCSF		
		Trueness (%)	Precision			Trueness (%)	Precision	
			Repeatability (%)	Intermediate precision (%)			Repeatability (%)	Intermediate precision (%)
Abacavir	5	91.7	6.0	9.0	2	101.8	5.8	8.5
	10	99.6	7.7	7.7	5	96.1	1.9	4.8
	30	103.4	9.1	9.1	10	95.7	1.5	3.1
	600	101.2	6.7	6.7	30	94.5	0.8	3.3
	2400	97.8	7.3	8.0	600	97.7	1.3	2.2
	4800	101.9	5.6	6.7	2400	95.9	7.5	7.5
	(8000/10)[a]	109.0	1.5	5.1	(3000/10)[a]	91.5	1.9	3.2
Emtricitabine					1	94.2	10.1	10.8
	5	99.7	9.7	9.7	2	93.2	6.8	6.8
	10	101.6	7.0	8.0	5	93.2	1.3	4.3
	30	104.0	10.7	10.7	10	95.4	0.8	2.9
	600	106.9	7.2	7.2	30	94.3	0.6	2.8
	2400	104.6	8.4	9.1	600	94.7	0.8	3.9
	4800	107.9	4.8	5.9	2400	95.4	0.9	2.2
	(8000/10)[a]	109.5	2.9	6.2	(3000/10)[a]	87.6	2.3	2.3
Lamivudine					2	93.7	7.1	7.1
	10	99.9	11.2	11.2	5	96.7	7.8	8.0
	30	103.4	7.9	10.8	10	97.7	4.1	5.4
	600	103.7	6.5	6.5	30	94.1	2.4	4.1
	2400	101.7	7.8	9.3	600	96.4	2.2	5.7
	4800	105.5	5.2	7.5	2400	97.4	2.6	3.1
	(8000/10)[a]	111.7	1.2	5.1	(3000/10)[a]	95.5	2.1	2.3
Tenofovir	5	95.3	10.9	10.9	2	112.1	15.1	15.1
	10	96.9	10.5	10.5	5	96.3	7.6	7.6
	30	99.4	9.7	9.7	10	93.7	5.5	7.1
	600	99.2	6.8	6.8	30	97.4	5.5	5.5
	2400	100.3	8.7	9.9	600	97.7	2.4	2.7
	4800	102.0	6.7	8.2	2400	97.7	2.0	2.0
	(8000/10)[a]	109.5	2.9	6.2	(3000/10)[a]	95.5	5.2	5.2
Zidovudine	10	110.4	12.6	13.9	5	100.0	5.0	5.9
	30	107.6	10.5	10.5	10	97.9	3.6	5.6
	600	105.5	5.5	6.5	30	92.7	2.8	4.8
	2400	103.2	8.1	10.2	600	96.4	1.9	4.2
	4800	105.2	6.0	7.1	2400	97.5	3.0	3.7
	(8000/10)[a]	101.7	2.5	5.8	(3000/10)[a]	97.7	2.6	2.6
Zidovudine glucuronide	30	99.5	10.0	10.0	10	97.2	10.5	11.3
	600	103.1	6.7	7.7	30	92.9	5.6	6.0
	2400	99.5	4.1	5.6	600	94.4	5.3	6.8
	4800	101.1	6.1	6.1	2400	95.4	4.3	4.7
	(8000/10)[a]	101.3	2.3	3.4	(3000/10)[a]	83.7	2.3	2.3

[a] Dilution integrity: spiked samples at high concentration were diluted 10-fold with the corresponding matrix.

observed for non-normalized parameters (see Supplementary material Table S2). The IS-nER was between 79 and 117%, with acceptable overall variability (i.e., RSD ≤ 14%). Finally, reasonable overall performance was achieved for IS-nPEs that were in the range of 71–116%, with RSD ≤ 13%. In sCSF samples, IS-nME, IS-nER and IS-nPE parameters were within the ± 15% allowances together with satisfactory variability (i.e., RSD < 15%) at all QC levels.

Finally, the lack of significant MEs impacting NRTIs quantitation in plasma and CSF was confirmed by the standard line slope approach [41]: relative standard deviations of standard line slopes constructed in seven different blank plasmas and sCSF did not exceed 3.4% for plasma or 2.5% for sCSF, except for Gln-ZDV in plasma (i.e., RSD 4.3%, though considered as acceptable).

3.2.3. Trueness, precision and accuracy profile

Response functions were obtained by plotting analyte/IS peak area ratios against analyte concentration in plasma from 1 to 6000 ng/mL and from 0.5 to 3000 ng/mL in sCSF. Given the relatively broad dynamic range required, the linear model was not expected to provide a reliable description of response function. Therefore, two non-linear regression models (i.e., quadratic log-log and quadratic weighted 1/x) were used to evaluate each analyte. For each compound, a calibration range was established that would allow a determination coefficient (R^2) greater than 0.99 and a back-calculated concentration of calibration samples within ± 15%, or ± 20% at the expected LLOQ. Concentrations of the validation standards were back-calculated using the daily prepared calibration curves and two different mathematical regression models. The 1/x weighted quadratic model was the most suitable in terms of trueness, precision and validation domain (i.e., lowest LLOQ). For both matrices, a calibration curve analyzed once per analytical series was finally selected, as the validation performances were similar to those obtained with triplicate analysis of calibrator. As reported in Table 3, satisfactory performances were obtained over the validated range in terms of trueness (plasma: 91.7–110.4%; CSF: 92.7–112.1%), repeatability (plasma: 4.1–12.6%; CSF: 0.6–15.1%) and intermediate precision (plasma: 5.6–13.9%; CSF: 2.0–15.1%). Both trueness and precision obtained after a 10-fold dilution of QC samples, with concentrations exceeding the highest calibrator, were within the acceptance criteria of ± 15%, except Gln-ZDV in sCSF at −16.3%. The high spiked concentrations for Gln-ZDV in CSF are, however, hardly

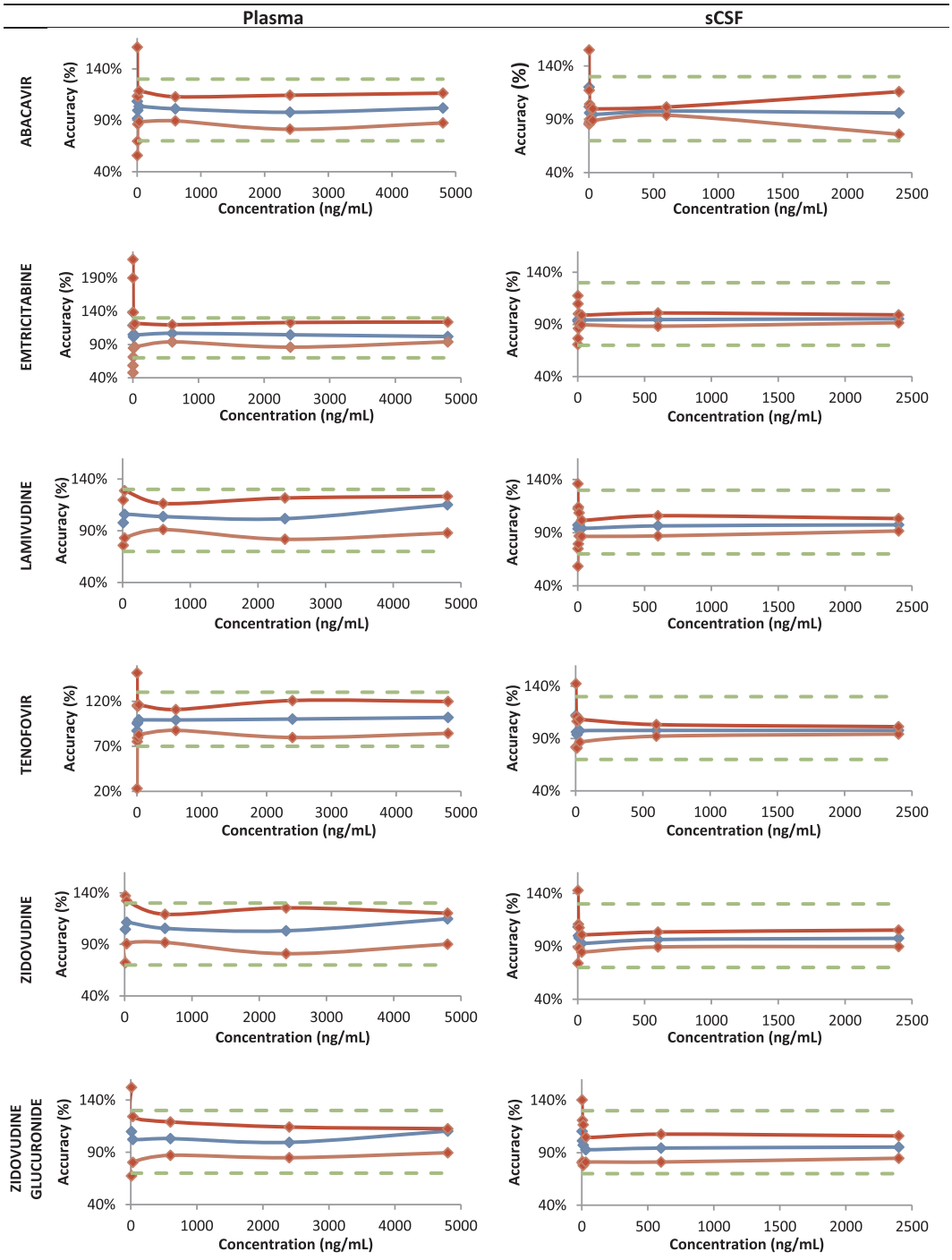

Fig. 4. Accuracy profiles over the investigated validation domain in plasma (left) and sCSF (right) of the five NRTIs and zidovudine glucuronide (Gln-ZDV). Trueness (blue, solid line), upper and lower β-expectation tolerance intervals (β = 90%) (red, solid lines) and acceptance limits (λ = ± 30%), green, dotted lines) are shown.

observed *in vivo*, and dilution integrity was therefore considered as globally satisfactory.

The accuracy profiles with β-expectation tolerance intervals were built using a β value of 90%, which represents the percentage of the future results expected to fall within the calculated tolerance intervals during routine application of the method. As depicted in Fig. 4, the accuracy profiles exceed the acceptance limits of ± 30% (established for biological samples), but only at very low concentrations [34].

The LLOQ values for the five NRTIs and Gln-ZDV correspond to the lowest concentration at which the tolerance limit crossed the acceptance limit by using absolute accuracy profiles [37]. The lowest level that can be measured in plasma and CSF with a defined total error (± 30%) were, respectively, 5 and 1.5 ng/mL for ABC, 3.7 and 1.2 ng/mL for FTC, 8.9 and 1.5 ng/mL for 3TC, 4.6 and 3.2 ng/mL for TFV, 26 and 2.6 ng/mL for ZDV, 19.6 and 3.4 ng/mL for Gln-ZDV. All LLOQ values were considered acceptable since they lie well below the trough concentrations (C_{trough}) commonly observed in clinical practice. Given its improved safety profile (i.e., bone and renal toxicities) as compared to tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) is increasingly prescribed. Plasma concentrations of TFV after TAF administration are known to be 90% lower than those obtained with TDF [62]. The TFV concentration range analyzed with our method is

Table 4
Stability of NRTIs and zidovudine glucuronide under different storage conditions.

Compound	Abacavir			Emtricitabine			Lamivudine			Tenofovir			Zidovudine			Zidovudine glucuronide		
QC level[a]	Low	Middle	High	Low	Middle	High	Low	Middle	High	Low	Middle	High	Low	Middle	High	Low	Middle	High
Room temperature for 24 h																		
Plasma	95	98	102	104	103	105	95	103	90	87	103	88	97	109	96	122	106	119
CSF	95	99	96	95	103	97	93	113	106	93	110	96	104	112	110	97	100	97
Room temperature for 48 h																		
Plasma	101	99	101	108	103	104	89	92	108	107	114	108	113	99	100	143	109	124
CSF	92	96	96	97	101	95	101	108	105	90	104	95	90	111	111	104	92	97
+4 °C for 24 h																		
Plasma	100	103	98	109	106	97	94	106	92	92	107	91	108	113	96	150	114	115
CSF	94	96	97	92	103	97	93	112	108	90	107	98	106	105	112	92	101	95
+4 °C for 48 h																		
Plasma	97	100	102	105	106	105	91	111	95	87	94	86	92	107	100	128	110	124
CSF	92	96	96	99	101	95	102	109	103	90	107	99	99	109	108	94	89	94
After 3 consecutive freeze-thaw cycles																		
Plasma	102	101	103	103	101	102	102	101	102	104	98	98	101	100	100	98	95	104
CSF	86	98	95	92	99	96	86	99	97	87	97	95	89	100	94	94	101	95
−20 °C during 6 weeks																		
Plasma	99	100	104	106	98	104	103	107	107	109	111	111	98	107	108	103	111	111
CSF	98	95	93	105	100	97	104	106	103	96	109	93	92	99	94	90	88	85
−80 °C during 6 weeks																		
Plasma	101	104	105	102	105	111	103	106	110	108	103	104	115	112	114	111	114	113
CSF	101	99	98	106	104	100	108	114	107	95	111	95	115	114	113	108	102	99
Thermal viro-inactivation[b]																		
Plasma	113	108	106	112	109	108	114	105	107	105	105	101	103	108	103	114	104	106

[a] Low concentration is defined as 30 ng/mL for plasma and 10 ng/mL for CSF, middle concentrations as 300 ng/mL for plasma and 100 ng/mL for sCSF, and high concentrations are defined as 3000 ng/mL.
[b] 60 min at +60 °C in a water bath.

suitable for the determination of TFV plasma levels over the entire dosing interval after either TDF 300 mg or TAF 25 mg administration. It must be acknowledged, however, that our current LLOQ for TFV would allow monitoring of TFV in plasma only until approximately 12 h after the administration of the 10 mg TAF dose, as such low doses yield TFV plasma levels higher than 10.2 ng/mL at a median time of 11.2 h (range 4.1–18.6 h) after dosing [63]. On the other hand, the concentration of the highest validation sample (i.e., 4800 ng/mL for plasma and 2400 ng/mL for sCSF) correspond to the ULOQ.

Linearity of trueness was determined by plotting back-calculated validation concentrations versus theoretical concentrations and by fitting it with a linear regression model (data not shown). Slopes ranged from 0.96 to 1.12 and 0.92 to 1.01, in plasma and CSF, respectively, and determination coefficients (R^2) were all greater to 0.99, indicating that satisfactory linearity was provided by the method.

Finally, the successful performance of our laboratory as part of the external quality proficiency program for NRTIs, as organized by Asqualab, Paris, France (http://www.asqualab.com/), constituted definitive demonstration that the proposed assay is able to provide sufficiently accurate analytical results.

3.2.4. Stability studies

The stability of NRTIs and Gln-ZDV in plasma and rCSF QC samples left at room temperature and at +4 °C was ascertained up to 48 h. The variation over time of each compound's levels at 24 h and 48 h (Table 4) was within ± 15% of nominal concentrations, except for Gln-ZDV in plasma.

Variation after three freeze–thaw cycles was less than 15%, indicating no significant loss after three cycles.

Stability studies up to six weeks at −20 °C and −80 °C demonstrate no NRTI or Gln-ZDV degradation during long-term storage of plasma and rCSF samples.

Finally, the variation in NRTI/Gln-ZDV levels estimated after heating plasma to +60 °C for 60 min was within ± 15% of nominal concentrations, indicating that plasma samples can be subjected to our thermization procedure without significant loss of analyte.

In summary, for all conditions tested, deviations from initial concentrations of NRTIs ranged between 86 and 115%, suggesting no significant sample deterioration in plasma and CSF, except Gln-ZDV, which can be considered globally less stable under the different storage conditions. A reliable analysis of Gln-ZDV requires, therefore, that plasma and CSF be stored after collection without delay at temperature lower than −20 °C. To our knowledge, this is the first report on the stability of NRTIs and Gln-ZDV in human plasma and CSF up to 48 h [12,16,64].

3.3. Clinical applications

The validated method has been applied for the measurement of antiretroviral drug levels in both plasma and CSF from patients with documented mild neurocognitive impairment who are part of the Swiss HIV Cohort Study (SHCS) project, NAMACO (Neurocognitive Assessment in the Metabolic and Aging Cohort study, SHCS #700). Comprehensive analyses of this ongoing prospective study will be published elsewhere. The chromatographic profiles of plasma and CSF samples from one patient receiving tenofovir disoproxil fumarate (300 mg once daily) and emtricitabine (200 mg once daily), in addition to ritonavir-boosted atazanavir, are shown in Fig. 5, patient A. Both plasma and CSF samples were collected simultaneously during a medical visit 15.5 h post-dose. This sampling design allowed determination of the CSF-to-plasma ratio for each drug. The measured concentrations in plasma were 116 ng/mL for TFV and 107 ng/mL for FTC, while concentrations in CSF were 6 ng/mL for TFV and 76 ng/mL for FTC, providing CSF-to-plasma ratios of 0.05 for TFV and of 0.71 for FTC. These results are in general agreement with previously published data [24,25,65].

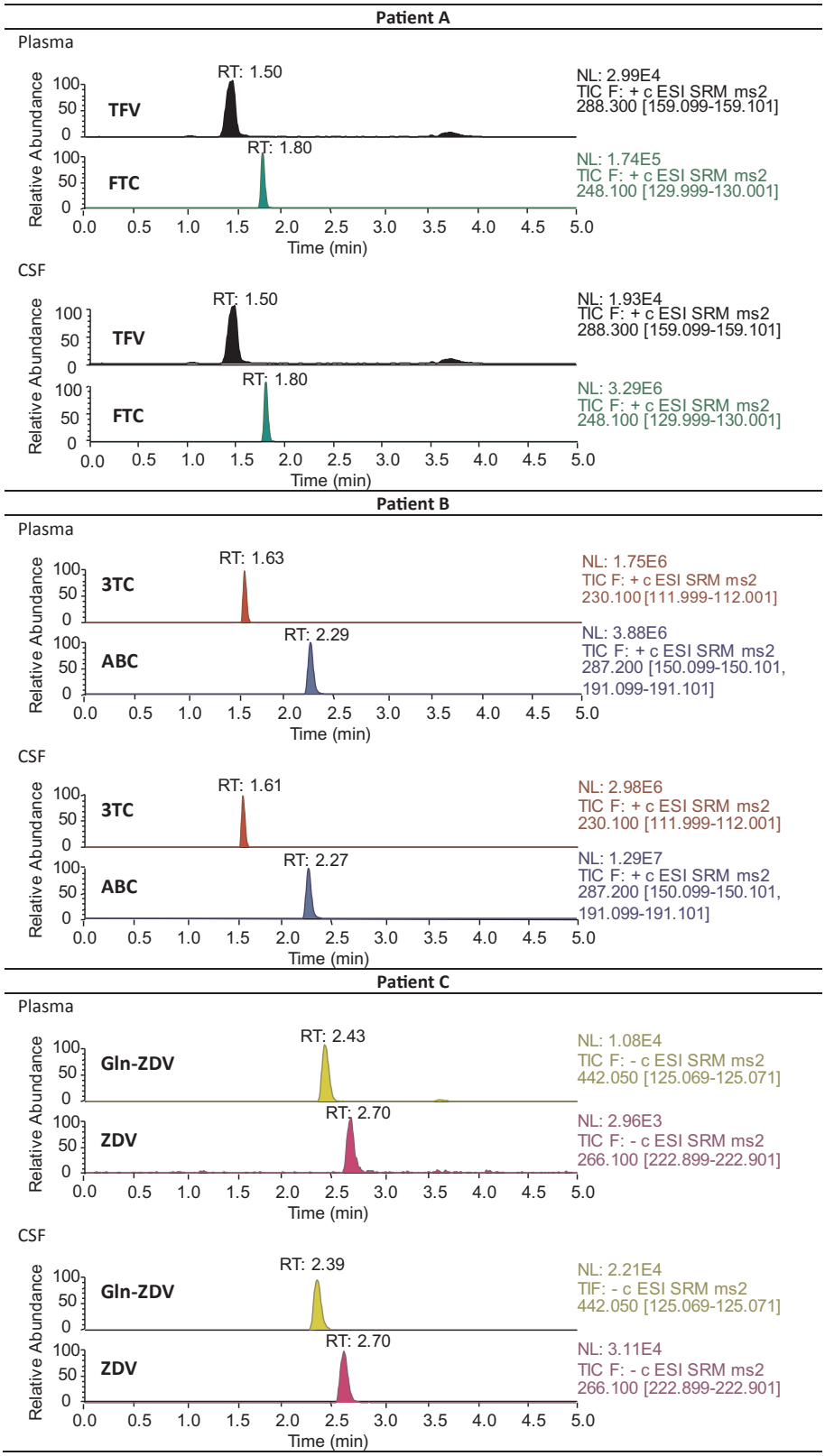

Fig. 5. Representative UHPLC–MS/MS analyses of plasma and CSF samples. In HIV-infected patient A receiving a backbone combination regimen of tenofovir disoproxil fumarate (TDF, 300 mg) and emtricitabine (FTC, 200 mg), plasma and CSF samples were collected 15.5 h after drug intake. For patient B treated with abacavir (ABC, 600 mg) and lamivudine (3TC, 300 mg), both samples were collected 15.75 h after the last drug intake. In HIV-infected patient C treated with zidovudine (ZDV, 300 mg), plasma and CSF samples were collected after 5.75 h. See Section 3.3 for details.

The chromatograms of a patient treated with ABC (600 mg once daily), 3TC (300 mg once daily), in addition to dolutegravir, are represented in Fig. 5, patient B. Both plasma and CSF samples were collected 15.75 h post-dose. The concentration of ABC was 94 ng/mL and 42 ng/mL in plasma and CSF, respectively. The concentration of 3TC was 466 ng/mL and 99 ng/mL in plasma and CSF, respectively. Hence, CSF-to-plasma ratios were 0.45 and 0.21, for ABC and 3TC, in accordance with previously published data [32,66].

Another example of application is shown in Fig. 5, patient C: UHPLC–MS/MS analyses of plasma and CSF samples collected 5.75 h post-dose in one HIV-patient receiving ZDV (300 mg once daily). The concentrations ZDV and Gln-ZDV in plasma were 52 and 606 ng/mL, respectively, whereas their concentrations in CSF were 72 and 185 ng/mL, respectively, giving CSF-to-plasma ratios of 1.38 and 0.31 for ZDV and Gln-ZDV, respectively. These results are in agreement with existing data for ZDV, which is known to have an effective CNS penetration with a CSF-to-plasma ratio increasing linearly with time after drug administration [33,67]. ZDV is also characterized by an important variability in that it is predominantly and selectively metabolized by the polymorphic UGT 2B7 to its inactive metabolite Gln-ZDV, making ZDV a probe substrate for this UGT isoform [57,68,69]. With the ability to simultaneously monitor ZDV and Gln-ZDV in both plasma and CSF, characterization of potential alterations in UGT 2B7 activity occurring in the liver and/or in the brain can be realized [70].

Following diligent internal and external quality control certification, this multiplex UHPLC–MS/MS assay was also integrated into our general routine TDM service for antiretrovirals allowing real-time plasma levels measurement of NRTIs in HIV patients, or, alternately, in individuals taking tenofovir disoproxil fumarate with emtricitabine in the context of Pre-Exposure Prophylaxis (PrEP) [71,72].

4. Conclusion

A multiplex assay method integrating a simplified sample processing protocol and a rapid, sensitive and robust UHPLC–MS/MS analysis was developed for the determination of concentrations of five NRTIs and Gln-ZDV in plasma and CSF. For both matrices, the quantitative performances of the method assessed during validation confirmed its suitability for routine application. The method was also applied to clinical research studies investigating the disposition of NRTIs in deep compartments, in order to increase our understanding of the ability of NRTIs to cross the blood brain barrier and their potential implication in the neuropsychological disorders observed in HIV-patients.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clinms.2018.04.001.

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Supplementary material

Table S1: Quantification of real CSF quality controls by using two different calibrations, *i.e.* in artificial CSF (aCSF) and in surrogate CSF (sCSF, *i.e.* 1:200 diluted human plasma). Results are expressed as percentage of nominal concentrations.

Compound	QC nominal concentration (ng/mL)	% of nominal concentration	
		Calibration in aCSF	Calibration in sCSF
Abacavir	2	101	93
	5	96	95
	10	94	96
	30	98	102
	600	98	101
	2400	101	101
Trueness (%)		**98.0**	**98.0**
RSD (%)		**2.7**	**3.8**
Emtricitabine	2	62	61
	5	97	97
	10	92	92
	30	97	98
	600	103	104
	2400	101	103
Trueness (%)		**92.0**	**92.5**
RSD (%)		**16.4**	**17.5**
Lamivudine	2	98	94
	5	98	96
	10	100	98
	30	101	101
	600	102	102
	2400	100	102
Trueness (%)		**99.8**	**98.8**
RSD (%)		**1.7**	**3.5**
Tenofovir	5	97	96
	10	103	104
	30	97	99
	600	100	103
	2400	100	103
Trueness (%)		**99.4**	**101.0**
RSD (%)		**2.3**	**3.1**
Zidovudine	5	115	98
	10	98	89
	30	99	95
	600	100	98
	2400	99	99
Trueness (%)		**102.2**	**95.8**
RSD (%)		**7.0**	**4.4**
Zidovudine glucuronide	5	87	79
	10	107	103
	30	93	92
	600	100	100
	2400	98	98
Trueness (%)		**97.0**	**94.4**
RSD (%)		**7.8**	**10.2**

Table S2: Matrix effects, extraction recoveries and process efficiencies for NRTIs, Gln-ZDV and IS in 7 different plasmas and surrogate CSF (*i.e.* 1:200 diluted plasma).

Compound	QC level	ME % (RSD)		ER % (RSD)		PE % (RSD)	
		Plasma	sCSF	Plasma	sCSF	Plasma	sCSF
Analyte[a]							
ABC	Low	12 (12)	10 (3)	-6 (24)	-3 (8)	5 (23)	7 (7)
	Middle	-6 (26)	8 (5)	-23 (38)	-5 (6)	-28 (31)	3 (2)
	High	-3 (27)	2 (3)	0 (38)	-11 (2)	-3 (23)	-10 (2)
FTC	Low	25 (11)	9 (4)	-4 (23)	-2 (9)	20 (23)	7 (8)
	Middle	-5 (23)	8 (6)	-22 (33)	-5 (8)	-27 (27)	2 (3)
	High	-6 (27)	0 (3)	-2 (37)	-11 (3)	-8 (22)	-11 (3)
3TC	Low	31 (10)	17 (3)	-3 (23)	-3 (10)	26 (22)	13 (8)
	Middle	-2 (22)	11 (6)	-21 (32)	-7 (6)	-23 (27)	3 (3)
	High	-6 (26)	0 (4)	-2 (36)	-12 (3)	-8 (21)	-12 (3)
TFV	Low	73 (11)	20 (4)	4 (22)	10 (10)	80 (21)	33 (11)
	Middle	03 (21)	9 (4)	-19 (31)	-3 (6)	-16 (26)	5 (3)
	High	0 (27)	5 (3)	-3 (37)	-12 (4)	-3 (22)	-8 (4)
ZDV	Low	28 (10)	4 (15)	-3 (20)	16 (10)	24 (19)	21 (8)
	Middle	-9 (25)	-12 (7)	-25 (36)	12 (7)	-31 (29)	-2 (2)
	High	-7 (30)	-6 (3)	-2 (43)	5 (4)	-9 (25)	-2 (2)
Gln-ZDV	Low	19 (10)	-13 (13)	-2 (25)	15 (16)	17 (24)	0 (9)
	Middle	-9 (24)	-14 (8)	-26 (36)	13 (7)	-33 (30)	-2 (3)
	High	-6 (32)	-4 (4)	-1 (44)	6 (5)	-8 (25)	2 (3)
Internal standards[b]							
	Low	17 (10)	3 (3)	-10 (23)	1 (9)	5 (21)	4 (8)
[$^{2}H_5$]-ABC	Middle	-3 (25)	1 (5)	-20 (38)	2 (6)	-12 (35)	3 (1)
	High	-10 (29)	-7 (3)	8 (38)	-6 (2)	-2 (23)	-12 (3)
	Low	33 (9)	2 (4)	-9 (21)	0 (9)	21 (20)	2 (9)
[$^{2}H_3$, ^{15}N]-FTC	Middle	-2 (22)	2 (6)	-19 (35)	2 (7)	-20 (32)	4 (3)
	High	-13 (28)	-5 (4)	5 (37)	-7 (3)	-9 (23)	-12 (3)
	Low	34 (9)	8 (3)	-8 (21)	-3 (10)	23 (19)	5 (9)
[^{13}C, $^{2}H_2$, $^{15}N_2$]-3TC	Middle	01 (21)	4 (6)	-17 (33)	0 (6)	-16 (31)	4 (1)
	High	-13 (26)	-5 (3)	5 (36)	-7 (3)	-9 (21)	-12 (3)
	Low	88 (12)	19 (3)	1 (22)	0 (8)	91 (18)	19 (8)
[$^{2}H_6$]-TFV	Middle	5 (20)	03 (6)	-15 (31)	3 (7)	-12 (30)	7 (2)
	High	-6 (27)	-1 (4)	7 (37)	-7 (3)	1 (22)	-8 (3)
	Low	29 (10)	2 (7)	-16 (23)	3 (8)	8 (20)	5 (2)
[^{13}C, $^{2}H_3$]-ZDV	Middle	01 (24)	0 (8)	-5 (35)	1 (8)	-4 (35)	0 (1)
	High	-14 (30)	-3 (3)	0 (41)	-7 (3)	-14 (25)	-10 (1)

[a] Low concentration was defined as 10 ng/mL, middle concentrations as 300 ng/mL for plasma and 100 ng/mL for sCSF, and high concentrations were defined as 3000 ng/mL

[b] The IS were tested at the concentration used in the method namely 80 ng/mL for [$^{2}H_5$]-ABC, 50 ng/mL for [$^{2}H_3$, ^{15}N]-FTC, 100 ng/mL for [^{13}C, $^{2}H_2$, $^{15}N_2$]-3TC and [$^{2}H_6$]-TFV and 600 ng/mL for [^{13}C, $^{2}H_3$]-ZDV.

III.2. UHPLC-MS/MS assay for simultaneous determination of amlodipine, metoprolol, pravastatin, rosuvastatin, atorvastatin with its active metabolites in human plasma, for population-scale drug-drug interactions studies in people living with HIV.

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UHPLC-MS/MS assay for simultaneous determination of amlodipine, metoprolol, pravastatin, rosuvastatin, atorvastatin with its active metabolites in human plasma, for population-scale drug-drug interactions studies in people living with HIV

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A B S T R A C T

Thanks to highly active antiretroviral treatments, HIV infection is now considered as a chronic condition. Consequently, people living with HIV (PLWH) live longer and encounter more age-related chronic co-morbidities, notably cardiovascular diseases, leading to polypharmacy. As the management of drug-drug interactions (DDIs) constitutes a key aspect of the care of PLWH, the magnitude of pharmacokinetic DDIs between cardiovascular and anti-HIV drugs needs to be more thoroughly characterized. To that endeavour, an UHPLC-MS/MS bioanalytical method has been developed for the simultaneous determination in human plasma of amlodipine, metoprolol, pravastatin, rosuvastatin, atorvastatin and its active metabolites. Plasma samples were subjected to protein precipitation with methanol, followed by evaporation at room temperature under nitrogen of the supernatant, allowing to attain measurable plasma concentrations down to sub-nanogram per milliliter levels. Stable isotope-labelled analytes were used as internal standards. The five drugs and two metabolites were analyzed using a 6-min liquid chromatographic run coupled to electrospray triple quadrupole mass spectrometry detection. The method was validated over the clinically relevant concentrations ranging from 0.3 to 480 ng/mL for amlodipine, atorvastatin and p-OH-atorvastatin, and 0.4 to 480 ng/mL for pravastatin, 0.5 to 480 ng/mL for rosuvastatin and o-OH-atorvastatin, and 3 to 4800 ng/mL for metoprolol. Validation performances such as trueness (95.4-110.8%), repeatability (1.5-13.4%) and intermediate precision (3.6-14.5%) were in agreement with current international recommendations. Accuracy profiles (total error approach) were lying within the limits of ±30% accepted in bioanalysis. This rapid and robust UHPLC-MS/MS assay allows the simultaneous quantification in plasma of the major currently used cardiovascular drugs and offers an efficient analytical tool for clinical pharmacokinetics as well as DDIs studies.

1. Introduction

Highly active antiretroviral treatments (ARTs) have transformed Human Immunodeficiency Virus (HIV) infection from a deadly disease into a chronic condition. Consequently, people living with HIV (PLWH) live longer and the proportion of older individuals within the HIV-infected population is constantly growing [1]. Notably, interactions between traditional health risk factors and HIV infection itself lead PLWH to a higher risk of adverse cardiovascular events [2,3]. A wide variety of cardiovascular drugs such as calcium channel blockers, β-blockers and statins, are consequently prescribed to PLWH along with their ARTs. Atorvastatin, rosuvastatin, and pravastatin are the most prescribed lipid-lowering agents in PLWH [4]. Atorvastatin is predominantly metabolized by cytochrome P450 3A4 (CYP3A4) into two major active metabolites (namely *ortho*-hydroxy-atorvastatin, and *para*-hydroxy-atorvastatin), and the inactive metabolite atorvastatin lactone [5]. Conversely, rosuvastatin and pravastatin undergo minor hepatic metabolism. However, all statins are actively transported in the liver by the organic anion transporting polypeptide 1B1 (OATP1B1) [6]. In the case of polymedicated PLWH, antiretroviral drugs (ARVs) such as

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atazanavir are strong inhibitors of OATP1B1 and are therefore expected to substantially increase statin exposure by both inhibiting the entry of the statin in the liver and by further inhibiting its biotransformation [7–9]. This drug-drug interaction (DDI) can lead to severe adverse drug reactions such as rhabdomyolysis [10].

Additionally, 26.1% of PLWH have been reported to suffer from high blood pressure in the Swiss HIV Cohort Study (SHCS) [11]. Calcium channel blockers, such as amlodipine, are the preferred first-line antihypertensive drugs for most patients, including PLWH [12]. Amlodipine is predominantly metabolized by CYP3A4/5 [13,14]. In consequence, the inhibition of CYP3A4 by ritonavir-boosted protease inhibitors (PIs) can increase amlodipine plasma concentrations, potentially leading to toxicity [13]. Finally, metoprolol, a β-blocker commonly used in the treatment of hypertension and heart failure, is predominantly metabolized by the polymorphic CYP2D6, and boosted ARVs with inhibitory properties for this isoform could potentially lead to increase metoprolol plasma concentrations, though reportedly to a moderate extent [15].

Cardiovascular drugs are commonly used in PLWH, particularly in the context of an aging population, and are subject to DDIs leading to substantial risks of iatrogenic toxicity. Bioanalytical methodologies are therefore needed to determine plasma concentrations of cardiovascular drugs in PLWH and help manage DDIs in clinical practice.

Various methods have been published for the quantification in human plasma of a wide variety of cardiovascular drugs including calcium channel blockers, β-blockers and statins. Previously published assays for statins in human plasma, imply a chromatographic step on C18 support [16–29], or on Zorbax-SB phenyl column such as proposed by Macwan *et al.* for atorvastatin or rosuvastatin and their metabolites [30,31]. While multiplex liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analyses of various combinations of statins, calcium-channel inhibitors and β-blocker agents have been previously reported [20,21,32,33], none of them have actually investigated the simultaneous assay of amlodipine, metoprolol, pravastatin, rosuvastatin and atorvastatin which represented the most prescribed drugs at risk of DDIs in the PLWH population under study [34]. In general, plasma clean-up procedure for cardiovascular drugs may be straightforward, using protein precipitation [18,24,31,33] or liquid-liquid extraction [20,32,35]. To our knowledge, only one study has reported the quantification by LC-MS/MS of atorvastatin and its metabolite in plasma by liquid-liquid extraction followed by evaporation to dryness [36]. Alternately, attractive -yet technically more demanding to set-up-solid-phase extraction (SPE) approaches have been also applied [17,19,23,25–27,29] to attain the required lower limits of quantification nearly or even well below the nanogram per milliliter levels, compatible with statins trough concentrations commonly encountered in clinical practice [37,38].

The aim of this project was to develop and validate a rapid and convenient multiplex ultra-high-pressure liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) assay using a simplified sample preparation for the simultaneous quantification in human plasma of amlodipine, metoprolol, pravastatin, rosuvastatin and atorvastatin (the latter with two active metabolites) all these drugs being the most prescribed cardiovascular drugs to PLWH and also those at high risk of DDIs (34).

2. Materials and methods

2.1. Chemicals and reagents

Amlodipine benzenesulfonate (chemical purity 98%), metoprolol tartrate (98%), pravastatin sodium salt (98%), rosuvastatin sodium salt (97%), atorvastatin calcium salt (98%), *ortho-* and *para*-hydroxy-atorvastatin (*o*-OH-atorvastatin and *p*-OH-atorvastatin) calcium salt (98% and 95%, respectively), and atorvastatin lactone (98%) were purchased from Alsachim (Strasbourg, France) (chemical structures are displayed in Fig. 1). Their stable isotopically labeled internal standards (IS) (*i.e.* [$^{13}C_6$]-amlodipine benzenesulfonate (chemical purity 95%), [$^{2}H_7$]-metoprolol hydrochloride (98%), [$^{2}H_9$]-pravastatin ammonium salt (98%), [^{13}C, $^{2}H_4$]-rosuvastatin sodium salt (98%), [$^{2}H_5$]-atorvastatin calcium salt (98%), [$^{2}H_5$]-*o*-OH-atorvastatin calcium salt (98%), [$^{2}H_5$]-*p*-OH-atorvastatin calcium salt (95%), [$^{2}H_5$]-atorvastatin lactone (98%)), were also obtained from Alsachim (Strasbourg, France).

Acetonitrile (ACN), methanol (MeOH), and formic acid (98–100%, FA), all of analytical grade, were purchased from Merck (Darmstadt, Germany). Dimethylsulfoxide (99.5%, DMSO) was purchased from Alfa Aesar (Kandel, Germany). Water was purified through a Milli-Q® UF-Plus apparatus (Millipore Corp., Burlington, MA, USA).

For method development and validation, human blank plasma samples were obtained either from citrated blood (1970*g i.e.* 3000 rpm, 10 min, +4 °C, Hettich model Rotanta 460RF centrifuge) withdrawn from TCS Bioscience® (Buckingham, UK) or from patients with Vaquez disease (CHUV, Lausanne, Switzerland).

2.2. Patients samples

Patients' samples have been collected within the framework of observational pharmacokinetics studies, aiming at examining the prevalence of polypharmacy and the potential drug-drug interactions (DDIs) between ARVs and frequently used comedications. For this purpose, a systemic collection of blood samples and pharmacokinetics (PK) information was performed during the medical visits of PLWH enrolled in the SHCS and followed at the HIV clinics in Lausanne and Basel. The study (SHCS project #815) has been approved by the SHCS Scientific Board and Executive Committee in 2017. In addition, an observational PK DDIs study with rich sampling over one dosing interval has been proposed to consenting SHCS patients from Lausanne and Basel centers. The protocol -still ongoing- was approved by Institutional Ethics committees in April 2018, and registered in clinicaltrials.gov (NCT03515772).

2.3. Analyte and internal standard stock solutions

Stock solutions of each analyte were prepared at 1 mg/mL, by accurate weighing and dissolving each analyte in the required volume of DMSO. Stock solutions were corrected for salt forms by applying the weighing factor. One single working solution (WS) containing the seven analytes at 10 µg/mL and metoprolol at 100 µg/mL was prepared in a mixture of H_2O:DMSO (3:1), and stored at −20 °C. Stock solutions of IS were prepared at 1 mg/mL in MeOH and stored at −20 °C. These solutions were further combined and diluted with MeOH containing 0.1% FA to obtain an internal standard WS (IS-WS) at 100 ng/mL for each IS except for [$^{2}H_9$]-pravastatin and [$^{2}H_7$]-metoprolol (1000 ng/mL). This IS-WS was subsequently diluted in MeOH (with 0.1% FA) to obtain the final plasma protein precipitation solution at 1 ng/mL (and 10 ng/mL for [$^{2}H_9$]-pravastatin and [$^{2}H_7$]-metoprolol).

2.4. Calibration/validation standards and quality controls (QCs) preparation

Further dilutions of the WS containing analytes were performed using a mixture H_2O:DMSO (3:1) and citrated blank plasma samples were spiked with these different diluted solutions to reach the required calibration/validation concentrations. For the calibration samples, nine concentration levels (k) were prepared in triplicate (n = 3): 0.05, 0.1, 0.2, 0.5, 2, 10, 50, 100, 500 ng/mL, except for metoprolol, having 10-fold higher values (*i.e.* 0.5, 1, 2, 5, 20, 100, 500, 1000, and 5000 ng/mL), with respect to the concentrations commonly observed in clinical practice. For the preparation of validation standard samples, eight concentrations levels were prepared in a mixture H_2O:DMSO (3:1) in triplicate (n = 3): 0.1, 0.2, 0.3, 0.6, 1.2, 12, 120, 480 ng/mL, except for metoprolol (10-fold higher values).

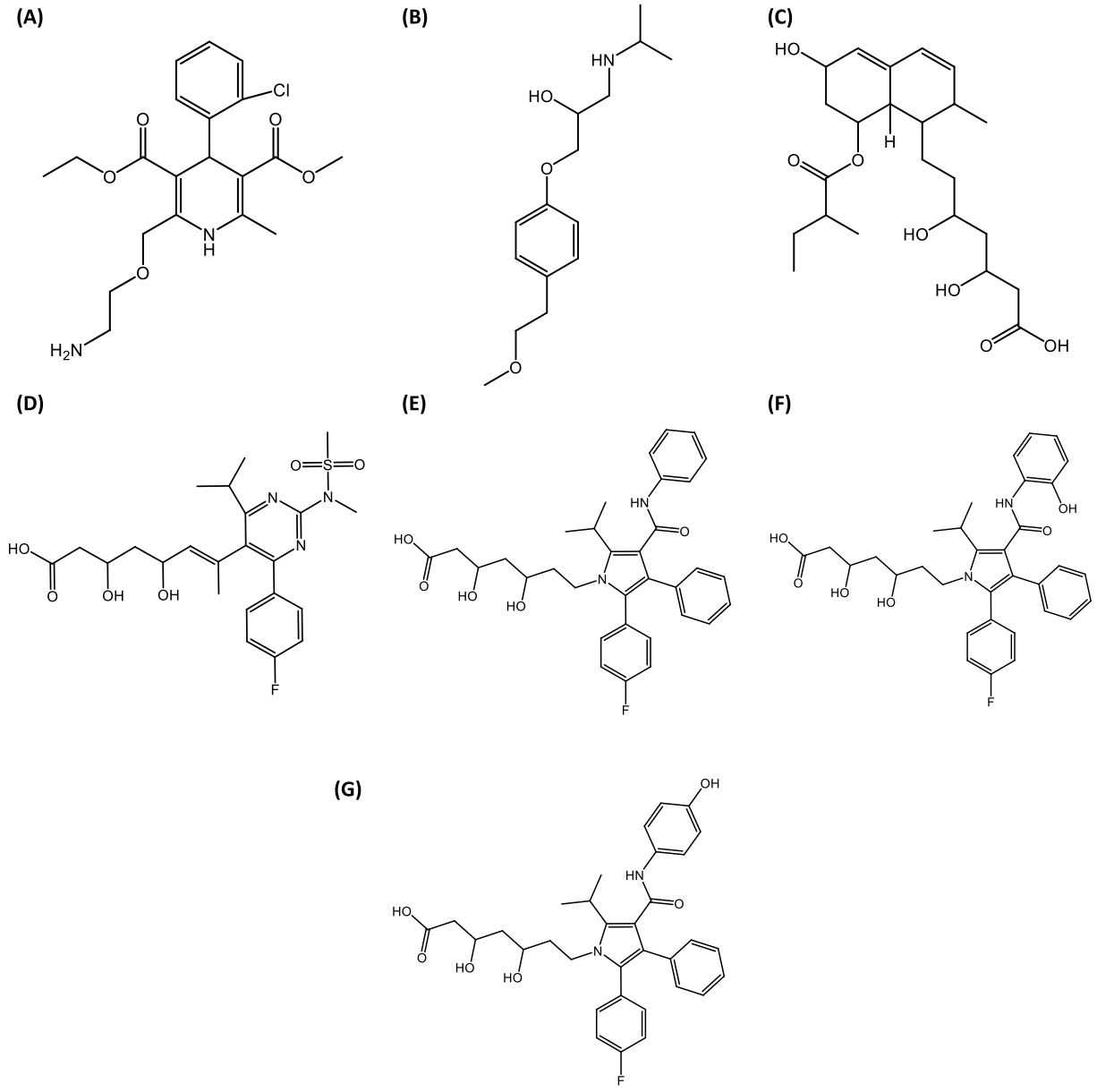

Fig. 1. Chemical structures of the selected cardiovascular drugs and relevant metabolites. (A) Amlodipine (logP = 3.0). (B) Metoprolol (logP = 1.9). (C) Pravastatin (logP = 0.6). (D) Rosuvastatin (logP = 0.1) (E) Atorvastatin (logP = 5.7). (F) *o*-OH-atorvastatin (logP = 4.6). (G) *p*-OH-atorvastatin (logP = 4.6). Chemicalize was used for compound properties, 2017, https://chemicalize.com/ developed by ChemAxon (http://www.chemaxon.com).

To accurately estimate the LLOQ values, one validation sample at the estimated LLOQ and one at 2–3-fold LLOQ were used for each analyte. Preparation of validation standards followed the recommendations for bioanalytical method validation stating that the total added volume must be <10% of the biological sample volume [39,40]. The concentrations ranges were selected to cover the expected patients' plasma concentrations encountered in clinical practice.

2.5. Plasma samples pre-treatment

For protein precipitation, 200 µL of calibration, validation or patient samples were mixed and vortexed with 600 µL of IS-WS. The mixture was centrifuged at 20,000*g* (14,000 rpm) at + 4 °C for 10 min with a benchtop centrifuge (Benchtop Mikro 220R centrifuge, Hettich, Bäch, Switzerland). A 600 µL aliquot of the supernatant was transferred into a 5 mL polypropylene tube before evaporation to dryness during 4 h (nitrogen flux, at room temperature). The dried extract was reconstituted in 60 µL of MeOH:H_2O 3:1 mixture containing 0.1% FA, vortexed, sonicated and centrifuged again. Finally, a 50 µL-aliquot of limpid supernatant was directly transferred into an HPLC vial with glass insert.

2.6. LC-MS/MS apparatus

The liquid chromatography system involved a Vanquish Flex Binary UHPLC system (Thermofisher Scientific, San Jose, CA, USA). The separation was performed on an Acquity UPLC CSH™ analytical column (Waters, Millford, MA, USA) with dimensions 2.1 × 50 mm and particle size 1.7 µm, thermostated at 25 °C by a column oven. The detector consisted of a triple-stage quadrupole TSQ Quantiva™ mass spectrometer equipped with an Ion Max NG™ electrospray ionization (ESI) source (Thermofisher Scientific, San Jose, CA, USA). Xcalibur software version 1.1 was used for system control and data acquisition (Thermofisher Scientific, San Jose, CA, USA).

2.7. LC-MS/MS analysis conditions

The mobile phase used for the chromatographic separation consisted in a multi-steps gradient of ultrapure H_2O with 0.1% FA (A) and ACN with 0.1% FA (B). The following elution program was used: isocratic stage at 2% B from 0 to 0.2 min, followed by a first linear gradient from 2% to 45% B in 0.8 min, up to 50% B in 0.1 min, followed by a second linear gradient from 50% to 60% B until 3 min. Then, B was increased to 100% in 0.1 min for intensive column rinsing during 0.9 min and, finally, returned to initial conditions (2% B) in 0.1 min for re-equilibration from 4.1 to 6 min (total analysis time). The flow rate and injection volume were 0.5 mL/min and 5 µL, respectively. The autosampler was thermostated at +5 °C for samples storage before injection.

The ionization conditions were set as follows: the ion transfer tube and vaporizer temperatures at 300 and 400 °C, respectively; ESI spray voltage at 3600 V and 3100 V for positive mode and negative mode, respectively; sheath, auxiliary and sweep gas flow rates at 50, 15 and 1 (arbitrary units), respectively. The instrument that we used has the capacity to enable positive and negative ionization mode alternatively, by programing specific transitions segments.

Mass resolution of both quadrupoles (Q1 and Q3) was set at 1.2 Da (*i.e.* m/z 1.2 full width at half maximum, FWHM). The cycle time was 0.2 s. The collision gas was argon and its pressure was set at 2 mTorr in the second quadrupole (Q2).

Identification of analytes was based on two specific MS/MS transitions (except for *o*-OH-atorvastatin), both used for quantification purposes. In addition, retention times obtained from the pure certified standard substances were considered as the reference retention times to appropriately identify analytes.

2.8. Analytical method validation

The strategy applied for the validation of the method was based on the recommendations by the Food and Drug Administration (FDA) [40], the European Medicine Agency (EMEA) [39] and the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) [41,42]. The authors also referred to other published articles dealing with bioanalytical method validation [43–47].

2.8.1. Selectivity and carry-over

Method selectivity toward matrix was assessed at the retention times of analytes and IS while monitoring the chromatograms obtained with ten different blank human plasma extracts.

A high concentration calibration sample of the analytes processed without IS (pure MeOH), and a blank plasma processed with the IS-WS were injected for the evaluation of cross-talk interferences between the different analytes and their respective IS.

Carry-over effect was evaluated for each analyte by injecting one or more blank solvent (MeOH) or blank plasma extract immediately after the injection of the most concentrated calibration sample (including IS at usual concentrations).

2.8.2. Matrix effect, extraction recovery, process efficiency

2.8.2.1. Qualitative assessment of matrix effect. Matrix effects (MEs) were first assessed qualitatively using the widely recognized technique proposed by Bonfiglio *et al.* [48]. Extracts from different blank plasma (n = 6) were simultaneously injected into the UHPLC–MS/MS system while a standard solution of analytes and ISs was continuously infused post-column directly into the MS [48]. The mixture was infused at a flow rate of 10 µL/min at the concentration of 50 ng/mL (500 ng/mL for metoprolol) and 75 ng/mL (750 ng/mL for metoprolol and pravastatin IS), for analytes and ISs, respectively. For each MS/MS transition, any alteration (suppression or enhancement) of the LC-MS signals at the analytes' respective retention time is suggestive for a ME [49].

2.8.2.2. Quantitative evaluation of matrix effect, extraction recovery and process efficiency. Subsequently, MEs, extraction recoveries (ER), as well as process efficiencies (PE) were also assessed quantitatively, at low (L, 2 ng/mL; 20 ng/mL for metoprolol), middle (M, 20 ng/mL; 200 ng/mL for metoprolol) and high (H, 200 ng/mL; 2000 ng/mL for metoprolol) QC concentrations in seven different human plasmas [50].

For that purpose, several solutions were prepared as follows: three neat solutions (MeOH:H_2O 3:1) spiked with analytes and ISs (=A), seven blank plasma samples precipitated, dried, reconstituted and spiked in duplicate with both analytes and ISs (=B), and seven blank plasma samples, spiked in duplicate with analytes and ISs before protein precipitation, drying and reconstitution (=C). MEs (B/A in %), ERs (C/B in %) and PEs (C/A in %) were finally calculated from the average peak areas obtained from samples A, B and C, at the three QC levels. Variability between the seven plasma samples was expressed as relative standard deviation (RSD), and a maximum RSD value of 15% was accepted for each concentration. The IS-normalized ME, ER and PE (*i.e.*, IS-nME, IS-nER and IS-nPE) were also calculated.

The assessment of matrix effect was also based on the calculation of RSD values of slopes from linear regressions estimated at L, M and H concentrations for seven samples of blank plasma [46]. A method is considered devoid of significant matrix effect if the RSD value does not exceed 3–4%.

2.8.3. Response function, trueness, precision, accuracy profiles and limit of quantification

Method precision and trueness determination was carried out on three different days/series. Based on previous assessment of response functions during the pre-validation phase, two regression models were fitted for the calibration curve: quadratic log-log regression, and weighted (1/x) quadratic regression. Accuracy profiles were plotted for each compound and each calibration model. The optimal regression model was determined according to the best total error profile obtained when covering the entire concentrations' range. In order to determine trueness, repeatability and intermediate precision, the QC samples were recalculated using the daily established response function. The trueness was expressed as the percentage of deviation between the mean value obtained from a series of measurements and the true value. Precision was defined as the dispersion level among replicated measurements and described by both repeatability and intermediate precision, expressed as RSD based on the nominal concentration at each level, as described in the SFSTP reports [41,51]. Both systematic and random errors defined the total error of a procedure and can be represented with accuracy profiles based on β-expectation tolerance intervals, as previously suggested by several authors [52,53]. Acceptance criteria would ensure that a high proportion (β%) of future observations lie within acceptable limits [45,54]. As stated in FDA and EMEA official documents [39,40], and in reference publication [55], total error should not exceed 30%. Based on the results obtained in the validation step, this approach allows a confident prediction of the future results that will be obtained during the daily use of the method. As described by Feinberg et al. [56], lower limit of quantification (LLOQ) was graphically interpolated from the absolute accuracy profile: they corresponded to the concentrations at the intersection of the β-expectation tolerance intervals and the acceptance limits (± 30%).

During the 3 days of validation, back-calculated validation concentrations were plotted *versus* nominal concentrations and were fitted with a linear regression model. This allowed assessing the linearity of trueness of the method, that's to say its ability to generate quantitative results directly proportional to known nominal analyte concentrations.

2.8.4. Short and medium stability studies

Bench-experiments samples stability as well as medium stability studies in human plasma were performed under different conditions:

1) at room temperature (RT) and at +4 °C in the fridge up to 48 h;

Table 1
MS/MS parameters and typical retention times for the analysis of five cardiovascular drugs, three of their metabolites and the respective stable-isotope-labeled ISs.

Compound	ESI polarity (+/−)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (eV)	Tube lens voltage (V)	Typical retention time (min)
Amlodipine	+	409	238	12	64	1.51
			294	11	64	
[$^{13}C_6$]-Amlodipine	+	415	244	10	65	1.51
			300	10	65	
Metoprolol	+	268	116	18	74	1.20
			74	22	74	
[$^{2}H_7$]-Metoprolol	+	275	123	19	74	1.20
			191	18	74	
Pravastatin	−	423	321	14	81	1.66
			303	15	81	
[$^{2}H_9$]-Pravastatin	−	432	321	14	85	1.66
			303	17	85	
Rosuvastatin	+	482	258	33	110	1.90
			272	34	110	
[^{13}C, $^{2}H_4$]-Rosuvastatin	+	487	263	34	122	1.90
			305	36	122	
Atorvastatin	+	559	440	20	93	2.50
			292	31	93	
[$^{2}H_5$]-Atorvastatin	+	564	440	21	91	2.49
			250	42	91	
o-OH-Atorvastatin	+	575.3	466	10	102	2.40
[$^{2}H_5$]-*o*-OH-Atorvastatin	+	580.1	445	22	100	2.39
			471	12	100	
p-OH-Atorvastatin	+	575.2	440	22	107	1.87
			466	12	107	
[$^{2}H_5$]-*p*-OH-Atorvastatin	+	580	445	21	99	1.87
			471	12	99	

2) three freeze/thaw cycles. Frozen samples were allowed to thaw at RT for 1 h and were then refrozen during for 2 h;
3) freezing at −20 °C and −80 °C during 6 weeks;
4) thermal viro-inactivation procedure (60 min at +60 °C in a water-bath) performed in our laboratory, prior to analysis. This treatment has been shown to efficiently inactivate HIV particles present in the samples [57–59].

Duplicate analyses at L (2 ng/mL and 20 ng/mL for metoprolol), M (20 ng/mL and 200 ng/mL for metoprolol) and H (200 ng/mL and 2000 ng/mL for metoprolol) QC levels were performed. For all stability tests, the variation in analyte's concentration was determined by dividing the concentration measured after storage or thermal viro-inactivation, by the initial value, and expressed in %.

Finally, some studies have reported that the inactive metabolite atorvastatin lactone may revert back to atorvastatin after hydrolysis at elevated temperature [17,60]. We have therefore examined the evolution of atorvastatin and its inactive lactone metabolite in plasma spiked with atorvastatin lactone subjected to the above thermization treatment.

3. Results and discussion

3.1. Bioanalytical method development

The most prescribed cardiovascular drugs in the SHCS#815 study (see Section 2.2), and also those with the highest potential for DDIs in polymedicated PLWH were amlodipine, metoprolol, pravastatin, rosuvastatin and atorvastatin and were therefore incorporated into the multiplex UHPLC-MS/MS assay along with the two active atorvastatin metabolites, *o*-OH and *p*-OH-atorvastatin. First, optimal MS/MS transitions of the seven analytes have been selected (Table 1) by directly infusing into the MS detector a standard solution of analytes (1 µg/mL in MeOH). Since analytes were mostly apolar (log P ranging from 0.13 to 5.7) -as previously reported in the literature- reverse-phase (RP) liquid chromatography was selected for the multiplex separation. The assay development design has included to test i) several RP-based stationary phases (*i.e.* Acquity UPLC HSS T3, Acquity UPLC CSH C18, or Acquity UPLC HSS cyano); ii) the nature of the organic solvent in the mobile phase (ACN or MeOH, both with 0.1% FA), and finally iii) column temperature (+10 °C, +25 °C or + 40 °C). The optimal compromise in term of resolution, peak shape, MS response and reasonable analysis time of the seven compounds was obtained with the Acquity UPLC CSH C18 column, using ACN + 0.1% FA in the mobile phase, and a column temperature set at +25 °C. Mobile phase gradient program was finely optimized (multi-steps elution) providing a satisfactorily retention of the seven compounds resolved in <3 min (Fig. 2). Despite a suboptimal separation of *p*-OH-atorvastatin and rosuvastatin with this 6-min gradient program, the use of deuterated IS for each analyte circumvent any potential reciprocal influence on their respective ionization efficiencies. Preliminary selectivity experiments revealed a major co-eluting matrix interference in the *o*-OH-atorvastatin quantitative transition using product ion *m/z* 440, which was the main ion used so far [16,61]. For this reason, the product ion *m/z* 466 was chosen in the transition used for quantification, leading to excellent selectivity with a very limited loss of sensitivity.

ESI source parameters (reported in the experimental section) were optimized for each analyte using the actual analytical conditions in order to obtain the best performance in terms of sensitivity and background noise minimization.

However, direct injection of supernatants obtained after generic plasma protein precipitation turned out to be insufficiently sensitive to detect low albeit clinically relevant plasma concentrations of some cardiovascular drugs. In fact, lower limits of quantification well below 1 ng/mL were required for some analytes, with respect to their reported plasma concentration ranges in clinical practice. The most convenient and cost-effective way to improve sensitivity down to sub-nanogram per milliliter levels was obtained *via* a combined solvent-based plasma protein precipitation followed by supernatant evaporation to dryness and reconstitution of solid residue in a suitable microvolume of solvent. Other published LC-MS/MS methodologies used complicated and time-consuming sample preparation mostly involving SPE processes. In our study, MeOH +0.1% FA was preferred as protein precipitation solvent over ACN + 0.1% FA or ACN:MeOH 1:1 (+ 0.1% FA) because it

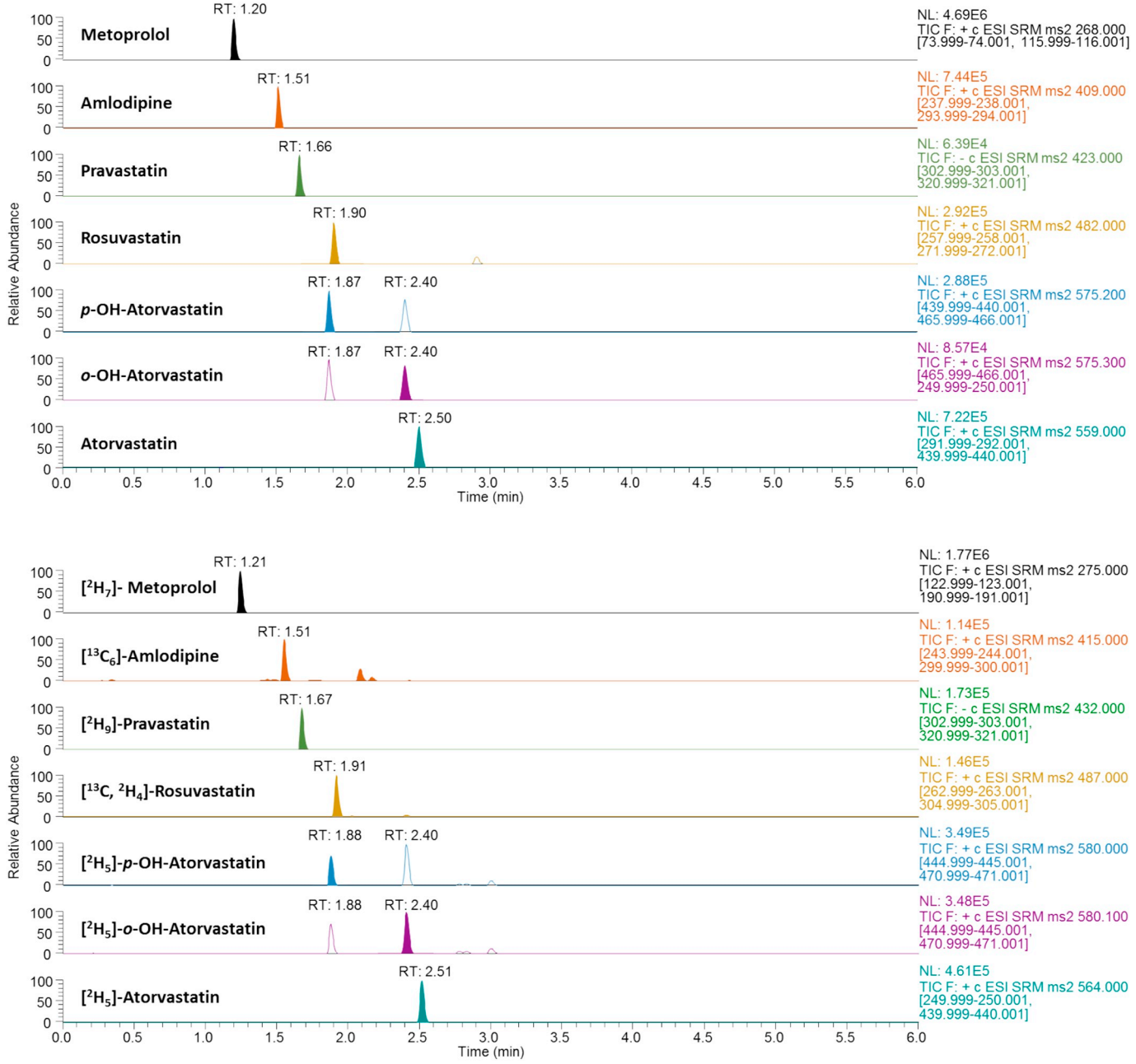

Fig. 2. UHPLC-MS/MS chromatograms of a calibrator sample containing the five cardiovascular drugs and two metabolites, at the concentration of 10 ng/mL for all the analytes except for metoprolol (100 ng/mL). Calibrator sample was prepared as described in the Materials and methods section.

provided better sensitivity. Of note, the addition of FA was required since several reports [31,62] have found that lowering the plasma pH was able to minimize the conversion of the -inactive- lactone form of statins into the corresponding hydroxy-acid form. After centrifugation, the supernatant was concentrated 10-fold *via* evaporation to dryness, reconstitution in MeOH:H_2O 3:1 (+ 0.1% FA) and sonication. Insoluble matrix components appeared after reconstitution of the dried residue and were removed by centrifugation to yield a clear supernatant directly injected for LC-MS/MS analysis.

Finally, for the preparation of calibration/QC samples, it was found that a low percentage of DMSO (H2O:DMSO 3:1) in WSs was necessary to obtain satisfactory linearity over the wide investigated calibration range while minimizing the organic solvent content to biological matrix, as recommended by guidelines [39,40].

3.2. Method validation

3.2.1. Selectivity and carry-over

When analyzing human blank plasma from 10 different sources, no endogenous or exogenous interferences were observed at the respective retention times of the seven analytes, demonstrating good selectivity of the chromatographic method.

Cross-talk was considered satisfactory as no significant signal was observed on the analytes' transitions when a blank plasma extract containing only IS was injected or conversely (absence of significant signal on the ISs' trace after the injection of the highest calibration standard without IS).

No carry-over was observed when analyzing blank matrix samples or MeOH directly after the highest calibration standard.

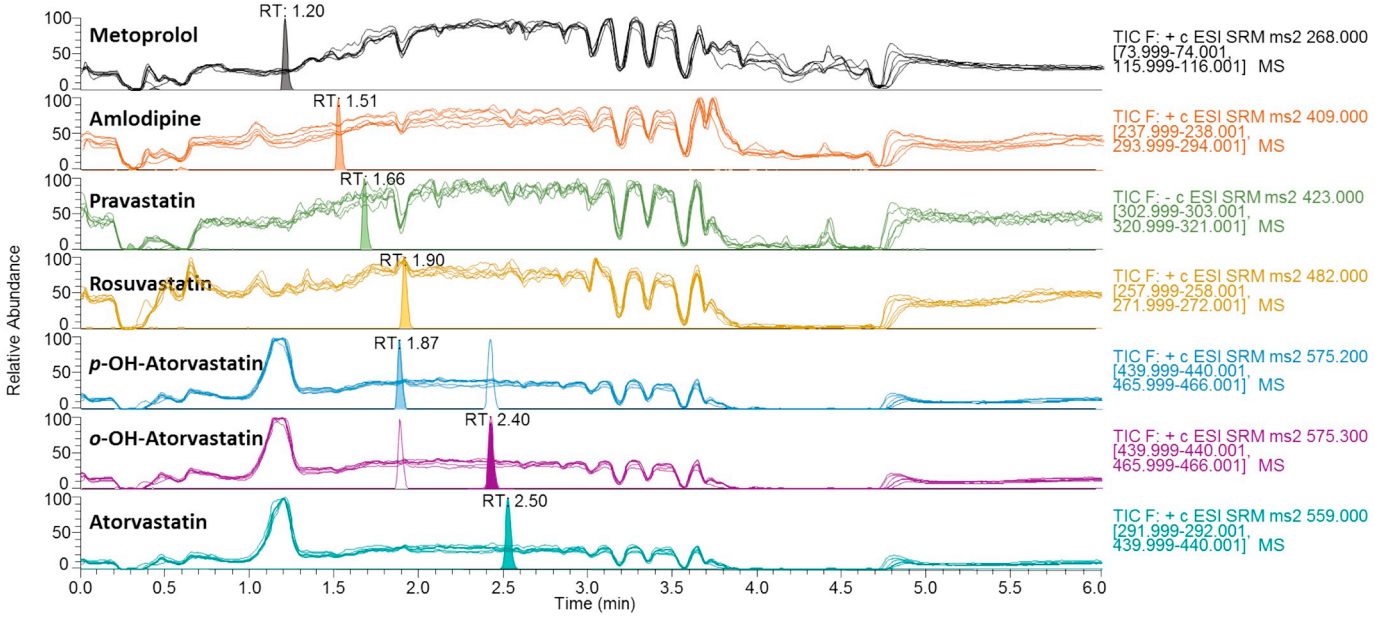

Fig. 3. Qualitative assessment of matrix effect in human plasma. Overlaid UHPLC-MS/MS traces obtained for the analysis of six blank plasma extracts during post-column infusion of a standard solution containing the seven analytes at 50 ng/mL (500 ng/mL for metoprolol). Retention times and chromatographic peaks obtained of the seven analytes were superimposed for interpretation.

Table 2
Internal Standard normalized Matrix Effect (IS-nME), extraction recovery (IS-nER) and process efficiency (IS-nPE) in human plasma.

Compound	QC level[a]	IS-nME % (RSD)	IS-nER % (RSD)	IS-nPE % (RSD)
Amlodipine/[$^{13}C_6$]-amlodipine	Low	0 (2)	−4 (5)	−4 (6)
	Middle	−2 (2)	3 (6)	1 (5)
	High	1 (1)	1 (5)	0 (5)
Metoprolol/[$^{2}H_7$]-metoprolol	Low	3 (1)	−7 (5)	−4 (5)
	Middle	1 (1)	−1 (5)	0 (5)
	High	−1 (1)	0 (3)	−2 (2)
Pravastatin/[$^{2}H_9$]-pravastatin	Low	5 (6)	−10 (5)	−6 (7)
	Middle	1 (2)	−1 (7)	0 (5)
	High	4 (1)	−3 (3)	2 (3)
Rosuvastatin/[^{13}C, $^{2}H_4$]-rosuvastatin	Low	2 (2)	−8 (4)	−7 (4)
	Middle	−4 (2)	−1 (5)	−5 (5)
	High	−4 (1)	−1 (4)	−5 (4)
Atorvastatin/[$^{2}H_5$]-atorvastatin	Low	12 (4)	−20 (7)	−11 (7)
	Middle	−2 (4)	−9 (7)	−10 (9)
	High	1 (4)	1 (5)	1 (3)
o-OH-atorvastatin/[$^{2}H_5$]-*o*-OH-atorvastatin	Low	7 (3)	−6 (4)	0 (5)
	Middle	2 (2)	3 (7)	5 (6)
	High	−3 (1)	−2 (2)	−5 (2)
p-OH-atorvastatin/[$^{2}H_5$]-*p*-OH-atorvastatin	Low	10 (4)	−14 (5)	−5 (6)
	Middle	7 (3)	−3 (7)	4 (6)
	High	−1 (3)	−5 (3)	−6 (3)

[a] Low concentration is defined as 2 ng/mL (20 ng/mL for metoprolol), middle concentrations as 20 ng/mL (200 ng/mL for metoprolol), and high concentrations are defined as 200 ng/mL (2000 ng/mL for metoprolol).

3.2.2. Matrix effect, extraction recovery and process efficiency

Matrix effects were qualitatively (post-column infusion) and quantitatively (Matuszewski's approach) determined.

Qualitatively, elution of the highly polar compounds present in plasma led to signal suppression at the beginning of the run (from 0 to 0.5 min), emphasizing the importance of the chromatographic step and sufficient analytes' retention such as obtained with the present method (*i.e.* first analyte eluted at 1.2 min). Signal interferences observed from 3 to 4.7 min (particularly total suppression between 3.6 and 4.7 min) were not problematic since localized after the last eluted peak (Fig. 3). A signal enhancement at the *m/z* transitions of atorvastatin and its metabolites was observed at 1.2 min, yet without consequences being far distant from their retention times. The MS response remained otherwise stable and constant, emphasizing the suitability of the developed LC program.

The quantitative internal standard normalized matrix effect (IS-nME), extraction recovery (IS-nER) and process efficiency (IS-nPE) parameters for each analyte are reported in Table 2. IS-nMEs ranged from 96 to 112% (RSD ≤ 6%), confirming that the ionization process of the analytes was not significantly affected by co-eluting plasma matrix components. The IS-nERs were satisfactorily comprised between 80 and 103%, with acceptable variability overall (RSD ≤ 7%). Finally, good performances were globally obtained for IS-nPEs that were in the range 89 to 105%, with satisfactory RSD ≤ 9%.

Finally, standard line slopes approach confirmed the absence of significant MEs which could alter the quantitation in plasma, *i.e.* RSDs of standard line slopes constructed in seven different blank plasma matrices did not exceed 3.8% for all analytes.

3.2.3. Trueness, precision and accuracy profile

Response functions were obtained by plotting analyte/IS peak area ratios over analyte concentration in human plasma. Calibration range of each compound was determined in order to obtain determination coefficient (R^2) of the calibration curves higher than 0.99 and back-calculated concentration of calibration samples within ±15% (± 20% at expected LLOQ). Concentrations of the validation samples were calculated thanks to the calibration curves obtained for each serie and used to investigate the different regression models to find out the best response function. Validation criteria in terms of trueness and precision were met over the whole calibration range of 0.1–500 ng/mL for amlodipine, 0.2–500 ng/mL for atorvastatin, *o*-OH-atorvastatin, *p*-OH-atorvastatin, pravastatin and rosuvastatin, and 0.5–5000 ng/mL for

Table 3
Trueness, repeatability and intermediate precision in human plasma over the validated range.

Compound	Conc. (ng/mL)	Plasma		
		Trueness (%)	Precision	
			Repeatability (%)	Intermediate precision (%)
Amlodipine	0.3	103.7	5.0	6.4
	0.6	99.6	6.4	7.4
	1.2	103.7	6.5	7.9
	12	110.1	3.8	4.9
	120	106.0	3.6	3.6
	480	97.9	5.0	5.0
Metoprolol	3	101.6	4.0	10.7
	6	102.4	4.4	7.4
	12	102.9	3.2	5.9
	120	100.1	2.0	3.6
	1200	98.2	3.5	3.9
	4800	98.3	3.7	4.4
Pravastatin	0.6	97.8	3.8	8.0
	1.2	95.5	5.7	8.9
	12	103.3	1.5	3.9
	120	102.7	3.0	4.6
	480	98.4	4.4	4.4
Rosuvastatin	0.6	96.6	10.5	14.2
	1.2	95.4	4.4	14.5
	12	101.6	2.4	10.5
	120	101.8	4.2	12.5
	480	96.7	3.8	9.3
Atorvastatin	0.3	110.8	5.5	5.8
	0.6	106.4	7.4	9.1
	1.2	109.8	3.2	4.6
	12	108.5	2.1	4.2
	120	105.9	5.3	6.0
	480	103.1	3.1	8.4
o-OH-atorvastatin	0.6	101.4	7.8	12.8
	1.2	103.2	8.0	9.6
	12	106.2	3.2	6.6
	120	108.6	5.0	6.0
	480	98.4	5.9	5.9
p-OH-atorvastatin	0.3	108.8	6.5	7.8
	0.6	99.5	13.4	13.4
	1.2	98.9	5.0	9.1
	12	105.4	3.3	4.3
	120	105.7	6.1	6.1
	480	101.0	4.7	6.0

metoprolol. The quadratic log-log regression model turned out to be most appropriate in terms of trueness, precision and validation domain (*i.e.* lowest LLOQ) for each analyte, except for metoprolol (1/x weighted quadratic regression model).

Satisfactory performances were obtained in terms of trueness (95.4–110.8%), repeatability (1.5–13.4%) and intermediate precision (3.6–14.5%) (Table 3) all over the validated concentration ranges.

The accuracy profiles with β-expectation tolerance intervals were built using a β value of 80%, which represents the percentage of future results that would be expected to fall within the obtained tolerance intervals during routine application of the method [63]. Accuracy profiles obtained for each compound did not exceed the acceptance limits for biological samples (± 30%) over the validated domain [40] (Supplementary file S1). The LLOQ values correspond to the lowest concentration at which the tolerance limit crossed the acceptance threshold in the absolute accuracy profile representation (Supplementary file S2) [42]. The lowest concentrations measurable in human plasma with an accuracy of ±30% (total error) were 0.3 ng/mL for amlodipine, atorvastatin and *p*-OH-atorvastatin, 0.4 ng/mL for pravastatin, 0.5 ng/mL for rosuvastatin and *o*-OH-atorvastatin, and 3 ng/mL for metoprolol. LLOQ were generally higher than those reported in published LC-MS/MS assays for the analytes of interest [16,17,64,65] but were considered satisfactory considering the expected range of plasma concentrations in clinical practice. ULOQ corresponds to the concentration of the highest validation sample (*i.e.* 480 ng/mL and 4800 ng/mL for metoprolol).

Linearity of trueness was satisfactory for all analytes, *i.e.* slopes ranged from 0.91 to 1.09 and determination coefficients (R^2) were all higher than 0.99.

3.2.4. Stability studies

Results of stability studies are summarized in Table 4.

The stability of QC samples left at RT and at +4 °C, ascertained up to 48 h indicated that human plasma samples could be stored at RT and +4 °C for up to 48 h, without significant degradation of the analytes (variation over time < ± 15% of nominal concentrations), except for atorvastatin. Atorvastatin plasma concentrations were 30% higher after 48 h at RT probably due to the hydrolysis of atorvastatin lactone into atorvastatin, as previously reported [17,60].

After three freeze–thaw cycles, the variations in plasma were below 15%, indicating acceptable stability for all analytes. In the similar way, stability studies up to 6 weeks at −20 °C and −80 °C demonstrated no degradation during the investigated long-term storage of human plasma samples.

The concentrations variations assessed after heating plasma at 60 °C for 60 min were comprised within ±15% of nominal concentrations for all analytes, except for atorvastatin which showed a significant increase in atorvastatin concentrations (up to 60%), again presumably because of the thermal lability reported for its (inactive) metabolite atorvastatin lactone [17,60]. Accordingly, plasma spiked with atorvastatin lactone subjected to the above thermization procedure showed a corresponding decrease of about 66% of its initial concentrations.

Considering the poor stability of atorvastatin lactone metabolite present in patients that is likely to yield spuriously elevated atorvastatin levels *ex vivo*, spiked and patient samples should be maintained at +4 °C after blood collection, centrifuged without delay at +4 °C, then immediately frozen and stored at −80 °C. In addition, the thermization procedure will be omitted for PWLH samples on atorvastatin and therefore optimal safety conditions for plasma handling have to be implemented (*i.e.* wearing gloves and samples treatment under class II biohazard laminar flow).

3.2.5. Clinical applications

The validated bioanalytical method was applied for the quantification of cardiovascular drugs and relevant metabolites concentrations in PLWH plasma samples collected during the above mentioned observational SHCS#815 study and for the currently ongoing pharmacokinetic study (see 2.2). A representative example of chromatogram of a patient treated with atorvastatin 10 mg, concurrently with cobicistat-boosted darunavir, is reported in Fig. 4A. Plasma sample was collected 6 hour post-dose and plasma concentrations were 7.4 ng/mL for atorvastatin and 0.9 and 0.4 ng/mL for *o*- and *p*-OH-atorvastatin, respectively. As expected, atorvastatin concentration was higher than the ones reported in absence of CYP3A4 and OATP1B1 inhibitors which would lie within 3 ng/mL for both atorvastatin and *o*-OH-atorvastatin [66]. Conversely, *o*-OH-atorvastatin concentration was lower while *p*-OH-atorvastatin concentration remained unchanged. An example of the chromatographic profile of a plasma from a PLWH receiving amlodipine 10 mg QD and pravastatin 40 mg QD in addition to his ART (*i.e.* rilpivirine, emtricitabine, tenofovir disoproxil fumarate) is shown in Fig. 4B. Plasma concentration of amlodipine and pravastatin were 7.6 ng/mL and 1.8 ng/mL, respectively, in a sample collected 9 h after the last doses of both comedications. Finally, Fig. 4C represents a

Table 4
Stability of the analytes under different storage conditions.

Compound	QC level[a]	Room temperature for 24 h	Room temperature for 48 h	+4 °C for 24 h	+4 °C for 48 h	After 3 consecutive freeze-thaw cycles	−20 °C during 6 weeks	−80 °C during 6 weeks	Thermal viro-inactivation[b]
Amlodipine	Low	−5.9	−2.4	−2.2	1.5	4.4	−6.3	−2.5	−5.9
	Middle	−3.4	−3.3	−1.1	−0.2	1.7	−3.7	−4.0	−1.0
	High	−5.3	−6.9	−0.2	−3.6	3.6	1.2	−3.0	−0.2
Metoprolol	Low	−1.1	1.6	0.9	1.3	1.6	−3.1	−2.8	2.3
	Middle	−0.7	1.0	1.0	1.0	2.3	−3.3	−3.3	−0.9
	High	−1.1	1.6	0.7	1.2	2.6	−4.0	−0.6	−0.2
Pravastatin	Low	−4.2	3.4	2.4	2.9	−0.6	−1.9	−3.3	−0.2
	Middle	−1.7	−0.3	0.6	1.3	5.4	−6.4	−5.0	1.3
	High	−1.6	0.3	−1.6	−0.5	6.4	−4.0	−0.3	2.7
Rosuvastatin	Low	1.5	−2.0	0.6	2.8	−8.5	7.1	−7.4	−5.2
	Middle	−2.7	1.2	−0.9	3.7	−8.3	−4.6	−4.3	−5
	High	−1.2	−0.2	3.8	2.3	−6.2	−4.2	−2.0	−4.8
Atorvastatin	Low	13.9	25.0	−0.3	1.2	−8.5	−1.7	−3.7	36.8
	Middle	22.1	33.7	4.6	5.6	2.8	−3.1	−3.8	55.8
	High	19.1	30.5	0.9	2.9	9.1	6.5	5.9	61.1
o-OH-atorvastatin	Low	−4.4	−5.5	−5.5	−1.8	−6.3	−12.8	−11.4	−6.4
	Middle	−0.8	−0.5	−2.4	0.0	1	−8.5	−3.4	−0.6
	High	1.4	−1.1	−3.0	−0.8	3.1	6.6	9.2	2.0
p-OH-atorvastatin	Low	−0.2	−2.2	−0.8	−2.3	−0.7	1.4	−3.6	−0.4
	Middle	0.7	1.9	2.1	3.3	0.9	0.4	2.1	2.8
	High	0.2	−0.3	−0.7	1.4	4.3	5.0	9.5	3.5

Data are reported as mean deviations (%) of investigated concentration (n = 3) from concentration measured at t_0.

[a] Low concentration is defined as 2 ng/mL (20 ng/mL for metoprolol), middle concentrations as 20 ng/mL (200 ng/mL for metoprolol), and high concentrations as 200 ng/mL (2000 ng/mL for metoprolol).

[b] 60 min at 60 °C in a water bath.

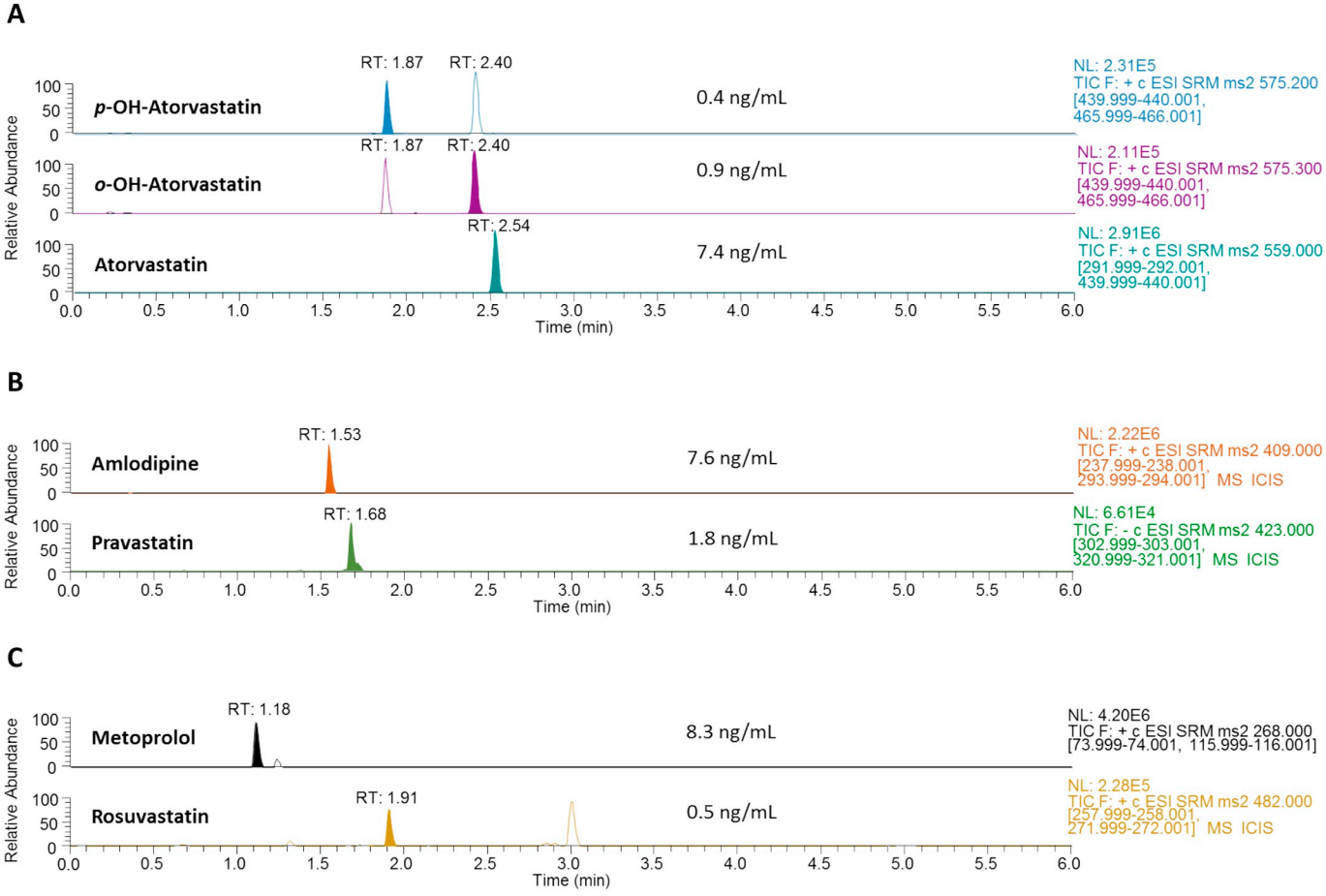

Fig. 4. Representative UHPLC-MS/MS analysis and measured concentrations in plasma sample from three PLWH receiving comedications concurrently to ARTs. See Section 3.2.5 for details.

chromatogram of a plasma sample collected 23.5 h after the last doses of metoprolol 12.5 mg QD and rosuvastatin 5 mg QD. The concentrations of metoprolol and rosuvastatin in this patient were 8.3 ng/mL and 0.5 ng/mL, respectively. Plasma concentrations of both analytes were similar to those reported in other published LC-MS/MS methods [32,67]. Comprehensive pharmacokinetic analyses are to be published elsewhere.

4. Conclusion

A sensitive and selective bioanalytical UHPLC-MS/MS assay was developed to enable the simultaneous quantification of the five cardiovascular drugs amlodipine, metoprolol, pravastatin, rosuvastatin and atorvastatin and two active metabolites in human plasma. Simple and convenient sample preparation consisting in protein precipitation followed by evaporation and reconstitution was found suitable to achieve satisfactory LLOQ. To the best of our knowledge, this is the first assay for cardiovascular drugs that combines the convenience of protein precipitation followed by supernatant preconcentration. This assay therefore offers to analytical scientists a simplified approach to quantify low levels of most frequently cardiovascular drugs, without the need of solid-phase extraction procedures that are delicate to develop and validate. Validation performances were in compliance with international recommendations and validation ranges covered the low plasma concentrations commonly observed in clinical practice. Finally, this assay can confidently be applied to clinical pharmacokinetic studies aiming at exploring the DDIs between these cardiovascular drugs and co-administered ARTs in PLWH.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2019.121733.

Declaration of Competing Interest

None.

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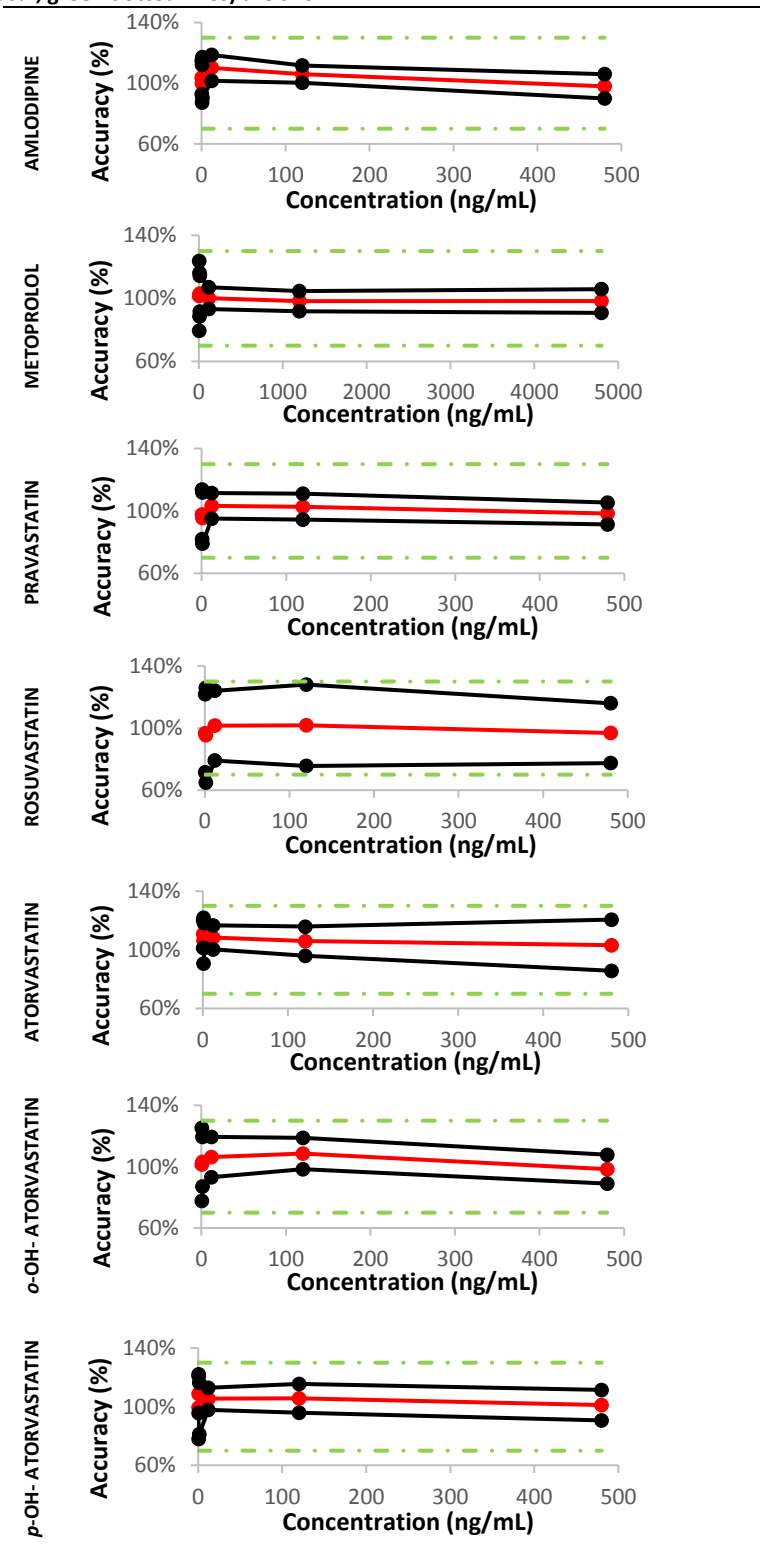
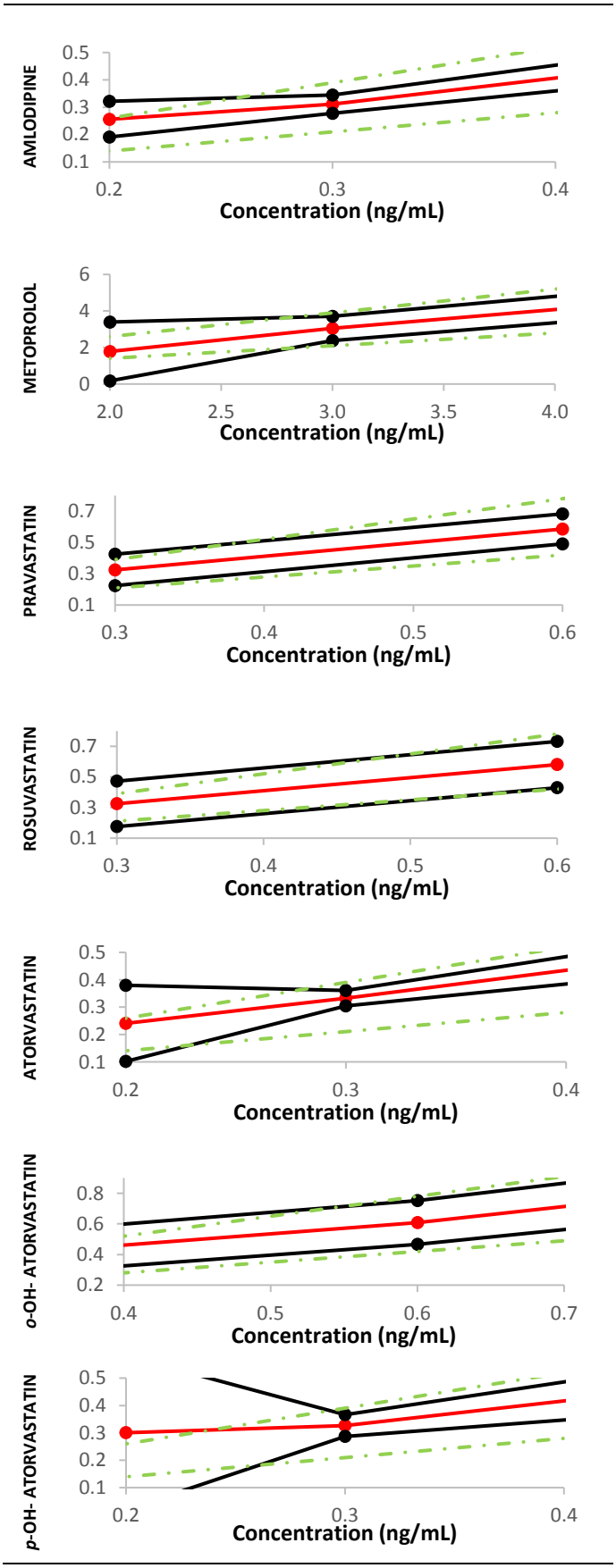
Supplementary file 1: Accuracy profiles over the validated domain in human plasma of the five comedications and the two metabolites. Trueness (red solid line), upper and lower β-expectation tolerance intervals (β = 80%) (black solid lines) and acceptance limits (λ = ± 30%, green dotted lines) are show.



Supplementary file 2: Focus on lowest validation samples reported in the absolute accuracy profiles in order to determine LLOQs. Absolute accuracy is defined as the relative accuracy multiplied by the nominal value of the validation sample and is represented in y-axis. Trueness (red solid line), upper and lower β-expectation tolerance intervals (β = 80%) (black solid lines) and acceptance limits (λ = ± 30%), green, dotted lines) are shown.



III.3. Development and validation of a multiplex UHPLC-MS/MS assay with stable isotopic internal standards for the monitoring of plasma concentrations of the antiretroviral drugs bictegravir, cabotegravir, doravirine and rilpivirine in people living with HIV

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Development and validation of a multiplex UHPLC-MS/MS assay with stable isotopic internal standards for the monitoring of the plasma concentrations of the antiretroviral drugs bictegravir, cabotegravir, doravirine, and rilpivirine in people living with HIV

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Abstract

The widespread use of highly active antiretroviral treatments has dramatically changed the prognosis of people living with HIV (PLWH). However, such treatments have to be taken lifelong raising issues regarding the maintenance of both therapeutic effectiveness and long-term tolerability. Recently approved or investigational antiretroviral drugs present considerable advantages, allowing once daily oral dosage along with activity against resistant variants (eg, bictegravir and doravirine) and also parenteral intramuscular administration that facilitates treatment adherence (eg, long-acting injectable formulations such as cabotegravir and rilpivirine). Still, there remains a risk of insufficient or exaggerated circulating exposure due to absorption issues, abnormal elimination, drug-drug interactions, and others. In this context, a multiplex ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) bioassay has been developed for the monitoring of plasma levels of bictegravir, cabotegravir, doravirine, and rilpivirine in PLWH. A simple and convenient protein precipitation was performed followed by direct injection of the supernatant into the UHPLC-MS/MS system. The four analytes were eluted in less than 3 minutes using a reversed-phase chromatography method coupled with triple quadrupole mass spectrometry detection. This bioassay was fully validated following international guidelines and achieved good performances in terms of trueness (94.7%-107.5%), repeatability (2.6%-11%), and intermediate precision (3.0%-11.2%) over the clinically relevant concentration ranges (from 30 to 9000 ng/mL for bictegravir, cabotegravir, and doravirine and from 10 to 1800 ng/mL for rilpivirine). This sensitive, accurate, and rapid UHPLC-MS/MS assay is currently applied in our laboratory for routine therapeutic drug monitoring of the oral drugs bictegravir and doravirine and is also intended to be applied for the monitoring of

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cabotegravir/rilpivirine levels in plasma from PLWH receiving once monthly or every 2-month intramuscular injection of these long-acting antiretroviral drugs.

KEYWORDS
antiretroviral therapy, long-acting injectables, pharmacokinetics, therapeutic drug monitoring, UHPLC-MS/MS

1 | INTRODUCTION

Optimal efficacy and good tolerability are key points during the development of antiretroviral (ARV) drugs.[1] Yet, besides therapeutic effectiveness and drug safety profile, long-term adherence is required to achieve viral suppression.[2,3] The development of long-acting injectable (LAI) formulations can overcome the adherence issue[4] by maintaining effective plasma concentrations over months. Thus, LAI has the potential to improve adherence thereby preventing drug resistance. In addition, LAI can improve patients' privacy and reduce social stigmas associated with daily intake of ARV drugs. It has been stated that about as much as 50% to 70% of people living with HIV (PLWH) would be interested in LAI formulations when available.[5]

Cabotegravir and rilpivirine are the first two drugs of LAI formulation, currently in final phase of clinical development.[6,7] Cabotegravir is a potent HIV integrase strand transfer inhibitor (INSTI),[8] while rilpivirine is non-nucleoside HIV reverse transcriptase inhibitor (NNRTI). Long plasma half-life of both substances made them good candidates for the development of LAI formulations administered monthly[9,10] or every 2 months.[11] In addition to HIV treatment, LAI-ARV drugs are also investigated separately in the indication of pre-exposure prophylaxis (PreP). Whether used for treatment or prevention, important pharmacokinetic variability was shown following intramuscular injection of cabotegravir and rilpivirine in clinical trials.[9,10,12-14] These clinical studies have generally included carefully selected PLWH, who may not reflect the complex situation in a real-life clinical setting. In particular, drug-drug interactions (DDIs) are likely to occur,[15] also with LAI-ARV drugs, and we have at present very limited information on their actual clinical importance, prompting the monitoring of ARV plasma levels when new comedications at risk of DDIs are introduced in patients on LAI-ARV drugs. Besides, inter-subject variability may be more pronounced particularly in special population (ie, underweight or obese patients, hepatic or renal impairment, aging, or pregnancy).

In addition to these novel injectable formulations, ARV developments are also focused on improving the safety and tolerability profile. The last-generation ARV drugs bictegravir (a potent unboosted INSTI[16]) and doravirine (a next-generation NNRTI[17]) represent attractive oral therapeutic options because of their improved tolerability profiles. Both bictegravir and doravirine are substrates of CYP3A4 and can consequently be victims of DDIs. However, there is currently a lack of data concerning the magnitude of DDIs with these novel ARV drugs. Yet, in the next few years, most PLWH in middle- and high-income countries will switch to one of these last-generation ARV therapies, either oral or LAI formulations.

The availability of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) methodologies for the determination of ARV concentrations in human plasma is a key aspect for drug pharmacokinetic studies and therapeutic drug monitoring (TDM) in patients. Several assays have been previously developed for the measurement of rilpivirine as oral formulation.[18-20] To the best of our knowledge, only two LC-MS/MS assays have been published for the quantification of bictegravir in human plasma.[21,22] In addition, although cabotegravir and doravirine plasma concentrations have been determined in several studies,[23,24] no publication has been dedicated to the development and the validation of such LC-MS/MS methodologies.

In this article, we aimed at developing and validating a simple and fast multiplex assay by ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) for the simultaneous determination of the latest generation ARV drugs bictegravir, cabotegravir, doravirine, and rilpivirine in human plasma.

2 | MATERIAL AND METHODS

2.1 | Chemical and reagents

Bictegravir (purity ≥98%) and cabotegravir (≥98%) were obtained from Alsachim (Strasbourg, France), while doravirine (98%) and rilpivirine (98.5%) were purchased from Toronto Research Chemical (Toronto, Canada). Chemical structures are depicted in Figure 1. Their stable isotopically labelled internal standards (ISs) (ie, [$^{13}C_3{}^2H_2{}^{15}N$]-bictegravir [purity ≥98%], [$^{13}C_3{}^2H_5$]-cabotegravir [≥95%], [$^{13}C_6$]-doravirine [≥95%], and [$^{13}C_6$]-rilpivirine [99.3%]), were obtained from Alsachim. In addition, cabotegravir O-β-D-glucuronide (purity 95%) was purchased from Alsachim.

All solvents (ie, acetonitrile [ACN], methanol [MeOH], and formic acid [FA] [98%-100%]) were of analytical grade and were obtained from Merck (Darmstadt, Germany). Dimethylsulfoxide (DMSO, 99.5%) was purchased from Alfa Aesar (Kandel, Germany). Ultrapure water was supplied by a Milli-Q UF-Plus apparatus (Millipore Corp, Burlington, MA, USA).

Human blank plasma samples used for method development and validation, as well as for the preparation of calibration samples and quality controls (QCs), were obtained according to institutional ethical standard from citrated blood from patients with *polycythemia vera* who underwent regular phlebotomy at the Center of Transfusion Medicine, Lausanne University Hospital, Lausanne, Switzerland, by centrifugation (1970*g*, ie, 3000 rpm, 10 min, +4°C, Hettich model Rotanta 460RF centrifuge) or from TCS Bioscience (Buckingham, UK).

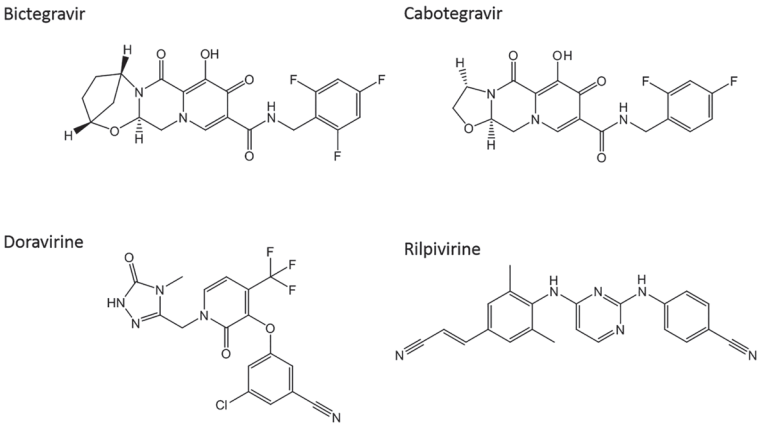

FIGURE 1 Chemical structures of the analyzed antiretroviral drugs

2.2 | Stock solutions preparation

Each analyte was weighed and dissolved in the required volume of solvent. Stock solutions of bictegravir (1 or 5 mg/mL), cabotegravir (1 mg/mL), and doravirine (2 mg/mL) were prepared in DMSO. Rilpivirine powder was dissolved in a mixture of DMSO:MeOH 1:1 to obtain the final concentration of 0.5 mg/mL. These stock solutions were stored at −20°C for bictegravir, cabotegravir, and doravirine. The stock solution of rilpivirine was stored at +4°C, as currently done for the routine monitoring of rilpivirine plasma concentrations in the framework of our TDM service.[18]

One working solution (WS) at 100 μg/mL for bictegravir, cabotegravir, and doravirine and 20 μg/mL for rilpivirine was prepared in a mixture of H_2O:DMSO (3:1) for calibration standards. Another WS for validation standards was independently prepared in the same solvent, at the following concentrations: 90 μg/mL for all analytes except for rilpivirine (18 μg/mL). Spiking solutions for calibration and validation samples were prepared at the appropriate concentrations by sequentially diluting the WSs in a mixture of H_2O:DMSO (3:1).

Stock solutions of each IS were prepared at 1 mg/mL in DMSO (isotopically labelled bictegravir and cabotegravir) or MeOH (isotopically labelled rilpivirine and doravirine) and stored at −20°C. An IS-WS was prepared at 250 ng/mL for all analytes, except for rilpivirine (50 ng/mL) by mixing the required volumes of the four IS stock solutions with a mixture of MeOH:ACN 1:1.

All solutions were stored at −20°C.

2.3 | Calibration and validation standards preparation

Spiked plasma was obtained by diluting tenfold the spiking solutions (100 μL) with blank plasma (900 μL). The total added volume was ≤10% of the biological sample volume to follow the recommendations for bioanalytical method validation.[25,26] Nine concentration levels (k) of calibration samples were prepared each validation day (n = 3) at the following concentrations: 10 000, 5000, 2500, 1000, 500, 250, 125, 50, and 25 ng/mL, except for rilpivirine with fivefold lower concentrations (ie, 2000, 1000, 500, 200, 100, 50, 25, 10, and 5 ng/mL), with respect to the values commonly observed in clinical practice.[14,27,28] Eight validation standards were prepared at the following concentrations: 9000, 4500, 1500, 600, 300, 150, 60, and 30 ng/mL, except for rilpivirine, having fivefold lower values. For each analyte, accurate determination of lower limits of quantifications (LLOQs) relied on the use of one validation sample at the estimated LLOQ and one at twofold to threefold LLOQ.

2.4 | Plasma pre-treatment procedure

Protein precipitation was operated by adding a 300-μL volume of the IS-WS to 100 μL of calibration, validation, or patient plasma samples. The mixture was vortexed and centrifuged at 18 620*g* (14 000 rpm) at +4°C for 10 minutes with a benchtop centrifuge (Benchtop Mikro 220R centrifuge, Hettich, Bäch, Switzerland). Three hundred microliters of the supernatant were directly transferred into an HPLC vial with insert.

2.5 | UHPLC-MS/MS instrumentation

UHPLC-MS/MS analyses were conducted using an Ultimate 3000 Rapid Separation (RS) LC system (Thermo Fisher Scientific, San Jose, CA, USA) composed of an Ultimate 3000 RS column compartment, an RS autosampler, and an RS binary pump. Chromatographic separation was carried out with a Xselect HSS T3 analytical column from Waters (Milford, MA, USA) with 3.5-μm particle size and dimensions of 2.1 × 75 mm. The UHPLC system was coupled with a TSQ Quantis triple quadrupole mass spectrometer from Thermo Fisher Scientific, equipped with an OptaMax NG ion source used in electrospray ionization (H-ESI) mode. Data acquisition, treatment, and instrument control were performed using the XCalibur software version 4.1.31.9 and Chromeleon version DCMS link (ThermoFisher Scientific).

2.6 | Analytic conditions

The mobile phases (ie, H_2O + 0.1% FA (A) and ACN + 0.1% FA (B)) were delivered at a flow rate of 300 μL/min, following this multistep gradient: first, linear gradient from 40% to 60% B in 3 minutes, up to 95% B in 0.2 minutes, followed by an isocratic stage at 95% B for 0.8 minutes. Then, solvent B was reduced to 40% (initial conditions) in 0.1 minute, followed by a re-equilibration step up to 5 minutes (total

analysis time). Samples were stored at +5°C in the autosampler, and the injection volume was 7 μL.

Polarity switching capability enabled ESI positive (spray voltage 3900 V) and negative (spray voltage 3400 V) analysis in the same sample injection. ESI source parameters were optimized as follows: the ion transfer tube and vaporizer temperatures at 300°C and 150°C, respectively; sheath, auxiliary, and sweep gas flow rates at 45, 25, and 0 (arbitrary units), respectively. The first (Q1) and third (Q3) quadrupoles operated with a mass resolution of 1.2 Da (ie, *m/z* 1.2 full width at half maximum, FWHM). The cycle time was 0.2 seconds. The pressure of the collision gas (argon) in the second quadrupole (Q2) was set at 1.5 mTorr.

2.7 | Validation procedure

2.7.1 | Selectivity

The selectivity of the method was evaluated by analyzing blank human plasma from 10 different donors processed with pure ACN:MeOH 1:1. Cross-talk interferences were then established by injecting a high concentration calibration sample processed with pure ACN:MeOH 1:1 (no ISs) and a blank plasma processed with the IS-WS. Finally, the injection of blank solvent (ACN:MeOH 1:1) or blank plasma extract immediately after a high calibration sample processed with IS-WS allowed the assessment of carryover effect.

In addition, since the INSTIs bictegravir and cabotegravir are metabolized by UDP-glucuronosyltransferase (UGT) to glucuronoconjugated metabolites, the separations between drug and their metabolites were investigated. This was particularly important to ascertain that these INSTIs and their respective glucuronides do not coelute that would give spuriously high drug levels because of the in-source dissociation of glucuronide to parent compound during the ionization step, such as previously reported for raltegravir.[29,30] Cabotegravir glucuronide was provided by Alsachim, whereas bictegravir glucuronide was not available at the time of the current development. MS/MS transition of cabotegravir glucuronide was assessed by direct infusion into the MS detector. Bictegravir glucuronide MS/MS transition was empirically reckoned, considering that bictegravir was the main fragment obtained from bictegravir glucuronide. The selectivity of the method regarding glucuronides was evaluating by injecting a plasma sample containing cabotegravir and cabotegravir glucuronide processed with blank MeOH:ACN (1:1) and a plasma sample from an HIV-infected patient receiving bictegravir.

2.7.2 | Matrix effect, extraction recovery, and process efficiency

Qualitative evaluation of matrix effect

The method proposed by Bonfiglio et al[31] allowed the evaluation of the potential impact of endogenous compounds on ionization process. A solution of analytes in MeOH (1100 ng/mL for bictegravir, cabotegravir, and doravirine and 200 ng/mL for rilpivirine) and ISs (200 ng/mL for all IS except for [$^{13}C_6$]-rilpivirine at 50 ng/mL) was continuously infused postcolumn, while seven different blank plasma extracts processed with pure ACN:MeOH 1:1 were injected into the UHPLC-MS/MS system. Each MS/MS transition was visually examined to check for any alteration (suppression or enhancement) at the analytes' retention times.

Quantitative assessment of matrix effect, extraction recovery, and process efficiency

Matrix effects (MEs), extraction recoveries (ERs), and process efficiencies (PEs) were quantitatively evaluated following Matuszeswski's approach.[32] Low (60 ng/mL for bictegravir, cabotegravir, and doravirine and 12 ng/mL for rilpivirine), medium (600 ng/mL for bictegravir, cabotegravir, and doravirine and 120 ng/mL for rilpivirine), and high (6000 ng/mL for bictegravir, cabotegravir, and doravirine and 1200 ng/mL for rilpivirine) concentrations were considered. Three sets of samples at the three concentration levels were prepared as follows: (A) three neat solutions (H_2O) with analytes and ISs; (B) seven postextraction spiked blank plasma in duplicate; (C) seven pre-extraction spiked blank plasma in duplicate. IS normalization was considered by using ratio of analyte peak areas to the corresponding IS peak area to calculate the following parameters: IS-normalized matrix effects (IS-nMEs) as B/A (in %), IS-normalized extraction recoveries (IS-nERs) as C/B (in %), and IS-normalized process efficiencies (IS-nPEs) as C/A (in %).

Relative standard deviation (RSD) of slopes from linear regressions estimated at L, M, and H concentrations were also calculated. An LC-MS/MS method is considered devoid of significant ME if RSD value is <4%.

2.7.3 | Trueness, precision, accuracy profiles, limits of quantification, and linearity

Trueness and precision of the method were assessed over three different days. Several regression models were fitted to adequately describe the response concentration profile. The selection of the best calibration model was based on the estimations of trueness and precision, the narrowest β-expectation tolerance interval, and the lowest LLOQ.[33]

Concentrations of the validation standards were back-calculated with the daily calibration curve. The trueness (systematic error) was defined as the percentage of deviation between the calculated concentrations of validation standards and the nominal value. The precision (random error) was estimated by two components: the repeatability (intraday variances) and intermediate precision (intraday and interday variances).[34-36] Precision parameters were reported as RSD at each concentration level.[33] The total error encompassed both systematic and random errors and was evaluated using accuracy profiles. β-expectation tolerance intervals represent the concentration range where β% of future results is expected to lie.[37-39] Using data obtained during the validation phase, this approach allows to confidently predict the future results that will be obtained during the routine use of the method. Based on the absolute accuracy profiles, LLOQ was graphically interpolated as the lowest concentration for which the β-expectation tolerance interval crosses the acceptance limits (±30%).[25,26,40]

Finally, the capacity of the method to give quantitative results proportional to nominal concentrations was evaluated by ordinary least square regression on the plot representing back-calculated concentrations vs nominal concentrations. This defines the linearity of trueness and was assessed each day of validation.

2.7.4 | Measurement uncertainty

An analytical result should also be reported with respect to its measurement uncertainty (MU). MU was evaluated by the type A estimation method, based on experimental measurements. Feinberg et al demonstrated that the β-expectation tolerance interval is directly related to the MU.[41] The accuracy profile validation methodology enables the estimation of MU without any additional experiments.[42] MU can be derived from the data collected during the validation phase, by fixing the β value at 0.95. Continuous models were developed in order to obtain values of MU as a function of the concentration of the analytes. Several models were tested to identify the one that fitted the data best, by visual inspection of the uncertainty profiles. Ordinary least squares regression was used to estimate the coefficients of the uncertainty function. This methodology allows easy calculation of the MU at any concentration within the validation domain. All calculations were performed using Excel.

2.7.5 | Stability studies

Stability studies included bench- and long-term stabilities. The stability of plasma at room temperature (RT) and in the fridge (+4°C) up to 48 hours was evaluated. In addition, stability after three freeze/thaw cycles was assessed by thawing frozen samples at RT for 1 hour and refreezing them during 1 hour, three times in a row. Furthermore, plasma samples were submitted to thermal viro-inactivation process (60 min at +60°C in a water bath) since this procedure has been shown to efficiently inactivate HIV particles present in the samples.[43,44] Finally, medium stability was evaluated with plasma samples frozen at −20°C and −80°C during 6 weeks. Analyses were performed in triplicate. The mean of the concentrations obtained after each stability study were compared with the mean concentration of samples prepared at time 0.

2.8 | Patients samples

Blood samples were collected from PLWH at the request of physicians during their usual follow-up visits. In the frame of the hospital routine TDM program for ARV drugs, TDM was performed rather liberally, being particularly recommended in case of suspicion of altered pharmacokinetics (eg, DDIs or impaired hepatic/renal functions) or to evaluate short-term adherence to oral ARV drugs. Blood samples were collected in EDTA-Monovettes. The preanalytical sample preparation was performed in our laboratory by centrifuging the Monovettes, transferring plasma into propylene tubes in class II biohazard hoods using standard biosafety precautions (gloves and others) and storing samples at −20°C until batch analyses.

3 | RESULTS

3.1 | Analytical method development

The optimization of the LC-MS/MS assay aimed at improving sensitivity while minimizing runtime. First, standard solutions of each analyte at 5 μg/mL in MeOH were directly infused into the MS detector in order to select optimal MS/MS, as reported in Table 1. LC-MS/MS transitions for bictegravir and rilpivirine differed from reported values.

TABLE 1 MS/MS parameters and typical retention times of the four ARV drugs and their respective stable isotope–labelled ISs

Compound	ESI polarity (+/−)	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Collision Energy (V)	Typical Retention Time, min
Bictegravir	−	448.4	286.2	25	2.16
	−		301.0	25	
[^{13}C,^{2}H$_2$,^{15}N]-Bictegravir	−	452.2	203.0	35	2.13
	−		287.1	25	
Cabotegravir	−	404.2	305.1	26	1.74
	−		374.1	22	
[^{13}C,^{2}H$_5$]-Cabotegravir	−	410.2	311.1	27	1.72
	−		380.0	23	
Doravirine	+	426.1	111.9	24	2.35
	+		315.1	18	
[^{13}C$_6$]-Doravirine	+	432.5	320.8	10	2.35
	+		416.4	11	
Rilpivirine	−	365.1	142.1	30	1.41
[^{13}C$_6$]-Rilpivirine	−	371.2	148.1	31	1.41
	−		329.3	25	

Abbreviations: ARV, antiretroviral; IS, internal standard; MS/MS, tandem mass spectrometry.

In the only published bioassays for bictegravir, mass spectrometer operated in ESI positive mode, while in our study, bictegravir sensitivity was higher in the negative mode.[21,22] In most of published methods for the quantification of rilpivirine, MS/MS transition was 367/195 in the positive mode.[18,20,45] However, the infusion of a rilpivirine solution into the MS detector revealed a higher sensitivity in the negative mode. This was certainly due to the fact that one main fragment with high intensity was observed in ESI− whereas multiple fragments with shared intensities were present after fragmentation in ESI+. Nevertheless, transition 367/195 in the positive mode was tested during method development, and the lower sensitivity was confirmed. Therefore, transition 365/142 in the negative mode was finally retained for the quantification of rilpivirine in this bioanalytical assay. Since no LC-MS/MS method had been yet reported for the determination of

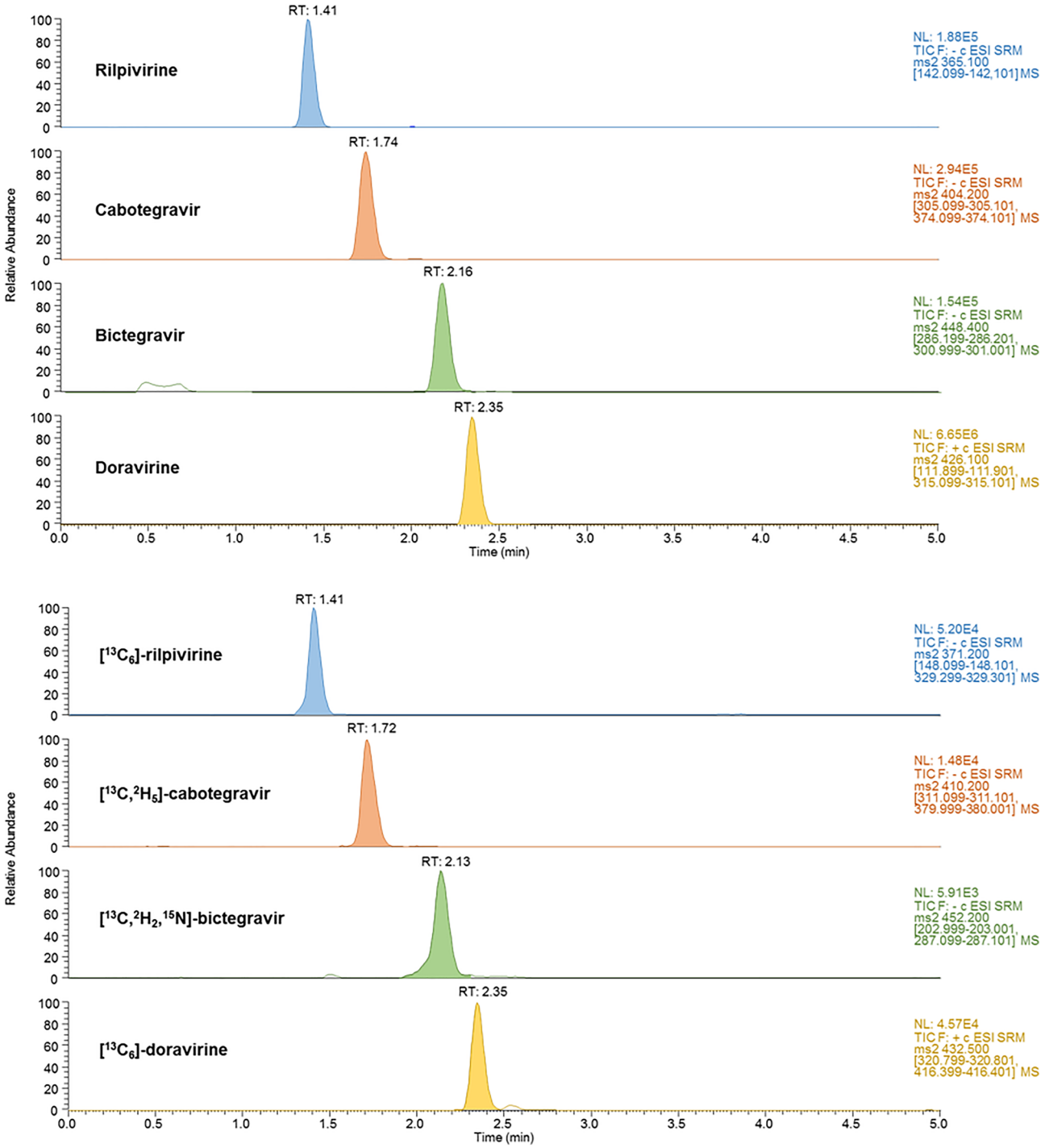

FIGURE 2 Ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) separation of a calibration sample containing the four antiretroviral drugs, at the concentration of 10 000 ng/mL for all the analytes except for rilpivirine (2000 ng/mL). Calibration sample was prepared as described in Section 2 [Colour figure can be viewed at wileyonlinelibrary.com]

cabotegravir and doravirine, no comparison between transitions could be made.

Concerning the chromatographic part of the method, analytical efforts have been made to achieve satisfactory separation and peak shape, in order to accurately quantify each analyte. For that purpose, conventional mobile (ie, H_2O + 0.1% FA and ACN + 0.1% FA) and stationary (Xselect HSS T3 column) phases were shown to be suitable. Mobile-phase gradient program was optimized to adequately separate each analyte in a minimal runtime. Sample preparation was limited to a convenient and fast protein precipitation, which was considered sufficient to accurately quantify the range of concentrations commonly observed in clinical practice. Sensitivity was compared between different protein precipitation solvents, and a mixture of MeOH:ACN 1:1 was selected instead of MeOH or

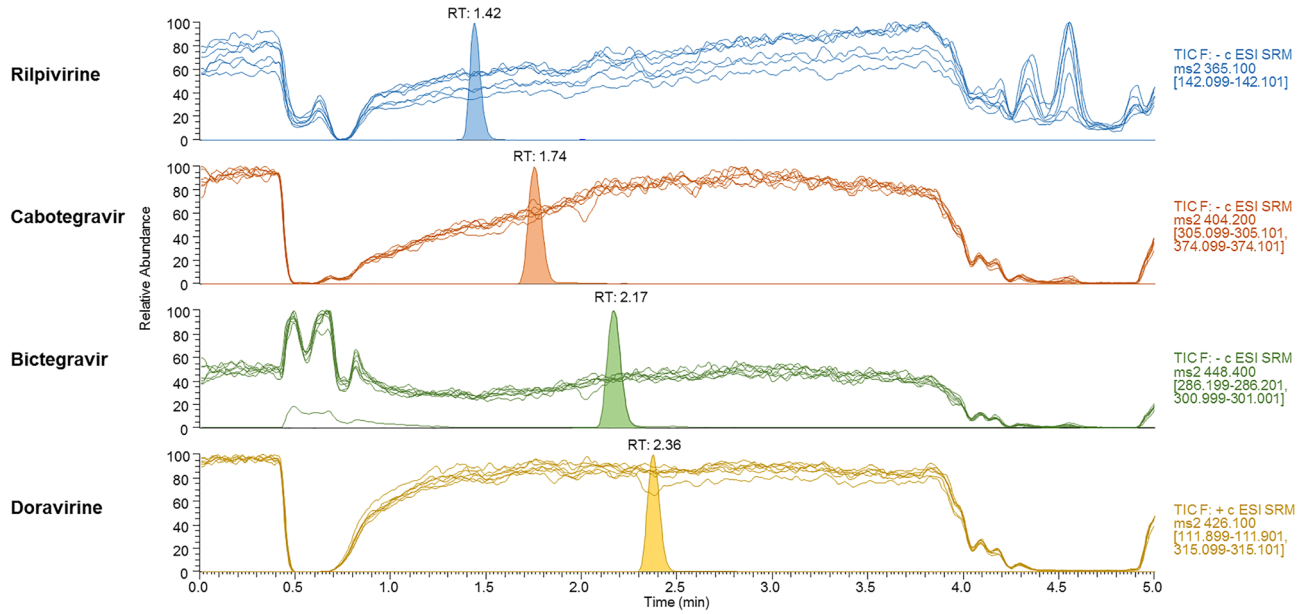

FIGURE 3 Qualitative assessment of matrix effect. Overlaid ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) profiles obtained from seven blank plasma extracts during postcolumn infusion of a solution containing the four analytes, as described in Section 2.7.2. Chromatographic peaks obtained during experiments were superimposed for interpretation [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Internal standard–normalized matrix effect (IS-nME), extraction recovery (IS-nER), and process efficiency (IS-nPE) in human plasma

		IS-nME	IS-nER	IS-nPE
Compound	**QC Level[a]**	**% (RSD)**	**% (RSD)**	**% (RSD)**
Bictegravir/[^{13}C,^{2}H$_2$,^{15}N]-Bictegravir	Low	0 (6)	−6 (6)	−6 (7)
	Middle	3 (3)	−5 (3)	−2 (2)
	High	−5 (3)	−8 (4)	−12 (3)
Cabotegravir/[^{13}C, ^{2}H$_5$]-Cabotegravir	Low	5 (4)	−6 (6)	−1 (5)
	Middle	−1 (3)	−6 (4)	−6 (3)
	High	−6 (3)	−7 (3)	−12 (3)
Doravirine/[^{13}C$_6$]-Doravirine	Low	3 (5)	−11 (5)	−8 (3)
	Middle	4 (3)	−8 (3)	−5 (2)
	High	−4 (2)	−10 (3)	−13 (3)
Rilpivirine/[^{13}C$_6$]-Rilpivirine	Low	12 (4)	−15 (10)	−5 (8)
	Middle	9 (5)	−4 (8)	5 (3)
	High	−6 (3)	−11 (6)	−16 (4)

Abbreviation: RSD, relative standard deviation.

[a]Low concentrations are defined as 60 ng/mL (12 ng/mL for rilpivirine), middle concentrations are defined as 600 ng/mL (120 ng/mL for rilpivirine), and high concentrations are defined as 6000 ng/mL (1200 ng/mL for rilpivirine).

ACN. Different injection volumes of pretreated samples ranging from 3 to 10 µL were tested, and a volume of 7 µL was finally chosen as the best compromise between suitable sensitivity and satisfactory peak shape. As shown in Figure 2, an adequate separation of the four analytes was achieved in less than 3 minutes, with satisfactory peak shapes.

The MS part of the analytical assay was optimized (as reported in Section 2.6) by choosing the appropriate ESI source parameters to improve sensitivity while minimizing background noise. Finally, IS concentrations were selected to obtain satisfying IS-normalized response functions, by avoiding variability due to low IS concentrations and by circumventing a significant contribution of IS signal to analyte signal in case of excessive IS concentrations.

3.2 | Validation of the method

3.2.1 | Selectivity and carryover

The good selectivity of the chromatographic method was demonstrated with the absence of interference at the retention times of the four analytes when analyzing human blank plasma from 10 different sources.

The injections of a blank plasma processed with IS-WS or the highest calibration standard processed with MeOH:ACN (1:1) did not reveal any significant signal on the analytes or IS transitions, respectively, demonstrating the absence of cross talks.

The additional experiments regarding the selectivity with the glucurono-conjugated metabolites demonstrated that retention times

TABLE 3 Trueness, repeatability, and intermediate precision in human plasma over the validated range

			Precision		
Compound	Concentration, ng/mL	Trueness, %	Repeatability, %	Intermediate Precision, %	Relative Uncertainty, %
Bictegravir	30	106.2	9.6	9.6	27.2
	60	107.5	6.2	6.2	17.6
	150	103.1	7.8	7.8	22.0
	300	97.8	3.4	3.8	11.3
	600	96.6	6.0	6.0	15.2
	1500	97.0	4.0	4.1	11.6
	4500	96.5	4.3	4.3	12.1
	9000	101.6	6.5	6.5	18.4
Cabotegravir	30	94.7	11.0	11.2	41.4
	60	101.3	6.7	11.1	41.4
	150	97.7	5.6	8.5	29.8
	300	101.5	4.0	6.6	24.1
	600	99.8	2.6	3.0	9.0
	1500	103.9	4.3	4.3	12.2
	4500	97.1	3.5	3.5	10.0
	9000	97.7	3.5	3.5	9.9
Doravirine	30	104.0	4.0	4.0	11.2
	60	102.2	2.8	3.7	12.3
	150	100.8	3.4	3.4	9.5
	300	100.7	3.2	3.5	10.3
	600	100.2	3.0	4.3	14.8
	1500	102.2	3.6	4.4	14.0
	4500	97.9	3.3	3.3	9.3
	9000	99.4	3.1	3.3	9.6
Rilpivirine	12	100.9	7.6	7.6	21.4
	30	107.5	5.4	6.2	18.8
	60	100.7	6.9	6.9	19.4
	120	101.5	4.3	4.3	12.1
	300	106.3	4.5	4.8	14.0
	900	101.1	3.7	3.9	11.3
	1800	100.1	3.7	3.9	11.1

for cabotegravir glucuronide and bictegravir glucuronide were 0.83 and 0.89 minutes, respectively. In consequence, they were adequately separated from their respective parent drug, cabotegravir and bictegravir, eluting at 1.74 and 2.16 minutes, respectively.

Carryover was considered satisfactory since the chromatogram of blank matrix samples or MeOH directly injected after the highest calibration standard was devoid of analytes' and IS traces.

3.2.2 | ME, ER, and PE

As shown in Figure 3, no major interferences (ie, ion suppression or enhancement) were observed at analytes' retention times. This result supports the suitability of the chromatographic method, preventing an impact of endogenous plasma components on the ionization process of the four analytes and ISs.

Quantitative results of the assessment of IS-nME, IS-nER, and is-nPE are summarized in Table 2. The IS-nME of the analytes was considered satisfactory and varied from −6% to 12%, while RSD values were lower than 6%. Regarding IS-nER and IS-nPE, acceptable results were observed with values ranging from −15% to 4% and −16% to 5%, respectively, with RSD lower than 10%. Overall, matrix-matched calibration along with the use of isotopically labelled IS was found to adequately limit MEs issues.

The lack of significant ME was corroborated by the standard line slopes approach, with RSD values of 2.0%, 1.7%, 2.3%, and 1.8% for bictegravir, cabotegravir, doravirine, and rilpivirine, respectively.

3.2.3 | Trueness, precision, and accuracy profile

Analyte/IS peak area ratios were plotted vs analyte concentrations to obtain response functions. The quadratic log-log regression model provided the best description of the response-concentration profile in terms of determination coefficient and back-calculated calibration samples (±15% and ±20% at expected LLOQ) and was finally retained for each compound. For each series, plasma levels of the validation standards were then calculated using the calibration curves. The validated calibration ranges varied from 25 to 10 000 ng/mL for bictegravir, cabotegravir, and doravirine and from 10 to 2000 ng/mL for rilpivirine. Trueness (94.7% to 107.5%), repeatability (2.6% to 11.0%), and intermediate precision (3.0% to 11.2%) were appropriate for quantifying plasma levels of the four ARV drugs of interest (Table 3).

A β value of 80% was chosen for the establishment of β-expectation tolerance intervals, representing the fraction of future results that would be expected to fall within the obtained tolerance intervals during routine application of the method.[46] As demonstrated in Figure 4, accuracy profiles obtained for each compound lie within the acceptance limits of ±30% for biological samples.[26]

Since the β-expectation tolerance interval of bictegravir, cabotegravir, and doravirine does not cross the acceptance limits of ±30%, the LLOQ was defined as the concentration of the lowest validation sample (ie, 30 ng/mL). Considering an accuracy of ±30% (total error), the lowest concentration measurable in human plasma (LLOQ) was 10 ng/mL for rilpivirine.

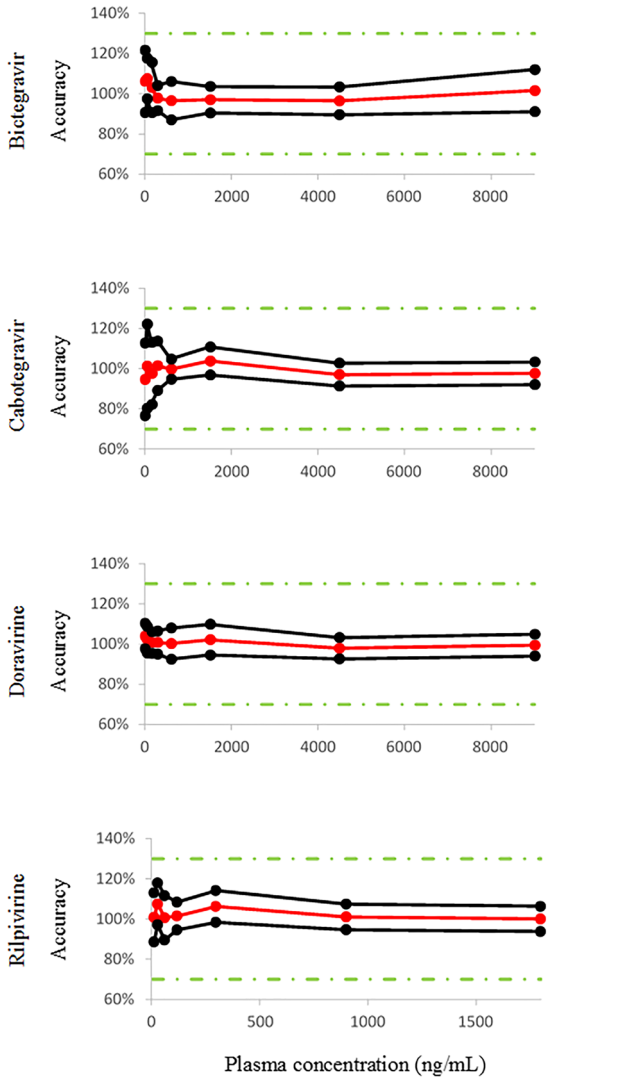

FIGURE 4 Accuracy profiles over the validated domain in human plasma of the five comedications and the two metabolites. Trueness (red solid line), upper and lower β-expectation tolerance intervals (β = 80%) (black solid lines) and acceptance limits (λ = ±30%, green dotted lines) are shown [Colour figure can be viewed at wileyonlinelibrary.com]

Linearity was considered satisfactory since slopes and intercepts ranged from 0.96 to 1.03 and −75.4 to 40.2, respectively. In addition, determination coefficient (R^2) were all higher than .99.

3.2.4 | Measurement uncertainty

The absolute uncertainty vs concentration profiles were best described by polynomial (bictegravir, cabotegravir, and doravirine) and power regression models (rilpivirine).

The relative uncertainty of each compound at each validation levels is shown in Table 3. With a confidence level of 95%, the unknown true value located at maximum ±27.2%, ±41.4%, ±14.8%, and ±21.4% around the measured result for bictegravir, cabotegravir, doravirine, and rilpivirine, respectively. Table 3 also demonstrates that the relative uncertainty is higher at the lowest concentrations of the validation domain.

3.2.5 | Stability studies

Stability of analytes in plasma is reported in Table 4. Results demonstrated that analytes did not significantly degrade after storage of plasma samples at room temperature and +4°C for up to 48 hours. In addition, no significant alteration of plasma concentrations was observed after three consecutive freeze-thaw cycles (variation < ±15%). The thermal viro-inactivation process had no significant influence on analytes concentrations, with variations comprised between −6.5% and 10.6%. Finally, medium-term stability studies showed no significant influence of degradation after 6 weeks of freezing at −20°C and −80°C.

3.3 | Clinical applications

The proposed LC-MS/MS assay has been applied to patient's samples obtained for clinical purposes, within the framework of our TDM service. A typical chromatographic profile of a plasma from an HIV-infected patient receiving bictegravir 50 mg once daily is shown in Figure 5A. Plasma sample was collected 17.25 hours after the last

TABLE 4 Stability studies

Compound	QC Concentration (ng/mL)	Room Temperature for 24 h	Room Temperature for 48 h	+4°C for 24 h	+4°C for 48 h	After 3 Consecutive Freeze-thaw Cycles	Thermal Viro-inactivation[a]	−2°C During 6 weeks	−80°C During 6 weeks
Bictegravir	60	−8.0	−10.4	−4.8	−9.3	−10.5	−6.3	−8.2	−11
	600	6.7	11.4	3.8	−4.3	−0.9	−2.6	8.3	11.9
	6000	5.1	9.6	4.9	0.7	−5.9	10.4	10.0	1.0
Cabotegravir	60	−5.4	−9.5	−10.3	−5.9	−12.9	−6.4	3.1	12.9
	600	2.8	5.0	10.1	5.0	8.2	9.8	14.4	13.6
	6000	6.2	6.8	6.3	7.3	6.4	7.1	12.5	13.4
Doravirine	60	−7.0	−6.2	−2.6	−6.4	−7.6	3.2	−8.9	−5.0
	600	−2.1	0.0	1.9	3.7	2.2	7.1	8.0	9.6
	6000	−3.0	3.9	4.0	0.0	0.5	2.7	−0.7	1.9
Rilpivirine	12	8.9	14.5	12.9	11.9	−2.1	10.5	−11.4	−5.3
	120	11.3	9.4	8.8	10.7	2.9	7.2	10.8	11.9
	1200	2.4	8.8	7.9	6.4	−0.3	1.9	5.4	2.1

Note. Data are reported as deviations (%) from concentration measured at t_0.
[a]60 min at 60°C in a water bath.

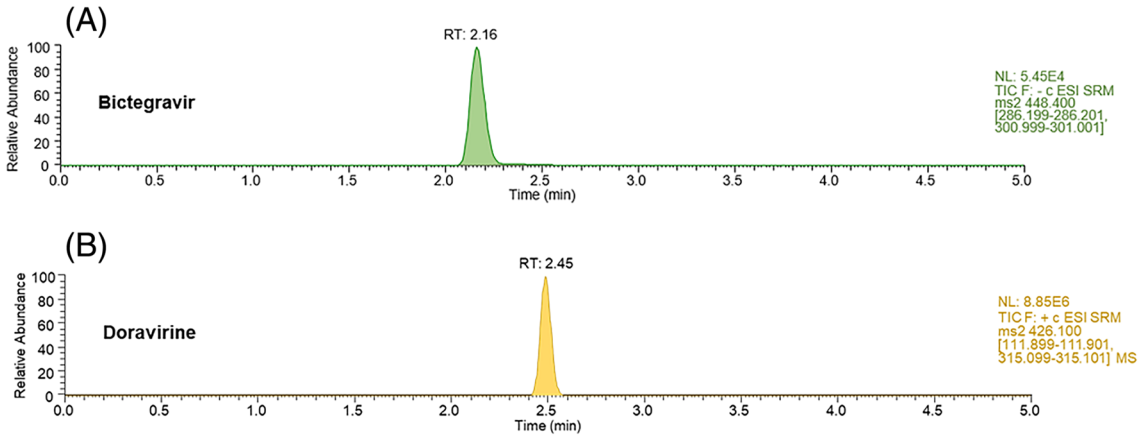

FIGURE 5 Chromatographic profile of a plasma from one HIV-infected individual receiving bictegravir 50 mg once daily (A) and from another patient receiving doravirine 100 mg once daily (B) [Colour figure can be viewed at wileyonlinelibrary.com]

drug intake, and plasma concentration of bictegravir was 3351 ± 340 ng/mL.

At present, 32 plasma samples from PLWH receiving bictegravir have been collected in our TDM service. The interpretation of these plasma concentrations was made possible thanks to the availability of a population pharmacokinetic model summarized in the European Public Assessment Report.[47,48] Using the Tucuxi software[49,50] developed by our service, the pharmacokinetic profile of bictegravir at steady state was simulated over the dosing interval, exploiting the reported intraindividual and interindividual variabilities. The 32 plasma concentrations determined using the proposed LC-MS/MS assay were compared with the simulated population pharmacokinetic profile to ascertain the expectedness of the result. As shown in Figure 6, 59% (n = 19) and 94% (n = 30) of the measured bictegravir plasma concentrations lied into the 50% and 95% prediction interval, respectively. This result demonstrates the ability of our LC-MS/MS methodology to quantify bictegravir and to replicate the manufacturer's findings regarding the rather large variability of plasma concentrations commonly observed in clinical practice.

In addition, the chromatographic profile of an HIV-infected individual receiving doravirine 100 mg once daily is shown in Figure 5B. Doravirine plasma concentration measured in this patient was 1139 ± 124 ng/mL, 15 hours after the last drug intake.

Finally, the combination of LAI cabotegravir/rilpivirine, injected monthly, has been demonstrated as effective as the daily, oral, three-drug regimen in maintaining HIV virus suppression throughout a 48-week period.[9,10,51] The LAI cabotegravir/rilpivirine formulation (Cabenuva) is therefore currently being reviewed by the Food and Drug Administration. Once used in the clinical setting, it is anticipated that physicians will be asking for cabotegravir and rilpivirine levels measurement in patients on LAI cabotegravir/rilpivirine, for the monitoring of their plasma drug exposure in special clinical situations such as the initiation of treatments for inaugural diseases with definite risk of DDIs (tuberculosis, epilepsy, HCV infection, or cancer). Finally, although no clear correlation has been established between cabotegravir and rilpivirine plasma concentrations and the emergence of resistance during the phase 2 study LATTE-2,[14] the management of failure of ARV drug remains crucial.[52] This bioanalytical assay offers clinicians the possibility to closely monitor the plasma levels of cabotegravir and rilpivirine in the special instances where LAI-ARV drug needs to be stopped and switched to oral intake of ARV drugs.

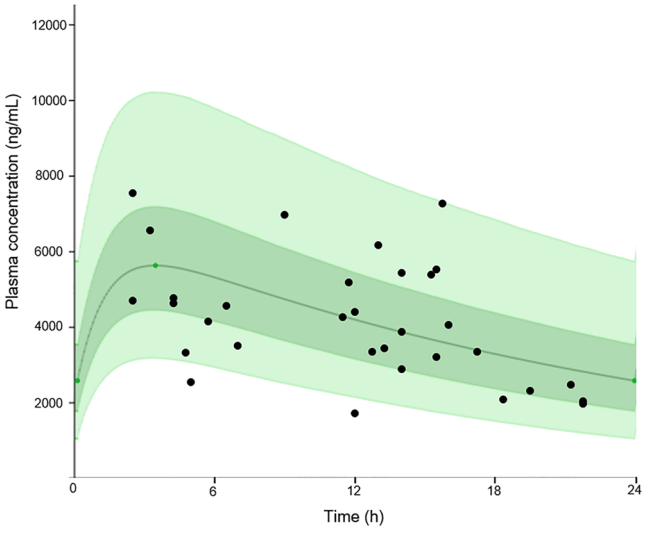

FIGURE 6 Steady-state pharmacokinetic profile of bictegravir when administered at 50 mg once daily. Continuous green line represents the population median prediction. Dark green and light green shaded area represent the 50% and 90% prediction interval, respectively. Bictegravir plasma concentrations obtained from our therapeutic drug monitoring (TDM) service in patients included in the Swiss HIV Cohort Study has been superimposed (black points) [Colour figure can be viewed at wileyonlinelibrary.com]

4 | CONCLUSION

A sensitive and selective LC-MS/MS assay was developed and validated, enabling the simultaneous quantification in human plasma of four newly approved ARV agents, or ARV drugs at the latest phase of their development. Validation performances met international recommendations for bioanalytical assay and were achieved over a large validation domain that covers the plasma concentrations commonly observed in clinical practice. The method could be easily implemented for both clinical and research purposes. Our assay thus provides important information on the plasma levels of these latest generation ARV drugs in PLWH patients and constitutes a useful TDM tool for ascertaining that they are always exposed to suitable systemic drug exposure in the various clinical situations that do occur in the real-life conditions.

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CONFLICT OF INTEREST

No conflicts of interest to declare. M.C., outside of this study, has received through his institution research grant from ViiV, Gilead, and offered expert testimony for Abbvie, MSD, Gilead, and Sandoz. H.F.G., outside of this study, received unrestricted research grants from Gilead and Roche; fees for data and safety monitoring board membership from Merck; and consulting/advisory board membership fees from Merck, ViiV, and Gilead.

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CHAPTER IV: POPULATION PHARMACOKINETIC ANALYSES

Chapter IV in the thesis context

The analytical method for the measurement of the most prescribed comedications in PLWH, or those with the highest risk of DDIs, once fully validated, was instrumental to conduct several clinical pharmacokinetics studies within the frame of the SHCS. The quantification of the magnitude of DDIs with ARVs drugs in the real-life situation (*i.e.* outside the stringent frame of controlled trials) has never been studied before and is therefore of definite interest for helping healthcare providers to manage appropriately DDIs that might result in drug toxicity or insufficient clinical response. The first section of this chapter focused on DDIs between ARV drugs and a central nervous system drug, since this therapeutic class is highly represented in both young and older PLWH. The following sections constitute direct applications of the developed methodology for the determination of cardiovascular drugs in plasma reported in chapter II.

In the first part of this chapter, pharmacokinetics of escitalopram was described for the first time in PLWH using population pharmacokinetic modelling. The influence of different covariates on drug concentrations in plasma were tested, with a focus on DDIs.

The second study describes the population pharmacokinetic profile of amlodipine in a real clinical setting. Since co-administration of amlodipine and boosted protease inhibitors have never been studied, the recommended half dosage when both drugs are coadministered is solely based on theoretical considerations and on DDI studies with other CYP3A4 inhibitors. In addition, no studies have reported the effect of coadministration of non-nucleoside reverse transcriptase inhibitors (which are known CYP3A4 inducers) on amlodipine disposition.

In the third study, population pharmacokinetics of atorvastatin and *o*-OH-atorvastatin (the major active metabolite) were described. The development of the model constituted a challenge because of the very high between-subject variability. Indeed, atorvastatin and its metabolite are substrates of CYP3A4 and of several drug transporters influenced by pharmacogenetic traits, and atorvastatin undergoes extensive first-pass and presystemic metabolism. We have therefore examined for the first time in the real life setting the influence of CYP3A4 and transporters inhibitors or inducers on atorvastatin and *o*-OH-atorvastatin plasma exposures.

The fourth study describes a population pharmacokinetic-pharmacodynamic (PK/PD) model that was developed for rosuvastatin plasma concentrations and non-HDL-cholesterol levels. Rosuvastatin PK/PD analyses have never been performed in a real clinical setting despite the fact that is highly prescribed in PLWH because of its lower potential for DDIs with ARV agents and its higher efficacy compared to other statins. PK/PD analyses of rosuvastatin are ongoing and still need to be refined.

Finally, the last part of this chapter is a project performed in collaboration with another PhD student of our service, which aims at evaluating the impact of various clinical factors and DDIs on dolutegravir

disposition. Dolutegravir population pharmacokinetic profiles were derived based on data gathered from routine blood concentrations measurements within the frame of TDM in our hospital in non-selected patients, most of them taking part of SHCS.

For each of these models (*i.e.* escitalopram, amlodipine, atorvastatin, rosuvastatin and dolutegravir), model-based simulations were performed to compare drug disposition between ARV regimens or perpetrators of DDIs.

Own contribution: Clinical protocols writing and organisation. Samples collection, initial samples analyses by mass spectrometry, data management, population pharmacokinetic and pharmacodynamic modeling, analysis and interpretation of the data. Writing of the manuscript. Dolutegravir manuscript: help with pharmacokinetic analysis and interpretation of the data.

IV.1. Escitalopram population pharmacokinetics in people living with HIV and in the psychiatric population: drug-drug interactions and probability of target attainment.

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ORIGINAL ARTICLE

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Escitalopram population pharmacokinetics in people living with human immunodeficiency virus and in the psychiatric population: Drug–drug interactions and probability of target attainment

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Aims: The aims of this study were to characterize escitalopram pharmacokinetic profile, to identify factors influencing drug exposure, notably drug–drug interactions with antiretrovirals, and to simulate expected exposure under standard dosage regimen.

Methods: A population pharmacokinetic analysis was performed using NONMEM. A total of 159 plasma concentration measurements were obtained from 39 human immunodeficiency virus (HIV)-infected and 71 uninfected psychiatric patients. The influence of age, weight, sex, HIV and psychiatric cohorts, racemic citalopram treatment, and comedications on oral clearance was examined. Simulations served to calculate the percentage of patients expected to be under- or over-exposed, considering established therapeutic targets (15–80 ng/mL).

Results: A 1-compartment model with first-order absorption and elimination described the data adequately. The average escitalopram clearance and volume of distribution were 23.1 L/h (interindividual variability 51%), and 920 L, respectively. Escitalopram disposition did not differ between HIV-infected and uninfected patients, and was not affected by antiretroviral treatments. Coadministration of at least 1 proton-pump inhibitor (CYP2C19 inhibitor) modestly influenced escitalopram elimination (clearance decreased by 19%), with limited clinical relevance. Model-based simulations showed that, under a standard regimen of 10 mg once daily, a significant proportion of patients (56%) might be under-exposed.

Conclusion: The variability in escitalopram disposition is large and poorly explained by demographic, clinical and environmental covariates, thus suggesting a role for dosage individualization based on therapeutic drug monitoring in case of poor clinical response. Escitalopram disposition is modestly impacted by comedications and therefore no a priori dosage adjustments are needed in patients receiving antiretroviral treatments, including boosted regimens.

KEYWORDS
pharmacokinetics, drug interactions < pharmacokinetics, HIV/AIDS < infectious diseases, NONMEM < pharmacodynamics

1 | INTRODUCTION

It is widely recognized that the rate of depression is higher in people living with human immunodeficiency virus (PLWH) than in the general population. The frequency of major depressive disorder is roughly 2 times higher in PLWH than in human immunodeficiency virus (HIV)-negative subjects, and up to 40–60% of HIV-individuals can report depressive symptoms.[1-3] As depression can play a key role in the treatment of HIV infection, affecting treatment adherence and thus efficacy, depressive symptoms deserve particular attention in this population.[4,5] Among antidepressants, selective serotonin reuptake inhibitors are agents of choice in PLWH, like in other psychiatric patients, due to their good efficacy, safety, and tolerability.[6]

Escitalopram is the pharmacologically active S-enantiomer of the racemic selective serotonin reuptake inhibitor citalopram (*rac*-citalopram).[7] The in vitro inhibition of serotonin uptake by escitalopram and its metabolite S-demethylcitalopram is 167 and 7 times more potent, respectively, than by the corresponding R-enantiomers.[7] The disposition of escitalopram administered as 20 mg tablets was reported to be similar to 40 mg of *rac*-citalopram, confirming the bioequivalence of both forms.[8,9] Although enantiospecificities related to their clearances were notified,[10,11] both enantiomers are metabolized by the same cytochrome P450 isoenzymes (CYP): **CYP2C19** (37%), **CYP3A4** (35%), and **CYP2D6** (28%).[12] These metabolic pathways are at risk of drug–drug interactions (DDIs), especially with antiretroviral treatments (ARTs), which are among the therapeutic agents with the highest potential for DDIs.

Current consensus guidelines for therapeutic drug monitoring (TDM) in neuropsychopharmacology recommend escitalopram trough plasma concentrations from 15 to 80 ng/mL as therapeutic reference range.[13] The target of 15 ng/mL was defined as the minimal plasma concentration needed to reach a serotonin transporter occupancy of 80%, required to achieve therapeutic efficacy.[14,15] The upper limit arises from the summary of product characteristics stating that escitalopram trough plasma concentrations expected under a dosage of 10 mg are 20–125 nmol/L (6.5–40 ng/mL). Considering linear pharmacokinetics, the expected concentration at the maximal dose of 20 mg once daily would be 40–250 nmol/L (13–80 ng/mL).[11] While data concerning the relationship between adverse events and escitalopram plasma concentrations are lacking, *rac*-citalopram and escitalopram demonstrate a dose-dependent QTc prolongation.[16-19] They are among antidepressants with the highest risk of torsade de pointes.[20,21] Overexposure could thus yield to an increased risk of QT prolongation and arrhythmias.[22]

The aims of this observational study were: (i) to develop a population pharmacokinetic model for escitalopram in PLWH and uninfected psychiatric individuals; (ii) to identify sources of variability that could influence drug exposure, and notably to evaluate DDIs involving antiretroviral agents; and (iii) to simulate expected exposures under standard dosage regimen and compare them with the established therapeutic reference range.

What is already known about this subject

- Escitalopram pharmacokinetics has been previously investigated in several cohorts, reporting large interindividual variability, which could imply subtherapeutic exposure in some patients.
- Drug–drug interactions are likely to occur in human immunodeficiency virus- infected or psychiatric patients but, due to multiple elimination pathways of escitalopram, they have been reported to be of limited clinical relevance.

What this study adds

- Escitalopram disposition is modestly impacted by comedications and no a priori dosage adjustments are needed in patients treated with antiretroviral treatments including boosted regimens.
- Model-based simulations show that, under a standard dose of 10 mg once daily, 56% of patients might present escitalopram trough concentrations below the established therapeutic range of 15–80 ng/mL.

2 | METHODS

2.1 | Study population

In the framework of a Swiss HIV Cohort Study (SHCS) project (www.shcs.ch), we have launched a comprehensive analysis of relevant DDIs between ARTs and comedications frequently used. The study involves the systematic collection of samples along with the record of information for both HIV and non-HIV comedications during SHCS biannual follow-up visits in the HIV clinics at the University Hospitals of Lausanne and Basel. Escitalopram concentrations from 39 ART treated PLWH and receiving either *rac*-citalopram (n = 25 plasma concentrations) or escitalopram (n = 25 plasma concentrations), collected between January and December 2017, were included in the analysis. In addition, data from 71 uninfected psychiatric consenting patients were obtained from an ongoing pharmacogenetic study (PsyMetab, described elsewhere[23]) or from patients included in the clinical

follow-up (PsyClin) at the University Hospital of Lausanne. Psychiatric data were collected between June 2010 and December 2017 (PsyMetab) and between June 2010 and December 2015 (PsyClin). Due to the noninterventional posthoc analysis study design, no informed consent needed to be requested from the patients who had clinical follow-up (PsyClin). Both studies were approved by the Ethics Committee of the Canton of Vaud.

Exclusion criteria were undetectable escitalopram plasma concentrations, suggestive of nonadherence to treatment, and nonreliable time information about blood sampling or last dose intake (missing information or time after dose intake >30 h).

2.2 | Analytical method

Plasma level measurements were performed in the unit of pharmacogenetics and clinical psychopharmacology at the University Hospital Centre of Lausanne. Plasma samples obtained from PLWH were isolated by centrifugation and stored at −20°C until batch analysis. Plasma *rac*-citalopram levels were determined by a nonenantioselective LC–MS/MS methodology after protein precipitation with acetonitrile. The method showed acceptable repeatability (CV 1.6–4.3%) and intermediate precision (CV 3.7–8.2%). The lower limit of quantification of citalopram was 1 ng/mL.[24]

2.3 | Model-based pharmacokinetics analysis

Escitalopram pharmacokinetics were analysed using the nonlinear mixed effect modelling (NONMEM) program (version 7.4.2, ICON Development Solutions, Ellicott City, MD, USA). According to manufacturer's specifications, the escitalopram dose of patients under *rac*-citalopram was set to half of the parent drug dose. Their plasma escitalopram concentrations were derived using the literature S/R enantiomer ratio of 0.45 of the total *rac*-citalopram concentrations,[25,26] since the analytical method was not enantioselective. This ratio was confirmed by analysing escitalopram and *rac*-citalopram data simultaneously. Log-transformed escitalopram concentration–time data were eventually fitted using the first-order conditional estimation with interaction (FOCEI). PsN v4.2.0 was used for automation of various model development and evaluation methods, Pirana v2.9.2 to structure and document model development and R v3.3.1 (Rstudio v.1.1.423) for data management, statistical analysis and graphical output.[27,28]

2.3.1 | Basic model

To find the model that fitted the escitalopram data best, we tested different structural models including 1- or 2-compartments, with first- or zero-order absorption, and with or without absorption lag time. Escitalopram absorption parameters could not be properly estimated due to the paucity of data within a few hours after drug administration. We thus fixed the absorption rate constant value (k_a) to 0.8 h^{-1}, based on literature data reporting k_a values from 0.4 to 2 h^{-1} and in accordance of T_{max} with the manufacturer's data.[11,29-33]

The interindividual variability of pharmacokinetic parameters was estimated using the relationship $\theta j = \theta \times e^{\eta j}$, where θ_j is the individual parameter value of the j^{th} individual, θ is the geometric average population value, and η_j is a between-subject random effect, assumed to follow a normal distribution with mean zero and variance ω^2. Additive, proportional and mixed error models were tested to select the most appropriate residual error model.

The final base escitalopram model was applied on escitalopram and *rac*-citalopram raw data estimating a fixed effect for the S/R ratio, which was then compared to the literature value. Since the estimated value was similar to the published value, the model developed on escitalopram data was kept for further analyses.

2.3.2 | Covariate model

The impact of continuous (age, weight) and categorical covariates (HIV *vs* psychiatric cohort, difference in formulation (racemic *vs* isomer), sex and comedications) was first assessed graphically. All potential and physiologically plausible relationships were tested following a stepwise insertion/deletion approach. Since infectious diseases can affect drug pharmacokinetics, a dummy dichotomous covariate for HIV or psychiatric patients was used to test their influence on escitalopram apparent clearance (CL/*F*, *F* representing the bioavailability).[34,35] Similarly, an indicator variable was assigned to patients receiving *rac*-citalopram and tested on clearance to detect a potential difference in escitalopram clearance. Several studies demonstrated that the magnitude of DDIs with escitalopram was weak or moderate.[36,37] As strong perpetrators influenced only moderately escitalopram exposure, the effect of weak perpetrators was supposed to be clinically nonsignificant and was therefore not included in the analysis. Since several studies have evaluated the effect of proton-pump inhibitors on escitalopram pharmacokinetics,[38,39] they were included in the analysis as moderate CYP2C19 inhibitors.[40] Comedications including ARTs were classified as moderate CYP2C19 inhibitors (12% of the patients) or inducers (10%), strong CYP3A4 inhibitors (16%) or inducers (2%), strong CYP2D6 inhibitors (2%) as well as p-glycoprotein inhibitors (15%), according to the lists published by the Food and Drug Administration and the University Hospital of Geneva.[41,42] The effect of receiving at least 1 perpetrator was first tested on clearance. Then, we evaluated the effect of the number of inhibitors of the same isoenzyme. Finally, the numbers of CYP inhibitors taken by each patient were added up to evaluate the effect of the inhibition of multiple metabolic pathways on escitalopram clearance. Covariates (continuous variables centred and normalized on their median value and categorical covariates being coded as indicator variables, as 0 or 1) were incorporated in the model, testing linear and nonlinear relationships. Missing values for weight (16% of the observations) were imputed to the population median value.

2.3.3 | Model selection

The criteria considered for model selection were log-likelihood ratio tests based on the reduction of the objective function value (ΔOFV

approximation of a χ^2 distribution for nested models), goodness-of-fit plots, precision of pharmacokinetic parameter estimates, and decrease in between-subject variability. In the base model building and in the forward inclusion of covariates, a decrease in OFV by more than 3.84 ($P < .05$) for 1 additional parameter was considered statistically significant. During the backward deletion step, a covariate was retained in the final multivariate model if its deletion from the full model led to a 7.88-point increase in the objective function ($P < .005$, 1 degree of freedom).

2.3.4 | Model evaluation

The stability and performance of the final population pharmacokinetic model were assessed by the bootstrap method using 2000 sampling with replacement. The final population pharmacokinetic model was fitted repeatedly to the bootstrapped samples and the median and 95% confidence intervals ($CI_{95\%}$) of each parameter were compared with the corresponding parameters obtained with the original dataset. The final model was also validated via prediction-corrected visual predictive check obtained from 1000 simulations of the database using the parameter values of the final population pharmacokinetic model.

2.3.5 | External validation

The established final model was externally validated using 149 additional samples collected from an independent group of 110 psychiatric individuals (among whom 2 were HIV-infected) followed routinely at the University Hospital of Lausanne between April 2008 and December 2015 (PsyClin). Escitalopram plasma concentrations were predicted by fixing the population parameters and the between- and within-subject variability to the final model estimates using post hoc Bayesian forecasting with the MAXEVAL = 0 option in NONMEM. The predictive performance of the model was assessed in terms of bias (mean prediction error $MPE = \exp\left(\frac{\sum Cpred - Cobs}{N}\right) - 1$ and precision (root mean square prediction error, $RMSE = \exp\left(\sqrt{\frac{\sum (Cpred-Cobs)^2}{N}}\right) - 1$ and the associated $CI_{95\%}$, where C_{pred} are log-transformed population and individual predictions, C_{obs} the observed concentrations of the validation group and N corresponds to the number of observations.[43]

2.4 | Simulations of dosage regimens

Simulations were performed for 1000 individuals under several dosage regimens based on the final model estimates, including between- and within-subject variability. Predicted average escitalopram minimal concentration together with predicted 2.5 and 97.5 percentiles were calculated and compared across dosage regimens. The percentage of patients with trough concentrations within the recommended therapeutic interval served for dosage regimen comparisons.[13]

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[44] and are permanently achieved in the Concise Guide to PHARMACOLOGY 2017/2018.[45]

3 | RESULTS

3.1 | Data

The median daily dose was 20 mg (range 5–20 mg) and 20 mg (10–40 mg) for escitalopram and *rac*-citalopram, respectively. Drugs were administered once (QD) or twice daily. A median of 1 sample per patient (range 1–2 for PLWH and 1–7 for uninfected psychiatric patients) of *rac*-citalopram or escitalopram was collected between 0.1 hours and 29 hours after the last dose intake (time after dose). As shown in Figure 1, data from PLWH were collected at unselected times after the last drug intake (38% of the concentrations were trough concentrations [time after dose >20 hours]) while data from psychiatric population were mostly trough concentrations (74%) to allow for TDM interpretation.

For the psychiatric cohort, dosage history was collected as part of the standard follow-up (PsyClin cohort) or within the PsyMetab study. Data about dosage modification or missing doses that occurred during the week before the blood sample of interest were included in the dataset, estimating that the steady state was not reached for these patients. Steady state was assumed for all PLWH due to the lack of dosage history in the HIV cohort.

3.2 | Basic and covariate models

A total of 159 plasma concentrations collected in 110 patients were available for the pharmacokinetic model development. Participant demographic and clinical characteristics of the model building and validation groups are summarized in Table 1. Eleven PLWH (28%) were treated with a protease inhibitor-based regimen and 15% with efavirenz or nevirapine, known to affect the activity of several CYP isoforms.

A 1-compartment model with first order absorption and elimination best described escitalopram pharmacokinetics. Models with zero-order absorption process or lag time absorption did not improve the model compared to first-order absorption, as well as the addition of a second compartment ($\Delta OFV > -3.4$, $P > .07$). Average escitalopram CL/F and volume of distribution (V/F) were 22.5 L/h and 926 L respectively. Between-subject variability was assigned only on CL/F and was estimated to be 53%. The intersubject variability on V/F was very low and the addition of a variance–covariance matrix of the variability resulted in a decrease in the precision of the parameter estimates, so they were not included in the model. In log-scale, an

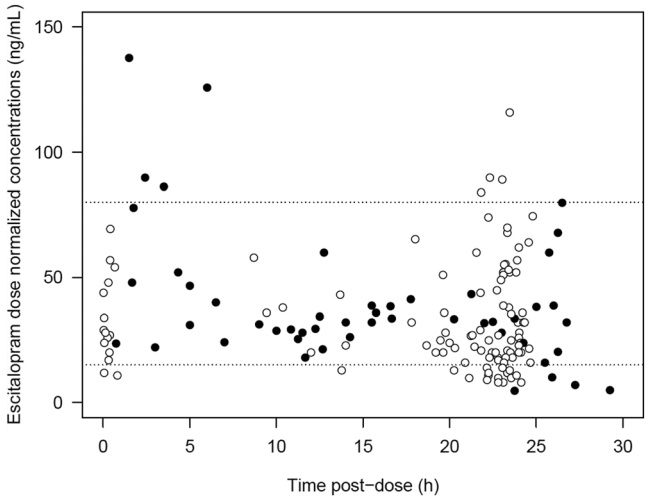

FIGURE 1 Observed escitalopram concentrations *vs* time after dose. Concentrations in people living with human immunodeficiency virus (full circles) and uninfected psychiatric patients (open circles) are shown. Dashed lines represent the therapeutic interval

TABLE 1 Demographic and clinic characteristics of the study populations

Characteristics	*n* (%) or median [IQR]		
	Model building group (*n* = 110)	Model validation group (*n* = 110)	*P*
People living with HIV	39 (36)	2 (2)	<.001
Male sex	55 (50)	68 (62)	.103
Age (years)	48 [36–56]	46 [35–58]	.887
Body weight (kg)	77 [62–85]	69 [56–81]	.041
Missing data	19 (17)	15 (14)	
Comedications			
≥ 1 CYP3A4 strong inhibitor[a]	17 (16)	2 (2)	.001
≥ 1 CYP3A4 moderate inducer[b]	2 (2)	0	.477
≥ 1 CYP2C19 moderate inhibitor[c]	13 (12)	21 (19)	.192
≥ 1 CYP2C19 strong inducer[d]	11 (10)	1 (1)	.008
≥ 1 CYP2D6 strong inhibitor[e]	2 (2)	11 (10)	.022
≥ 1 P-glycoprotein strong inhibitor[f]	16 (15)	9 (8)	.202

[a]atazanavir, cobicistat, darunavir, ritonavir.

[b]efavirenz.

[c]esomeprazole, lansoprazole, omeprazole.

[d]ritonavir.

[e]bupropion, haloperidol, levomepromazine.

[f]cobicistat, haloperidol, lopinavir quetiapine, ritonavir.

Continuous variables were described by their medians and interquartile ranges (IQR) and compared between groups using the Mann–Whitney *U* or Wilcoxon tests. Categorical variables were described by proportions and compared with the χ^2 test.

additive model was selected to describe the residual error, and was estimated at 27%.

When simultaneously analysing escitalopram and *rac*-citalopram rough data, the S/R ratio was estimated at 0.47 (relative standard error 11%). This value was very close to the reported ratio of 0.45 shown by previous studies.[25,26] The development and validation of the model was therefore pursued with the concentrations calculated using the S/R ratio of 0.45.

The coadministration of at least 1 CYP2C19 inhibitor, described by a proportional model, decreased escitalopram clearance by 19% and explained 4% of the original between-subject variability on CL/*F* (ΔOFV = −4.8, *P* = .03). Neither other CYP inhibitors or inducers, nor demographic covariates or the difference in formulation (racemic or isomer) showed any influence on escitalopram pharmacokinetics (ΔOFV > −3.049, *P* > .08). Extent of η-shrinkage was low (8% in the final model). The final model parameters and their precisions are presented in Table 2. Diagnostic plots of the final model showed good fit of the model to the observed data (Figure 2).

3.3 | Model validation

All the 2000 replicates analysed during the bootstrap analysis were included when calculating the bootstrap results. Model reliability was supported by all bootstrap median parameter estimates values contained within the $CI_{95\%}$ (Table 2). All parameters differed by <6% from the population estimates. The prediction-corrected visual predictive check revealed an adequate description of the observed data: the observed concentrations were homogeneously distributed around the 50th percentile of simulated concentrations (Figure 3).

TABLE 2 Parameter estimates of the final escitalopram pharmacokinetic model and bootstrap results.

Parameters	Final escitalopram pharmacokinetic model		Bootstrap (*n* = 2000 samples)	
	Estimate	RSE (%)	Median	$CI_{95\%}$
CL/*F* (L/h)	23.1	6	23.0	20.1–26.2
V/*F* (L)	920	17	903	547–1294
k_a (h^{-1}) FIX	0.8			
$\theta_{CYP2C19\ inh}$	−0.19	45	−0.20	−0.39 to −0.04
$BSV_{CL/F}$ (%)	51	8	49	39–60
Additive residual error (%)	27	18	27	22–33

$CI_{95\%}$; 95% confidence interval; CL/F, apparent clearance; V/F, apparent volume of distribution; k_a, first-order absorption rate constant fixed to 0.8 h^{-1}; $\theta_{CYP2C19\ inh}$, relative influence of CYP2C19 inhibitors on CL/F using a power function; BSV, between-subject variability defined as coefficient of variation (%); RSE, relative standard error defined as SE/estimate, with SE directly retrieved from NONMEM.

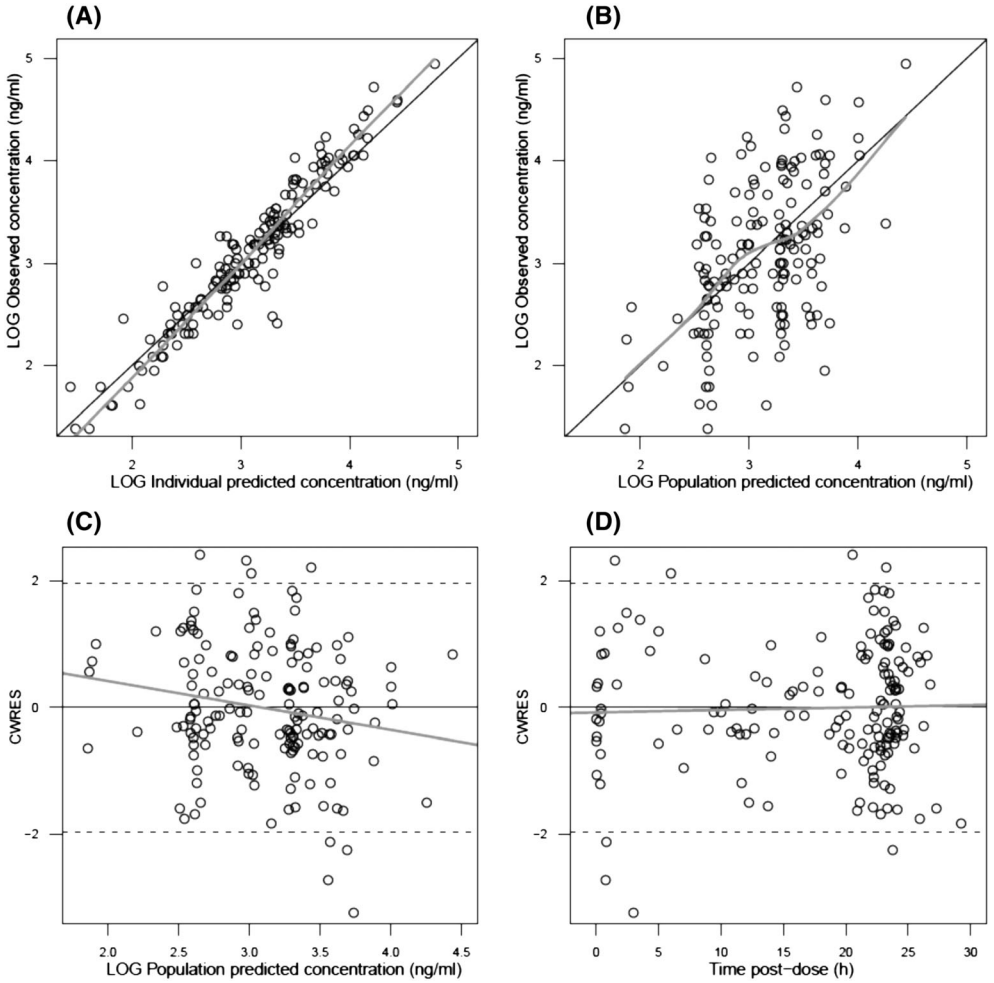

FIGURE 2 Goodness-of-fit plots of escitalopram final population pharmacokinetic model. Loess smooth curves of the ordinate values are printed in grey. (A) Log observed concentrations *vs* log individual predictions; line of identity is printed in black. (B) Log observed concentrations *vs* log population predictions; identity line is printed in black. (C) Conditional weighted residuals (CWRES) *vs* log population predictions; ordinate value zero is printed in black. (D) CWRES *vs* time postdose; ordinate value zero is printed in black

3.4 | External validation

A total of 149 additional escitalopram concentrations from an independent group of psychiatric patients were included in the external model validation. Individual predicted concentrations agreed well with observed concentrations with a nonsignificant bias (MPE) of −1% ($CI_{95\%}$ −4 to 2%). The precision (RMSE) was 21%, comparable to the model residual error.

3.5 | Simulations

We simulated escitalopram steady-state plasma concentrations for 1000 individuals with the recommended dosages from 10 to 20 mg QD, with and without coadministration of CYP2C19 inhibitors (Figure 4). Simulated median escitalopram minimal concentration (95% prediction interval) after 10 and 20 mg daily dose were 13.8 (3.2–43.6) and 27.6 (6.5–87.2) ng/mL, respectively, and 18.1 (4.7–55.1) and 36.2 (9.5–110.2) ng/mL when coadministered with CYP2C19 inhibitors. Accounting for the variability in escitalopram clearance, 56% of patients receiving a standard regimen of 10 mg QD without CYP2C19 inhibitors had predicted trough concentrations below the suggested target of 15 ng/mL,[13] whereas 18% are expected to be below target after 20 mg QD. These proportions would decrease to 38% and 9% in case of coadministration of a CYP2C19 inhibitor, following daily dosages of 10 and 20 mg, respectively. Following a daily dose of 10 mg, none of the patients would present trough concentrations above the upper limit of the therapeutic interval (80 ng/mL). Only a very limited proportion of patients (4 or 8% without or with coadministration of CYP2C19 inhibitors) would exceed it after the 20-mg QD dose.

4 | DISCUSSION

Our study provides a description of the population pharmacokinetic profile of escitalopram in a real patient setting in 2 cohorts of patients at risk of drug–drug interactions.

Our results are in good accordance with previously reported data. Escitalopram clearance and volume of distribution are close to

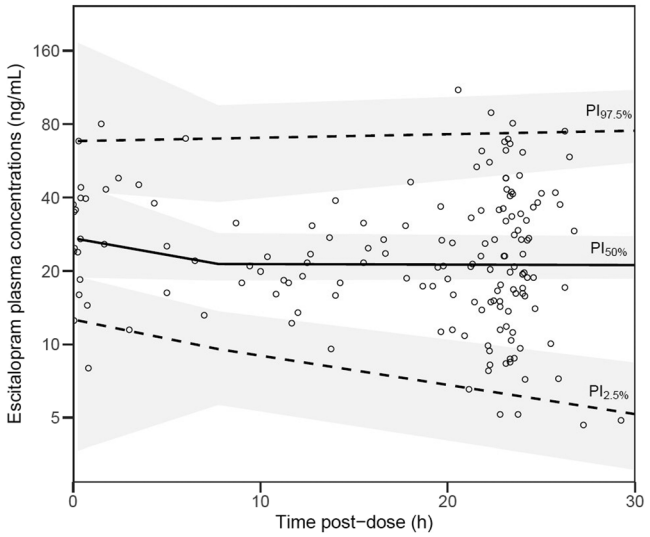

FIGURE 3 Prediction-corrected visual predictive check of escitalopram final model. Open circles represent escitalopram plasma concentrations. The continuous line represents the population median prediction from the final model and the broken lines and shaded areas represent the 95% prediction intervals and corresponding 95% confidential intervals. PI, prediction interval

reported values of 25.5 and 22.1 L/h and of 1020, 947 and 1390 L, respectively.[29-31] Due to the paucity of data available within a few hours after drug administration, the escitalopram absorption phase could not be described. Previously published pharmacokinetic studies reported k_a values varying from 0.8 h^{-1} to 3.6 h^{-1}, but most of them used values close to 0.8 h^{-1}.[29-33] Moreover, this value is in line with the T_{max} of 4.5 hours reported in the summary of product characteristics.[11]

None of the tested demographic covariates appeared to have a significant impact on escitalopram elimination. Escitalopram disposition did not differ between PLWH and uninfected psychiatric patients, indicating that HIV-infection does not affect escitalopram pharmacokinetics. Age was not significantly associated with a decrease in escitalopram elimination. Actually, our results suggested a nonsignificant 14% decrease in escitalopram clearance every 20 years of age, which was less than previous estimations of a decrease by 30–42%.[29,30] This might arise from our narrow age interquartile range (36–56 years) and the absence of extremes ages comparing to other studies, compromising our power to detect an effect of age. An influence of sex on the pharmacokinetics of *rac*-citalopram is controversial in the literature.[29,46-49] A recent study showed that sex seems to affect only the disposition of the R-enantiomer, which could explain the absence of sex effect in our study.[29] Finally, several publications revealed an effect of CYP2C19 genotype on escitalopram exposure,[29,30,50-52] but the lack of genotyping data in our study prevented the replication of these results.

Our results are consistent with earlier findings regarding the risk of DDIs between escitalopram and other treatments. Owing to the contribution of multiple CYPs in the metabolic pathways of escitalopram, impaired activity of any 1 of these isoforms due to DDIs or genetic polymorphism is unlikely to have a large effect on escitalopram clearance, as metabolism can still occur through unaffected routes.[12] The only variable causing a detectable, yet weak decrease in escitalopram clearance was the coadministration of CYP2C19 inhibitors. These comedications were all represented by proton-pump inhibitors. Although several publications already mentioned this interaction, its magnitude varies from weak to moderate.[38,39,53] The decrease of 19% in escitalopram clearance when coadministered with at least 1 CYP2C19 inhibitor found in our study is in good agreement with a prediction based on theoretical considerations provided in the DDI-predictor tool.[54] Moreover, this value is close to the clearance increase reported in extensive or CYP2C19 rapid metabolizers,[29,30] indicating that DDI with CYP2C19 inhibitors and CYP2C19 polymorphism could lead to similar pharmacokinetic consequences.

The risk of QT prolongation and torsade de pointes following escitalopram administration and the reported dose–response relationship with QT prolongation has raised concerns,[55-57] with escitalopram dosage limitations from the European medicines agency and the UK medicines and healthcare products regulatory agency, but not from the Food and Drug Administration.[58-60] Taking into account the large

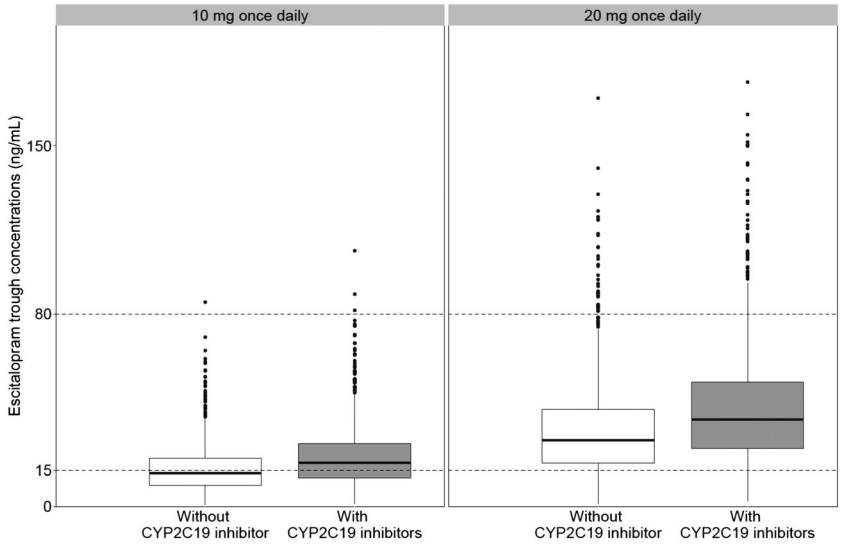

FIGURE 4 Simulations of 2 dosage regimens, without and with CYP2C19 inhibitors. Dashed lines represent the therapeutic interval

between-subject variability in escitalopram exposure, the modest influence of CYP2C19 inhibitors is not expected to put patients at increased risk of QT prolongation. Still, the risk of QT prolongation could be potentiated in case of coadministration with medication presenting the same risk, and notably ARTs such as atazanavir, lopinavir or saquinavir.

A recently published study demonstrated that clinicians tend to overestimate the impact of the inhibitory effect of ritonavir/cobicistat, resulting in the use of too low doses of psychotropic drugs in PLWH due to fear of DDIs.[61] While ARTs are indeed among therapeutic agents with the highest potential for DDIs,[62] they did not appear to affect escitalopram exposure, confirming previous observations.[36,37,63] The absence of effect of CYP3A4 inhibitors on escitalopram clearance is in good agreement with previous data showing a lack of effect of ketoconazole, a strong CYP3A4 inhibitor.[63] Furthermore, no influence of ritonavir, another strong inhibitor of CYP3A4, weak inhibitor of CYP2D6 and potent inducer of CYP2C19, was observed in healthy volunteers.[36] The opposite strong inhibitory/inducing effects on CYP3A4 and CYP2C19 might compensate each other and thereby mitigate the magnitude of DDI. Our evaluation of the inhibition of multiple metabolic pathways did not reveal significant decrease in escitalopram clearance, probably due to the limited number of patients under such conditions. Only 1 PLWH received 2 CYP3A4 inhibitors in combination with 1 CYP2C19 inhibitor and 1 CYP2C19 inducer. His escitalopram clearance was decreased by 45% compared to patients without CYP2C19 inhibitors. This observation suggests that the inhibition of multiple elimination pathways might lead to a clinically relevant increase in escitalopram exposure. Lorenzini *et al*.[64] reported a serotonin syndrome following initiation of ritonavir-boosted darunavir and esomeprazole in a patient treated with escitalopram 10 mg twice daily. Investigations showed that this patient was a poor metabolizer of CYP2D6 and CYP2C19, resulting in a 13-fold increase in escitalopram concentrations. This case report indicates that clinically significant DDIs can occur when most metabolic pathways involved in escitalopram elimination are blocked, and that pharmacogenetics can impact the magnitude of DDIs.[64]

Escitalopram is also a substrate of P-gp in vitro.[65] No significant association was found between escitalopram clearance and coadministration of P-gp inhibitors, in agreement with a previously published study.[65] Although no effect is seen on systemic concentrations, P-gp modulators might still impact on the amount of escitalopram reaching the brain through the blood–brain barrier.

The median trough concentration simulated under 10 mg QD was in good agreement with the summary of product characteristics.[11] Our model predicted that about half of the patients receiving the recommended dose of 10 mg QD could be underexposed to escitalopram, considering the minimal therapeutic target of 15 ng/mL.[13] This proportion decreased to 18% when increasing the dosage to 20 mg QD. This result is in agreement with a study demonstrating that escitalopram doses above 20 mg once daily could be clinically necessary in patients with major depressive disorders.[66] In addition, this may arise from the high proportion of individuals with the rapid metabolizer status in Europe and can not be generalized to the whole population.[67] By contrast, a small proportion of patients is expected to present trough concentrations above the upper limit of 80 ng/mL. However, the relationship between adverse events and escitalopram plasma concentrations is not clearly defined. Therefore, it should be noted that the decision to adjust the dose should be based not only on the attainment of therapeutic concentration targets, but also on the monitoring of clinical and psychiatric status, recognized as key criteria for antidepressant dosage adjustment.

This study suffers from some limitations that must be acknowledged. First, for patients receiving *rac*-citalopram, both enantiomers were administered simultaneously in 1 pill containing the racemic mixture (50/50) and plasma concentrations were analysed using a nonchiral methodology. Escitalopram plasma concentrations were therefore calculated using a S/R ratio of 0.45,[25] constituting a limitation of our study. Although discordances exist concerning the value of S/R ratio, several studies showed a S/R ratio <1, meaning that enantiomers of citalopram may be metabolized at different rates.[10,29,68] The use of a fixed ratio supposes the absence of between-subject variability on *rac*-citalopram enantiospecific metabolism. Our findings supported an S/R ratio of 0.45 when simultaneously analyzing escitalopram and *rac*-citalopram data. Of note, we could not estimate between-subject variability because of few patients receiving *rac*-citalopram. However, in-house studies demonstrated that between-subject variability can affect S/R ratios, with a coefficient of variation of 33%. Proper enantiomers pharmacokinetics comparison would require separate measurement of both R- and S-citalopram. Another limitation is that only 1 sample for escitalopram concentration determination was available in most patients, thus limiting our capacity to well differentiate inter- from intrasubject variability, although η-shrinkage value appeared acceptable. Finally, the weak number of patients receiving several CYP inhibitors prevented the estimation of the effect of the inhibition of multiple pathways on escitalopram pharmacokinetics.

In conclusion, this pharmacokinetic model confirmed that escitalopram exhibits important between-subject variability. Simulations revealed that the standard 10 mg once-daily regimen may lead to trough concentrations below the established therapeutic target of 15 ng/mL, with a risk of suboptimal antidepressant efficacy. Dosage adjustment may benefit from both TDM and monitoring of clinical efficacy and tolerability, taking into account other risks factors for QT prolongation, especially in elderly patients. These results emphasize the importance of TDM under specific conditions, such as therapeutic failure or suspected DDIs, for a proper individualization of dosing regimens. This study finally brings reassuring data concerning the risk of DDIs between escitalopram and others medications, including ARTs.

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COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTORS

P.C. performed the population pharmacokinetic analyses, interpreted the data and drafted the manuscript. M.G. supervised the pouplation pharmacokinetic analyses and reviewed the manuscript. C.C. supervised the population pharmacokinetic work. L.A.D. was the principal investigator of the study proposal and reviewed the manuscript. A.G. contributed to the mathematical evaluation. SA participated in data collection. C.B.E. supervised the bioanalytical data analysis. M.C., C.M., T.B. and C.B.E. brought their clinical expertise to interpret the results and reviewed the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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IV.2. Population pharmacokinetic modelling to quantify the magnitude of drug-drug interactions between amlodipine and antiretroviral drugs.

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IV.2.1. Abstract

Background and objectives

Drug-drug interactions (DDIs) with antiretroviral drugs (ARVs) constitutes an important issue in elderly people living with HIV (PLWH). Amlodipine is a highly prescribed antihypertensive drug metabolized by CYP3A4, thus predisposed to the risk of DDIs. Guidance on the management of DDIs is mostly based on theoretical considerations or derived from coadministration with other CYP3A4 inhibitors. This study aimed at characterizing the magnitude of DDIs between amlodipine and ARV drugs in order to establish dosing recommendations.

Methods

A population pharmacokinetic analysis was developed using non-linear mixed effect modelling (NONMEM) and included 163 amlodipine concentrations from 55 PLWH. Various structural and error models were compared to characterize adequately the concentration-time profile of amlodipine. Demographic and clinical characteristics as well as comedications were tested as potential influential covariates. Model-based simulations were performed to compare amlodipine exposure (*i.e.* area under the curve) between coadministered ARV drugs.

Results

Amlodipine concentration-time profile was best described using a one-compartment model with first-order absorption and a lag-time. Amlodipine apparent clearance was influenced by CYP3A4 inhibitors and efavirenz (CYP3A4 inducer). These covariates explained 48% of the variance on clearance. Model-based simulations revealed that amlodipine area under the curve was increased by 96% when coadministered with CYP3A4 inhibitors while efavirenz decreased drug exposure by 59%.

Conclusion

Coadministered ARV drugs significantly contributed to the variability in amlodipine disposition in PLWH. Clinicians should adjust amlodipine dosage accordingly, by initiating a half-dosage in PLWH receiving ARV with inhibitory properties, whereas amlodipine dose should be doubled when coadministered with efavirenz, with monitoring of clinical response and adverse reactions.

IV.2.2. Introduction

The advent of antiretroviral therapy (ARVs) in the 1990's has revolutionized HIV care, now considered as a manageable chronic condition. Meanwhile, the management of HIV-infection is becoming more challenging, with an ageing HIV-infected population affected by physiological changes and age-related comorbidities. People living with HIV (PLWH) are predisposed to the risk of polypharmacy, thus increasing the burden of drug-drug interactions (DDIs). Indeed, ARV drugs are part of the therapeutic

agents with the higher potential for DDIs due to the inhibition or induction of several cytochromes P450 (CYP) isoforms.

Despite its predisposition to be victim of DDIs with ARV drugs, amlodipine is a calcium channel blocker commonly prescribed in PLWH. It is predominantly metabolized by CYP3A4/5 [1, 2] leading to potential DDIs with strong CYP3A4 inhibitors such as darunavir/ritonavir, or with inducers such as efavirenz. However, the magnitude of DDIs with ARV drugs remained to be explored. Although the simple monitoring of blood pressure allow the evaluation of the clinical response to amlodipine, the knowledge of the magnitude of DDIs could help clinicians to adjust drug dosage regarding the associated ARV drugs. The product information label of amlodipine mentions an increase in amlodipine exposure (+60%) in presence of diltiazem, a CYP3A4 inhibitor [3]. The label also indicates that a more pronounced increase is expected with other strong inhibitors like ritonavir without neither further detail on the magnitude of the interaction nor guidance on how to adjust amlodipine dosage. Moreover, DDIs between amlodipine and several antiviral agents for the treatment of chronic hepatitis C infection or old ARV drug (*e.g.* ritonavir-boosted indinavir, which is no longer prescribed) have been evaluated using non-compartmental analyses [1, 4]. Authors demonstrated a 2-fold increase in amlodipine exposure when coadministered with these drugs, suggesting that a half dosage should be prescribed in individuals receiving such regimens. However, available data on DDIs are mostly collected in healthy young volunteers and therefore may not reflect the complex situation in a real-life clinical setting.

Published population pharmacokinetic (PK) models investigate amlodipine clearance in different populations (*i.e.* healthy volunteers, children, adolescents, patients living in nursing homes) and identified body weight, gender and age as the most important factors beyond pharmacokinetic heterogeneity [5-9]. However, to our knowledge, no population PK models have been developed in PLWH.

The objectives of this study were to develop a population PK model of amlodipine in aging PLWH and to perform model-based simulations to compare amlodipine exposure between concomitantly prescribed ARV regimens, thus allowing the establishment of dosage recommendations.

IV.2.3. Methods

IV.2.3.1. Study design and participants

Plasma samples collected in PLWH from Lausanne and Basel enrolled in our prospective Swiss HIV Cohort study project #815 designed to evaluate clinically significant DDIs between ARVs and frequently prescribed comedications were included in the analysis. In addition, PLWH participating in the pharmacokinetic study NCT03515772 (registered in clinicaltrials.gov) contributed with intensive

sampling. Undetectable amlodipine plasma concentrations, suggestive of non-adherence to treatment, or missing information about drug administration or sampling times or unclear dose schedule were excluded from the analysis. For all PLWH, age, bodyweight, gender, liver function tests (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), albumin), creatinine clearance (calculated with the Cockroft and Gault formula [10]), and comedications (HIV and non-HIV medications) were recorded.

IV.2.3.2. Plasma concentrations determination

All blood samples were collected and centrifuged in EDTA-containing tubes. Plasma samples were stored at −80 °C until batch analysis by an ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) methodology [11]. Plasma samples were subjected to protein precipitation with methanol, followed by evaporation at room temperature under nitrogen of the supernatant. Lower limit of quantification was 0.3 ng/mL, well below trough concentrations commonly observed in clinical practice.

IV.2.3.3. Model-based pharmacokinetic analysis

Structural and error model

A population PK analysis was performed using non-linear mixed effect modelling (NONMEM version 7.3, ICON Development Solutions, Ellicott City, MD, USA) to characterize amlodipine concentration-time profile in PLWH. PsN v4.2.0 was used for automation of various model development and evaluation methods, Pirana v2.9.2 to structure model development and R v3.3.1 (Rstudio v.1.1.423) for data management, statistical analysis and graphical output [12, 13]. One- and two-compartment models were compared while evaluating the absorption phase by several models: zero-, first-order or mixed model absorption, with or without lag-time, or transit compartments models. Between subject variability (BSV) was described by exponential errors following a log-normal distribution with mean zero and variance ω^2. Several error model (*i.e.* proportional, additive and mixt) were tested to describe the residual variability.

Covariate model

First, visual inspection of the correlation between post hoc individual estimates of the PK parameters and the available biologically plausible covariates was carried out. Covariates considered as potentially influent were then sequentially included into the model using a stepwise insertion/deletion approach. ARV drugs were classified as moderate CYP3A4 inhibitors (*i.e.* ritonavir-boosted darunavir, cobicistat-boosted darunavir, ritonavir-boosted atazanavir, cobicistat-boosted elvitegravir) or moderate inducers (*i.e.* efavirenz, etravirine), according to the lists published by the FDA [14] and the paper of Molto *et al*. [15]. The effect of the weaker CYP3A4 inducers nevirapine was also tested. Linear or non-

linear functions were used as appropriate (categorical variables coded as 0 and 1 and continuous variables centered on their median values). Missing values for continuous covariates were imputed to the population median value.

Model selection and parameter estimation

Amlodipine concentration-time profiles were fitted using the first-order conditional estimation (FOCE) method with interaction. Discrimination between hierarchical models was based on the variation on the objective function value (ΔOFV, (−2 log likelihood, approximate Chi-square distribution) using the log-likelihood ratio test. For one additional parameter, a decrease of the ΔOFV exceeding 3.84 (p<0.05) or 6.63 (p<0.01) was considered statistically significant during the model building and backward deletion steps, respectively. Reliability of the results was evaluated using diagnosis plots, along with precision of the estimation of pharmacokinetic parameters and eta-shrinkage.

Model evaluation and assessment

The bootstrap method implemented in PsN was employed to validate the stability and performance of the final population PK model, using 2000 bootstrap sampling with replacement [12]. Median parameter values with their 95% confidence interval ($CI_{95\%}$) generated with the bootstrap method were compared with the original model estimates. The predictive performance of the final pharmacokinetic model was evaluated with the normalized prediction distribution errors (NPDEs). In addition, prediction-corrected visual predictive check (pcVPC) was performed.

Model-based simulations

Amlodipine maximum (C_{max}) and trough concentrations (C_{trough}), along with area under the curve from time 0 to 24h (AUC_{0-24}) were computed in 1000 simulated PLWH per different ARV regimen (CYP3A4 inhibitors, efavirenz or ARVs devoid of interaction potential with amlodipine). Average C_{max}, C_{trough} and AUC_{0-24} between ARV groups were compared using the Mann-Withney test.

IV.2.4. Results

IV.2.4.1. Data

A total of 163 amlodipine concentrations were available from 55 PLWH, eight of whom participated in the PK study with rich sampling and provided 84 concentrations. PLWH in the SHCS#815 project provided a median (range) of one sample (1 to 3) while the median was 11 (8 to 11) for individuals included in the rich PK study. Amlodipine was administered at a dose ranging from 2.5 to 10 mg once daily. Three PLWH had amlodipine at the dosage of 5 mg twice daily. The measured plasma concentrations varied from 0.4 to 70 ng/mL. Since dosage history was not recorded during the studies, steady state was assumed for all PLWH. Characteristics of the study population are presented in table 1.

Table 1: Demographic and clinical characteristics of PLWH included in the model development dataset.

[1]considered as moderate CYP3A4 inducers.

Patients' Characteristics (N=55)	Median [IQR] or n (%)
Age (years)	61 [53-70]
Male sex	41 (75)
Body weight (kg)	79 [71-91]
Missing data	9 (16)
Systolic blood pressure (mmHg)	140 [130-150]
Missing data	9 (16)
Diastolic blood pressure (mmHg)	83 [75-93]
Missing data	9 (16)
ALAT (IU/L)	27 [20-41]
Missing data	11 (20)
ASAT (IU/L)	26 [20-35]
Missing data	11 (20)
Albumin (g/L)	42 [41-44]
Missing data	11 (20)
Creatinine clearance (mL/min)	82 [50-101]
Missing data	17 (31)
Comedications (N=163)	**n (%)**
CYP3A4 inhibitors	
Ritonavir-boosted darunavir	27 (17)
Cobicistat-boosted darunavir	1 (1)
Ritonavir-boosted atazanavir	1 (1)
Cobicistat-boosted elvitegravir	7 (4)
CYP3A4 inducers	
Efavirenz	7 (4)
Others	
Etravirine[1]	18 (11)
Nevirapine[1]	8 (5)
Rilpivirine	2 (1)
Dolutegravir	103 (63)
Raltegravir	8 (5)
Anti-hypertensives agents	137 (84)

IV.2.4.2. Structural, statistical and covariate models

A one-compartment model provided the best fit for amlodipine pharmacokinetics. The addition of an absorption lag-time (ALAG) significantly improved the fit (ΔOFV=-7.39, p=0.007).

BSV was assigned only on CL as the addition of BSV on the other parameters did not improve data description (ΔOFV>-1.84, p>0.17). The population estimates and variability (CV%) of the PK parameters with the base model were as follow: an absorption rate constant (k_a) of 0.66 h^{-1}, an ALAG of 0.86 h, a volume of distribution of 980 L and a clearance of 15.7 L/h (61%).

An additive error model adequately described the intra-patient variability. Univariate analyses revealed an effect of CYP3A4 inhibitors (*i.e.* ritonavir-boosted darunavir, cobicistat-boosted darunavir, ritonavir-boosted atazanavir, cobicistat-boosted elvitegravir, ΔOFV=-20.9, p<0.001) and efavirenz (ΔOFV=-10.8, p=0.001) on CL/F. Our results showed that CL/F is decreased by 49% and increased by 40% in case of coadministration of CYP3A4 inhibitors or efavirenz, respectively. These two covariates explained 48% of the variance on CL/F [16]. In contrast, age, sex, weight, albumin, ASAT, ALAT and creatinine clearance were not associated with amlodipine PK (ΔOFV>-1.1, p>0.29). Finally, coadministration of ritonavir-boosted darunavir in two patients (13 amlodipine concentrations) receiving etravirine prevented the estimation of the effect of etravirine on amlodipine disposition.

IV.2.4.3. Model evaluation

The final model parameter estimates are summarized in table 2, along with their bootstrap estimations. The parameter estimates of the final model lied within the bootstrap $CI_{95\%}$ and differed less than 7% from the median bootstrap parameters except for ka (17%), supporting the reliability of the model. Goodness of fit plots are presented in supplementary material 1. NPDE distribution was not found to significantly differ from a normal distribution. As shown in figure 1, pcVPC confirmed the good predictive performance of the model.

Table 2: Final population parameter estimates of amlodipine with the bootstrap results.

ka: first-order absorption rate constant, ALAG: absorption lag-time, CL: mean apparent amlodipine clearance, V: mean apparent volume of distribution, $CI_{95\%}$ 95% confidence interval, CYP: cytochrome P450, CVs: coefficients of variation, RSEs, relative standard errors defined as SE/estimate and expressed as percentages. CYP3A4 inhibitors included ritonavir-boosted darunavir, cobicistat-boosted darunavir, ritonavir-boosted atazanavir, cobicistat-boosted elvitegravir.

	Final pharmacokinetic model		**Bootstrap** (n=2000 samples)	
Parameters	**Estimate**	**RSE (%)**	**Median**	**CI 95%**
ka (h^{-1})	0.69	25	0.80	0.44-2.15
ALAG (h)	0.87	28	0.90	0.24-2.41
V/*F* (L)	1000	16	985	777-1472
CL/*F* (L/h)	17.0	9	16.9	14.6-20.5
BSV_{CLator} (CV%)	42	19	40	27-59
$\theta_{CYP3A4\ inhibitors}$	-0.49	12	-0.49	-0.60 to -0.34
$\theta_{efavirenz}$	1.40	37	1.46	0.55-3.35
σ_{add} (ng/mL)	2.85	11	2.77	2.17-3.49

Final model: CL/*F*=17.0 x (1-0.49 x CYP3A4inhibitors) x (1+1.40 x efavirenz)

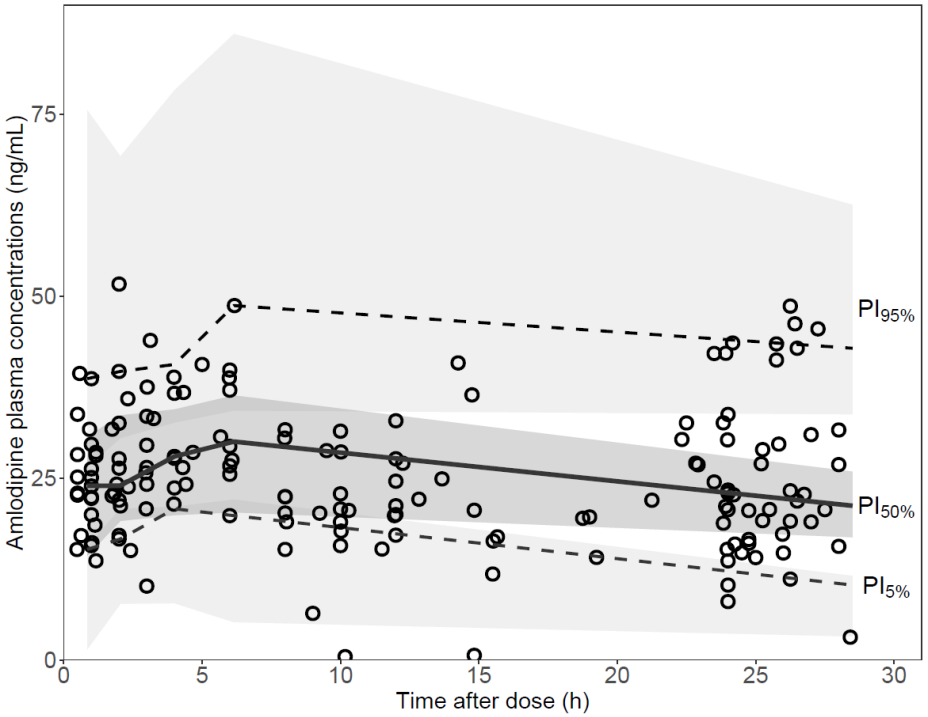

Figure 1: pcVPC of amlodipine final model with amlodipine prediction-corrected concentrations (open circles), median of the observed concentrations (solid line) with 90% prediction interval (dashed lines). Grey fields represent the model-based 90% confidence interval of the simulated median and PI_{90}%.

IV.2.4.4. Model-based simulations

Simulations revealed a 96% increase and a 59% decrease of amlodipine $AUC_{0\text{-}24}$ in patients taking the initial recommended dosage of 5 mg of amlodipine QD with CYP3A4 inhibitors and efavirenz, respectively, with respect to amlodipine at the standard dosage alone.

Figure 2 compares amlodipine concentration-time profiles under the standard posology with alternative amlodipine dosage regimens in presence of CYP3A4 inhibitors and efavirenz. The predicted concentration-time profile of 2.5 mg of amlodipine with CYP3A4 inhibitors almost entirely overlaps with the curve of 5 mg once daily alone (8% and 2% decrease in C_{max} and $AUC_{0\text{-}24,}$ respectively, while C_{trough} was increased by 8% in the alternative *vs* standard regimen, table 3). On the other hand, the dosage of 10 mg QD in presence of efavirenz seems to provide lower exposure (C_{max} increased by 1%, C_{trough} and $AUC_{0\text{-}24}$ decreased by 38 and 17%, respectively, in the alternative *vs* standard regimen, Table 3). Increasing the dose to 15 mg QD in presence of efavirenz would result in a 50% and 25% increase in C_{max} and AUC, respectively, and a 7% decrease in C_{min}.

None of PLWH receiving CYP3A4 inhibitors were concomitantly treated with efavirenz. However, model-based simulations demonstrated that PLWH concomitantly receiving 5 mg of amlodipine with CYP3A4 inhibitors and efavirenz reached amlodipine exposure 18% lower than those receiving 5 mg of amlodipine alone.

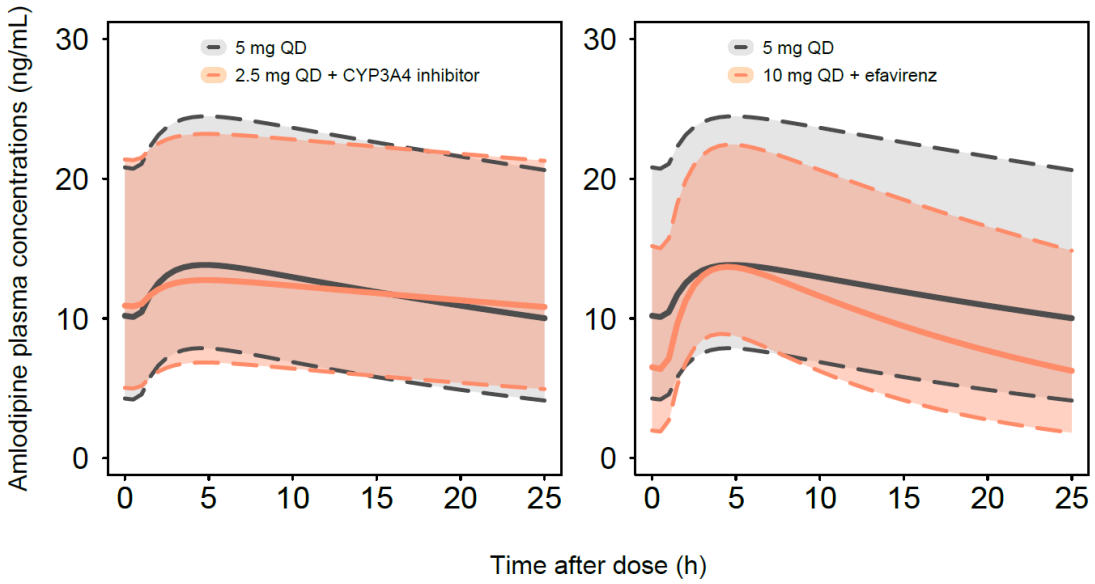

Figure 2: Simulated amlodipine plasma concentrations for dosage regimens of 2.5 mg QD with CYP3A4 inhibitors (*i.e.* ritonavir-boosted darunavir, cobicistat-boosted darunavir, ritonavir-boosted atazanavir, cobicistat-boosted elvitegravir) (left) or 10 mg with efavirenz (right), compared with standard dosage of 5 mg QD. Continuous line represent the population median prediction for standard (grey) and alternative regimens (orange) while shaded areas and dashed lines represent the 90% prediction interval based on 1000 simulated PLWH.

Table 3: Summary of amlodipine PK parameters following several dosage regimens, in presence or absence of CYP3A4 inhibitors or efavirenz, derived from model-based simulations. PK values are presented as median (95% prediction interval).

C_{max}: maximal concentrations, C_{trough}: residual concentrations (24h after the last drug intake), AUC_{0-24}: area under the concentration-time curve from 0 to 24h calculated as dose/CL, GMR: geometric mean ratio compared with the standard regimen of 5 mg QD.

	Standard dosage of 5 mg QD	**2.5 mg QD with CYP3A4 inhibitors**	**10 mg QD with efavirenz**
C_{max} (ng/mL)	13.6 (7.3-29.0)	12.7 (6.2-27.8)	13.7 (8.4-26.4)
GMR		0.92	1.01
C_{trough} (ng/mL)	10.2 (3.6-25.5)	10.9 (4.3-26.0)	6.5 (1.5-19.0)
GMR		1.08	0.62
AUC_{0-24} (ng.h/mL)	290.8 (129.3-658.4)	285.7 (127.0-646.8)	242.3 (107.8-548.7)
GMR		0.98	0.83

IV.2.5. Discussion

This study investigated the magnitude of DDIs between amlodipine and ARV drugs. For this purpose, the effect of the inhibitory or inducing potential of ARVs on amlodipine population pharmacokinetic profile was assessed in Swiss PLWH enrolled in two pharmacokinetic studies. The pharmacokinetic parameters of amlodipine are in good accordance with reported values, while lower apparent clearance in our analysis [5-8]. Several studies reported a 2-compartments model to describe amlodipine concentration-time profile [6, 9, 17]. However, the sampling design of our study with blood samples collected no more than 28h after the last drug intake prevented us to capture adequately the terminal elimination of this drug with such a long half-life [18]. Despite the important BSV, none of the tested demographic covariates showed an influence on the pharmacokinetics of amlodipine. Of note, age was not significantly associated with amlodipine clearance, indicating that dosage adjustment is not required in elderly PLWH. In the present study, we report a 49% decrease in amlodipine clearance in case of coadministration of CYP3A4 inhibitors. This result is in good agreement with a pharmacokinetic study conducted in healthy volonteers, where amlodipine exposure was increased by 90% when coadministered with ritonavir-boosted indinavir [1]. In addition, two physiologically-based pharmacokinetic models reported a 2-fold increase in amlodipine AUC when coadministered with ritonavir [19, 20]. Two studies showed a more pronounced increase in amlodipine exposure when coadministered with anti-HCV agents (2.6-fold increase in amlodipine AUC with ombitasvir/paritaprevir/ritonavir/dasabuvir and 2.8-fold increase with telaprevir). Finally, the DDI-predictor website reports an AUC ratio (AUC in presence of interactor/AUC in absence of interactor) of 2.66 based on several pharmacokinetic studies [21]. While magnitude of DDI between amlodipine and CYP3A4 inhibitors vary between studies, our results supports the proposed 50% reduction in amlodipine dosage in case of coadministration with CYP3A4 inhibitors [22].

Although no plasma concentrations upper threshold has been established for amlodipine, an increase in amlodipine exposure is not devoid of clinical consequences. Indeed, serious adverse events such as severe hypotension, oedema and bradycardia have been described in case reports of PLWH receiving both calcium-channel blockers and ARVs with inhibitory potential [23-26]. This suggests that caution is needed when prescribing in elderly PLWH receiving boosted ARV regimens, also considering pharmacodynamics alterations and presence of comorbidities.

To the best of our knowledge, coadministration of amlodipine and ARVs with inducing properties has not been studied until now. Our results demonstrated a 59% decrease in amlodipine exposure when coadministered with efavirenz. The lack of plasma concentrations from PLWH receiving etravirine prevented the estimation of the effect of etravirine on amlodipine PK. Visually, this effect appeared less pronounced with etravirine than with efavirenz. This is in line with a study demonstrating a

weaker magnitude of DDIs between etravirine and erlotinib or gefitinib (both CYP3A4 substrates) than with efavirenz [26]. However, the comparison of the inducing potential of etravirine and efavirenz is controversial in the literature [19, 26]. Pharmacokinetic-pharmacodynamic relationship has been established, indicating an impact of amlodipine plasma concentrations on antihypertensive effect [6, 8]. Our results indicated that amlodipine dosage should be doubled when coadministered with efavirenz, to reach comparable plasma concentrations to individuals not receiving interacting drugs, and therefore to achieve similar antihypertensive effect. Nevertheless, amlodipine dosage adjustment should be firstly based on the clinical monitoring of blood pressure.

Finally, model-based simulations revealed a 18% decrease in amlodipine exposure when coadministered with both CYP3A4 inhibitors and efavirenz, compared to amlodipine alone. This effect was not considered clinically significant and indicates that the inhibitory effect of boosted ARV drugs is compensated by the inducing effect of efavirenz. Furthermore, one PLWH included in the PK study with rich sampling and receiving both ritonavir-boosted darunavir and etravirine had relatively high amlodipine plasma concentrations. This indicates a predominance of the CYP3A4 inhibitory effect compared to the lower inducing potential of etravirine. This observation is in line with a previously published study demonstrating a 3-fold increase in maraviroc exposure when coadministered with ritonavir-boosted darunavir and etravirine compared to that obtained when maraviroc was administered alone [27].

Limitations of the present work should be acknowledged. First, CYP3A4 inhibitors and efavirenz were the only significant covariates while an effect of age, gender and body weight has been demonstrated in other population pharmacokinetics studies [5, 7, 8]. The sparse sampling approach applied to the majority of data may have been insufficient to detect some covariate effects. In addition, the low number of PLWH receiving each ARV CYP3A4 inhibitor prevented us not only to establish an interaction model considering ARV drugs plasma concentrations but also to discriminate the effect of different CYP3A4 inhibitors. This could have been of interest as ritonavir and cobicistat may have different interaction potential [28].

Despite these limitations, these data provide robust information on the magnitude of DDI between amlodipine and ARV drugs to guide clinicians for drug dosage adjustment. Our results confirm the proposed half-dosage in case of coadministration of CYP3A4 inhibitors and suggest doubling the dose when coadministered with efavirenz. Finally, no dosage adjustment is recommended when amlodipine is coadministered with both CYP3A4 inhibitors and efavirenz.

In conclusion, we showed that DDIs between amlodipine and ARV regimens containing boosted agents or those with inducing potential may markedly alter amlodipine exposure. The clinical relevance of

high or low amlodipine exposure is unclear but a particular attention must be paid to elderly PLWH in order to improve tolerability and clinical outcome.

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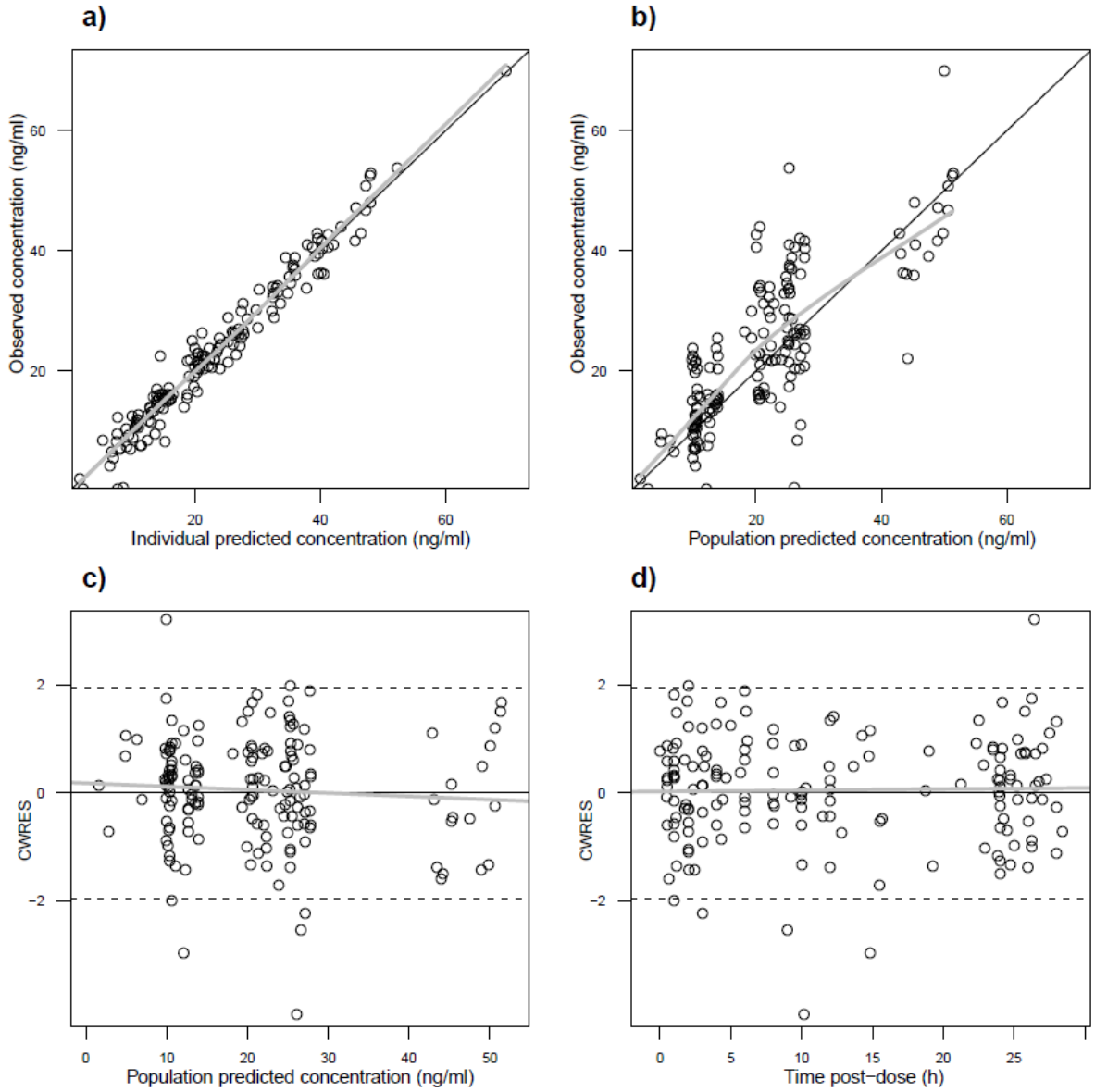
IV.2.7. Supplementary material



Supplementary 1: Goodness-of-fits plots for amlodipine final population PK model. a) Observed concentrations vs. individual predictions. b) Observed concentrations vs. population predictions. c) Conditional weighted residuals (CWRES) vs. population predictions. d) CWRES vs. time after the last drug intake.

IV.3. Influence of drug-drug interactions on the pharmacokinetics of atorvastatin and its major active metabolite *ortho*-OH-atorvastatin in aging people living with HIV.

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ORIGINAL RESEARCH ARTICLE

Influence of Drug–Drug Interactions on the Pharmacokinetics of Atorvastatin and Its Major Active Metabolite *ortho*-OH-Atorvastatin in Aging People Living with HIV

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Abstract

Background People living with HIV (PLWH) are aging and experience age-related physiological changes and comorbidities. Atorvastatin is a widely prescribed lipid-lowering agent metabolized by cytochrome P450 (CYP) 3A4, whose hepatocyte uptake is facilitated by organic anion transporting polypeptide (OATP) 1B1/1B3. Inhibition or induction of this enzyme and hepatic transporter can increase or decrease atorvastatin exposure, respectively.

Objective This study aimed to describe the pharmacokinetic profile of atorvastatin and its major metabolite, and to evaluate drug–drug interactions (DDIs) with antiretrovirals (ARVs).

Methods The atorvastatin pharmacokinetic profile was best described by a two-compartment model with first-order absorption and elimination. Metabolite concentrations were described by considering both linear metabolism from atorvastatin and presystemic metabolism. The influence of demographic and clinical covariates on drug and metabolite pharmacokinetics was assessed using NONMEM®. Model-based simulations were performed to evaluate the magnitude of DDIs with ARVs.

Results Full pharmacokinetic profiles (98 atorvastatin + 62 *o*-OH-atorvastatin concentrations) and sparse concentrations (78 and 53 for atorvastatin and *o*-OH-atorvastatin, respectively) were collected in 59 PLWH. Interindividual variability was high. The coadministration of boosted ARVs decreased atorvastatin clearance by 58% and slowed down *o*-OH-atorvastatin formation by 88%. Atorvastatin clearance increased by 78% when coadministered with CYP3A4 inducers. Simulations revealed a 180% increase and 44% decrease in atorvastatin exposure (area under the curve) in the presence of ARVs with inhibiting and inducing properties, respectively.

Conclusion This study showed an important interindividual variability in atorvastatin pharmacokinetics that remains largely unexplained after the inclusion of covariates. Since boosted ARVs double atorvastatin exposure, the initial dosage might be reduced by half, and titrated based on individual clinical targets.

1 Introduction

People living with HIV (PLWH) live longer and experience age-related physiological changes and comorbidities, notably cardiovascular diseases. Polypharmacy is frequent in elderly PLWH, leading to an increased risk for drug–drug interactions (DDIs), which may harm this vulnerable population. Antiretroviral drugs (ARVs) are among the therapeutic agents with the highest potential for DDIs. Protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) can indeed inhibit and/or induce cytochrome P450 (CYP) isoforms [1] as well as drug transporters [2].

Atorvastatin is a widely prescribed lipid-lowering agent that undergoes extensive first-pass metabolism [3]. It is predominantly metabolized by CYP3A4 into two active metabolites: the major *ortho*-hydroxy atorvastatin (*o*-OH-atorvastatin) and the minor *para*-hydroxy atorvastatin (*p*-OH-atorvastatin). Both atorvastatin and its active metabolites can undergo lactonization and thus exist in equilibrium with their respective inactive lactone forms. A study has

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Key Points

Our findings highlight the high variability in atorvastatin pharmacokinetics, which is partially explained by drug–drug interactions with antiretroviral (ARV) treatments.

Simulations revealed a 180% increase and 44% decrease in atorvastatin exposure (area under the curve) in the presence of ARVs with inhibiting and inducing properties, respectively.

The present model provides a rationale for the selection of initial atorvastatin dosage, taking into account the associated ARV regimen.

suggested that most of the acid metabolites present in human plasma results from an interconversion of lactone metabolites [4]. It has been reported that about 70% of the HMG-CoA reductase inhibition is attributable to *o*-OH-atorvastatin and *p*-OH-atorvastatin, while the lactone forms are inactive [3]. Nevertheless, the latter may be incriminated for statin-induced myotoxicity [5, 6].

Importantly, the organic anion transporting polypeptide (OATP1B1/1B3) facilitates the entry of atorvastatin in the liver (i.e. the site of action) [7]. PIs inhibit OATP1B1 in addition to CYP3A4 and are therefore expected to substantially increase atorvastatin exposure, both by inhibiting the entry of the statin in the liver and by further inhibiting its biotransformation. According to the summary of product characteristics, atorvastatin exposure could increase by three- to fourfold in the presence of ritonavir-boosted darunavir [8]. This interaction can lead to serious adverse effects, such as rhabdomyolysis [9]. The current recommendations indicate to initiate atorvastatin at a low dosage in the presence of boosted darunavir and not to exceed a daily dose of 20 mg. However, formal DDI studies have not been performed, particularly in the elderly, leading to a lack of knowledge on the magnitude of DDIs.

To date, several studies have evaluated the factors influencing atorvastatin pharmacokinetics (PK). The effect of age is controversial, with some authors reporting an age-related increase in atorvastatin exposure [10–12], while others did not find any significant influence [13, 14]. One non-compartmental PK study showed an effect of sex (11% decrease in area under the curve [AUC] in women) on atorvastatin disposition [11]. Moreover, population PK studies indicate a body weight-related decrease in atorvastatin clearance [13], an influence of liver enzymes (aspartate aminotransferase [AST] and lactate dehydrogenase) on atorvastatin disposition [14, 15], and an effect of polymorphisms in the intestinal breast cancer resistance protein (BCRP) on atorvastatin bioavailability [16]. However, to our knowledge, no study investigated the effect of ARVs on atorvastatin disposition in a real-life setting.

The aims of this observational study were to develop a population PK model for atorvastatin and its major active metabolite in aging PLWH, and to quantify the effect of ARVs and other covariates on their disposition.

2 Material and Methods

2.1 Data Collection

Full PK investigations were performed in the framework of a study evaluating DDIs in PLWH enrolled in the Swiss HIV Cohort Study (SHCS), as described elsewhere [17]. All study participants gave written informed consent before entering the study. The study protocol was reviewed and approved by the Ethics Committee of Vaud and northwest/central Switzerland (CER-VD 2018-00369) and registered in ClinicalTrials.gov (NCT03515772). In addition, sparse plasma samples were collected at the patient's biannual cohort visits (SHCS project #815), at unselected times after the last drug intake. Undetectable atorvastatin plasma levels during the SHCS follow-up visits, suggestive of non-adherence to treatment, were excluded from the analysis. In addition, samples with non-reliable time information (i.e. time of blood sampling or last dose intake) were excluded from the analysis. Information on concurrent comedications (HIV and non-HIV medications), bodyweight, sex, age and liver function tests (AST and alanine aminotransferase [ALT]) were also available.

2.2 Analytical Method

Blood samples were collected in EDTA-containing tubes, immediately placed at + 4 °C. Shortly afterwards, blood samples were centrifuged and the plasma was stored at − 80 °C until analysis. Atorvastatin, *o*-OH-atorvastatin and *p*-OH-atorvastatin concentrations were determined by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) using a multiplex method developed and validated purposely for this research project [18]. The assay showed appropriate repeatability and intermediate precision for the quantification of atorvastatin and its two active metabolites *o*-OH-atorvastatin and *p*-OH-atorvastatin (coefficient of variation [CV] 2.1–13.4% and 4.2–13.4%, respectively) and trueness (98.4–110.8%). Lower limits of quantification (LLOQs) were 0.3 ng/mL for atorvastatin and *p*-OH-atorvastatin, and 0.5 ng/mL for *o*-OH-atorvastatin. In addition, darunavir and ritonavir plasma concentrations were measured using previously published LC-MS/MS methodology [19].

2.3 Model-Based Pharmacokinetic Analysis

Population PK analyses were performed using the non-linear mixed-effect modeling (NONMEM®) program (version 7.4.2, ICON Development Solutions, Ellicott City, MD, USA). PsN v4.2.0 was used for automation of various model development and evaluation methods, Pirana v2.9.2 was used to structure and document model development, and R v3.6.1 (1.2.1335) was used for data management, statistical analysis and graphical output [20, 21].

2.3.1 Base Model

Since a substantial proportion (42%) of *p*-OH-atorvastatin concentrations were below the LLOQ (BQL), population PK modeling was pursued for atorvastatin and its major measurable active metabolite *o*-OH-atorvastatin. *o*-OH-atorvastatin concentrations were mainly BQL over the full PK (i.e. 'rich') investigations and were distributed throughout the dosing interval. The population PK analysis was first performed using the samples collected during the full PK investigations for the parent atorvastatin, and subsequently using all available samples for the parent atorvastatin and the metabolite, assuming linear metabolism and integrating the first-pass effect of atorvastatin. Administered doses, atorvastatin and *o*-OH-atorvastatin plasma concentrations were converted into nanomoles (nmol) and nanomoles per liter (nmol/L), respectively, for the analyses of drug and metabolite data. Pharmacodynamic properties of atorvastatin and its active metabolites are generally considered equivalent [22] and the sum of both substances was defined as the 'active moiety'. With the exception of PLWH who reported missing atorvastatin doses during the last week before their blood intake, steady state was assumed for all PLWH.

A stepwise procedure was used to find the model that adequately fitted the data. The two-compartment model for atorvastatin, with an additional compartment for *o*-OH-atorvastatin, schematically depicted in Fig. 2, was finally retained for data description. Presystemic metabolism was modeled by estimating a proportional coefficient ($FR_{ator\text{-}oOH}$) between total atorvastatin and *o*-OH-atorvastatin absorption rate constants (k_a). This parameter allowed the description of atorvastatin presystemic metabolism by both considering the fraction of the dose directly converted into metabolite and by adjusting the *o*-OH-atorvastatin k_a. The total k_a was fixed to the value estimated during the analysis of rich parent PK data (2.59 h^{-1}), and $(1-FR_{ator\text{-}oOH})\times k_a$ and $FR_{ator\text{-}oOH}\times k_a$ are the atorvastatin and *o*-OH-atorvastatin k_a—k_{12} and k_{13}, respectively. Owing to identifiability problems, both compounds were assumed to have the same apparent volume of distribution. Since atorvastatin was administered orally, apparent PK parameters were estimated. Exponential errors were used to describe between-subject variability for all PK parameters, with the exception of $FR_{ator\text{-}oOH}$. Individual $FR_{ator\text{-}oOH}$ were constrained to vary between 0 and 1 by using the logit of $FR_{ator\text{-}oOH}$, and its interindividual variability was calculated as previously reported [23, 24]. Finally, several error models (i.e. proportional, additive and mixed) were compared to describe the residual variability for both drug and metabolite. The correlation between atorvastatin and its metabolite concentration measurements was tested using the L2 function in NONMEM®.

2.3.2 Covariate Model

The analysis of each covariate was sequentially examined using a stepwise insertion/deletion approach. First, correlation between post hoc individual estimates of the PK parameters and the covariates of interest were visually inspected. Potentially influential covariates were then incorporated sequentially into the model using linear or non-linear functions as appropriate. Categorical variables (sex and comedications, classified as the presence or absence of a boosted regimen or CYP3A4 inducers [25]) were coded as 0 and 1, and continuous covariates (age, weight, AST and ALT) were centered on their median value. Missing values for weight, AST and ALT were imputed to the population median value. Darunavir and ritonavir area under the concentration-time curves from zero to 24 h (AUC_{24}) were calculated using previously published population PK models [26]. Non-competitive interaction models including darunavir and ritonavir AUC_{24} on CL_{ator} and $FR_{ator\text{-}oOH}$ were tested using linear, power or exponential functions.

2.3.3 Model Selection and Parameter Estimation

Atorvastatin and metabolite concentrations were fitted by using the first-order conditional method (FOCE) with interaction using the ADVAN5 subroutine. BQL plasma levels for sparse data were excluded from the analysis. BQL concentrations for rich PK data were treated using the M6 approach, replacing BQL by LLOQ/2 at the first BQL observation and ignoring the following ones [27, 28]. The model was selected based on the likelihood ratio test (based on the reduction of the objective function value [ΔOFV]), visual inspection of diagnostic plots, and evaluation of estimates of population fixed and random parameters, along with their precision. Since an ΔOFV between two models approximates a Chi-square distribution, a decrease in the OFV > 3.84 ($p < 0.05$) for one additional parameter was considered statistically significant in the model building and the forward inclusion of covariates. During the backward deletion step, a covariate was retained in the final multivariate model if its deletion from the full model led to a 6.63-point increase in the objective function ($p < 0.01$, 1 degree of freedom).

2.4 Model Evaluation

A sensitivity analysis was carried out to assess the possible leverage effect on significant covariates due to potential outlier concentrations. Population parameters obtained when excluding data were compared with those obtained using the full dataset. In addition, 2000 datasets were generated by resampling from the original dataset for the evaluation of the final model by the bootstrap method implemented in PsN [20]. Stratification was based on the presence of boosted ARVs, CYP3A4 inducers and on rich PK sampling. Mean parameter values with their 95% confidence intervals ($CI_{95\%}$) were compared with the original model estimates. Finally, prediction- and variability-corrected visual predictive checks (pvcVPCs) were also performed on the final PK model with variability using the PsN-Toolkit and the R package Xpose4, to visually compare observed concentrations with 5th, 50th and 95th prediction percentiles [20, 29, 30].

2.5 Model-Based Simulations

Simulations of 1000 individuals with different ARV regimens based on the final model with between-subject variability were conducted to derive the average AUC_{24} with 95% prediction intervals ($PI_{95\%}$) for atorvastatin, *o*-OH-atorvastatin and the active moiety (AUC_{ator}, $AUC_{o\text{-}OH}$, $AUC_{active\ moiety} = AUC_{ator} + AUC_{o\text{-}OH}$).

3 Results

3.1 Data

Nine PLWH contributed to 98 atorvastatin and 62 *o*-OH-atorvastatin plasma concentrations collected in a rich sampling design. In addition, 78 sparse atorvastatin and 53 sparse *o*-OH-atorvastatin concentrations from 55 PLWH were included in the analysis. Overall, 176 atorvastatin and 115 *o*-OH-atorvastatin plasma concentrations were available from 59 PLWH who were receiving atorvastatin at a dose ranging from 5 to 40 mg once daily. Characteristics of the study population are presented in Table 1. The median (range) of samples available per study individual was 11 (10–11) for PLWH included in the full PK study, and 1 (1–2) for PLWH whose samples were collected during the SHCS follow-up visits. Plasma concentration measurements varied from 0.3 to 106 ng/mL (0.5–190 nmol/L), and from 0.5 to 24 ng/mL (0.9–42 nmol/L), for atorvastatin and *o*-OH-atorvastatin, respectively. None of the atorvastatin concentrations and 38% ($n = 69$) of *o*-OH-atorvastatin levels were below their respective LLOQs. Figure 1 represents concentration-time profiles for atorvastatin and *o*-OH-atorvastatin according to concurrent ARV drugs.

In addition, darunavir and ritonavir plasma concentrations were available for PLWH receiving such ARV regimens concomitantly to atorvastatin.

3.2 Base and Covariate Model

Atorvastatin full PK profiles were best described by a two-compartment model with first-order absorption and elimination. The addition of a second compartment to describe atorvastatin disposition significantly improved the fit (variation in OFV, $\Delta OFV = -113$; $p < 0.001$) and the individual plots of rich PK data. The k_a was estimated at 2.59 h^{-1} and was fixed to this value for subsequent model development to allow precise estimation of the other model parameters during the analysis of the full dataset. The model presented in Fig. 2 adequately described atorvastatin and *o*-OH-atorvastatin data. The inclusion of the factor $FR_{ator\text{-}oOH}$ describing presystemic metabolism of atorvastatin improved the description of the data. Residual variabilities on atorvastatin and *o*-OH-atorvastatin were satisfactorily described using proportional and mixed-error models, respectively. The additive part of the metabolite error model was estimated at 0.44 nmol/L. Parameter estimates and between-subject variability (CV%) of the base PK model were a k_a fixed to 2.59 h^{-1} (239%), an $FR_{ator\text{-}oOH}$ of 11% (131%), an atorvastatin clearance (CL_{ator}) of 204 L/h (94%), a central volume of distribution of atorvastatin and *o*-OH-atorvastatin (Vc_{ator}=Vc_{met}) of 3170 L (137%), a peripheral volume of distribution (Vp_{ator}) of 591 L, an intercompartmental clearance (Q) of 104 L/h, a metabolic rate constant (k_{23}) of 0.0096 h^{-1}, and a metabolite clearance ($CL_{o\text{-}OH\text{-}ator}$) of 118 L/h. Drug and metabolite concentrations were found to be correlated (59%).

Due to substantial eta shrinkage on $FR_{ator\text{-}oOH}$ (46%) in the base model, the graphic exploration was interpreted cautiously between the parameter estimate and covariates. Since visual inspection of exploratory plots did not reveal an effect of sex, weight, AST and ALT on atorvastatin and *o*-OH-atorvastatin PK, these covariates were not tested in the model.

The coadministration of boosted ARVs decreased atorvastatin clearance by 58% and logit($FR_{ator\text{-}oOH}$) by 225% ($\Delta OFV < -26$; $p < 0.001$). Consequently, *o*-OH-atorvastatin was formed 88% slower in the presence of boosted ARVs than in the absence of such a regimen. Atorvastatin clearance increased by 78% in PLWH receiving CYP3A4 inducers ($\Delta OFV = -21$; $p < 0.001$). The inclusion of covariates decreased original between-subject variability on CL_{ator} and $FR_{ator\text{-}oOH}$, by 34 and 46%, respectively. Aging did not significantly influence $FR_{ator\text{-}oOH}$ or CL_{ator} and was not retained

Table 1 Demographic and clinical characteristics of the study population

Patient characteristics [$n = 59$]	Median [IQR] or n (%)
Age, years	64 [58–71]
Male sex	46 (78)
Body weight, kg	73 [65–84]
Missing data	3 (5)
ALT, IU/L	27 [21–37]
Missing data	6 (10)
AST, IU/L	27 [22–32]
Missing data	6 (10)
HDL-cholesterol, mmol/L	1 [1–2]
Missing data	6 (10)
LDL-cholesterol, mmol/L	3 [3–4]
Missing data	5 (8)
Triglycerides, mmol/L	2 [1–3]
Missing data	7 (12)
Comedications ($n = 176$)[a]	n (%)
Ritonavir-boosted darunavir[b]	50 (28)
Cobicistat-boosted darunavir[b]	24 (14)
Ritonavir-boosted atazanavir[b]	2 (1)
Cobicistat-boosted elvitegravir[b]	2 (1)
Efavirenz[c]	12 (7)
Etravirine[c]	29 (17)
Nevirapine[d]	4 (2)
Rilpivirine	2 (1)
Dolutegravir	80 (46)
Raltegravir	26 (15)
Verapamil[b]	2 (1)

ALT aspartate aminotransferase, *AST* alanine aminotransferase, *HDL* high-density lipoprotein, *IQR* interquartile range, *LDL* low-density lipoprotein, *CYP* cytochrome P450, *OATP* organic anion transporting polypeptide

[a]Values are reported according to the number of atorvastatin plasma concentrations

[b]Strong CYP3A4 and OATP1B1 inhibitors

[c]Strong CYP3A4 inducers

[d]Moderate CYP3A4 inducers [25]

in the final model ($\Delta OFV > -0.4$; $p > 0.1$). The parameter estimates of the final model and their precisions are presented in Table 2. Interaction models integrating darunavir or ritonavir AUC_{24} on atorvastatin clearance or $FR_{ator\text{-}oOH}$ did not improve the description of the data. Diagnostic plots for the final model are shown in electronic supplementary Fig. S1. While the model seemed to adequately describe the absorption phase, a bias remained for very high atorvastatin concentrations, as shown on the observed concentrations versus population predictions plot. This bias was entirely due to a single individual, who was the only one treated with ritonavir-boosted darunavir concomitantly with atorvastatin at a dose of 40 mg once daily. Reassuringly, this extremely high atorvastatin dose is not recommended by the US product label of darunavir in patients treated with ritonavir-boosted darunavir and is therefore not expected to be usually encountered in clinical practice.

3.3 Model Evaluation

The sensitivity analysis performed while removing one individual with extremely high atorvastatin concentrations (11 blood samplings) did not reveal any significant influence on the estimated PK parameters, but the effect of boosted regimens on the logit($FR_{ator\text{-}oOH}$) parameter decreased by 15%. *o*-OH-atorvastatin was formed 83% slower in the presence of boosted ARVs than in the absence of such a regimen when removing this individual. This effect was not considered significantly different compared with the results obtained with the full dataset (the *o*-OH-atorvastatin formation rate was reduced by 88% in the presence of boosted ARVs) and this individual was therefore maintained in the dataset.

The final model parameters, together with their bootstrap estimations, are displayed in Table 2. The model was considered reliable since the parameters were within the bootstrap $CI_{95\%}$ and differed less than 9% from the bootstrap estimations, with the exception of between-subject variability on k_a and $FR_{ator\text{-}oOH}$, which were 13% and 25%, respectively. $CI_{95\%}$ of the peripheral volume was very large, despite good precision of its estimate (27%). In addition, while the bootstrap was stratified by rich sampling data, including several drug concentrations in the absorption phase, $CI_{95\%}$ of the between-subject variability on the k_a remains very wide. This reflects the difficulty of the model in accurately describing the highly variable absorption phase.

Finally, predictive performance of the model was supported by the results of the pvcVPCs (Fig. 3).

3.4 Simulations

Model-based simulations were performed to estimate and compare AUC_{ator}, $AUC_{o\text{-}OH}$ and the sum of both, the $AUC_{active\ moiety}$, for individuals receiving atorvastatin 10 mg with different ARV regimens (Fig. 4). The simulated average AUC_{ator} indicated a 180% increase in atorvastatin exposure in PLWH receiving boosted ARVs compared with those receiving ARVs devoid of interaction potential with atorvastatin. Conversely, $AUC_{o\text{-}OH}$ decreased by 12% in PLWH treated with boosted regimens. In total, $AUC_{active\ moiety}$ increased by 110% in PLWH receiving boosted ARVs compared with PLHW receiving ARVs without interaction potential. Following coadministration of CYPA4 inducers, AUC_{ator} and $AUC_{active\ moiety}$ decreased by 44 and 31%, respectively, compared with PLWH receiving non-interacting ARV treatments.

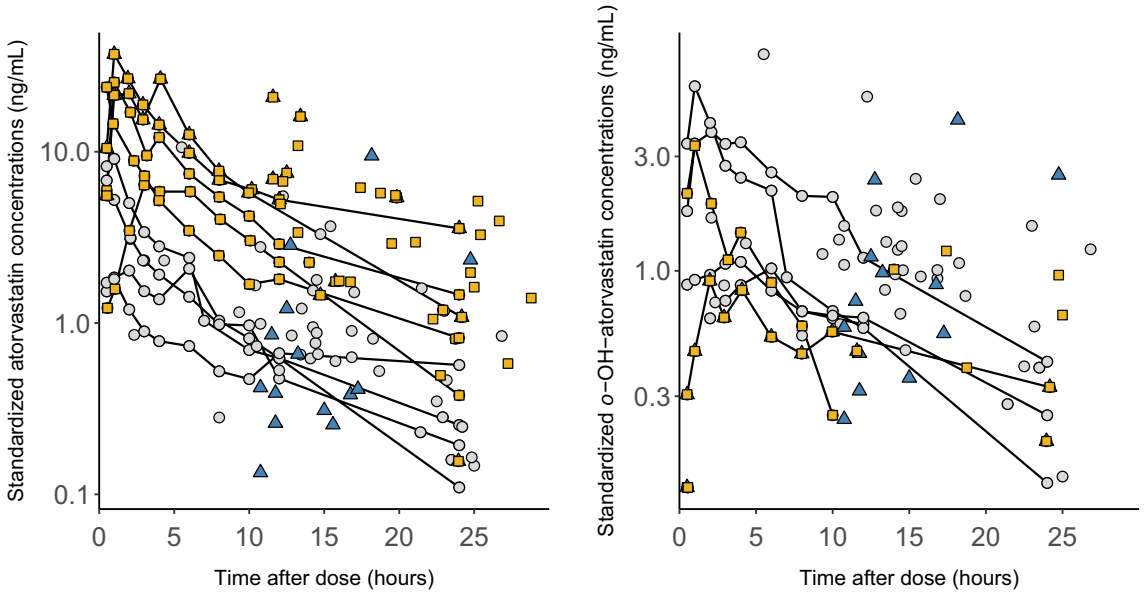

Fig. 1 Observed atorvastatin (left) and *o*-OH-atorvastatin concentrations (right) [log-scale] versus time after dose. Concentrations were standardized for a daily dose of 10 mg. Concentrations for PLWH receiving neither CYP3A4 inhibitors nor CYP3A4 inducers (grey circles), or receiving boosted ARVs (yellow squares) or strong CYP3A4 inducers (blue triangles) are shown. Plasma drug concentrations of PLWH who participated in the pharmacokinetic study with rich sampling are joined by black lines. *PLWH* people living with HIV, *CYP* cytochrome P450, *ARVs* antiretrovirals

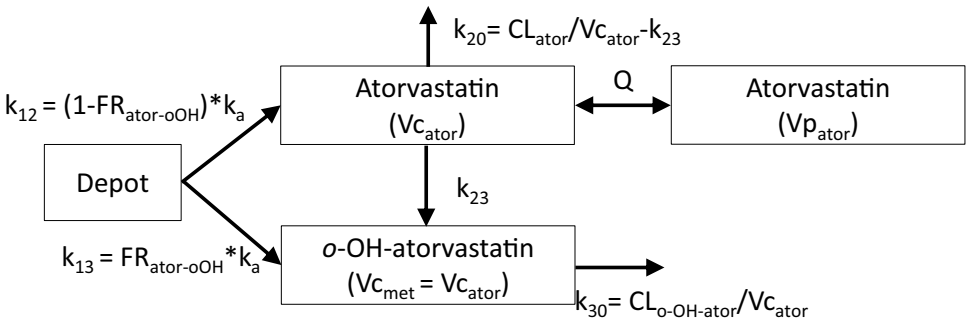

Fig. 2 Compartmental model used to describe atorvastatin and *o*-OH-atorvastatin plasma concentration-time profiles. k_{12} absorption rate constant from depot to the atorvastatin compartment, k_{13} absorption rate constant from depot to the *o*-OH-atorvastatin compartment, k_a total absorption rate constant, $FR_{ator\text{-}oOH}$ proportional coefficient between total atorvastatin and *o*-OH-atorvastatin absorption rate constants, k_{20} atorvastatin elimination rate constant, k_{30} *o*-OH-atorvastatin elimination rate constant, CL_{ator} apparent atorvastatin clearance, $CL_{o\text{-}OH\text{-}ator}$ apparent *o*-OH-atorvastatin clearance, Vc_{ator} apparent atorvastatin volume of distribution, Vc_{met} apparent *o*-OH-atorvastatin volume of distribution, Vp_{ator} peripheral atorvastatin volume of distribution, Q intercompartmental clearance

In PLWH receiving both boosted regimens and CYP3A4 inducers, AUC_{ator} and $AUC_{active\ moiety}$ increased by 61 and 21%, respectively.

4 Discussion

Our study provides a description of the population PK profile of atorvastatin and *o*-OH-atorvastatin, and quantifies the magnitude of DDIs with ARVs in real-life situations. Although parameter estimates widely differ between published population PK analyses, the reported PK parameters estimated in the present study were generally in fair concordance with overall reported values [13, 14].

The present model revealed large interindividual variability in atorvastatin PK, notably during the absorption phase, known to be affected by multiple factors. First, food has been reported to decrease atorvastatin peak concentration (C_{max}) and increase time to C_{max} (T_{max}) [3]. Although all full PK samples were obtained under standardized conditions, this

Table 2 Parameter estimates of the final atorvastatin and *o*-OH-atorvastatin pharmacokinetic model and bootstrap results

Parameters	Final model		Bootstrap ($n = 2000$ samples)	
	Estimate	RSE (%)[a]	Median	$CI_{95\%}$
k_a (h^{-1})[b]	2.59 FIX			
ω_{ka} (CV%)	246	29	214	25 to 2103
Logit $FR_{ator\text{-}oOH}$	−1.56	10	−1.52	−2.2 to −1.0
$\omega_{logitFR_{ator\text{-}oOH}}$ (CV%)	70	16	62	25 to 92
$\theta_{boosted\ ARVs}$	−2.25	28	−2.27	−7.9 to −0.9
CL_{ator} (L/h)	230	12	233	162 to 312
ω_{CLator} (CV%)	63	17	61	37 to 84
$\theta_{boosted\ ARVs}$	−0.58	9	−0.58	−0.7 to −0.4
$\theta_{CYP3A4\ inducers}$	0.78	39	0.76	0.05 to 1.30
Vc_{ator}=Vc_{met} (L)	2910	33	2902	1215 to 6023
ω_{Vcator} (CV%)	131	10	127	87 to 173
Vp_{ator} (L)	617	27	676	322 to 11,687
Q (L/h)	98	14	92	40 to 428
k_{23} (h^{-1})	0.0072	14	0.0075	0.0035 to 0.019
$CL_{o\text{-}OH\text{-}ator}$ (L/h)	116	10	117	62 to 196
$\sigma_{ator,\ prop}$ (CV%)	38	22	37	30 to 46
$\sigma_{o\text{-}OH\text{-}ator,\ prop}$ (CV%)	28	16	28	22 to 34
$\sigma_{o\text{-}OH\text{-}ator,\ add}$ (nmol/L)	41	51	41	4 to 60
Correlation ator/*o*-OH-ator	66	18	69	47 to 77

Final model: $TVCL_{ator} = 230 \times (1 - 0.58 \times \text{boosted ARVs}) \times (1 + 0.78 \times \text{CYP3A4 inducers})$

$TVLogit(FR_{ator\text{-}oOH}) = -1.56 - 2.25 \times \text{boosted ARVs}$

k_a first-order absorption rate constant, ω between-subject variability reported as CV (%), $LogitFR_{ator\text{-}oOH}$ logit transformation of proportional coefficient between total atorvastatin and *o*-OH-atorvastatin absorption rate constants, CL_{ator} mean apparent atorvastatin clearance, Vc_{ator}=Vc_{met} mean apparent atorvastatin and *o*-OH-atorvastatin volume of distribution, Vp_{ator} mean peripheral atorvastatin volume of distribution, Q intercompartmental clearance, k_{23} metabolic rate constant, $CL_{o\text{-}OH\text{-}ator}$ mean apparent *o*-OH-atorvastatin clearance, $CI_{95\%}$ 95% confidence interval, *CYP* cytochrome P450, *CV* coefficient of variation, *RSE* relative standard error, *SE* standard error

[a]Defined as SE/estimate, and expressed as percentages

[b]Based on preliminary analysis of atorvastatin rich pharmacokinetic data

parameter was not controlled for samples collected during the follow-up visits. Since, atorvastatin is exposed to intestinal CYP3A4 during the absorption phase, CYP3A4 inhibitors and inducers may further contribute to the observed important variability in this parameter. In our PK model, this has been captured by integrating the effect of boosted regimens (all CYP3A4 inhibitors) on the absorption parameter $FR_{ator\text{-}oOH}$. Finally, several transporters are involved in the disposition of atorvastatin and its metabolites. Genetic polymorphisms can affect the intrinsic activity and/or expression of transporters and the observed variability in atorvastatin absorption could therefore be explained by the genetic background [10, 31]. Shitara et al. showed that OATP could play a significant role in atorvastatin absorption [32]. In addition, ABCG2 and SLCOB polymorphisms have been shown to affect atorvastatin C_{max}, with no effect on elimination half-life [33–35], supporting an effect of transporter genotypes on atorvastatin mainly during the absorption phase. However, the lack of genotyping data in our study prevented the estimation of such an effect. In our model, due to the complexity of the absorption phase, k_a was fixed to the value obtained during the analysis, using atorvastatin rich PK data to obtain a reasonable value of T_{max}. Predicted T_{max} values ranged from 0.5 to 3.7 h, with a median of 1.3 h, in accordance with the manufacturer's data [36]. Studies also reported T_{max} values varying from 0.5 to 2 h [37–41]. In addition, the k_a value of 2.59 h^{-1} is in the range of values reported in published population PK models, varying from 0.2 to 3.5 h^{-1}.

The present study also identified large between-subject variability in atorvastatin clearance and central volume of distribution. Although non-compartmental analyses showed an effect of age on atorvastatin disposition [10, 11], the majority of previously published population PK analyses did not report any significant influence [13, 14, 16], while one of the studies found an effect in men only [12]. In our study, this association did not reach statistical significance, although visual inspection of the plots evaluating the effect of age on atorvastatin clearance suggested a slight decrease

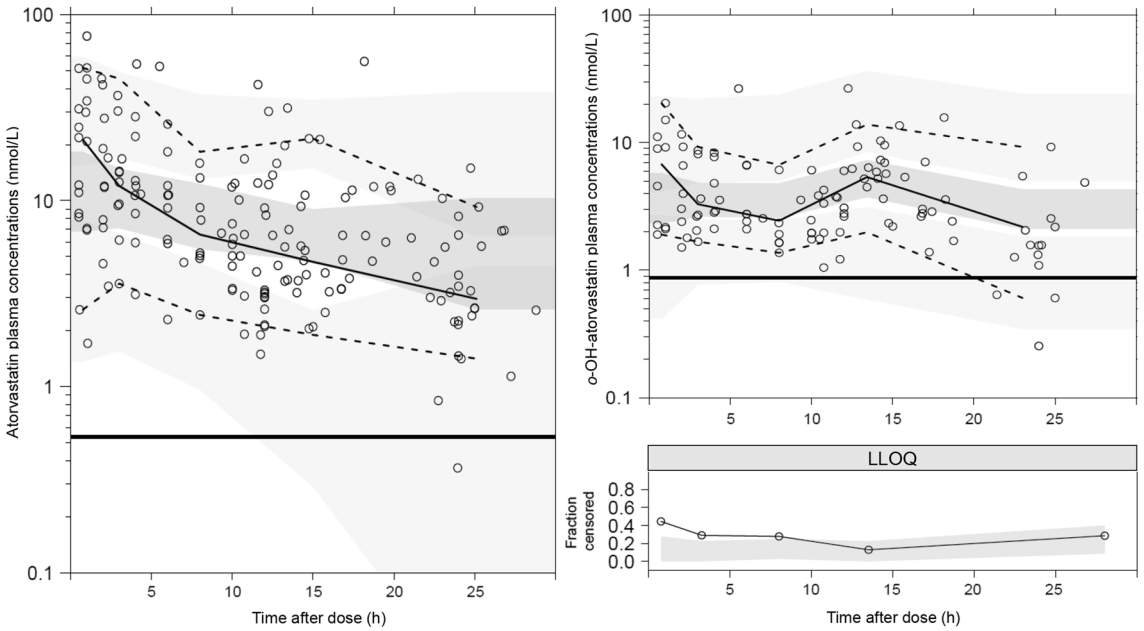

Fig. 3 Prediction- and variability-corrected visual predictive check of the final model of atorvastatin (left) and *o*-OH-atorvastatin (right). Open circles represent prediction- and variability-corrected observed plasma concentration; black solid and dashed lines represent the median and $PI_{90\%}$ of the observed data; shaded surfaces represent the model-predicted 90% confidence interval of the simulated median and $PI_{90\%}$; horizontal black lines are the LLOQ of atorvastatin (0.54 nmol/L) and *o*-OH-atorvastatin (0.87 nmol/L). In the lower panel, shaded areas represent the $PI_{90\%}$ of the simulated (shaded surface). BQL data and close circles show the fraction of observed BQL data. *LLOQ* lower limit of quantification, *BQL* below the LLOQ, $PI_{90\%}$ 90% prediction interval

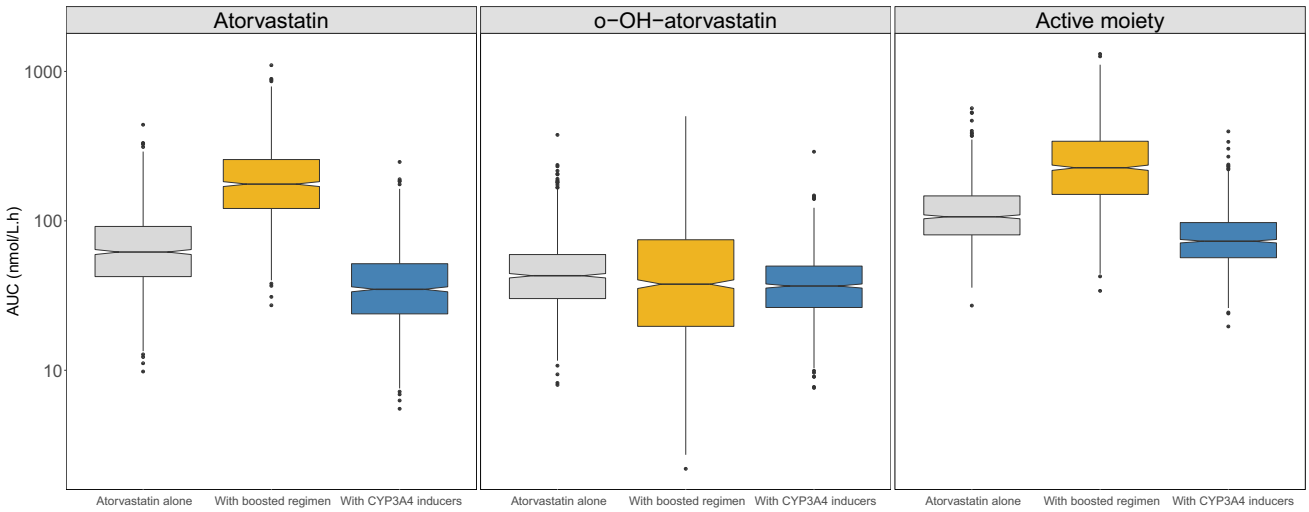

Fig. 4 Simulated estimates of AUC_{24} for atorvastatin, *o*-OH-atorvastatin and the active moiety in PLWH receiving ARVs not interacting with atorvastatin (grey boxes), receiving boosted ARVs (yellow boxes), or CYP3A4 inducers (blue boxes). *AUC* area under the concentration–time curve, *PLWH* people living with HIV, *ARVs* antiretrovirals, *CYP* cytochrome P450

in clearance for PLWH older than 60 years of age. This absence of age effect could result from the narrow interquartile range of age (58–71 years) in our population.

This model allowed for the evaluation of the impact of DDIs that are encountered in clinical practice. The dual inhibition of cytochromes and transporters is expected to

substantially increase atorvastatin exposure [42]. Inhibition of the hepatic uptake transporter OATP1B1 is expected to reduce the entry of atorvastatin in the liver, whereas inhibition of hepatic BCRP and P-gp decreases the hepatobiliary excretion of atorvastatin. A previously published study demonstrated that inhibition of hepatic transporters of atorvastatin might yield to DDIs with the same magnitude as enzyme inhibition [38], with potential occurrence of atorvastatin toxicity. Indeed, adverse effects such as rhabdomyolysis appeared to be at least partially related to atorvastatin plasma concentrations [43, 44], and several cases of rhabdomyolysis have been reported with the simultaneous administration of moderate or strong CYP3A4 inhibitors [45–48]. Studies suggested that myotoxicity may be related to either atorvastatin lactone or hydroxylated metabolites, or both [6, 39]. However, regardless of the actual incriminated species, its formation critically depends on the disposition and the circulating concentrations of the parent statin, which keeps a determinant interest. No atorvastatin target plasma trough concentrations have been clearly established to avoid toxicity, but caution is needed when co-prescribing enzyme and transporter inhibitors with atorvastatin.

The magnitude of DDIs with atorvastatin differs between boosted regimens. Atorvastatin AUC was shown to be increased by 822% when coadministered with ritonavir-boosted atazanavir, while atorvastatin AUC increased by 200–300% and 700–800% when coadministered with ritonavir-boosted darunavir or ritonavir-boosted tipranavir, respectively [37]. Differences in the magnitude of DDIs have been attributed to differences in the ability of PIs to inhibit OATP1B1 [49]. In our study, the lack of data prevented us from differentiating the effect of different boosted regimens on atorvastatin and *o*-OH-atorvastatin exposure. Model-based simulations revealed a 2.8-fold increase in AUC_{ator} when coadministered with boosted regimens that were mostly boosted darunavir. This result is in good agreement with the manufacturer's data reporting a three- to fourfold increase in atorvastatin AUC when coadministered with ritonavir-boosted darunavir. In addition, another study showed that atorvastatin AUC increased by 290% in cases of coadministration of cobicistat-boosted darunavir [50]. To our knowledge, no study has reported the effect of boosted regimens on the active moiety, which is modulated by the decrease in *o*-OH-atorvastatin exposure. Our results demonstrated that PLWH receiving concomitantly boosted regimens and atorvastatin at a daily dose of 10 mg obtained an atorvastatin exposure 29% lower than PLWH receiving atorvastatin alone at a daily dose of 40 mg. This result is slightly different, with manufacturer's data reporting a difference of 15% [8]. Conversely, AUC_{ator} and $AUC_{active\ moiety}$ were 44 and 31% lower, respectively, in PLWH receiving CYP3A4 inducers compared with PLWH receiving ARVs not involved in DDIs with atorvastatin. This is in perfect agreement with studies reporting a moderate magnitude of DDIs between atorvastatin and NNRTIs [51, 52]. Finally, the inducing effect of NNRTIs on CYP3A4 partly compensates for the magnitude of DDIs with boosted ARVs. Of interest, when coadministered together, the inhibitory effect of boosted regimens was shown to be stronger than the concurrent inducing effect of NNRTIs, as evidenced by the fact that AUC_{ator} and $AUC_{active\ moiety}$ increased by 61 and 21%, respectively.

This study has some limitations. First, the small sample size prevented us from differentiating the effect of different boosted regimens. However, atorvastatin PK data in PLWH are limited in the literature and this work aims to expand the current knowledge on DDIs in a real-life setting. In addition, the effect of boosted ARVs on the magnitude of DDIs could have been slightly attenuated as among the 80 concentrations obtained in PLWH treated with boosted regimens, 12 (15%) were also influenced by CYP3A4 inducers.

Despite these limitations, our study is the first to describe atorvastatin and *o*-OH-atorvastatin disposition by considering the first-pass and presystemic metabolism. The availability of rich PK data with concentrations collected in the absorption phase allowed for a satisfactory description of the entire concentration-time profile of atorvastatin and its metabolite. In addition, data collected in a real-life setting evidenced the high between-subject variability, which is partly explained by DDIs.

5 Conclusions

The present study showed an important between-subject variability in atorvastatin PK that remained largely unexplained after the inclusion of covariates. Since atorvastatin exposure doubles in the presence of boosted ARV drugs, the initial dosage might be reduced by half, and titrated based on clinical risk factors and targets.

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Compliance with Ethical Standards

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Conflict of interest Perrine Courlet, Laurent A. Decosterd, Susana Alves Saldanha, Felix Stader, Thierry Buclin, Catia Marzolini, Chantal Csajka, and MoniaGuidi have no conflicts of interest to declare. Matthias Cavassini has, through his institution, received research grants from ViiV and Gilead, and offered expert testimony for Abbvie, MSD, Gilead, and Sandoz. Marcel Stoeckle received advisory board fees from Gilead, ViiV, MSD, Sandoz and Mepha, as well as grants for conferences from Gilead and MSD, however these were unrelated to the present study.

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IV.4. Pharmacokinetic/pharmacodynamic modelling to describe non-HDL cholesterol lowering effect of rosuvastatin in people living with HIV.

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IV.4.1. Abstract

Background

Rosuvastatin is a widely prescribed lipid-lowering agent in people living with HIV (PLWH) actively transported in the liver, and a potential victim of drug-drug interactions (DDIs) with antiretroviral agents.

Objectives

The aims of this study were to characterize the pharmacokinetic (PK) profile of rosuvastatin and to describe the relationship between rosuvastatin PK and non-HDL-cholesterol levels.

Methods

A population PK model (NONMEM) was developed to quantify the influence of demographic, clinical characteristics and comedications on rosuvastatin PK. This model was combined with an indirect effect model to describe non-HDL-cholesterol profiles.

Results

A two-compartment model with sequential zero- and first-order absorption best fitted the 154 rosuvastatin concentrations provided by 65 PLWH. None of the tested covariate was significantly associated with rosuvastatin PK. A total of 403 non-HDL cholesterol were available for the pharmacokinetic-pharmacodynamic (PK/PD) modelling. Baseline non-HDL cholesterol was decreased by 14% and increased by 12% with etravirine or ARV drugs with a known impact on lipid profile (*i.e.* PIs, efavirenz, cobicistat), respectively. Baseline value was surprisingly decreased by 43% between PLWH aged 40 to 80 years-old. Model-based simulations revealed that, under standard rosuvastatin doses of 5 mg and 20 mg once daily, 31% and 64% of PLWH might achieve non-HDL-cholesterol targets, respectively.

Conclusion

A high between-subject variability has been observed in both rosuvastatin pharmacokinetic and pharmacodynamic profiles that remains unexplained after the inclusion of covariates. Considering its limited potency for DDIs with ARV agents and its effective lipid-lowering effect, rosuvastatin treatment should be favoured in PLWH.

IV.4.2. Introduction

The aging of people living with HIV (PLWH) and the higher risk for cardiovascular disease in this population resulted in an increased use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors (*i.e.* statins) [1, 2]

. The management of dyslipidemia in PLWH is complicated by the high potential of antiretroviral drugs (ARVs) for drug-drug interactions (DDIs), which may increase statin plasma concentrations, thus

potentially leading to clinically significant adverse events such as rhabdomyolysis [3, 4]. In addition, the decline in organs functions with age may affect statin pharmacokinetics (PK) and thereby the magnitude of DDIs.

Rosuvastatin is a widely prescribed lipid lowering agent, which undergoes minor metabolism. Nevertheless, rosuvastatin is actively transported in the liver by the organic-anion-transporting polypeptide OATP1B1/3. It is also a substrate of breast cancer resistance protein (BCRP), a transporter present in the intestine and in the liver where it limits the absorption and the biliary elimination of drug substrates [5]. By inhibiting both OATP1B1/3 and BCRP, protease inhibitors (PIs) are then expected to increase rosuvastatin exposure, although to a minor to moderate extent. Coadministration of ritonavir-boosted darunavir has been shown to increase rosuvastatin area under the curve (AUC) and maximal concentration (C_{max}) by 48% and 144% [6], respectively, while coadministration of cobicistat-boosted darunavir increased rosuvastatin AUC by 93% and C_{max} by 277% [7]. Current recommendations indicate to initiate rosuvastatin at the lowest possible dose in presence of boosted darunavir and not to exceed 20 mg once daily. Studies have been conducted mainly in young HIV-negative individuals (median age of about 25-30 years-old), and no guidance is available on how to adjust rosuvastatin dosage in elderly people.

In addition to their potential for DDIs, ARV treatments and notably PIs may cause metabolic complications such as lipid disorders, thus complicating the management of dyslipidemia in PLWH [8]. A study reported a 10% and 56% increase in total cholesterol and triglycerides in individuals receiving rosuvastatin with ritonavir-boosted darunavir *vs.* rosuvastatin alone, while HDL-cholesterol levels decreased by 13%, highlighting the potential of PIs to trigger lipid disorders [6].

The purposes of the present study were to characterize the PK profile of rosuvastatin in PLWH in real-life settings, and to quantify the effect of demographic and clinical covariates on its disposition. Secondly, this work aimed at describing the relationship between rosuvastatin plasma concentrations and non-HDL-cholesterol levels.

IV.4.3. Methods

IV.4.3.1. Study population and design

Rosuvastatin PK data were collected in PLWH from the Swiss HIV Cohort Study (SHCS) enrolled in two studies conducted in Lausanne and Basel. First, PK investigations with rich sampling were conducted in aging PLWH, as described elsewhere [9] (clinicaltrials.gov, NCT03515772). The second study involved the collection of sparse plasma samples during patient's biannual cohort visits, at unselected times after the last drug intake. Exclusion criteria from the analysis included undetectable rosuvastatin plasma concentrations and non-reliable time information (*i.e.* date and hour about last drug intake or

blood sampling). The following data were also recorded at the same time as blood intake for PK measurements: age, gender, body weight, total cholesterol (TC), HDL-cholesterol, triglycerides (TG), aspartate amino transferase (AST), amino alanine transferase (ALT), serum creatinine concentration, presence of diabetes and concomitant medications. Recorded comedications included antiretroviral treatment as well as medications for comorbidities. Although rosuvastatin is primarily excreted in the feces, creatinine clearance was estimated by the Cockcroft-Gault formula [10] since severe renal insufficiency has been reported to impact rosuvastatin disposition, while mild to moderate renal impairment did not affect affect rosuvastatin PK [11].

Total and HDL-cholesterol levels before initiation of rosuvastatin treatment were retrieved from the SHCS database. Since time of blood intake was not recorded, it was arbitrarily fixed at 8 am, considering the low circadian variation of cholesterol [12] compared to the expected statin lipid-lowering effect. At the same date, the following data were also reported for the pharmacodynamic (PD) analysis: weight, AST, ALT, presence of diabetes, antiretroviral treatment and comedications.

IV.4.3.2. Rosuvastatin and non-HDL cholesterol quantifications

Blood samples for determination of rosuvastatin plasma levels were collected on EDTA-containing tubes. Plasma were aliquoted, shipped frozen (Basel samples) and stored at -80°C until analysis. All rosuvastatin plasma level measurements were performed at the laboratory of clinical pharmacology at Lausanne University Hospital by ultra-high performance liquid chromatography coupled with tandem mass spectrometry [13].

For the PK study with rich sampling, total cholesterol and HDL-cholesterol levels were analysed using enzymatic reactions catalysed by cholesterol oxidase as routinely performed at the laboratory of clinical chemistry of the Lausanne University Hospital. For plasma samples collected during the sparse sampling study and plasma samples collected before rosuvastatin initiation, cholesterol levels were measured by each centre according to the method used routinely (*i.e.* enzymatic test for both centres). Since PLWH have often higher than normal TG values, LDL values cannot be reliably derived using the Friedewald formula [14]. Non-HDL-cholesterol levels were thus calculated by subtracting HDL-cholesterol to total cholesterol levels.

IV.4.3.3. Pharmacokinetic/pharmacodynamic analysis

Population PK/PD analyses were conducted using non-linear mixed-effect modelling (NONMEM) (version 7.4.2, ICON Development Solutions, Ellicott City, MD, USA), supplemented by the PsN-Toolkit (version 4.2.0) and Pirana version 2.9.2 [15, 16]. Data management, statistical and graphical analyses and graphical were performed using R (version 3.6.1) (http://www.r-project.org).

PK structural model

Log-transformed rosuvastatin plasma concentrations were fitted using the first-order conditional estimation with interaction (FOCEI), with the subroutine ADVAN4 TRANS4. Analyses were first performed using full PK profiles, and subsequently adding sparse data. The model that best fitted the data was identified using a stepwise procedure, comparing one- and two-compartment models, with first-, zero-order or mixed (sequential or simultaneous) absorption process, potentially including a lag time. Between-subject variability was assumed to follow a log-normal distribution and was described by exponential errors. An additive error model on the log scale was used to describe the residual variability.

PK/PD model

The final PK model was combined with an indirect effect model to describe non-HDL-cholesterol data (figure 1). Considering that the decrease in non-HDL-cholesterol plasma levels following rosuvastatin treatment is mediated trough HMG-CoA reductase inhibition, the variation of non-HDL-cholesterol over time was described as follows:

$$\frac{dnonHDLchol}{dt} = K_{in} \times \left(1 - \frac{C_t}{IC_{50} + C_t}\right) - K_{out} \times nonHDLchol$$

where k_{in} and k_{out} denote the production and elimination rate of non-HDL-cholesterol, respectively; C_t the PK model predicted rosuvastatin plasma concentration at time t and IC_{50} the rosuvastatin concentration that leads to a 50% inhibition of non-HDL-cholesterol production.

Non-HDL-cholesterol compartment was initialized with a baseline level, and K_{out} was defined as K_{in}/baseline. Exponential errors following log-normal distributions were assumed for the description of between-subject variability of PD parameters. Additive, proportional and mixed error models were compared to capture at best the residual unexplained variability.

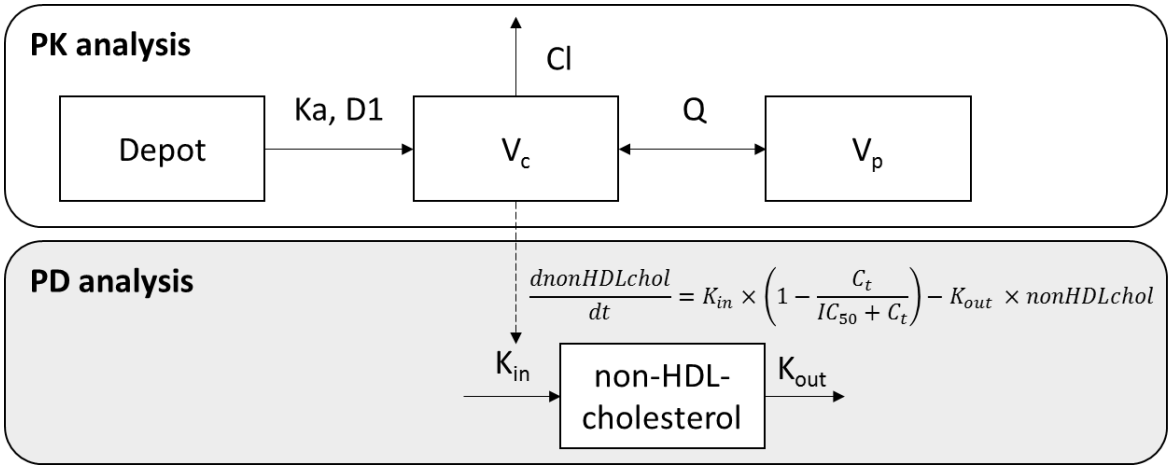

Figure 1: Compartmental model used to describe rosuvastatin PK and PD data. Ka: absorption rate constant, D1: duration of zero-order absorption, V_c: apparent central volume of distribution, Cl: apparent rosuvastatin clearance, Q: inter-compartmental clearance, V_p: apparent peripheral volume of distribution, K_{in}: production rate of non-HDL-cholesterol, K_{out}: elimination rate of non-HDL-cholesterol, C_t: rosuvastatin plasma concentration predicted by the model; IC_{50}: rosuvastatin concentration that produced a 50% inhibition of non-HDL-cholesterol production.

Covariate analysis

All potential and physiologically plausible associations were first graphically explored and then tested in univariate analyses in both PK and PD models. Patient's characteristics investigated for their impact on the PK parameters in the PK analyses were: gender, age, body weight, creatinine clearance, ALT, AST and presence of boosted PI. The effect of boosted PIs was tested on the absorption phase as studies reported a more pronounced effect of boosted PIs on rosuvastatin C_{max} than AUC [6, 7, 17]. Since the absorption rate constant (ka) and duration of zero-order absorption (D1) were fixed in the model, the effect of boosted PIs was tested on the volume of distribution (V_c), which also reflects the absorption phase.

In the PD model, covariates have been tested only on the baseline, unique parameter with quantifiable between-subject variability that can be explained with covariate inclusions [18]. The association between individual baseline levels and age, gender, body weight, AST, ALT, diabetes, antiretroviral treatment and presence of additional lipid-lowering agents was explored. Statistically significant covariates were then included in a stepwise forward inclusion and backward deletion approach. Continuous variables were centred on their median value and tested using linear and allometric relationships, as appropriate. For the PD part of the model, age was also tested as a time-varying covariate to account for differences in the within- and between-subject covariate effect, either by estimating a baseline covariate effect and a difference from baseline covariate effect, or by including between-subject variability on the covariate effect [19].

Further exploration of the effect of age on baseline was carried out by testing the effect of age at rosuvastatin treatment start, and of the follow-up period (*i.e.* time between the first and the last non-HDL-cholesterol value). Categorical covariates were coded as indicator variables, as 0 or 1. Missing values for weight, AST, ALT and creatinine clearance were imputed to the population median value. The effect of ARV drugs on PK/PD parameters was evaluated either by independently testing each ARV agent or grouping them considering their potential for DDIs with rosuvastatin (PK part of the model) or their impact on lipids according to the European AIDS Clinical Society (EACS) (20).

Parameter estimation and model selection

Hierarchical models were discriminated using the log likelihood ratio test, based on changes in the objective function value (ΔOFV). Goodness-of-fit plots, precision and plausibility of model parameters were also considered to evaluate the reliability of the model. In univariate analyses and forward inclusion of covariates, a decrease in OFV greater than 3.84 (p<0.05) was considered statistically significant. During backward deletion step, a covariate was retained in the final model if its deletion from the full model led to a 7.88-point increase in the OFV (p<0.005).

Model evaluation

A sensitivity analysis was performed to evaluate the potential leverage effect of outliers' concentrations on significant covariates. The comparison between parameter estimates obtained with the complete *vs.* reduced (*i.e.* after exclusion of these concentrations) dataset allowed deciding on the inclusion/removal of the data into/from the dataset.

The stability of the final PK and PK/PD models was assessed by the non-parametric bootstrap method using 2000 sampling with replacement to generate median parameters along with their 95% confidence intervals ($CI_{95\%}$), and to compare them with the final model estimates. In addition, prediction-corrected visual predictive checks (pcVPC) of final PK and PK/PD models were built using 1000 simulations [21].

Model-based simulations

Model-based simulations were performed to compare rosuvastatin maximal concentrations (C_{max}), minimal concentrations (C_{min}), and area under the concentration-time profile from 0 to 24h (AUC_{0-24}) under different ARV drugs. Non-HDL-cholesterol levels were also simulated and compared to a target value of 2.8 mmol/L, calculated by adding 0.8 mmol/L to the LDL target recommended by the EACS (2.0 mmol/L) [14, 20].

IV.4.4. Results

IV.4.4.1. Study population and data

The six PLWH enrolled in the PK study with rich sampling provided 65 rosuvastatin plasma concentrations. Additionally, 89 rosuvastatin plasma concentrations were collected in 62 PLWH in a sparse sampling design (figure 2). A median of 11 (range 10-11) and 1 (range 1-3) samples par patient was collected during the rich and sparse sampling studies, respectively, from 0.2 to 38.5 hours after the last drug intake. Rosuvastatin daily dose varied between 5 and 20 mg, with a median of 10 mg.

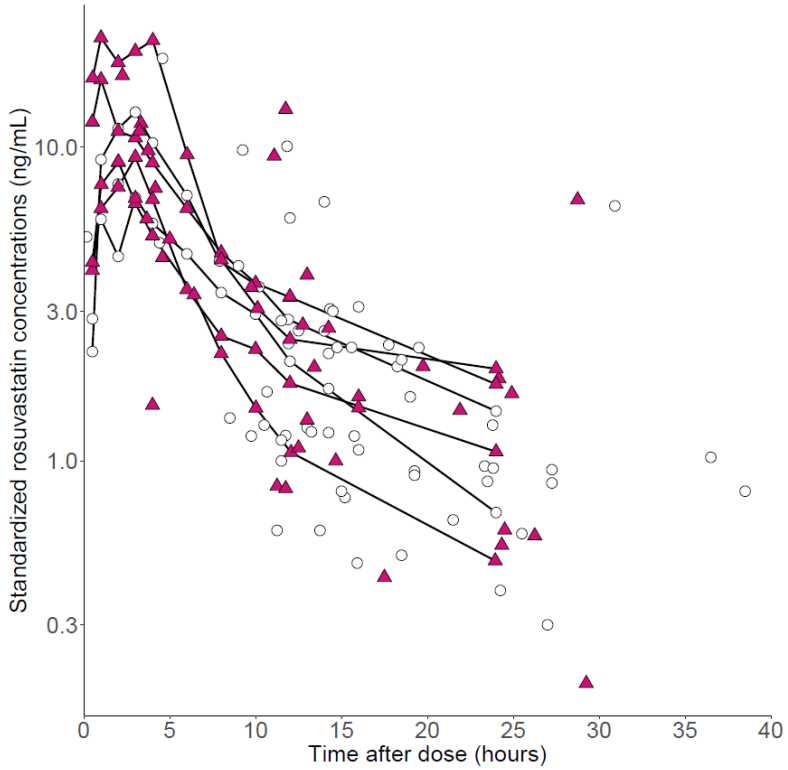

Figure 2: Standardized observed rosuvastatin plasma concentration-time profiles. Rosuvastatin plasma concentrations were standardized for a daily dose of 10 mg once daily and are presented in log-scale. Concentrations in PLWH receiving boosted PIs are presented in pink triangles while concentrations observed in PLWH receiving ARV drugs devoid of interaction potential in white circles. Rosuvastatin plasma concentrations observed in PLWH enrolled in the PK study with rich sampling are joined with black lines.

Characteristics of the study population are presented in table 1. Median age of participants was 61 years-old and ritonavir-boosted darunavir was the most frequent coadministered antiretroviral agent.

Table 1: Demographic and clinical characteristics of the study population.

Patient's characteristics at baseline (N=65)	**Median [IQR] or n (%)**
Age (years)	55 [49-64]
Women	8 (12)
Body weight (kg)	75 [66-85]
Missing data	2 (3)
ALT (UI/L)	29 [23-45]
Missing data	4 (6)
AST (UI/L)	28 [23-36]
Missing data	4 (6)
Creatinine clearance ($mL.min^{-1}.1.73m^{-2}$)	87 [79-119]
Missing data	4 (6)
Comedications (N=154)	**n (%)**
Ritonavir-boosted darunavir*	70 (46)
Cobicistat-boosted darunavir*	4 (3)
Ritonavir-boosted atazanavir*	1 (1)
Cobicistat-boosted atazanavir*	2 (1)
Cobicistat-boosted elvitegravir	14 (9)
Etravirine	38 (25)
Efavirenz	21 (14)
Nevirapine	9 (6)
Rilpivirine	2 (1)
Dolutegravir	47 (31)
Raltegravir	38 (25)

*Considered as boosted PIs

A total of 403 non-HDL cholesterol values were available for the PK/PD modelling (253 and 150 values before and after rosuvastatin beginning, respectively). Fifty-five PLWH had at least one baseline non-HDL-cholesterol level, collected in a median of 1.1 years (range 0-3.7) before rosuvastatin treatment start. The number of baseline non-HDL-cholesterol values per individual varied between 0 and 5, with a median of 5. After rosuvastatin initiation, data were collected for a median of 3.7 years (range 0-20.4). Characteristics of the study population before and after rosuvastatin initiation are presented in supplementary material 1.

IV.4.4.2. PK/PD analysis

PK analysis

A two-compartment model with sequential zero- and first-order absorption adequately described rosuvastatin full PK profiles (figure 1). Ka and D1 were estimated at 0.306 h^{-1} and 0.461 h. Fixing these parameters to these values during subsequent model development allowed a precise and plausible estimation of the other PK parameters when analysing the full dataset. Between-subject variability was estimated on clearance (Cl) and central volume of distribution (V_c). An additive error model in the log-scale adequately described the residual variability.

In univariate analyses, no covariate showed any influence on Cl, and coadministration of boosted PIs decreased V_c by 65% when coadministered with boosted PIs (ΔOFV=-3.88), and increased by 3-fold in women (ΔOFV= -6.12). None of the other tested covariates showed any significant influence on Cl or V_c (ΔOFV ≥−2.63; p>0.10). Overall, no covariate was retained in the final model due to a lack of statistical significance during the multivariate analysis. Of note, the sensitivity analysis performed excluding one individual (11 plasma samples) with high rosuvastatin plasma concentrations justified the maintenance of this individual in the dataset, *i.e.* no clinically significant changes in the parameter estimates or in the covariate impacts were observed.

Parameter estimates of the final rosuvastatin PK model along with bootstrap results are presented in Supplementary material 2. Model reliability was supported by the bootstrap results showing that median values differed less than 15% compared to the population estimates. In addition, pcVPC (supplementary material 3) demonstrated an adequate description of the data by the model.

PK/PD analysis

In the PK/PD model, between-subject variability was estimated with good precision on baseline and IC_{50}. A proportional error model adequately captured the residual variability. Univariate analyses revealed that etravirine, ARV drugs with a known negative impact on lipids (*i.e.* PIs, efavirenz and cobicistat) and age had a significant effect on baseline non-HDL-cholesterol (ΔOFV<−11.9; $p<5.10^{-4}$). The effect of age was modelled using a linear function and the addition of a time-varying covariate effect did not improve the fit (ΔOFV=−2.69; p=0.10). The decrease in the OFV was more pronounced when including the effect of age compared to the inclusion of both the age at start of rosuvastatin treatment and of the follow-up period (ΔOFV=3.4; p=0.07).

All the covariates were retained after multivariate analyses. Coadministration of etravirine was associated with a 14% decrease in baseline non-HDL-cholesterol value. Conversely, coadministration of PIs, efavirenz or cobicistat increased baseline level by 12%. Finally, baseline value was surprisingly decreased by 43% between PLWH aged 40 to 80 years-old.

Parameter estimates from the final PK/PD model are presented in table 2. All parameters were estimated with good precision (RSE≤39%), except for the effect of ARV with negative impact on lipids on baseline value (RSE=65%). The latter was however retained in the final model because of its known impact on non-HDL-cholesterol values. Bootstrap analyses are ongoing for model validation.

Table 2: Parameter estimates of the final PK/PD model.

	Final model	
Parameter	**Estimate**	**RSE (%)**
Pharmacokinetics		
Ka (h^{-1})	0.306 FIX	
D1 (h)	0.461 FIX	
CL ($L.h^{-1}$)	122	9
ω_{CL} (CV%)	51	13
V_c (L)	144	47
ω_{Vc} (CV%)	94	30
V_p (L)	1610	33
Q ($L.h^{-1}$)	69	19
Additive residual error[1]	0.30	14
Pharmacodynamics		
K_{in} ($mmol.L^{-1}.h^{-1}$)	0.02	20
Baseline ($mmol.L^{-1}$)	3.6	7
$\omega_{baseline}$ (CV%)	20	9
θ_{ARV}	0.12	65
θ_{ETV}	-0.14	39
θ_{age}	-0.81	24
IC_{50} (ng/mL)	15.8	30
ω_{IC50} (CV%)	101	17
Proportional residual error (%)	42	5

RSE: relative standard error, defined as SE/estimate, CI: confidence interval, Ka: absorption rate constant, D1: duration of zero-order absorption, CL: rosuvastatin clearance, V_c: rosuvastatin central volume of distribution, V_p: rosuvastatin peripheral volume of distribution, Q: inter-compartmental clearance, K_{in}: production rate of non-HDL-cholesterol, θ_{ARV}: effect of ARV with negative impact on lipids (*i.e.* boosted PIs, cobicistat and efavirenz) on baseline, θ_{ETV}: effect of etravirine on baseline, θ_{age}:

effect of age on baseline, IC_{50}: rosuvastatin concentration that led to a 50% inhibition of non-HDL-cholesterol production, ω: between-subject variability

[1]Additive residual error in log scale, reported as standard deviation

Goodness of fit plots for the final PK/PD model are shown in supplementary material 4. Finally, the pcVPC indicated an adequate description of the observed data by the final model (figure 3).

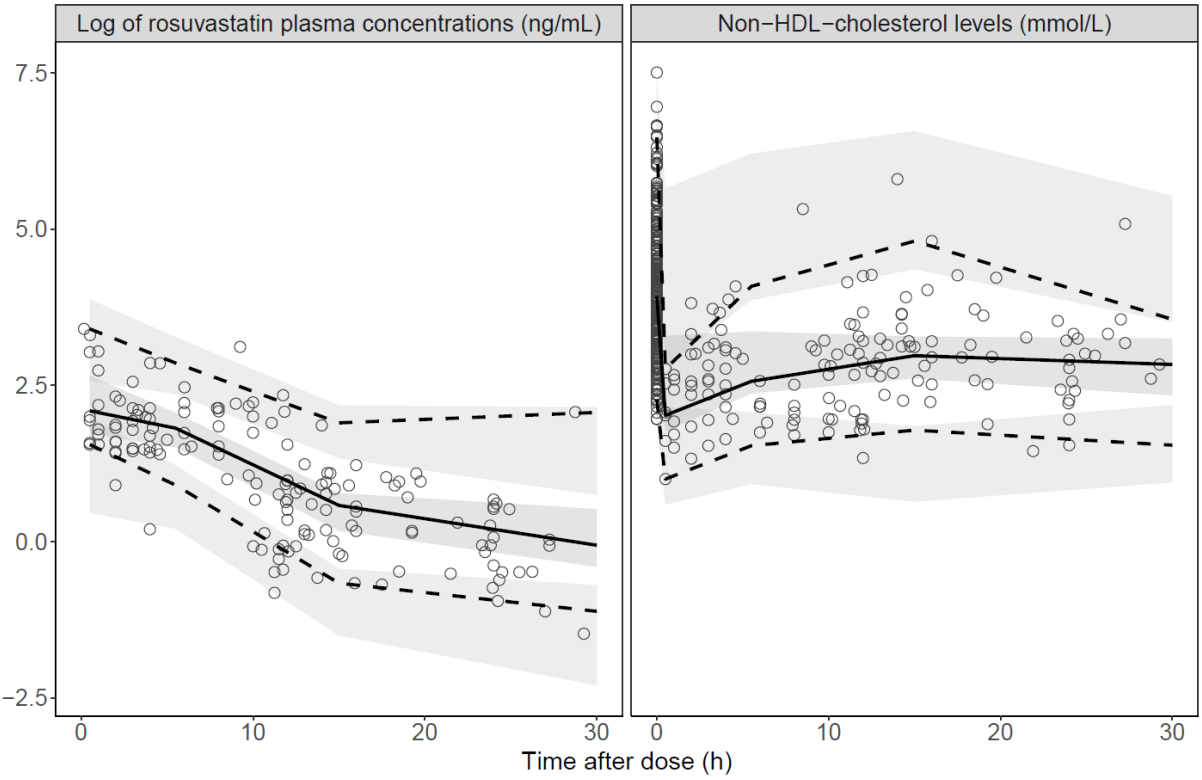

Figure 3: Prediction-corrected visual predictive check of the final PK/PD model. Open circles represent log rosuvastatin plasma concentrations (left) and non-HDL cholesterol values (right). The continuous line represents the median observed concentration and the dashed lines represent the observed 2.5% and 97.5% percentiles. Shaded areas represent the model-based 95% confidence interval for the median, the 2.5% and 97.5% percentiles.

Model-based simulations

Even though coadministration of boosted PIs was not retained as a covariate in the final PK model, model-based simulations were performed to compare rosuvastatin disposition when coadministered with boosted PIs or ARV drugs devoid of interaction potential. As demonstrated in figure 4, while rosuvastatin $AUC_{0\text{-}24}$ did not differ between ARV regimens, rosuvastatin C_{max} and C_{min} were increased by 29% and decreased by 6%, respectively when coadministered with boosted PIs.

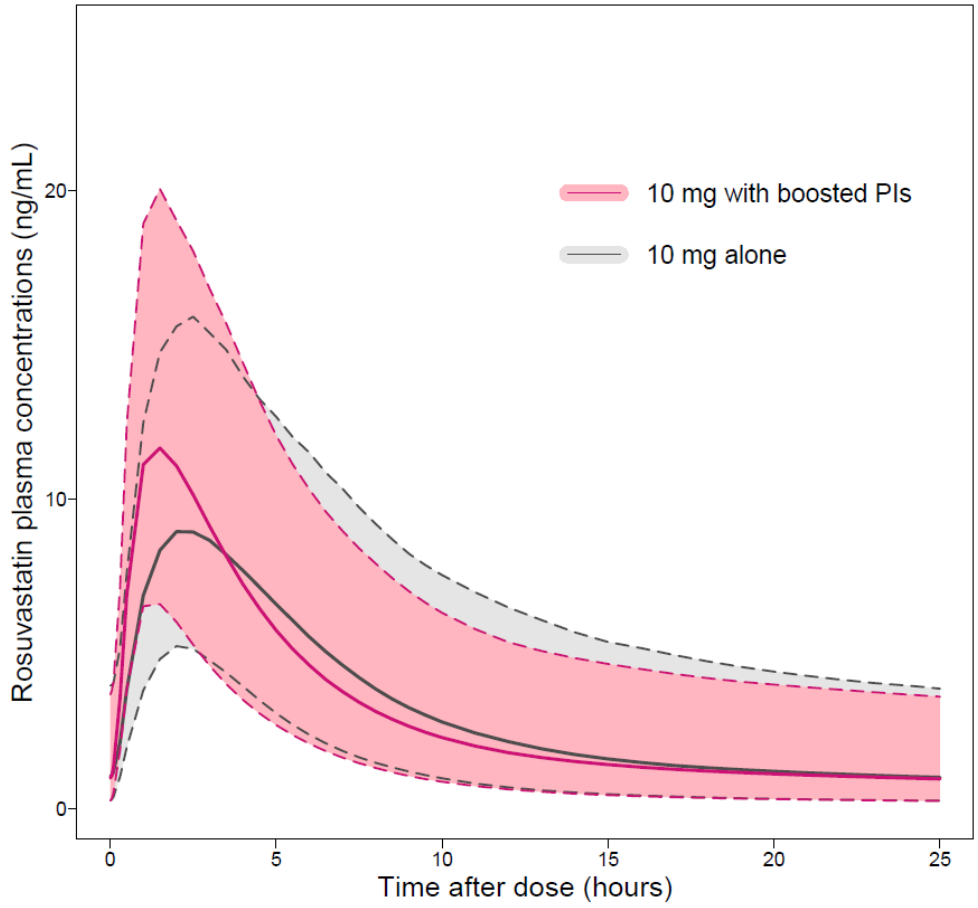

Figure 4: Rosuvastatin simulated plasma concentrations (n=1000) after administration of a standard dose of 10 mg once daily, alone (pink) or with boosted PIs (grey). Continuous lines represent the population median prediction and shaded areas represent 95% prediction interval for rosuvastatin alone (grey) or with boosted PIs (pink).

Simulations based on the structural PK/PD model (covariate-free model) showed that non-HDL-cholesterol targets were achieved in 31% of PLWH receiving a rosuvastatin dose of 5 mg once daily while this proportion reached 64% after the administration of a rosuvastatin daily dose of 20 mg (figure 5). The coadministration of inhibitors or inducers are not expected to have a clinical impact on the achievement of therapeutic targets.

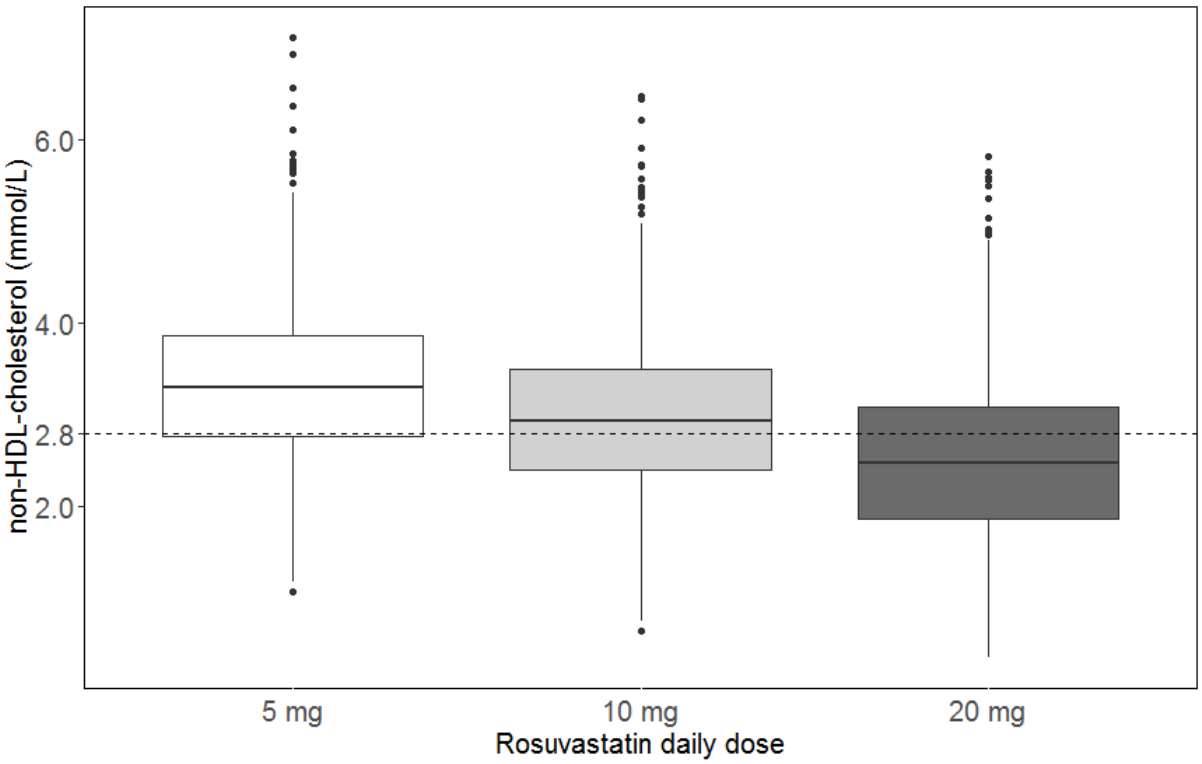

Figure 5: Distribution of non-HDL-cholesterol values, 24h after administration of rosuvastatin dose at steady state, simulated in 1000 individuals using the base PK/PD model. Dashed line represents the non-HDL-cholesterol target according to EACS guidelines [20].

IV.4.5. Discussion

Our study presents rosuvastatin exposure in a real-life setting of PLWH. To date, rosuvastatin population PK studies have been performed in healthy volunteers or pediatric patients [11, 22], but not in an HIV-infected population. PK parameters are in good accordance with previously published studies, while large fluctuations have been observed in the literature with values of volume of distribution varying from 1255 to 4870 L [11, 23, 24]. Our study reports a high between-subject variability on rosuvastatin Cl and V_c. This can be attributed to OATP1B1 and BCRP genetic polymorphism which have been shown to strongly affect rosuvastatin PK, mainly during the absorption phase [25, 26]. Despite this high variability, none of the tested covariates was retained in the final PK model. Although creatinine clearance and ethnicity have been shown to significantly influence rosuvastatin disposition in a previously published paper [11], the narrow interquartile range of creatinine clearance and the low percentage of non-white PLWH in our population prevented us to replicate these results.

The effect of ARV agents on rosuvastatin PK has already been described. PK studies reported a 243% and 148% increase in rosuvastatin C_{max} and AUC_{0-24} when coadministered with ritonavir-boosted darunavir (6) and a 277% and 93% increase in rosuvastatin C_{max} and AUC_{0-24} when coadministered with

cobicistat-boosted darunavir [7]. In addition, coadministration of ritonavir-boosted atazanavir and ritonavir-boosted lopinavir have been shown to increase rosuvastatin C_{max} by 600% and 370% respectively, while AUC_{0-24} was increased by 213% and 110%, respectively [17, 27]. The reported differences in the magnitude of DDIs between rosuvastatin and several boosted PIs have been attributed to their different potency to inhibit OATP1B1 [28]. In our study, the small sample size when individually considering each boosted PI prevented us to differentiate each of their effect on rosuvastatin PK. Our results demonstrated that coadministration of boosted PIs increased rosuvastatin C_{max} by 29%, without any influence on rosuvastatin exposure. The difference in the magnitude of DDIs compared to the above results may be related to the lack of data and to the high between-subject variability, notably during the absorption phase. Indeed, previously published studies were conducted in healthy volunteers [6, 7, 17, 27], who do not reflect the complex situation in a real-life clinical setting. In our non-selected population of PLWH, the 29% increase in rosuvastatin C_{max} when coadministered with boosted PIs was considered non-clinically significant. Our results are in line with the EACS guidelines, in which the maximum recommended daily dose of rosuvastatin when coadministered with boosted PIs does not differ from the maximal recommended dose in the general population. However, since some cases of rhabdomyolysis have been reported in PLWH concomitantly rosuvastatin and boosted PIs, clinical signs of adverse reactions should be cautiously monitored [3, 4].

To our knowledge, no PK/PD analysis describing rosuvastatin lipid-lowering effect has been published. The PD analysis revealed a large between-subject variability on the IC_{50} parameter, which could be related to the genetic polymorphism in transporters involved in the entry of rosuvastatin in the liver, thus regulating its concentration in the hepatocyte, the site of action. Between-subject variability on the baseline was smaller and remained unexplained after the inclusion of covariates. The absence of significant effect of gender and body weight on the PD parameters is in good agreement with a population PK/PD model developed for atorvastatin, simvastatin and fluvastatin [29]. Our study showed a negative impact of PIs, efavirenz and cobicistat on lipid profile, increasing baseline value by 12%. Although estimated with poor precision, this parameter was maintained in the model since this adverse effect is largely described in the literature for each of these drugs [8]. The magnitude of this effect was similar to the improvement of non-HDL-cholesterol when switching from a PI-containing to a dolutegravir-based regimen (neutral effect on lipids) [30]. On the other hand, coadministration of etravirine was significantly associated with a decrease in non-HDL-cholesterol baseline values. This effect may result either from a positive impact of etravirine *per se* on lipid profile, or from an improvement of lipid parameters after switching from ARV drugs with negative impact on lipid to etravirine treatment. Indeed, a previous study demonstrated that switching to an etravirine-

containing regimen in PLWH on stable ARV treatment (mainly efavirenz and PIs) was associated with a significant improvement on lipid parameters [31]. Although etravirine treatment was not necessarily preceded by an efavirenz- or PI-containing ARV regimen in the present study, this may have occurred before the inclusion in the study. Finally, our study showed a significant decrease in non-HDL-cholesterol levels when aging, whereas older age was associated with higher total cholesterol levels in previously published studies [32, 33]. This effect could result from a close and frequent monitoring of PLWH included in the SHCS, and therefore from a better management of cardiovascular risk factors throughout their follow-up. HIV clinicians insist on lifestyle and dietary measures by promoting physical activity and balanced diet which could improve the lipid profile of PLWH. Similar conclusions were drawn when observing a higher life expectancy of PLWH compared to the general population. Such results were attributed to the better management of chronic disease risk factors and earlier diagnosis of other diseases compared to the general population [34]. In addition, lifestyle and dietary measures to reduce cholesterol could be followed more closely by elderly patients, who have already experienced a cardiovascular event and in whom statin is introduced as a secondary prevention, compared to the youngest who receive statin as primary prevention.

Given the PK/PD model parameters, non-HDL-cholesterol values after different doses of rosuvastatin could be simulated. Simulations based on the base PK/PD model revealed that the majority (64%) of PLWH receiving a rosuvastatin dose of 20 mg once daily would achieve non-HDL-cholesterol targets [20]. This result highlights the effective lipid-lowering effect of rosuvastatin in PLWH and is in line with previously published studies reporting a better efficacy of rosuvastatin in lowering LDL cholesterol and raising HDL cholesterol levels in PLWH compared to other statins [35, 36].

In conclusion, the weak magnitude of DDI between rosuvastatin and ARV agents and the efficient lipid-lowering effect of rosuvastatin support the safe and effective prescription of rosuvastatin in PLWH.

IV.4.6. References

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IV.4.7. Supplementary material

Supplementary material 1: Comparison of demographic and clinical characteristics, before and after rosuvastatin initiation.

Characteristics	Median [IQR] or n (%)	
	Before rosuvastatin treatment (N=253)	After rosuvastatin treatment (N=150)
Age (years)	55 [50-64]	64 [60-70]
Body weight (kg)	74 [66-82]	76 [71-95]
Missing data	3 (1)	33 (22)
Non-HDL-cholesterol (mmol/L)	4.0 [3.2-4.8]	2.6 [2.1-3.2]
HDL cholesterol (mmol/L)	1.3 [1.0-1.6]	1.2 [1.1-1.4]
Triglycerides (mmol/L)	2.0 [1.4-2.9]	1.9 [1.4-3.0]
Missing data	31 (12)	1 (0.7)
AST (UI/L)	28 [23-34]	25 [22-33]
Missing data	1 (0.4)	67 (45)
ALT (UI/L)	27 [21-44]	24 [21-39]
Missing data	1 (0.4)	67 (45)
Associated lipid-lowering agent	4[1] (2)	23 (15)[2]
Antiretroviral treatment		
Ritonavir-boosted darunavir[3]	66 (26)	70 (47)
Cobicistat-boosted darunavir[3]	0	3 (2)
Ritonavir-boosted atazanavir[3]	16 (6)	1 (1)
Cobicistat-boosted atazanavir[3]	0	2 (1)
Lopinavir[3]	15 (6)	0
Saquinavir[3]	5 (2)	0
Cobicistat-boosted elvitegravir[3]	2 (1)	13 (9)
Etravirine	44 (17)	37 (25)
Efavirenz[3]	120 (47)	19 (13)
Nevirapine	23 (9)	9 (6)
Rilpivirine	5 (2)	2 (1)
Dolutegravir	9 (4)	46 (31)
Raltegravir	54 (21)	37 (25)

[1]3 ezetimibe, 1 fenofibrate

[2]16 ezetimibe, 8 fenofibrate

[3]Considered with negative impact on lipids

Supplementary material 2: Parameter estimates of the final rosuvastatin PK model with bootstrap results

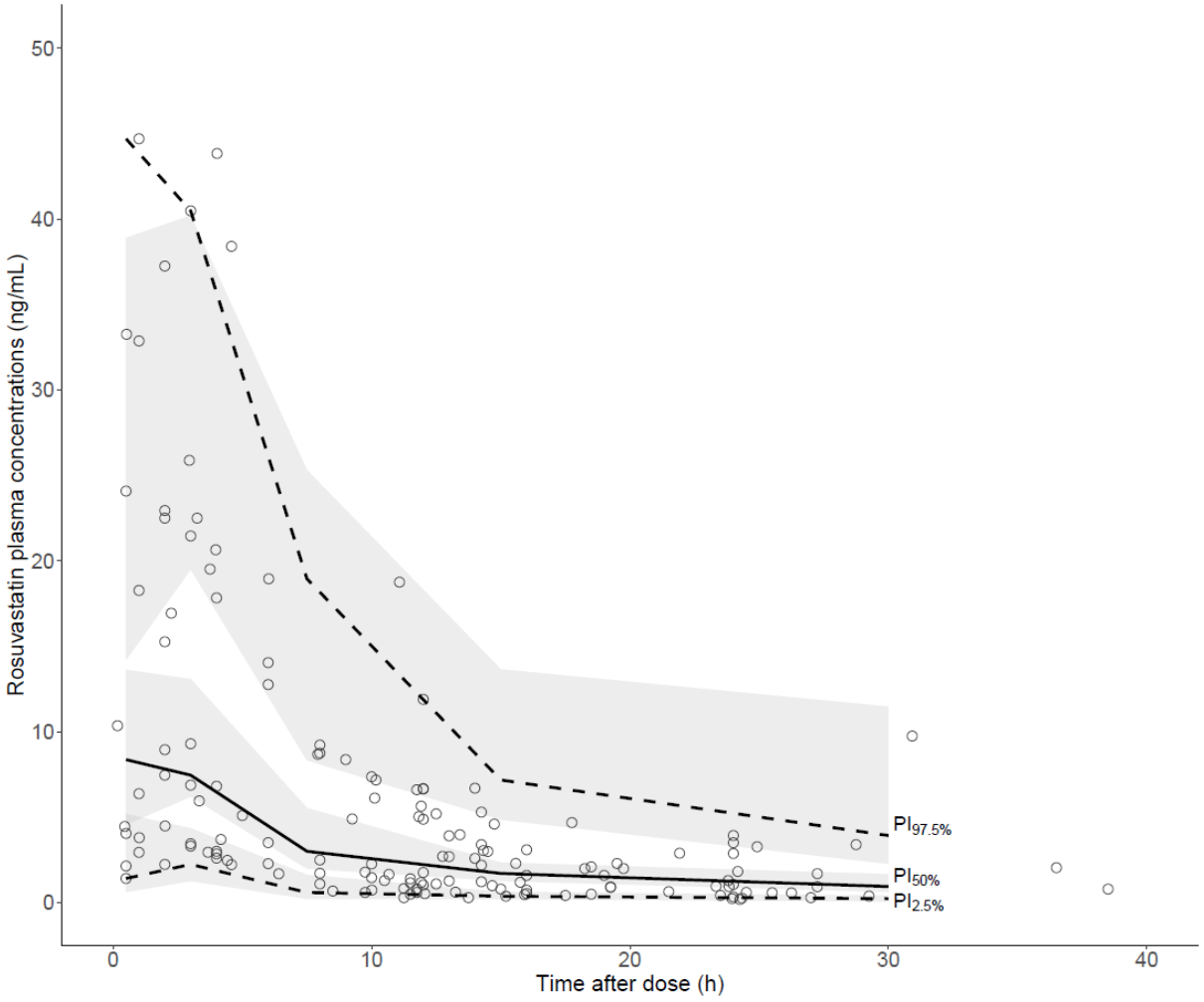
	Final model		Bootstrap (n=2000 samples)	
Parameter	**Estimate**	**RSE[1] (%)**	**Median**	**$CI_{95\%}$**
Ka (h^{-1})	0.306 FIX			
D1 (h)	0.461 FIX			
CL ($L.h^{-1}$)	122	8	122	103-144
ω^2_{CL} (CV%)	50	13	49	34-64
V_c (L)	153	23	144	46-266
ω^2_{Vc} (CV%)	97	42	87	24-526
V_p (L)	1660	22	1595	940-3868
Q ($L.h^{-1}$)	67	17	69	46-95
Additive residual error[2]	0.29	27	0.29	0.20-0.36

[1]Relative standard error, defined as SE/estimate.

[2]Additive residual error in log scale, reported as standard deviation

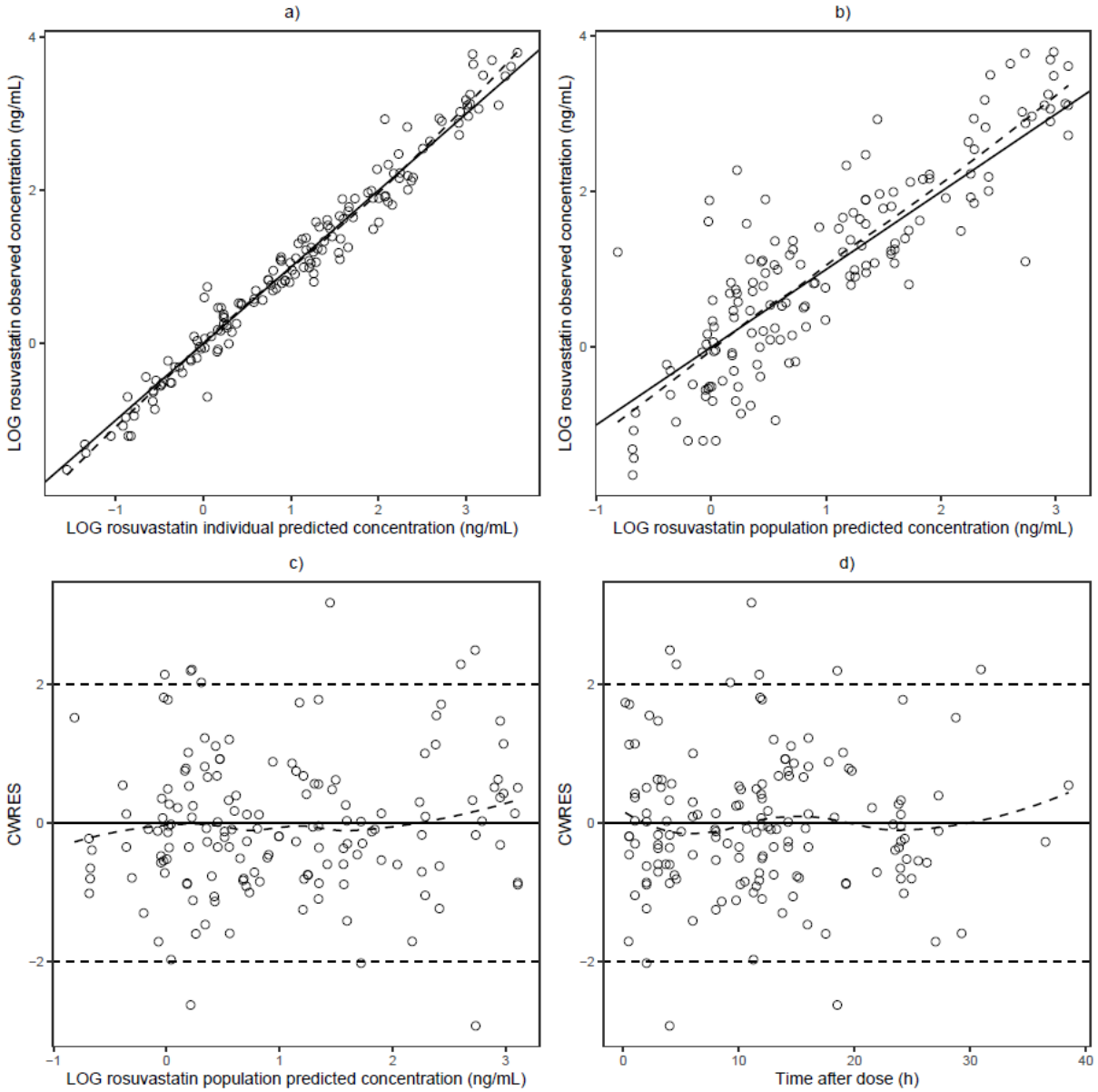
ω^2 between-subject variability

Supplementary material 3: pcVPC of the final PK model (n=154 rosuvastatin concentrations). Open circles show rosuvastatin plasma concentrations. The continuous and dashed line represents the median of the observed plasma concentrations, and the observed 2.5% and 97.5% percentiles respectively. Shaded areas show the model predicted 95% confidence interval for the median, 2.5% and 97.5% percentiles.



Supplementary material 4: Goodness-of-fit plots of the final PK (1) and PD (2) model. Loess smooth curves are printed in dashed lines. a) Observed concentrations vs. individual predictions; line of identity is printed in black. b) Observed concentrations vs. population predictions; identity line is printed in black. c) Conditional weighted residuals (CWRES) vs. population predictions; ordinate value zero line is printed in black. d) CWRES vs. time post-dose; ordinate value zero line is printed in black.

1



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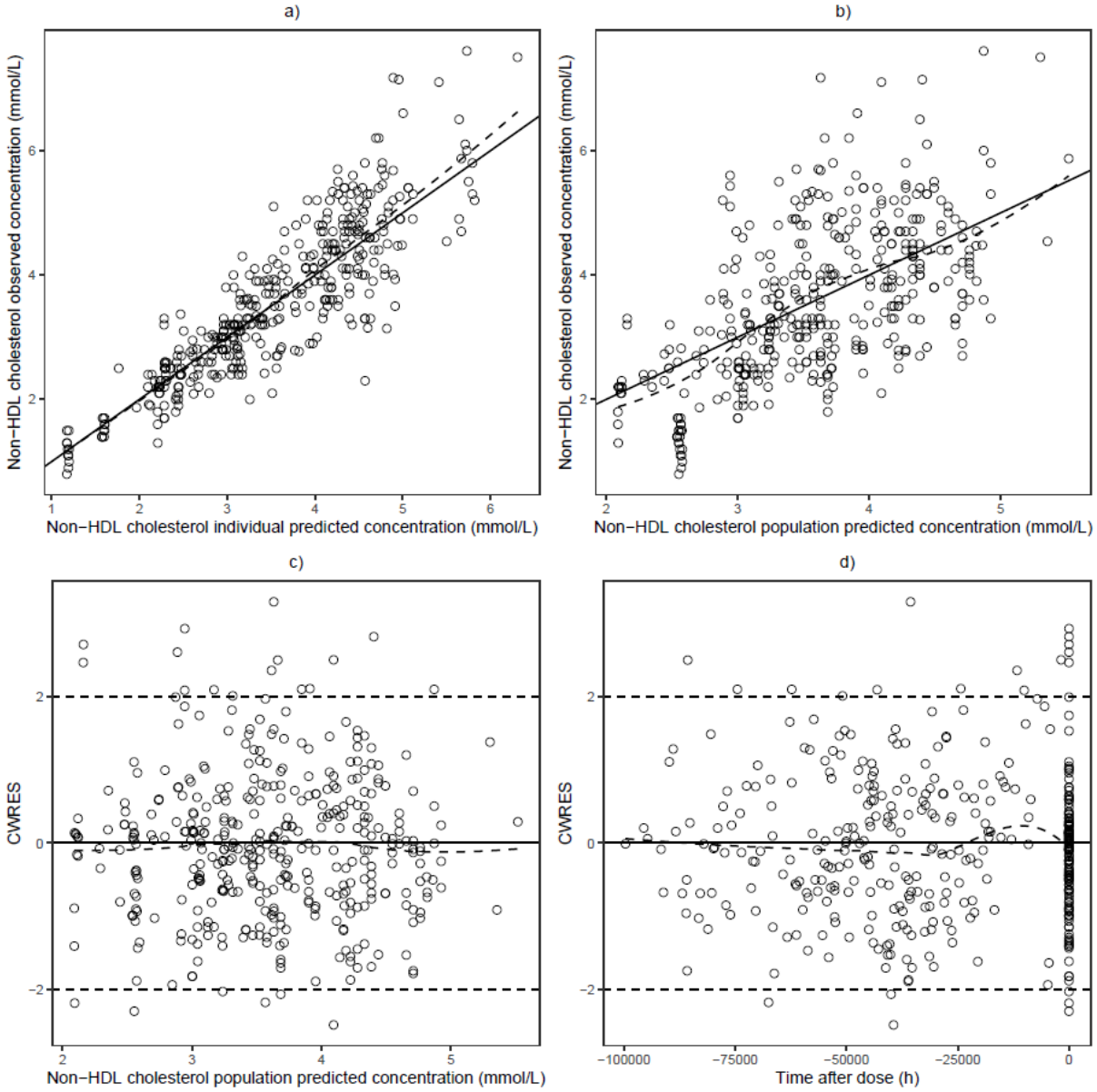
a)
Non−HDL cholesterol observed concentration (mmol/L)
Non−HDL cholesterol individual predicted concentration (mmol/L)
b)
Non−HDL cholesterol observed concentration (mmol/L)
Non−HDL cholesterol population predicted concentration (mmol/L)
c)
CWRES
Non−HDL cholesterol population predicted concentration (mmol/L)
d)
CWRES
Time after dose (h)

IV.5. Population pharmacokinetics of dolutegravir: influence of drug–drug interactions in a real-life setting.

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Population pharmacokinetics of dolutegravir: influence of drug–drug interactions in a real-life setting

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†Equal contribution to the work.
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Objectives: Dolutegravir is widely prescribed owing to its potent antiviral activity, high genetic barrier and good tolerability. The aim of this study was to characterize dolutegravir's pharmacokinetic profile and variability in a real-life setting and to identify individual factors and co-medications affecting dolutegravir disposition.

Methods: A population pharmacokinetic model was developed using NONMEM®. Relevant demographic factors, clinical factors and co-medications were tested as potential covariates. Simulations based on the final model served to compare expected dolutegravir concentrations under standard and alternative dosage regimens in the case of drug–drug interactions.

Results: A total of 620 dolutegravir plasma concentrations were collected from 521 HIV-infected individuals under steady-state conditions. A one-compartment model with first-order absorption and elimination best characterized dolutegravir pharmacokinetics. Typical dolutegravir apparent clearance (CL/*F*) was 0.93 L/h with 32% between-subject variability, the apparent volume of distribution was 20.2 L and the absorption rate constant was fixed to 2.24 h^{-1}. Older age, higher body weight and current smoking were associated with higher CL/*F*. Atazanavir co-administration decreased dolutegravir CL/*F* by 38%, while darunavir modestly increased CL/*F* by 14%. Rifampicin co-administration showed the largest impact on CL/*F*. Simulations suggest that average dolutegravir trough concentrations are 63% lower after 50 mg/12 h with rifampicin compared with a standard dosage of 50 mg/24 h without rifampicin. Average trough concentrations after 100 mg/24 h and 100 mg/12 h with rifampicin are 92% and 25% lower than the standard dosage without rifampicin, respectively.

Conclusions: Patients co-treated with dolutegravir and rifampicin might benefit from therapeutic drug monitoring and individualized dosage increase, up to 100 mg/12 h in some cases.

Introduction

Dolutegravir is an integrase strand-transfer inhibitor (INSTI) recommended as a first-line treatment in treatment-naive and experienced HIV-infected individuals. The combination of dolutegravir with abacavir/lamivudine or tenofovir disoproxil fumarate/emtricitabine has proven to be an efficacious and convenient once-daily regimen with a favourable safety profile and a high barrier to resistance.[1–5]

Dolutegravir is readily absorbed after oral administration, with a median maximum concentration achieved between 0.5 and 2.5 h post-dose. According to the manufacturer's prescribing information, dolutegravir may be taken with or without food. However,

dolutegravir exposure is increased when administered with a meal, particularly with high fat content (AUC increase ranged from 33% to 66% depending on the fat components of the meal).[1,6] Once absorbed, 99% of the circulating dolutegravir is bound to plasma proteins.[7] While many antiretroviral drugs are metabolized by the cytochrome P450 3A (CYP3A) pathway, dolutegravir is extensively metabolized via glucuronidation by the phase II enzyme uridine glucuronosyltransferase 1A1 (UGT1A1; nearly 80%), with a minor contribution of CYP3A, UGT1A3 and UGT1A9.[8] Then, dolutegravir is excreted mainly via faeces, while metabolites are principally eliminated in urine.[1,9] Dolutegravir is also a substrate of the transporter proteins P-glycoprotein and breast cancer resistance protein.[1]

A recent population pharmacokinetic analysis, using pooled data from three clinical trials in treatment-naive patients, reported a low to moderate between-subject variability [BSV; expressed as the coefficient of variation (CV)] on dolutegravir clearance (24%), volume of distribution (14%) and absorption rate (50%).[10] In routine clinical practice, dolutegravir is often combined with additional antiretroviral drugs or co-medications, and its dosage empirically adjusted to treat complex HIV infections. Dolutegravir potential drug–drug interactions have been analysed in relatively small pharmacokinetic studies including healthy volunteers and, recently, in a population pharmacokinetic study using real-life cohort data.[11–18] The objective of this study was to develop a population pharmacokinetic model to characterize dolutegravir pharmacokinetic variability in a real-life setting and to assess the impact of potential drug–drug interactions and other factors influencing dolutegravir disposition.

Methods

Ethics

This study was conducted within the frame of the Swiss HIV Cohort Study (SHCS; http://www.shcs.ch) in accordance with the Declaration of Helsinki and national and institutional standards. Written informed consent was obtained from all study participants.

Study population

Dolutegravir plasma concentration data were obtained as part of therapeutic drug monitoring performed in several Swiss centres between October 2014 and February 2015. At treatment initiation and during usual follow-up visits, a blood sample (4.9 mL) was collected from patients receiving dolutegravir-based therapy. Exclusion criteria were undetectable dolutegravir plasma concentrations, suggestive of non-adherence to treatment, and non-reliable time information about blood sampling or last dose intake.

Analytical method

Plasma level measurements were performed at the Laboratory of Clinical Pharmacology of the University Hospital Centre of Lausanne. Plasma samples obtained from HIV-infected individuals were centrifuged and stored at −20°C until batch analysis. On the day of the analysis, samples were inactivated for virus at 60°C for 60 min. Dolutegravir plasma levels were determined by LC-MS/MS after protein precipitation with acetonitrile according to our previously reported analytical method, which was adapted to include dolutegravir.[19] The method showed acceptable inter-day and intra-day precision (2.4%–5.7% CV and 0.3%–1.3% CV, respectively) and trueness (−7.4% to 7.1% bias). The calibration standard curve was fitted with a quadratic log–log regression, and the lower limit of quantification was 40 ng/mL. The laboratory participates in an international external quality assurance programme for analysis of antiretroviral drugs [Kwaliteitsbewaking Klinische Geneesmiddelanalyse en Toxicologie (KKGT) ('Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology'), The Hague, the Netherlands; http://www.kkgt.nl/], showing suitable performances (i.e. trueness −3.5% and precision 4.4% at KKGT External Quality Control Proficiency Program).

Population pharmacokinetic analysis

Pharmacokinetic data were analysed using a non-linear mixed-effect modelling approach with NONMEM® (v7.4.0, ICON Development Solutions, Ellicott City, MD, USA), together with PsN v4.2.0 for automation of various model development and evaluation methods, Pirana v2.9.2 to structure and document model development and R v3.3.1 (Rstudio v.1.1.423) for data management, statistical analysis and graphical output.[20,21]

Structural model

One- and two-compartment models with first- and/or zero-order absorption with and without absorption lag time were tested. Exponential errors following a log-normal distribution were assumed for the description of BSV of the pharmacokinetic parameters, described by the equation $\theta_j=\theta\times e^{\eta j}$, where θ_j is the individual pharmacokinetic parameter of the jth individual, θ is the geometric average population value and η_j is a between-subject random effect, which follows an independent, normal distribution with mean zero and variance ω^2.

Covariate model

The association between demographic characteristics, clinical characteristics and dolutegravir individual pharmacokinetic estimates was first explored graphically. All potential and physiologically plausible relationships were tested with a univariate analysis followed by a stepwise forward-inclusion and backward-deletion approach, and using linear or non-linear functions as appropriate (categorical covariates were coded as 0 and 1; continuous covariates were centred and normalized on their median value). Missing data in continuous covariates were imputed to the population median value, and missing data in categorical covariates were first coded as an additional category and then regrouped according to their parameter estimates. Typical bioavailability, *F*, was fixed to 1 and the impact of gastric bypass was estimated using a logit transformation.

Parameter estimation and model selection

The first-order conditional estimation method with interaction (FOCEI) was used for model fitting. The criteria considered for model selection were the log-likelihood ratio test, based on the reduction of the objective function value (ΔOFV), goodness-of-fit plots, pharmacokinetic parameter precision and BSV decrease. In the univariate analysis of covariates, a decrease in OFV >3.84 ($P<0.05$) for one additional parameter was considered statistically significant (ΔOFV between any two nested models approximates a χ^2 distribution), while a decrease/increase in OFV >7.88 ($P<0.005$) was considered statistically significant in the multivariate forward-inclusion and backward-deletion approach.

Model evaluation

The final model was evaluated using non-parametric bootstrapping (2000 replicates) to generate 95% CIs for parameter estimates. Additionally, a prediction- and variability-corrected visual predictive check (pvcVPC) was used to visually compare observed concentrations with 5th, 50th and 95th prediction percentiles.[22] An external validation was also performed with data collected as part of therapeutic drug monitoring performed in several

Swiss centres between March 2015 and December 2017.[23] Dolutegravir individual concentrations were predicted based on the final models using the MAXEVAL=0 option in NONMEM®. The predictive performance of the model was assessed in terms of bias [mean prediction error (MPE)], $\text{MPE} = \frac{\sum C_{\text{pred}} - C_{\text{obs}}}{N}$, and precision [root mean square prediction error (RMSE)], $\text{RMSE} = \sqrt{\frac{\sum (C_{\text{pred}} - C_{\text{obs}})^2}{N}}$, with the associated 95% CIs, where C_{pred} are population and individual predictions, C_{obs} are the observed concentrations of the validation group and N corresponds to the number of observations.[24]

Simulations of dosage regimens

Dolutegravir steady-state plasma concentrations were simulated with NONMEM® based on the final model estimates, including BSV for 1000 individuals under dolutegravir at 50 mg once daily, 50 mg twice daily, 100 mg once daily and 100 mg twice daily co-administered with rifampicin. Predicted average dolutegravir minimal concentration (C_{min}), maximal concentration (C_{max}) and AUC from 0 to 24 h (AUC_{0-24}), calculated as dose/CL/*F* at steady-state, together with 2.5th and 97.5th predicted percentiles were calculated and compared across dosage regimens. Simulated C_{min} values were compared with the *in vitro* protein-adjusted IC_{90} of 64 ng/mL and the suggested alternative target of 300 ng/mL, corresponding to trough concentrations observed with 10 mg of dolutegravir once daily that attained similar virological responses at week 24 to 50 mg of dolutegravir once daily in Phase 2 efficacy studies.[5,25]

Results

A total of 620 dolutegravir plasma concentrations from 521 HIV-infected individuals were obtained for the population pharmacokinetic model building, 17 of which were from 4 patients undergoing bypass surgery. A median of one sample per individual (range=1–7) was collected between 0.25 and 45.8 h after the last dose intake under steady-state conditions. Most patients received 50 mg of dolutegravir once daily, except for 15 participants whose dosage was adapted to increase dolutegravir exposure (Table 1). Two of the four individuals with gastric bypass presented an adjusted dosage of 50 mg of dolutegravir twice daily at some point and subsequently switched back to 50 mg once daily.[26] Participant demographic and clinical characteristics of the model building and validation groups are summarized in Table 1. Concerning potential drug–drug interactions, darunavir was the most frequent antiretroviral co-medication.

Table 1. Demographic and clinic characteristics of the study populations

Characteristic	Model building (*N*=521)	Model validation (*N*=155)
Male, *n* (%)	403 (77)	123 (79)
Age (years), median (range)	47 (17–79)	49 (24–76)
Body weight (kg), median (range)	73 (37–131)	74 (39–131)
missing data, *n* (%)	3 (0.6)	0
Current smoking, *n* (%)	190 (36)	64 (41)
missing data, *n* (%)	11 (2)	0
ALT (IU/L), median (range)	29 (6–792)	27 (10–96)
missing data, *n* (%)	15 (3)	0
AST (IU/L), median (range)	28 (5–343)	27 (14–387)
missing data, *n* (%)	17 (3)	0
Total bilirubin (μmol/L), median (range)	7 (0.2–55)	14 (3–122)
missing data, *n* (%)	461 (88)	119 (77)
Gastric bypass, *n* (%)	4 (0.8)	0
Co-medication[a], *n* (%)		
darunavir/ritonavir	94 (18)	22 (14)
atazanavir/ritonavir	10 (2)	3 (2)
atazanavir	4 (0.8)	4 (3)
lopinavir/ritonavir	2 (0.4)	1 (0.6)
tipranavir	1 (0.2)	0
efavirenz	15 (3)	1 (0.6)
etravirine	14 (3)	5 (3)
nevirapine	10 (2)	1 (0.6)
rilpivirine	4 (0.8)	0
maraviroc	5 (1)	2 (1)
rifampicin	9 (2)	1 (0.6)
Dolutegravir dosage[a], *n* (%)		
50 mg/24 h	515 (99)	151 (93)
50 mg/12 h	13 (2)	9 (6)
100 mg/24 h	3 (0.6)	1 (0.6)
100 mg/12 h	4 (0.8)	1 (0.6)

[a]Values reported as number of participants with at least one observation (% regarding total participant count) with a certain co-medication or dolutegravir dosage. Some individuals in the model building population received rescue antiretroviral regimens such as abacavir/lamivudine or tenofovir disoproxil fumarate/emtricitabine combined with dolutegravir/darunavir/ritonavir/etravirine (*n*=8), with dolutegravir/darunavir/ritonavir/nevirapine (*n*=3), with dolutegravir/darunavir/ritonavir/etravirine/maraviroc (*n*=2), with dolutegravir/tipranavir/ritonavir/maraviroc (*n*=1) and with dolutegravir/etravirine/maraviroc (*n*=2).

Dolutegravir structural and covariate model

Dolutegravir pharmacokinetics were best described by a one-compartment model with apparent clearance (CL/*F*), apparent volume of distribution (*V*/*F*), first-order absorption rate (k_a) fixed to 2.24 h^{-1} and typical bioavailability (*F*) fixed to 1. The value of k_a was fixed to the value of a previous pharmacokinetic study owing to the difficulty in estimating this parameter with acceptable precision (relative standard error ≥67%).[10] The addition of a second compartment resulted in poor precision estimates. BSV was only estimated with acceptable precision on CL/*F*. A combined proportional and additive error model adequately captured the residual variability.

Models with alternative absorption processes, including zero-order, combined first- and zero-order or lag time absorption, did not successfully fit the data (ΔOFV greater than −3.6). The presence of a gastric bypass significantly reduced dolutegravir bioavailability by 33%, but with poor estimate precision (95% CI=17% to 53%, *P*=0.049). Due to lack of power to adequately estimate the impact of gastric bypass on *F*, this condition was not retained in the final model.

Age, actual body weight, current smoking and co-administration of darunavir/ritonavir, atazanavir with or without ritonavir, and rifampicin showed a significant influence on dolutegravir CL/*F* and together explained 17% of the original BSV on CL/*F* (Table 2). Co-administration of nevirapine increased dolutegravir CL/*F* by 36%, but did not reach statistical significance in the multivariate forward covariate inclusion (95% CI=13% to 59%,

Table 2. Parameter estimates of the final dolutegravir pharmacokinetic model and bootstrap results

	Final dolutegravir pharmacokinetic model		Bootstrap (n=2000 samples)	
Parameter	estimate	RSE (%)	median	95% CI
CL/*F* (L h^{-1})	0.93	3	0.93	0.88 to 0.98
V/*F* (L)	20.2	9	20.2	17.2 to 23.4
k_a (h^{-1})	2.24		2.24	
Age∼CL/*F*	0.22	31	0.22	0.08 to 0.35
Body weight∼CL/*F*	0.31	31	0.31	0.12 to 0.49
Current smoking∼CL/*F*	0.19	23	0.18	0.10 to 0.27
ATV∼CL/*F*	−0.38	26	−0.38	−0.54 to −0.16
DRV/r∼CL/*F*	0.14	36	0.14	0.04 to 0.24
RIF∼CL/*F*	2.61	31	2.69	1.29 to 4.68
$BSV_{CL/F}$ (%)	32	31	31	25 to 36
Proportional residual error (%)	28	8	28	23 to 33
Additive residual error (ng/mL)	220	23	215	90 to 345

Final dolutegravir model: $CL/F = 0.93 \times \frac{age^{0.22}}{47} \times (1 + 0.31 \times \frac{body\ weight - 73}{73}) \times (1 + 0.19^{smoking}) \times (1 - 0.38^{ATV}) \times (1 + 0.14^{DRV}) \times (1 + 2.61^{RIF})$.
CL/*F*, apparent clearance; *V*/*F*, apparent volume of distribution; k_a, first-order absorption rate constant fixed to 2.24 h^{-1} according to published data; Age∼CL/*F*, relative influence of age, normalized by the median age in the population (47 years old), on CL/*F* using a power function; Body weight∼CL/*F*, relative influence of body weight, centred and normalized by the median body weight in the population (73 kg), on CL/*F* using a linear function; Current smoking∼CL/*F*, relative influence of current smoking on CL/*F*; ATV∼CL/*F*, relative influence of atazanavir with or without ritonavir co-administration on CL/*F*; DRV/r∼CL/*F*, relative influence of darunavir/ritonavir co-administration on CL/*F*; RIF∼CL/*F*, relative influence of rifampicin co-administration on CL/*F*; BSV, between-subject variability defined as CV (%); RSE, relative standard error defined as standard error/estimate, with standard error directly retrieved from NONMEM®.

P=0.02). Co-administration of etravirine modestly increased dolutegravir CL/*F* by 28%, but was not retained in the final model because of poor estimate precision and insufficient statistical significance in the multivariate forward covariate inclusion (95% CI=−12% to 45%, *P*=0.13). The parameter estimates of the final dolutegravir pharmacokinetic model are reported in Table 2. Covariate forward-inclusion and backward-deletion results are shown in Table S1, Table S2 and Table S3 (available as Supplementary data at *JAC* Online). Diagnostic plots for dolutegravir structural and final models are shown in Figures S1 and S2.

Model evaluation

Model reliability was supported by all bootstrap median values contained within the 95% CI and differing by <10% from the population estimates. The pvcVPC revealed an adequate description of the observed data, with <10% of points outside the 90% prediction interval (Figure 1). A total of 162 dolutegravir plasma concentrations from 155 HIV-infected individuals were obtained for the population pharmacokinetic model validation (Table 1). The external validation analysis showed a non-significant bias of −2% (95% CI= −6% to 2%) with a precision of 31% for dolutegravir individual predictions, similar to the proportional part of the model residual error, as expected.

Model simulations

Compared with the standard dosage of 50 mg once daily without rifampicin, simulations of 50 mg of dolutegravir twice daily co-administered with rifampicin showed an average predicted C_{min}, C_{max} and AUC_{0-24} that decreased by 63%, 28% and 40%, respectively. With dolutegravir at 100 mg once daily with rifampicin, average predicted C_{min} and AUC_{0-24} decreased by 92% and 40%, while C_{max} increased by 24%. When doubling the dosage to 100 mg of dolutegravir twice daily with rifampicin, average predicted dolutegravir C_{min} decreased by 25%, while C_{max} and AUC_{0-24} increased by 44% and 20%, respectively (Table 3). None of the simulated individuals with 50 mg of dolutegravir once daily without rifampicin presented a C_{min} below the protein-adjusted IC_{90} of 64 ng/mL.[25] Simulations with 50 mg twice daily, 100 mg once daily and 100 mg twice daily, all three co-administered with rifampicin, predicted 3%, 40% and 0.7% of individuals, respectively, with C_{min} below 64 ng/mL. Considering the alternative target C_{min} of 300 ng/mL, these percentages would increase to 32%, 81% and 12%, respectively. As shown in Figure 2, the predicted concentration–time course of 100 mg of dolutegravir twice daily co-administered with rifampicin overlaps with the curve of the 50 mg once daily without rifampicin regimen, whereas the recommended dosage of 50 mg twice daily and 100 mg once daily co-administered with rifampicin seems to provide lower exposure.

Discussion

Our study provides a description of the population pharmacokinetic profile of dolutegravir in a non-selected clinical setting, with dosage adjustment hypothesis testing, which could help the choice of dosage regimen under certain clinically relevant drug–drug interactions. The CL/*F* and *V*/*F* estimates are in good agreement with recently published population pharmacokinetic analyses.[10,18] The dolutegravir absorption phase could not be as exhaustively described, due to scarce measurements available within a few hours after drug administration. However, the time of maximal concentration derived from the final pharmacokinetic parameters (T_{max}=1.8 h) is in accordance with reported values.[10]

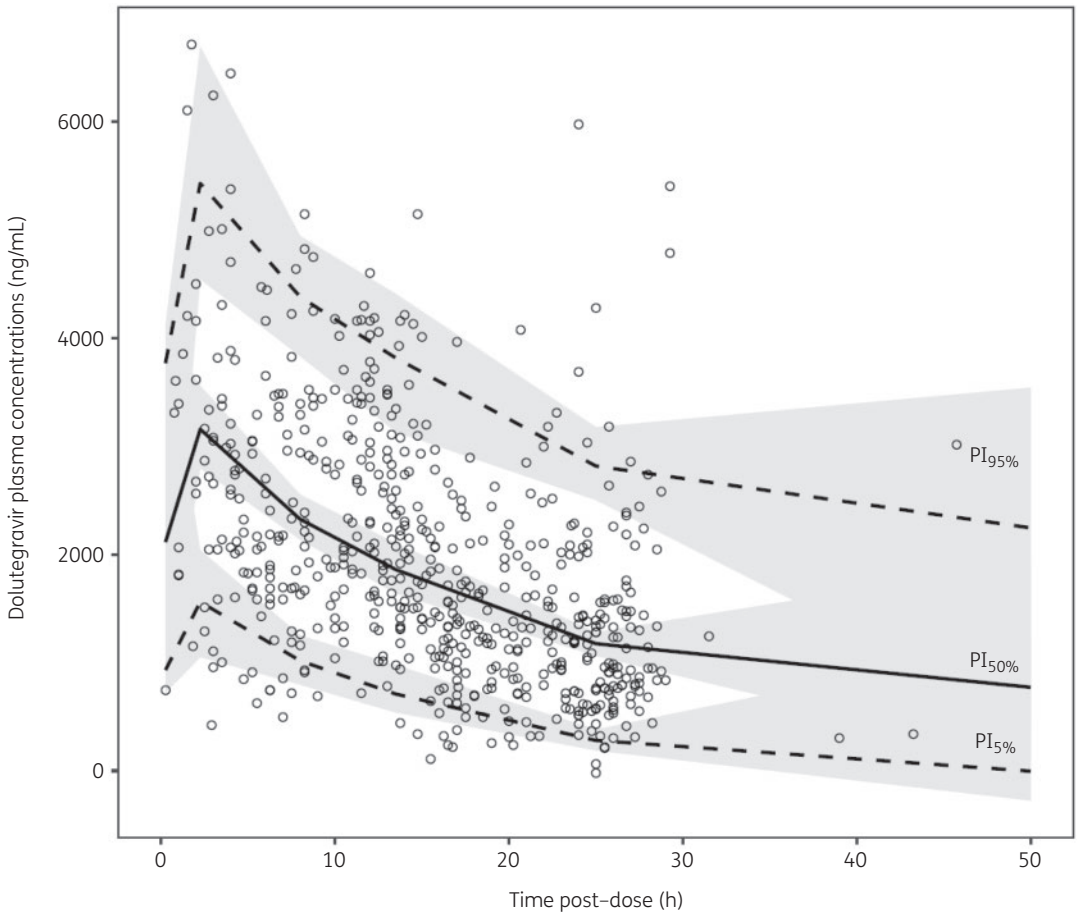

Figure 1. pvcVPC of the dolutegravir final model (n=521). Open circles represent dolutegravir plasma concentrations. The continuous line represents the population median prediction from the final model, and the broken lines and shaded areas represent the 90% prediction intervals and corresponding 95% CIs. PI, prediction interval.

Table 3. Summary of dolutegravir pharmacokinetic parameters at different dosage regimens with or without rifampicin co-administration, derived from model simulations

	50 mg/24 h	50 mg/12 h+RIF	100 mg/24 h+RIF	100 mg/12 h+RIF
C_{max} (ng/mL)	3249 (2367–5393)	2348 (1803–3448)	4064 (3563–5212)	4685 (3698–6863)
$GMR_{50\ mg/24\ h}$		0.72	1.24	1.44
C_{min} (ng/mL)	1077 (269–3186)	424 (54–1448)	94 (3–949)	840 (146-2864)
$GMR_{50\ mg/24\ h}$		0.37	0.08	0.75
AUC_{0-24} (ng h/mL)	49016 (25057–102381)	29794 (14746–58059)	29138 (15381–63819)	59322 (32032–115309)
$GMR_{50\ mg/24\ h}$		0.60	0.60	1.20

RIF, rifampicin; C_{max}, maximal concentration; C_{min}, minimal concentration; AUC_{0-24}, calculated with the equation AUC_{0-24}=dose/CL/F; $GMR_{50\ mg/24\ h}$, geometric mean ratio (compared with the standard regimen of 50 mg/24 h).
Pharmacokinetic values are reported as median (95% prediction interval).

Probably due to the non-selected population included in the present study, the estimated BSV on CL/F was moderate and slightly higher than in previous studies where dolutegravir pharmacokinetic variability was reported to be lower than for other INSTIs.[10,25,27]

In good accordance with other dolutegravir population analyses, older age, higher body weight and current smoking were associated with significantly higher dolutegravir elimination.[10,18] Yet, the magnitude of the effects of these covariates on dolutegravir exposure remains relatively small and does not require dose

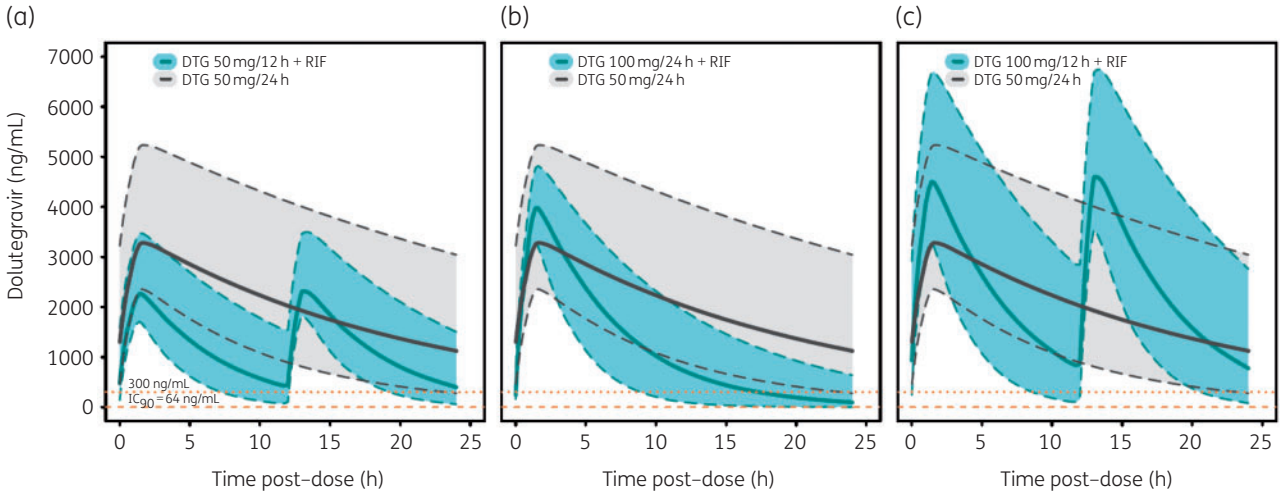

Figure 2. Dolutegravir simulated plasma concentrations for dosage regimens of 50 mg twice daily (a), 100 mg once daily (b) and 100 mg twice daily (c) using rifampicin co-administration as covariate on dolutegravir clearance, compared with the standard dosage of 50 mg once daily. Continuous lines represent the population median prediction for standard (black) and adjusted (turquoise) regimens based on 1000 simulated individuals. Shaded areas and dashed lines represent the 95% prediction intervals for standard (black) and adjusted (turquoise) regimens based on 1000 simulated individuals. Dashed and dotted orange lines represent the protein-adjusted IC_{90} of 64 ng/mL and the suggested target C_{min} of 300 ng/mL, respectively. DTG, dolutegravir; RIF, rifampicin. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

adjustment. Similarly, the lower degree of bioavailability under gastric bypass should not affect dosing: firstly, because the magnitude of this effect is similar to the BSV; and, secondly, because drug concentrations in these patients were much higher than the protein-adjusted IC_{90} of 64 ng/mL, thus limiting the risk of underexposure. Although these results are reassuring, further data with more gastric bypass patients are needed to confirm this finding.[26]

The present study also evaluated the impact of a variety of drug–drug interactions that might be encountered in routine clinical practice. Both nevirapine and etravirine co-administration showed a modest increase in dolutegravir clearance in the univariate analysis, probably due to UGT1A1 induction.[28] The number of individuals with concomitant etravirine and darunavir/ritonavir (n=8; Table 1) could explain the lesser effect on dolutegravir exposure compared with the reported effect of etravirine alone (71% decrease in dolutegravir AUC_{0-24}).[15] The influence of atazanavir on dolutegravir CL/*F* is explained by the known atazanavir-mediated inhibition of UGT1A1, the primary metabolic pathway of dolutegravir. Even if co-administration with atazanavir/ritonavir has been reported to increase dolutegravir AUC_{0-24} between 62% and 121%, no dose adjustment is currently recommended.[13,25] While several authors did not find any significant correlation between dolutegravir exposure and adverse events, others reported a higher prevalence of neuropsychiatric events in dolutegravir-treated patients, sometimes leading to treatment discontinuation.[29–34] A recent study showed that UGT1A1 poor metabolizers presented significantly higher dolutegravir trough concentrations, and trough concentrations were also significantly higher in patients presenting neuropsychiatric adverse events (P<0.01).[35] Genetic data on UGT1A1 polymorphisms and adverse events data were not available in the current analysis. Close monitoring should be advised for patients treated with atazanavir owing to the potential risk of concentrations higher than the required levels without any substantial benefit, but potential undesirable consequences.

An increase in dolutegravir elimination induced by darunavir boosted with ritonavir has also been described previously.[25,36] Although the mechanism is unclear, a possible explanation might be the induction of UGT1A1 by ritonavir.[15,16] The decrease in dolutegravir exposure is not considered clinically relevant, to the extent that the combination of once-daily dolutegravir/darunavir/ritonavir is being studied as a rescue NRTI-free regimen.[37]

In the current study, rifampicin co-administration showed the largest impact on dolutegravir CL/*F* in accordance with a recent study.[18] The recommended dosage when dolutegravir is co-administered with potent enzyme inducers, such as rifampicin, carbamazepine and efavirenz, is 50 mg twice daily.[11,12,17] Simulations based on the present model predicted comparable dolutegravir exposure to a previous study on 50 mg of dolutegravir twice daily with rifampicin.[11] Even though dolutegravir trough concentrations were strongly correlated with antiviral activity, a clinical target value has yet to be validated.[25] Our simulations predicted a relatively low proportion of subjects with C_{min} below the protein-adjusted IC_{90} and below the alternative target of 300 ng/mL, with either 50 or 100 mg twice daily, suggesting a low risk of suboptimal drug coverage at the end of the dosing interval in patients co-treated with rifampicin. A dose of 100 mg once daily with rifampicin could represent an improvement in terms of adherence to treatment, while attaining acceptable coverage in individuals located in the upper percentiles of expected concentrations. Dolutegravir at 100 mg twice daily provided greater coverage along the entire dosing interval and C_{min} concentrations more similar to 50 mg once daily without rifampicin. Nevertheless, due to the considerable increase in AUC_{0-24} and C_{max} with this empirical dose adjustment, precaution and close monitoring are advised until the

relationship between dolutegravir exposure and neurological toxicity is clarified. These findings support the benefits of therapeutic drug monitoring and dose individualization in the case of dolutegravir and rifampicin co-administration.

In conclusion, the variability in dolutegravir pharmacokinetics remains moderate after accounting for the influence of different individual characteristics and drug–drug interactions. Co-administration of UGT1A1 inhibitors or inducers, such as atazanavir or rifampicin, might lead to inadequate dolutegravir levels, and concentration measurement and adjustment by therapeutic drug monitoring should be envisaged in such situations. This population pharmacokinetic model will be implemented in a Bayesian tool to facilitate therapeutic drug monitoring and dose adjustment, if necessary.

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Members of the Swiss HIV Cohort Study

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Supplementary data

Supplementary data, including Tables S1 to S3 and Figures S1 to S2, are available at *JAC* Online.

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Supplementary data

Table S1. Covariate univariate forward-inclusion results

Univariate analyses (*P* value=0.05)							
Parameter - covariate	**Model**	**Estimate (RSE%)**	**OFV**	**ΔOFV**	**IIV CL (RSE%)**	***Prop. Residual error* (RSE%)**	***Add. Residual error* (RSE%)**
Structural model	-	-	9110.68	-	38.0% (9.3)	0.300 (9.0)	198 (22.1)
CL – rifampicin	$CL_{TV} \cdot (1 + RIF \cdot \Theta_b)$	Θ_b: 2.40 (30.6)	9044.61	-66.07	35.7% (6.9)	0.283 (7.8)	212 (26.6)
CL – smoke	$CL_{TV} \cdot (1 + Smoke \cdot \Theta_b) \cdot (1 + NA\text{-}Smoke \cdot \Theta_c)$†	Θ_b: 0.16 (27.4) Θ_c: 1.73 (39.7)	9047.86	-62.81	36.0% (6.6)	0.284 (7.1)	207 (23.3)
CL – atazanavir and ritonavir	$CL_{TV} \cdot (1 + ATV\&RTV \cdot \Theta_b) \cdot (1 + ATV \cdot \Theta_c)$	Θ_b: -0.19 (66.8§) Θ_c: -0.67 (4.9)	9077.97	-32.70	31.9% (9.9)	0.335 (8.5)	182 (20.4)
CL – atazanavir	$CL_{TV} \cdot (1 + ATV \cdot \Theta_b)$	Θ_b: -0.40 (27.3)	9092.31	-18.37	32.9% (10.6)	0.334 (9.6)	182 (23.6)
CL – age allometric	$CL_{TV} \cdot (age/47)^{\Theta b}$	Θ_b: 0.23 (31.8)	9099.94	-10.74	34.7% (10.7)	0.324 (10.3)	180 (23.0)
CL – darunavir/r	$CL_{TV} \cdot (1 + DRV \cdot \Theta_b)$	Θ_b: 0.153 (35.8)	9101.86	-8.81	37.6% (10.7)	0.302 (10.7)	192 (21.7)
CL – age linear	$CL_{TV} \cdot (1 + \Theta_b \cdot (age-47)/47)$	Θ_b: 0.21 (42.6)	9103.65	-7.02	37.4% (15.2)	0.302 (16.0)	198 (24.4)
CL – body weight linear	$CL_{TV} \cdot (1 + \Theta_b \cdot (BW-73)/73)$	Θ_b: 0.25 (40.5)	9104.24	-6.44	37.7% (9.5)	0.298 (9.5)	207 (19.9)
CL – body weight allometric	$CL_{TV} \cdot (BW/73)^{\Theta b}$	Θ_b: 0.25 (41.3)	9104.40	-6.28	37.7% (9.5)	0.298 (9.4)	207 (21.2)
CL – nevirapine	$CL_{TV} \cdot (1 + NVP \cdot \Theta_b)$	Θ_b: 0.42 (29.8)	9104.57	-6.11	37.7% (10.9)	0.304 (10.4)	196 (21.5)
CL – etravirine	$CL_{TV} \cdot (1 + ETV \cdot \Theta_b)$	Θ_b: 0.28 (63.4)	9106.52	-4.16	38.0% (9.2)	0.298 (9.1)	200 (21.3)
CL – bypass	$CL_{TV} \cdot (1 + BYP \cdot \Theta_b)$	Θ_b: 0.19 (96.9)	9109.86	-0.82	37.9% (9.6)	0.3 (9.2)	200 (21.2)
CL – lopinavir/r	$CL_{TV} \cdot (1 + LPV \cdot \Theta_b)$	Θ_b: -0.12 (275.7)	9110.52	-0.15	38.0% (9.3)	0.299 (9.0)	199 (22.2)

†All NA-smokers (not available) had rifampicin co-administration. Therefore, in the full model NA-smokers were grouped with non-smokers; §Not enough power to estimate ATV+RTV different from ATV alone
CL_{TV}: dolutegravir apparent clearance typical value; darunavir/r: ritonavir-boosted darunavir; lopinavir/r: ritonavir-boosted lopinavir

Table S2. Covariate multivariate forward-inclusion results

Multivariate analyses – forward (*P* value=0.005)							
Parameter - covariate	**OFV**	**ΔOFV**	**CL (RSE%)**	**IIV CL (RSE%)**	**V (RSE%)**	***Prop. Residual error* (RSE%)**	***Add. Residual error* (RSE%)**
CL – rifampicin	9044.61	-	1.0 (2.7)	35.7% (6.9)	20.3 (8.9)	0.283 (7.8)	212 (26.6)
CL – rifampicin – smoke	9029.05	-15.557	0.946 (2.9)	34.9% (6.9)	20.3 (8.8)	0.283 (7.6)	210 (25.6)
CL – rifampicin – smoke – atazanavir	9010.05	-19.001	0.953 (2.9)	32.9% (7.1)	19.8 (8.5)	0.29 (7.4)	212 (23.2)
CL – rifampicin – smoke – atazanavir – age	8994.31	-15.743	0.955 (2.9)	32.2% (7.3)	19.8 (8.5)	0.288 (7.5)	214 (22.4)
CL – rifampicin – smoke – atazanavir – age – darunavir/r	8987.28	-7.028	0.938 (2.9)	32.1% (7.4)	20 (8.6)	0.288 (7.8)	208 (24.2)
CL – rifampicin – smoke – atazanavir – age – darunavir/r – body weight	8975.58	-11.705	0.928 (2.8)	31.6% (7.4)	20.2 (8.5)	0.284 (8.1)	220 (23.8)
CL – rifampicin – smoke – atazanavir – age – darunavir/r – body weight – nevirapine	8970.01	-5.56	0.922 (2.8)	31.1% (7.6)	20.0 (8.5)	0.286 (8.0)	219 (23.6)
CL – rifampicin – smoke – atazanavir – age – darunavir/r – body weight – etravirine	8973.30	-2.273	0.928 (2.8)	31.6% (7.3)	20.3 (8.4)	0.282 (7.9)	224 (21.8)

darunavir/r: ritonavir-boosted darunavir

Table S3. Covariate multivariate backward-deletion results

Multivariate analyses – backward (*P* value=0.005)							
Parameter - covariate	**OFV**	**ΔOFV**	**CL (RSE%)**	**IIV CL (RSE%)**	**V (RSE%)**	***Prop. Residual error* (RSE%)**	***Add. Residual error* (RSE%)**
CL – rifampicin – smoke – atazanavir – age – darunavir/r – body weight	8975.58	-	0.928 (2.8)	31.6% (7.4)	20.2 (8.5)	0.284 (8.0)	220 (23.4)
CL – rifampicin – smoke – atazanavir – age – darunavir/r	8987.28	11.705	0.938 (2.9)	32.1% (7.4)	20 (8.8)	0.288 (8.1)	208 (27.3)
CL – rifampicin – smoke – atazanavir – age – body weight	8984.24	8.667	0.947 (2.9)	31.8% (7.3)	20 (8.5)	0.284 (7.7)	226 (23)
CL – rifampicin – smoke – atazanavir – darunavir/r – body weight	8987.08	11.508	0.923 (2.8)	32.1% (7.1)	20.3 (8.4)	0.286 (7.9)	219 (23.1)
CL – rifampicin – smoke – age – darunavir/r – body weight	8994.95	19.376	0.919 (2.9)	33.4% (7.1)	20.7 (8.7)	0.278 (7.8)	217 (23.6)
CL – rifampicin – atazanavir – age – darunavir/r – body weight	8997.72	22.147	0.985 (2.7)	32.4% (7.4)	20.1 (8.8)	0.288 (8.4)	217 (26.3)
CL – smoke – atazanavir – age – darunavir/r – body weight	9048.69	73.117	0.958 (3.3)	30.1% (9.2)	19.5 (8.2)	0.337 (8.0)	180 (19.1)

darunavir/r: ritonavir-boosted darunavir

Figure S1. Goodness-of-fit plots of dolutegravir structural population pharmacokinetic model. Loess smooth curves of the ordinate values are printed in grey. a) Observations vs. individual predictions; line of identity is printed in black. b) Observed concentrations vs. population predictions; identity line is printed in black. c) Conditional weighted residuals (CWRES) vs. population predictions; ordinate value zero is printed in black. d) CWRES vs. time post-dose; ordinate value zero is printed in black.

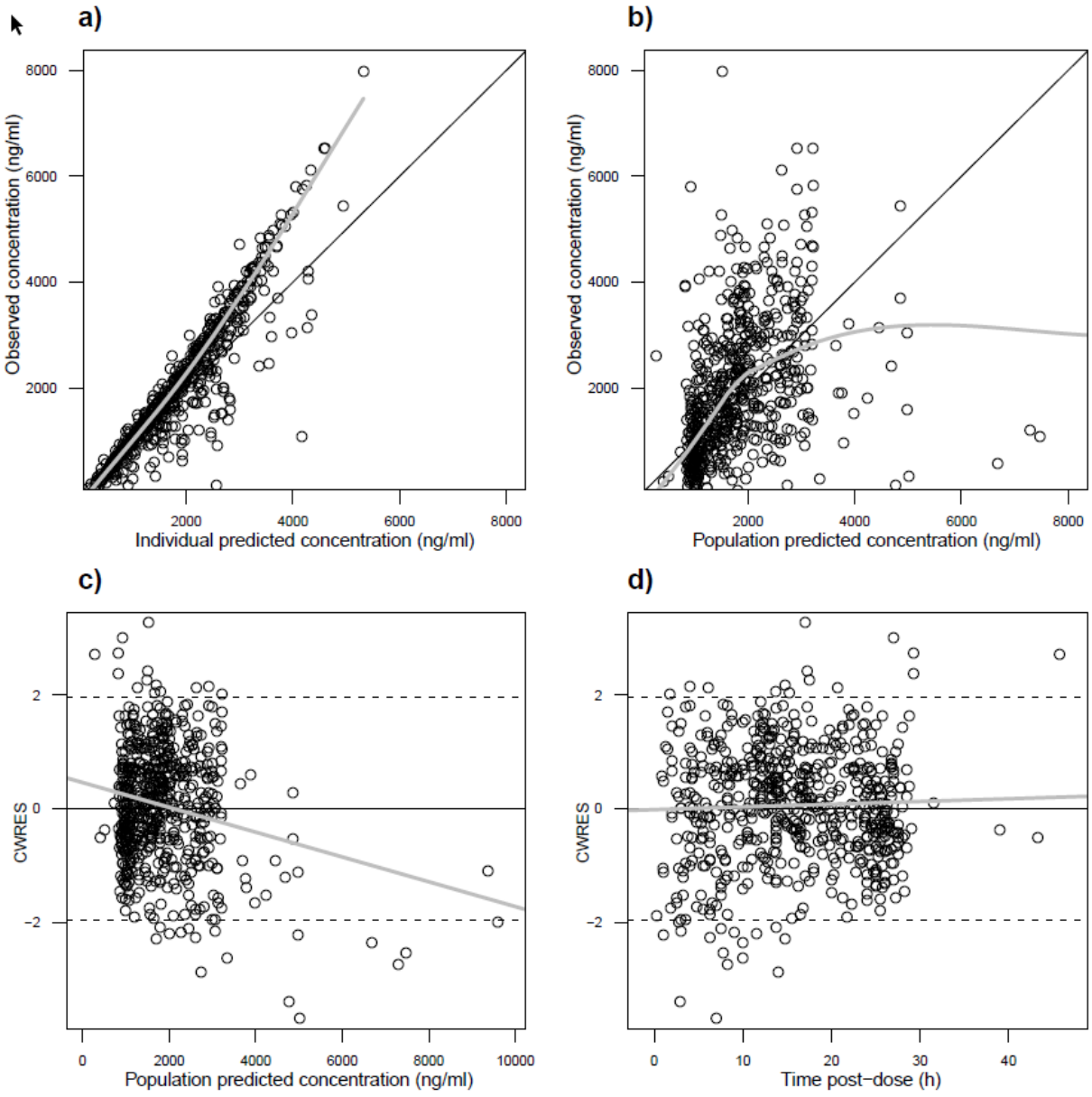
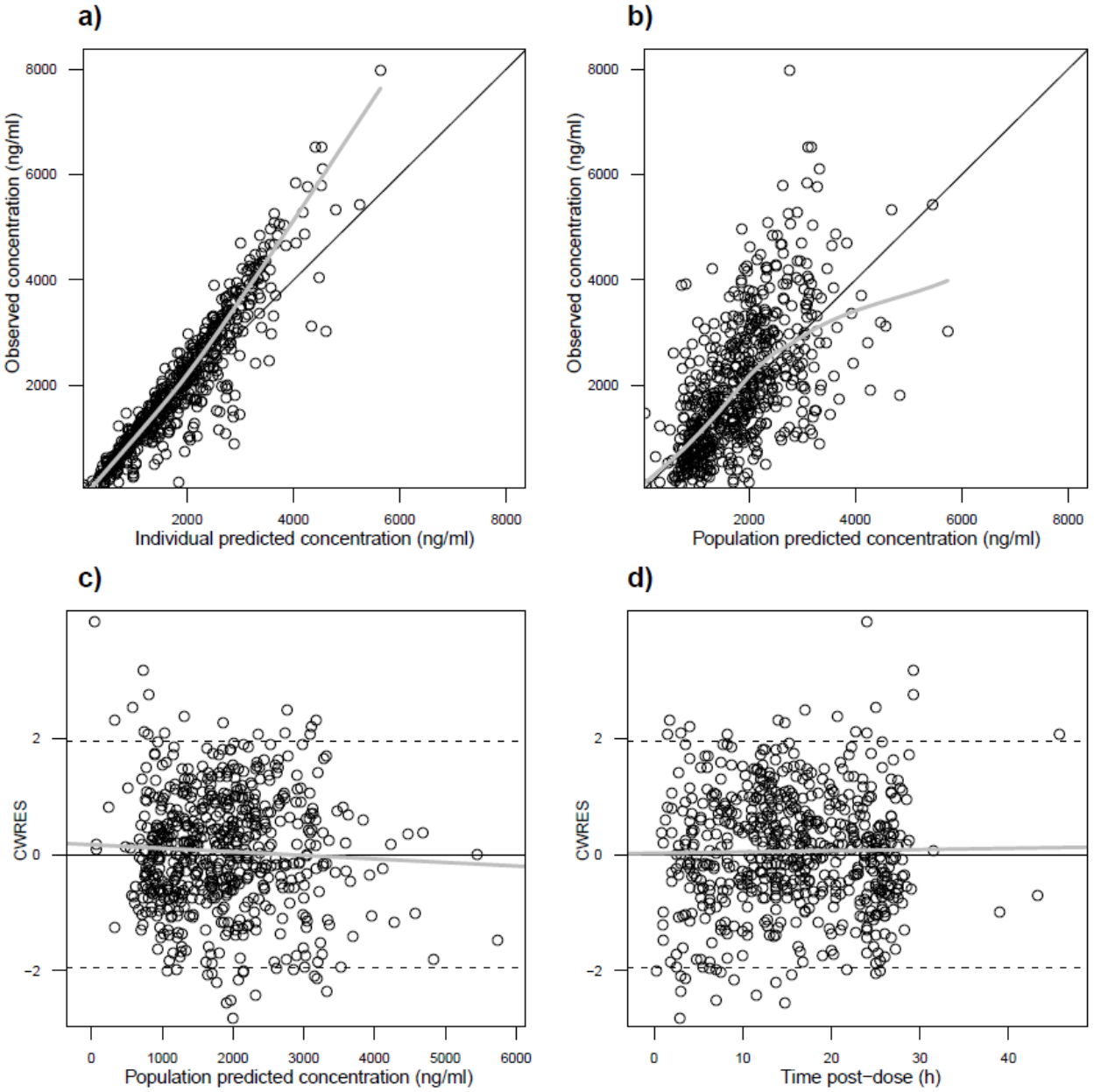


Figure S2. Goodness-of-fit plots of dolutegravir final population pharmacokinetic model. Loess smooth curves of the ordinate values are printed in grey. a) Observations vs. individual predictions; line of identity is printed in black. b) Observed concentrations vs. population predictions; identity line is printed in black. c) Conditional weighted residuals (CWRES) vs. population predictions; ordinate value zero is printed in black. d) CWRES vs. time post-dose; ordinate value zero is printed in black.



NONMEM 7.4 code for the final dolutegravir pharmacokinetic model

```
$PROBLEM DTG PK
$INPUT ID TUBE DAT1=DROP TIME AMT CMT SS II INTER DV SEX AGE BW ATV DRV CIG RIF
$DATA dataset.csv IGNORE=@
$SUBROUTINES ADVAN2 TRANS2
$PK
Q=0
IF(DRV.EQ.1) Q=1
Q1=0
IF(ATV.EQ.1) Q1=1
Q6=0
IF(RIF.EQ.1) Q6=1
Q7=0
IF(CIG.GT.0) Q7=1
MBW=73
BW1=BW
IF (BW.EQ.-99) BW1=MBW
FBW=(BW1-MBW)/MBW
RAGE=AGE/47
TVCL=THETA(1)*(1+Q6*THETA(6))*(1+Q1*THETA(7))*(1+Q7*THETA(8))*RAGE**THETA(9)
                    *(1+Q*THETA(10))*(1+ THETA(11)*FBW)
CL= TVCL* EXP(ETA(1))
TVV  = THETA(2)
V=TVV* EXP(ETA(2))
TVKA = THETA(3)
KA=TVKA* EXP(ETA(3))
S2 = V/1000
$ERROR
IPRED = F
W = SQRT(THETA(4)**2*IPRED**2 + THETA(5)**2)
Y = IPRED + W*EPS(1)
IRES = DV-IPRED
IWRES = IRES/W
```

```
$THETA
(0, 0.928) ; CL
(0, 20.2) ; V
(2.24) FIX ; KA
(0, 0.284) ; Proportional residual error
(0, 220) ; Additive residual error
(-10, 2.61) ; Rifampicin
(-10, -0.375) ; Atazanavir
(-10, 0.185) ; Smoker
(-10, 0.219) ; Age allometric
(-10, 0.138) ; Darunavir
(-10, 0.31) ; Body weight linear
$OMEGA
 0.0951 ; IIV CL
 0 FIX  ; IIV V
 0 FIX  ; IIV KA
$SIGMA
 1 FIX  ; Proportional error PK
$EST METHOD=1 INTER MAXEVAL=9000 NOABORT SIG=3 PRINT=1 POSTHOC
$COV
```

CHAPTER V: IMPACT OF AGING ON DRUG PHARMACOKINETICS

Chapter V in the thesis context

While most of our studies on the DDI between ARVs and comedications have been based on population PK modelling approaches, non-compartmental analyses (NCA) have been also carried-out for comparison. Although recognised to be less robust that modelling approaches, these analyses generally allow to provide to clinicians directly accessible, real-life clinical data that can be more easily understandable in clinically-oriented publications.

The first part of this chapter aims therefore at quantifying using the alternate NCA approach, the magnitude of DDIs between the three cardiovascular medications of interest (*i.e.* amlodipine, atorvastatin and rosuvastatin) and ARV agents in aging PLWH. Results demonstrated a marginal impact of aging on DDI magnitudes.

The second paper of this chapter fill the knowledge gap about the influence of aging on ARVs PK. Rich (NCT03515772) and sparse data (SHCS #815 study described in the first part of this thesis) were used to compare ARV disposition between younger and aging PLWH and also revealed a limited impact of age on ARV pharmacokinetics.

Own contribution: generation of PK data, data management, PK analyses, drafting of the manuscript

V.1. Aging does not impact drug-drug interaction magnitudes with antiretrovirals: a Swiss HIV Cohort Study.

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Conflicts of interest

There are no conflicts of interest.

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Aging does not impact drug–drug interaction magnitudes with antiretrovirals

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The risk of drug–drug interactions (DDIs) is elevated in aging people living with HIV (PLWH) because of highly prevalent age-related comorbidities leading to more comedications. To investigate the impact of aging on DDI magnitudes between comedications (amlodipine, atorvastatin, rosuvastatin) and boosted darunavir, we conducted a clinical trial in aging PLWH aged at least 55 years. DDI magnitudes were comparable with those reported in young individuals supporting that the clinical management of DDIs in aging PLWH can be similar.

Combined antiretroviral treatments (ARVs) have increased the life expectancy of people living with HIV (PLWH) close to the general population [1]. Consequently, PLWH have an identical high prevalence for age-related comorbidities, such as cardiovascular conditions, leading to complex drug associations with a higher risk for drug–drug interactions (DDIs) [2]. One current issue is the lack of knowledge concerning the

magnitude and clinical management of DDIs in aging PLWH. However, despite the high DDI potential of ARVs, it is neither feasible nor ethically possible to conduct clinical studies for every single drug combination. Additionally, elderly PLWH are underrepresented in clinical studies. The decline in hepatic and renal blood flow and in the glomerular filtration rate drives age-related pharmacokinetic changes of non-HIV drugs and likely impacts ARV pharmacokinetics [3]. The impact of aging on metabolizing enzymes and drug transporters activity is either controversially discussed or lacking in the literature [4], which in addition to pharmacokinetic alterations could both affect the magnitude of DDIs in the elderly.

The aim of this study was to quantify the DDI magnitudes between cardiovascular drugs (i.e. amlodipine, atorvastatin, rosuvastatin) and ARVs in aging PLWH to provide guidance on DDI management in this fragile population.

This was a prospective clinical study including PLWH aged at least 55 years in Lausanne and Basel that are enrolled in the Swiss HIV Cohort Study [5]. Included PLWH received amlodipine, atorvastatin and/or rosuvastatin with a dolutegravir or a boosted darunavir-containing regimen. PLWH were excluded if they had severe comorbidities, such as advanced renal impairment (KDOQI 4-5); heart failure (NYHA 3-4); cirrhosis (Child-Pugh score C) or if they were receiving comedications with inhibitory or inducing properties. Consenting PLWH came to the HIV clinic in the morning for the collection of serial blood samples over 24 h. The Ethics Committee of Vaud and Northwest/Central Switzerland approved the study protocol (CER-VD 2018-00369), which is registered at ClinicalTrials.gov (NCT03515772). Written informed consent was collected for each participant.

Plasma samples were isolated by centrifugation and stored at -80 °C until batch analysis. Plasma levels determination was performed in the Laboratory of Clinical Pharmacology in Lausanne, using liquid chromatography coupled with tandem mass spectrometry [6].

All doses were normalized as amlodipine, atorvastatin, and rosuvastatin exhibit dose-proportional pharmacokinetics. Pharmacokinetic parameters were calculated noncompartmentally from the measured concentration–time profiles in Matlab 2017a. The mean and standard deviation (SD) of the area under the curve (AUC) were calculated for the comedication received with either dolutegravir or boosted darunavir. The DDI magnitudes were calculated as the AUC of the comedication in the presence of boosted darunavir [inhibitory effects on cytochrome P450 3A4 (CYP3A4) and/or hepatic/intestinal transporters] divided by the AUC of the comedication in the presence of dolutegravir (no inhibitory effects).

A structured literature search was performed using the MEDLINE database to screen for studies investigating the same DDI magnitudes in young adults to evaluate the impact of aging.

A total of 21 white PLWH (four women) aged 56–80 years were included in the study. Amlodipine was taken by eight PLWH (dolutegravir: $n = 6$; boosted darunavir: $n = 2$) aged 64.8 ± 7.0 years. The AUC of amlodipine (dose-normalized to 5 mg) was 1155 ± 414 and 2425 ± 739 ng h/ml in combination with dolutegravir and boosted darunavir, resulting in an AUC-ratio of 2.10 ± 0.99 (Table 1). In young adults aged 20–50 years, the increase in amlodipine exposure in the presence of boosted indinavir was 1.89 [7] and in the presence of ritonavir was 2.11 [8].

Atorvastatin was investigated in nine aging PLWH (dolutegravir: $n = 4$; boosted darunavir: $n = 5$) aged 64.1 ± 8.0 years at a dose of 10 mg. The AUC of atorvastatin in the presence of dolutegravir was 31.4 ± 4.7 ng h/ml and increased to 193 ± 133 ng h/ml in PLWH receiving boosted darunavir. The resulting AUC ratio was 6.16 ± 4.35. No study could be identified in young individuals for atorvastatin in the presence of boosted darunavir, but for boosted saquinavir (AUC ratio: 3.93) [9] and boosted tipranavir (AUC ratio: 9.36) [10].

Rosuvastatin was administered to six PLWH (dolutegravir: $n = 2$ boosted darunavir: $n = 4$) aged 67.7 ± 5.3 years and concentrations were dose-normalized to 10 mg. The rosuvastatin AUC in the presence of dolutegravir and

Table 1. Comparison of drug–drug interaction magnitudes of amlodipine, atorvastatin, and rosuvastatin combined with boosted darunavir in young (20–50 years) and aging individuals (55–80 years).

	n	Age (years)	AUC-ratio of aging individuals (55–80 years)	AUC-ratio of young individuals (20–50 years)	Ratio aging/young
Amlodipine + boosted darunavir	8	64.8 ± 7.0	2.10 ± 0.99	2.11 (ritonavir alone) [8]	1.00
				1.89 (boosted indinavir) [7]	1.11
Atorvastatin + boosted darunavir	9	64.1 ± 8.0	6.16 ± 4.35	3.93 (boosted saquinavir) [9]	
				9.36 (boosted tipranavir) [10]	–
Rosuvastatin + boosted darunavir	6	67.7 ± 5.3	1.60 ± 0.88	1.57 ± 0.54 [11]	1.02

AUC, area under the curve.

boosted darunavir was 104.2 ± 32.6 and 166.9 ± 75.5 ng h/ml. The resulting AUC ratio was 1.60 ± 0.88. One clinical study investigated rosuvastatin exposure in the presence of boosted darunavir in young adults aged 20–50 years and reported an AUC ratio of 1.57 ± 0.54 [11].

Clinical studies concerning the impact of aging on DDI magnitudes involving ARVs do not exist, leading to uncertainty concerning the clinical management of DDIs in aging PLWH. To our knowledge, our study is the first to investigate DDIs of commonly used comedications (amlodipine, atorvastatin, rosuvastatin) and boosted darunavir in PLWH aged at least 55 years. The obtained AUC ratios in aging PLWH were in the same range as DDI magnitudes reported in young individuals aged 20–50 years, thus, demonstrating that aging has a marginal impact on DDI magnitudes.

Two clinical studies with midazolam and clarithromycin (inhibition) or rifampicin (induction) elucidated no age-related changes of the DDI magnitudes, which support our study results [12–14].

Several limitations should be acknowledged. Firstly, the small number of patients led to an observational study design. Nevertheless, the obtained clinical data show the real-life scenario of amlodipine, atorvastatin, and rosuvastatin in aging PLWH receiving boosted darunavir. Secondly, concentration–time profiles for the comedications in the presence of dolutegravir and boosted darunavir came from two different groups of patients because of medical and ethical reasons. Thirdly, clinical data for our investigated DDIs in young individuals were not obtained in the same study but gathered from published clinical trials that did not administer the same protease inhibitors as in our study. In the case of amlodipine, ritonavir itself is enough to inhibit CYP3A4 completely and thus, the second protease inhibitor can be neglected [15]. In the case of atorvastatin, the inhibition of OATP1B1 (organic anion transporting polypeptide) and P-gp (P-glycoprotein) adds to the CYP3A4 inhibition. Saquinavir and ritonavir show the least impact on OATP1B1 followed by darunavir and tipranavir with the latter one being a strong P-gp inhibitor [16,17]. Therefore, our results for atorvastatin and boosted darunavir are in line with published studies using either saquinavir or tipranavir.

In conclusion, our clinically observed data demonstrate that DDI magnitudes between ARVs and comedications appear to be similar in aging PLWH compared with young individuals and thus, the clinical management of DDIs can be similar. Further research is warranted in the future to investigate more DDI scenarios with a larger study population including more women to further support the clinical management of DDIs in aging PLWH.

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Conflicts of interest

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V.2. Pharmacokinetic profiles of boosted darunavir, dolutegravir and lamivudine in aging people living with HIV.

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CONCISE COMMUNICATION

Pharmacokinetic profiles of boosted darunavir, dolutegravir and lamivudine in aging people living with HIV

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Objectives: The pharmacokinetics of antiretroviral drugs may differ in elderly people living with HIV (PLWH) because of age-related physiological changes. We aimed to assess the pharmacokinetics of several antiretroviral drugs in aging PLWH enrolled in the Swiss HIV Cohort (SHCS).

Design: Full pharmacokinetic profiling nested in a multicenter, observational, prospective cohort study. Additional collection of single point pharmacokinetic data during SHCS follow-up visits (unselected PLWH).

Methods: PLWH were eligible for the full pharmacokinetics investigation if they were over the age of 55 years, on a stable boosted darunavir-containing or dolutegravir-containing regimen. Single point measurements were prospectively collected during SHCS follow-up visits to compare antiretroviral drug exposure in aging (≥65 years) and younger (<65 years) PLWH.

Results: Nineteen PLWH with a median age of 64 years participated in the full pharmacokinetic investigations. Single point pharmacokinetic data were collected for 804 PLWH with a median age of 52 years. Boosted darunavir clearance was 40% lower in aging (≥65 years) compared with younger (<65 years) PLWH, consistent with other drugs predominantly metabolized by CYP3A. Dolutegravir exposure was similar between age groups whereas lamivudine exposure increased by 11% in aging PLWH. Median boosted darunavir, dolutegravir and lamivudine $t_{1/2}$ were 148%, 45% and 32% higher in aging compared with younger PLWH.

Conclusion: Advanced age did not affect boosted darunavir exposure to a clinically significant extent despite the observed high variability in exposure. Age minimally

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affected dolutegravir and lamivudine exposure. Thus, dose adjustment based on age is a priori not warranted.

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Keywords: aging, antiretroviral drug, HIV, pharmacokinetics

Introduction

Effective antiretroviral therapy has reduced mortality significantly; thus, increasing life expectancy of people living with HIV (PLWH) closer to the general population [1]. At the end of 2016, more than 40% of active patients in the Swiss HIV Cohort Study (SHCS) were over the age of 50 years [2]. Mathematical models informed by data from United States, Italian, and Dutch HIV cohorts have projected that 40% of the HIV population will be greater than 60 years in 2030 [3,4]. As older PLWH are usually underrepresented in clinical trials [5], the impact of aging on the pharmacokinetics of antiretroviral drugs (ARVs) and related need for a dose adjustment are unclear.

Advanced age is characterized by anatomical, physiological and biological changes that have the potential to alter drug pharmacokinetics [6,7]. A major characteristic is the decreased hepatic and renal clearance and hence declined drug elimination with advanced age [7]. Available, studies suggest that the concentrations of raltegravir and efavirenz are not significantly altered in PLWH 45–79 years and more than 60 years whereas protease inhibitors were shown to be mostly increased [8–10]. Finally, dolutegravir maximal concentrations were increased by 25% in PLWH at least 60 years [11].

A major limitation for most of these studies is the inclusion of individuals below 65 years. Although age of 50 years has been commonly accepted in the HIV field to define an 'elderly' [12,13], the WHO recommends 65 years as an age-cut-off [14]. A pharmacological or clinical definition of an 'elderly' remains challenging because the aging process is not uniform across the population [15].

Therefore, a knowledge gap currently exists about the impact of older age on ARV pharmacokinetics and there is a lack of real-life data on aging PLWH. Polymorbidity and polymedication are poorly accounted for in-treatment guidelines, which are largely elaborated for single diseases [16]. However, the elderly might be more susceptible to drug toxicity including ARVs than younger people. Additionally, data from other therapeutic areas suggest that age should be considered when prescribing in the elderly as they may require lower doses to achieve therapeutic efficacy and to avoid adverse events [17–19].

This work aims to compare boosted darunavir, dolutegravir and lamivudine plasma exposures between aging (≥65 years) and younger (<65 years) PLWH involved in two observational studies to better document pharmacokinetics of ARVs in this growing vulnerable population.

Methods

Study design and participants

Full pharmacokinetic investigations

These investigations were performed using a prospective and observational design and included PLWH enrolled in the SHCS and followed up in the centers of Lausanne and Basel. Male and female PLWH were eligible if they were aged 55 years or older and treated with a boosted darunavir or dolutegravir-containing regimen. Exclusion criteria were the coadministration of inhibiting or inducing comedications as well as the presence of severe comorbidities [i.e. cirrhosis (Child-Pugh score C), heart failure (NYHA 3–4), advanced kidney impairment (KDOQI 4–5)]. Participants arrived in the clinic in the morning of the full pharmacokinetic investigation and took their ARV treatment in front of the study nurse. Serial blood samples were collected at steady state, at the following time-points: $t = 0$ (just before the drug intake) and 30 min, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after the drug intake.

All study participants gave written informed consent before entering the study. The study protocol was reviewed and approved by the Ethics Committee of Vaud and northwest/central Switzerland (CER-VD 2018–00369) and registered in ClinicalTrials.gov (NCT03515772).

Single point measurements

Single point measurements for therapeutic drug monitoring (TDM) of boosted darunavir and dolutegravir were collected in the framework of SHCS follow-up visits for PLWH attending the HIV clinics in Lausanne and Basel. One week before their biannual cohort visit, PLWH received a reminding letter with a form to fill out all their current medications and date/time of the last drug intake. Clinical nurses collected the forms, performed and documented blood sampling. TDM concentrations were measured at unselected times after the last drug intake.

Plasma concentration determination

All plasma level measurements were performed at the Laboratory of Clinical Pharmacology of the University Hospital of Lausanne. Blood samples were collected and

centrifuged in EDTA-containing tubes. Plasma were aliquoted and shipped frozen (Basel samples) and were stored at −80 °C until analysis by several liquid chromatography tandem mass spectrometry (LC-MS/MS) methodologies, reported in refs. [20–22]. LC-MS/MS assay for plasma determination of elvitegravir and rilpivirine was adapted to include dolutegravir [20]. Lamivudine plasma concentrations were only measured in PLWH participating in the full pharmacokinetic study.

Pharmacokinetic analysis

Concentration–time profiles of boosted darunavir, dolutegravir and lamivudine, the most prescribed ARV drugs in the full pharmacokinetic study were plotted to visually compare drug disposition between aging and younger PLWH. Single point concentrations of boosted darunavir and dolutegravir, obtained during TDM and representing mostly younger PLWH, were overlaid to show the effect of aging on pharmacokinetics and to visualize interpatient variability more adequately.

Plasma pharmacokinetic parameters were calculated noncompartmentally using data from the pharmacokinetic study with rich sampling and the PKNCA package in R [23]. The area under the concentration–time curve over a dosing interval (from 0 to τ, $AUC_{0\text{-}\tau}$) was calculated using the linear-up log-down method. Peak concentration (C_{max}) and time to peak plasma concentration (t_{max}) were directly retrieved from the R output. Half-life ($t_{1/2}$) was calculated as $\ln(2)/\lambda_z$ with λ_z being the elimination rate constant; apparent clearance (CL/F) as dose/$AUC_{0\text{-}\tau}$; and apparent volume (V/F) as $(CL/F)/\lambda_z$. Pharmacokinetic parameters were reported as median and range.

Results

Nineteen PLWH (17 men) with a median age of 64 years (range 56–80 years) participated in the full pharmacokinetic investigations and contributed to 97, 107 and 127 boosted darunavir ($n = 9$ PLWH), dolutegravir ($n = 10$ PLWH), and lamivudine ($n = 12$ PLWH) plasma concentrations, respectively. Darunavir was boosted either with 100 mg of ritonavir ($n = 7$ PLWH) or with 150 mg of cobicistat ($n = 2$ PLWH). Boosted darunavir dose varied between participants: 600 mg once daily (one PLWH), 800 mg once daily (two PLWH), 1200 mg once daily (four PLWH) and 600 mg twice daily (two PLWH). Dolutegravir dosage was always 50 mg once daily. Lamivudine dosage was 300 mg once daily except one individual receiving 150 mg once daily. Overall, boosted darunavir, dolutegravir and lamivudine plasma concentrations ranged from 12 to 10 652, 623–6445 and 51–3546 ng/ml, respectively.

In addition, 804 PLWH with a median age of 52 (range 20–86) contributed to the single point TDM measurements, thus adding 244 boosted darunavir and 560 dolutegravir plasma concentrations for visual inspection of data.

No distinct separation was observed between boosted darunavir, dolutegravir and lamivudine plasma concentrations of aging (≥65 years) and younger (<65 years) PLWH (Fig. 1). Although a high variability in boosted darunavir pharmacokinetic parameters was noticed in both age groups, boosted darunavir clearance was 40% lower in elderly compared with younger PLWH. Dolutegravir and lamivudine CL/F were similar between age groups (differences of 13% and 11% for dolutegravir and lamivudine, respectively). Thus, dolutegravir and

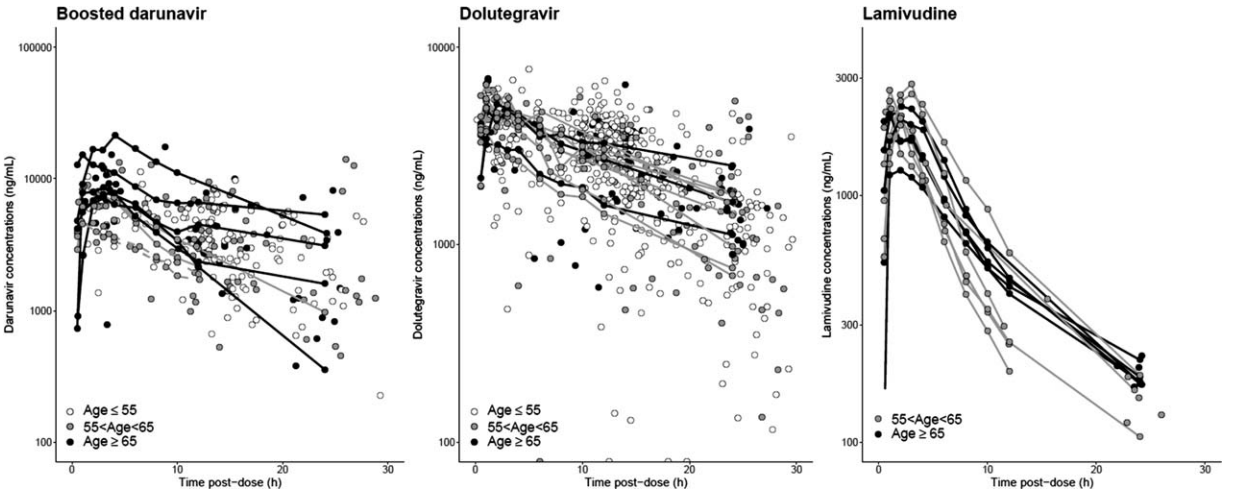

Fig. 1. Concentration–time profiles of antiretroviral drugs. Black lines/points: full concentration–time profiles and single point concentrations, respectively of aging PLWH (≥65 years old); grey lines/points: full concentration–time profiles and single point concentrations of PLWH younger than 65 years old; white points: single point concentrations of PLWH younger than 55 years old. Boosted darunavir plasma concentrations of PLWH participating in the full pharmacokinetics study and receiving ritonavir-boosted darunavir at the dosage of 600/100 mg twice daily are joined with dotted lines.

Table 1. Pharmacokinetic parameters in younger (<65 years old) and aging (≥65 years old) people living with HIV calculated from the full pharmacokinetic profiles using noncompartmental analyses.

	Boosted darunavir		Dolutegravir		Lamivudine	
	Younger ($n=4$)	Aging ($n=5$)	Younger ($n=7$)	Aging ($n=3$)	Younger ($n=6$)	Aging ($n=6$)
Age	63 (62–64)	73 (67–76)	60 (56–64)	74 (70–80)	61 (56–64)	72 (67–80)
C_{max} (ng/ml)	[a]7963 (7139–8787) [b]4651 (4558–4743)	7809 (5695–10 652)	4933 (3812–6445)	4831 (3564–5116)	2498 (1928–2840)	2092 (634–3546)
T_{max} (h)	1.6 (0.9–4)	3 (1–4)	1 (0.5–2)	1 (0.5–2)	1.6 (1–3)	2 (1–3)
$AUC_{0-\tau}$ (ng.h/ml)	[c]68 197 (57 790–78 605) [d]34 658 (33 431–35 885)	77 500 (55 541–121 893)	65 994 (40 132–81 548)	69 270 (45 406–80 442)	14 290 (11 570–22 378)	15 910 (6678–24 978)
$t_{1/2}$ (h)	7.1 (4.9–9.7)	17.6 (4.4–44.4)	12.4 (8.3–22.0)	18.0 (16.2–34.5)	6.4 (4.2–8.5)	8.5 (6.5–11.6)
CL/F (l/h)	17.3 (15.3–20.8)	10.3 (6.6–21.6)	0.8 (0.6–1.2)	0.7 (0.6–1.1)	21.3 (13.4–25.9)	18.9 (12.0–23.6)
V/F (l)	170.3 (145.6–235.1)	371.5 (109.0–441.3)	15.7 (9.1–22.8)	28.6 (16.9–30.9)	167.1 (121.3–300.3)	247.9 (111.9–374.4)

Pharmacokinetic values are reported as median (range).
[a]Boosted darunavir 1200 mg daily.
[b]Boosted darunavir 600 mg twice daily.
[c]Boosted darunavir 1200 mg daily: AUC_{0-24}.
[d]Boosted darunavir 600 mg twice daily: AUC_{0-12}.

lamivudine exposures were respectively only 5% and 11% higher in aging PLWH compared with younger individuals. Overall, median boosted darunavir, dolutegravir and lamivudine $t_{1/2}$ were 148%, 45% and 32% higher in elderly than in younger PLWH (Table 1).

Discussion

Advanced age does not affect boosted darunavir, dolutegravir, and lamivudine pharmacokinetics to a clinically significant extent.

For the first time, our clinical study evaluated boosted darunavir concentration–time profiles in PLWH at least 65 years. Boosted darunavir exposure tend to be higher in aging PLWH based on visual inspection, as clearance was decreased by 40%. This observation is in line with other drugs being predominantly metabolized by CYP3A in the liver, such as simvastatin [24], midazolam [25] and triazolam [26]. In contrast to our results, two population pharmacokinetic analyses of sparse sampling data did not show any significant influence of age on darunavir/ritonavir disposition, but the studied population was skewed towards younger PLWH in both studies [27,28]. Nevertheless, in our study, median clearance values of both investigated age groups were in the range of values reported from compartmental analysis varying from 11 to 20 l/h [27,28]. Between-individual variability of boosted darunavir pharmacokinetics was generally high for all analysed parameters, especially for $t_{1/2}$, which varied from 4.4 to 44.4 h in aging PLWH. In the individual with the highest observed $t_{1/2}$, darunavir elimination appeared to be altered with plasma concentrations being relatively stable between 12 and 24 h, but no explanation was found. Median $t_{1/2}$ in the younger group was similar to that reported in population pharmacokinetic analyses [27] whereas median $t_{1/2}$ in aging PLWH is close to the value reported by the manufacturer [29]. Finally, boosted darunavir V/F differed between the two age groups, but variability was also noticed in the literature with values varying from 120 to 1200 l [27,28]. Despite the large observed variability of boosted darunavir pharmacokinetics, aging did not alter pharmacokinetics to a clinically significant extent.

Dolutegravir pharmacokinetic parameters were similar to those reported by the manufacturer [30] and to a clinical study investigating dolutegravir exposure in PLWH aged 60–79 years [11]. There was no difference in pharmacokinetic parameters except for $t_{1/2}$, which was 18 h in aging compared with 12.4 h in younger PLWH. Nevertheless, this difference should lead to minor clinical consequences as dolutegravir is usually administered once daily. Between-subject variability was also comparable to population pharmacokinetic analysis of sparse sampling data ranging from 23 to 32% [31–33]. Those models demonstrated a counterintuitive 10% increase in dolutegravir clearance between the age of 40 and 65 years [31,32], which does not result in clinically significant changes considering dolutegravir overall variability.

Our lamivudine pharmacokinetic values are in good accordance with previously published population pharmacokinetic models [34,35]. Clearance distribution is close to that reported by Moore *et al.* [35] and $t_{1/2}$ (median: 7.2 h, range: 4.2–9.2 h) is similar to the value of 5–7 h reported in the summary of product characteristics [36]. Lamivudine is mainly cleared by renal elimination and reduced glomerular filtration rate with advanced age could guide clinicians to adjust lamivudine dosage in the elderly. However, our study suggests only a marginal impact of age on lamivudine pharmacokinetics (median clearance of 21.3 and 18.9 l/h in younger and aging PLWH, respectively).

The main limitation of this study is the relatively small number of full pharmacokinetic investigations. However, the comparison with a cohort of younger PLWH allows to demonstrate a lack of significant effect of aging on the pharmacokinetics of ARVs when considering elderly PLWH without severe comorbidities. Furthermore, this study fills the knowledge gap in real-life clinical pharmacokinetic data of ARVs in aging PLWH, which are essential for safe prescribing in this population.

In conclusion, boosted darunavir exposure was highly variable but modestly increased in aging PLWH. Dolutegravir and lamivudine exposure was minimally affected by age. Nevertheless, there is still a need for studies to allow a better understanding of ARV pharmacokinetics in this vulnerable growing population.

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Conflicts of interest

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CHAPTER VI: VARIOUS CLINICAL APPLICATIONS

Chapter VI in the thesis context

The numerous LC-MS/MS methodologies developed within the framework of this thesis have been either integrated in our routine TDM service and have also been applied for several research projects in collaboration with Swiss Hospitals. They all benefited from our expertise in clinical pharmacokinetics analyses.

The first part of this chapter describes the ARV PK profiles after post gastric bypass surgery period in an HIV-infected women from the University Hospital of Basel. The impact of such surgery on drug PK especially in the absorption phase is poorly documented, thus leading to uncertainty about the management of treatment in such conditions. This case report highlights the importance of TDM in such particular clinical situations.

The second study evaluates the suitability of saliva as a non-invasive surrogate marker to identify non-adherent patients to ART. This pilot study is part of a broader project initiated by the University Hospital of Geneva about optimization of HIV care in low-income countries.

The last part of this chapter emphasises the role of TDM in complex clinical situations involving both multi-organ failure and suspicion of DDIs. Although first developed for research purposes, the LC-MS/MS methodology for the determination of cardiovascular drugs presented in chapter III of this thesis was applied to plasma samples collected in a 85-old patient hospitalized in Bellinzona and helped clinicians to identify the cause of rhabdomyolysis.

Own contribution: generation of PK data, pharmacological interpretation, writing (saliva paper) or revision (gastric bypass, rosuvastatin accumulation and dolutegravir monotherapy papers) of the articles.

VI.1. Boosted darunavir, emtricitabine and tenofovir pharmacokinetics in the early and late post gastric bypass surgery period.

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Correspondence

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Boosted darunavir, emtricitabine and tenofovir pharmacokinetics in the early and late postgastric bypass surgery periods

The impact of bariatric surgery on antiretroviral pharmacokinetics is poorly documented. Common surgical interventions for morbid obesity include diversionary procedures such as Roux-en-Y gastric bypass (RYGB) and restrictive procedures such as sleeve gastrectomy. Both procedures consist of creating a small portion of the stomach resulting in decreased gastric volume and increased gastric pH, which may impair the absorption of drugs whose solubility relies on low gastric pH (e.g. rilpivirine, atazanavir). In the case of RYGB, the gastric pouch is attached to the small intestine, thereby partially bypassing the duodenum and jejunum. Consequently, RYGB may further impact drug absorption due to a reduced exposure to the intestinal mucosa. Maintenance of sufficient antiretroviral drug exposure is critical given the risk of viral relapse. Thus, the characterization of antiretroviral drugs pharmacokinetics after bariatric surgery is of clinical interest to provide dosage recommendations both in the early and late postoperative periods.

We report a 43-year-old HIV-infected woman undergoing RYGB (BMI: 47.5 kg/m^2). At the time of surgery, the patient was virologically suppressed with a CD4$^+$ cell count of 1040 cells/μl under once-daily treatment with darunavir/ritonavir (800/100 mg), emtricitabine (200 mg) and tenofovir disoproxil (245 mg). Routine therapeutic drug monitoring (TDM) performed 3 months before surgery showed plasma levels of darunavir/ritonavir, emtricitabine and tenofovir more than 75th percentile (5314/450; 428 and 121 ng/ml, 14 h after drug intake). We anticipated that RYGB might alter antiretroviral drug exposure; therefore, darunavir/ritonavir was increased immediately postsurgery to 600/100 mg twice daily, whereas emtricitabine and tenofovir dosages were unchanged. This dosing recommendation followed published data reporting adequate single-point measurement of these antiretrovirals, although 1-year post-RYGB [1]. Thus, the reported levels might not reflect the early postoperative period as the intestine could have adapted to compensate for the altered physiology. Due to the paucity of data, we decided to document the pharmacokinetic profiles of darunavir/ritonavir, emtricitabine and tenofovir in the early and late post-RYGB periods. During the morning of the pharmacokinetic investigations, the patient took darunavir/ritonavir with a small breakfast then blood samples were drawn at defined time points over 12 h. Given the once-daily administration of emtricitabine and tenofovir, their pharmacokinetics was measured from 12 to 24-h post-intake. Drug levels were quantified using validated liquid chromatography coupled to tandem mass spectrometry methods [2,3]. The drug profiles drawn from compartmental analyses and the pharmacokinetic parameters calculated using noncompartmental analysis are presented in Fig. 1.

Compared with reference pharmacokinetic profiles and parameters [4,5], darunavir area under the curve $(AUC)_{0-12h}$, C_{max} and C_{min} 3 days postsurgery were decreased by 50, 14 and 67%, whereas darunavir exposure 10 weeks postsurgery was comparable with reference (Fig. 1a). Ritonavir AUC_{0-12h}, C_{max} and C_{min} 3 days postsurgery were decreased by 67, 67 and 75% compared with reference [4,6], whereas ritonavir pharmacokinetic parameters were increased by 104, 119 and 135% 10 weeks postsurgery (Fig. 1b). Compared with references [7,8], emtricitabine exposure was at percentile 10th and close to 50th 3 days and 10 weeks postsurgery (Fig. 1c), whereas tenofovir exposure was at percentile 50th and above 75th, respectively (Fig. 1d). Given the normal drug exposures on the second pharmacokinetic investigation, the patient was changed back to a once-daily darunavir/ritonavir (800/100 mg) schedule. One-year postsurgery TDM showed good levels of darunavir/ritonavir, emtricitabine and tenofovir (changed to tenofovir alafenamide) (3140/298; 147 and 11 ng/ml, 14 h after drug intake).

This report describes for the first time the full pharmacokinetic profiles of darunavir/ritonavir in the early and late postsurgery periods. Darunavir exposure underwent a marked transient reduction as explained by the impaired absorption in the early postsurgery period of darunavir and notably of ritonavir, thus altering in turn its boosting effect on darunavir pharmacokinetics. Despite this decrease, darunavir levels remained well above the protein-adjusted concentration inhibiting viral replication by 90% for the wild-type virus (55 ng/ml) [4] and the patient remained virologically suppressed throughout. The administration of once-daily darunavir/ritonavir could likely have been possible in this patient given the good presurgery drug exposure. The extrapolated $C_{min,\ 24h}$ is ≈410 ng/ml, thus still within the observed darunavir C_{min} range for once-daily administration [median (95% prediction interval): 981 (110–4449) ng/ml] [4]. However, the observed 67% decrease in darunavir C_{min} early postsurgery could be problematic in patients with resistant viruses, particularly if the presurgery level is below percentile 50th. The transient decrease in tenofovir exposure with maintenance of viral suppression is in line with published data [9]. This case

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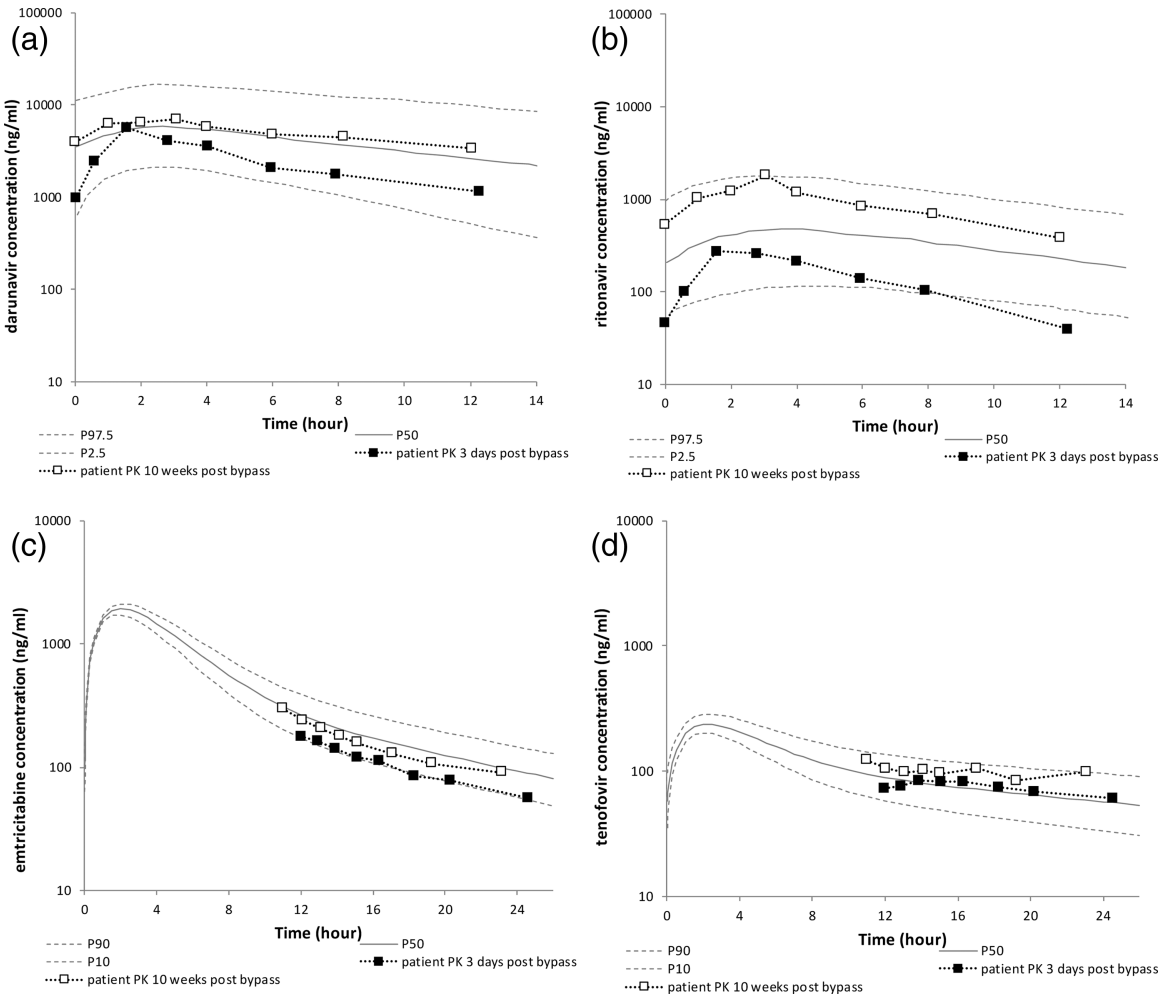

Fig. 1. Drug plasma profiles measured in the patient with the corresponding reference profiles issued from published population pharmacokinetic analyses. Darunavir [600 mg twice a day (BID)] (a), Ritonavir (100 mg BID) (b), emtricitabine [200 mg once a day (QD)] (c) and tenofovir (245 mg QD) (d) pharmacokinetic profiles 3 days (filled squares) and 10 weeks (open squares) post gastric bypass surgery. Grey dashed lines represent the 2.5 or 10, 97.5 or 90th percentile and grey continuous lines 50th percentile concentration curves derived from published population-based pharmacokinetic models of darunavir/ritonavir [4], emtricitabine [7] and tenofovir [8]. The area under the curve $(AUC)_{0-12h}$, C_{max}, C_{min}, half-life of darunavir 3 days and 10 weeks after surgery were 30 996 and 60 031 ng h/ml, 5574 and 6967 ng/ml, 1166 and 3350 ng/ml, 7.4 and 11.6 h, respectively. For ritonavir, the AUC_{0-12h}, C_{max}, C_{min}, half-live were 1717 and 10 620 ng h/ml, 277 and 1819 ng/ml, 40 and 384 ng/ml, 3.4 and 5.2 h, respectively.

highlights the utility of TDM to guide dosage recommendations in patients undergoing bariatric surgery [10].

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Conflicts of interest

There are no conflicts of interest.

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First reported case of integrase (R263K, G163R) and reverse transcriptase (M184V)-transmitted drug resistance from a drug-naive patient failing Triumeq

We report a case of confirmed transmission of R263K integrase mutation, which has not previously been described. R263K is a rare nonpolymorphic mutation, selected by raltegravir/dolutegravir *in vivo* and elvitegravir/dolutegravir *in vitro*. It confers low-level phenotypic reductions in elvitegravir susceptibility (~5-fold) and minimal reductions in raltegravir and dolutegravir susceptibility. The Stanford database classes raltegravir and dolutegravir susceptibility as low-level, which increases to intermediate-level and low-level resistance when G163K is also present [1].

Patient Y, a 40-year-old woman, presented to our clinic for testing as a heterosexual sexual contact of HIV. A fourth generation test was completed 6 weeks following exposure. She was found to be HIV-positive, subtype C. Baseline bloods showed $CD4^+$ 925 cells/μl (42%) and viral load 1723 copies/ml. Her avidity index of 0.628 indicated recent infection. She was treatment-naive and last tested HIV-negative 3 months prior.

Her contact (male patient Z) was known to have developed R263K, M184V and G163K/R as a result of poor adherence to Triumeq with subsequent virological failure. This was identified on genotypic testing using Sanger. Patient Z had fully susceptible subtype C virus at baseline (viral load 26 366 copies/ml), although integrase resistance testing was not performed at this point in accordance with national British HIV Association (BHIVA) guidelines [2]. He denied previous antiretroviral (ARV) therapy prior to starting Triumeq in spite of the low baseline viral load. He achieved an undetectable viral load after just 2 months of therapy. However, 8 months later, his viral load rebounded (909 267 copies/ml) because of poor adherence, and he disengaged from regular follow-up. Interestingly, the rebound viral load was much higher than his baseline and it could be argued that the patient may not have disclosed previous ARV therapy at baseline.

Baseline resistance on female patient Y confirmed all three drug resistance mutations (G163K/R, R263K and M184V) had been transmitted. She was commenced on Truvada and Rezolsta and her viral load became undetectable after 2 months on treatment.

R263K has been described in the SAILING trial in two patients who failed dolutegravir therapy who were integrase-naive but treatment-experienced [3]. Studies have identified that R263K reduces strand-transfer activity by decreasing the affinity of integrase for target DNA. In tissue culture, both viral infectivity and replication were also reduced. These effects have been found to be more pronounced in subtype C HIV-1 compared with subtype B [4]. Furthermore, R263K in combination with M184V has been shown to substantially reduce infectivity compared with a single substitution [5,6].

We believe this is the first case to describe sexual transmission of R263K and M184V mutations. Our case is also unique as we describe a drug-naive patient failing Triumeq with three resistance mutations. Patient Z had a high viral load prior to transmission, most probably because of poor adherence, which is likely to have increased the risk of transmission. However, it is interesting that the resistant virus transmitted out of his mixed population (Table 1) raising the question whether these mutations do indeed reduce viral fitness. Patient Y who had resistant virus population as her wild-type virus

VI.2. Emtricitabine and lamivudine concentrations in saliva: a simple suitable test for treatment adherence.

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Emtricitabine and lamivudine concentrations in saliva: a simple suitable test for treatment adherence

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Sir,

ART constitutes the cornerstone of HIV treatment and prevention. Successful ART results in suppressed viral load (VL) levels. Unsuppressed VL is either a result of acquisition of drug resistance mutations or insufficient drug concentrations due to poor adherence. Since clinical and immunological parameters are poor predictors of successful viral suppression, the WHO recommends regular VL monitoring for all individuals on ART.[1] However, due to cost and infrastructure issues, the implementation of routine and frequent VL monitoring remains slow in the countries with the highest numbers of individuals on ART. Although measures such as pill count and self-reporting have been reported in several studies to be a good guide to viral suppression, they remain error prone.[2] In this context, therapeutic drug monitoring (TDM) is frequently requested. Indeed, low plasma drug concentrations have been shown to predict unsuppressed VL.[3] In addition, plasma drug concentration constitutes the definitive marker of short-term drug exposure. Emtricitabine and lamivudine are part of virtually all currently used fixed-dose regimens, thus undetectable emtricitabine/lamivudine levels would be a good proxy for recent poor adherence. Non-invasive saliva sampling could therefore represent an alternative or clinically useful addition to VL measurements and genotype determination among patients suspected of non-adherence. If available as a point-of-care test, it would provide the clinicians with an objective assessment of recent adherence during the clinical visit.

To date, very few publications have evaluated saliva in the context of ART TDM.[4–7] Nevirapine was found to reach high saliva concentrations, indicating that saliva could be suitable for TDM. In Tanzania, nevirapine saliva concentrations have shown good agreement with self-reported adherence, demonstrating the value of such a TDM approach.[8] Concerning the other studied drugs (i.e. lamivudine and zidovudine), the excessive variability in saliva concentrations may preclude any reliable plasma level predictions from saliva drug measurements. Two studies reported a good penetration of emtricitabine into saliva in the context of pre-exposure prophylaxis.[9,10]

We aimed to evaluate whether saliva would constitute a suitable matrix for ART adherence monitoring, by examining the correlation between emtricitabine and lamivudine levels simultaneously measured in plasma and saliva. A total of 73 patients participating in the Swiss HIV Cohort Study (SHCS) #815 study agreed to donate a non-stimulated saliva sample simultaneously to the blood sample collected as part of the biannual SHCS follow-up visit. Emtricitabine and lamivudine levels were quantified by an LC/MS-MS assay, using stable isotopically labelled internal standards also adapted for saliva concentration determination.[11]

Overall, 47 and 26 paired plasma and saliva concentrations were collected for emtricitabine and lamivudine determination, respectively. In addition, six saliva samples were collected from three SHCS patients not receiving emtricitabine or lamivudine, and from three HIV-negative individuals, to evaluate the selectivity of the method.

Emtricitabine plasma and saliva concentrations ranged from 8 to 2471 ng/mL and 23 to 1017 ng/mL, respectively. The time between sampling and last drug intake (time after dose; TAD) ranged from 0.8 to 37.3 h. For lamivudine, plasma and saliva concentration varied between 45 and 3183 ng/mL and between 15 and 718 ng/mL, respectively, with a TAD of 1.3–27 h. Our results show that emtricitabine and lamivudine saliva concentrations are moderately correlated with plasma concentrations (correlation coefficient 0.63 and 0.58 for emtricitabine and lamivudine, respectively) (Figure 1).

Median saliva/plasma ratios were 0.61 for emtricitabine and 0.35 for lamivudine, with high variability (coefficient of variation 89% and 101% for emtricitabine and lamivudine, respectively). This variability can be explained by multiple factors involving drug

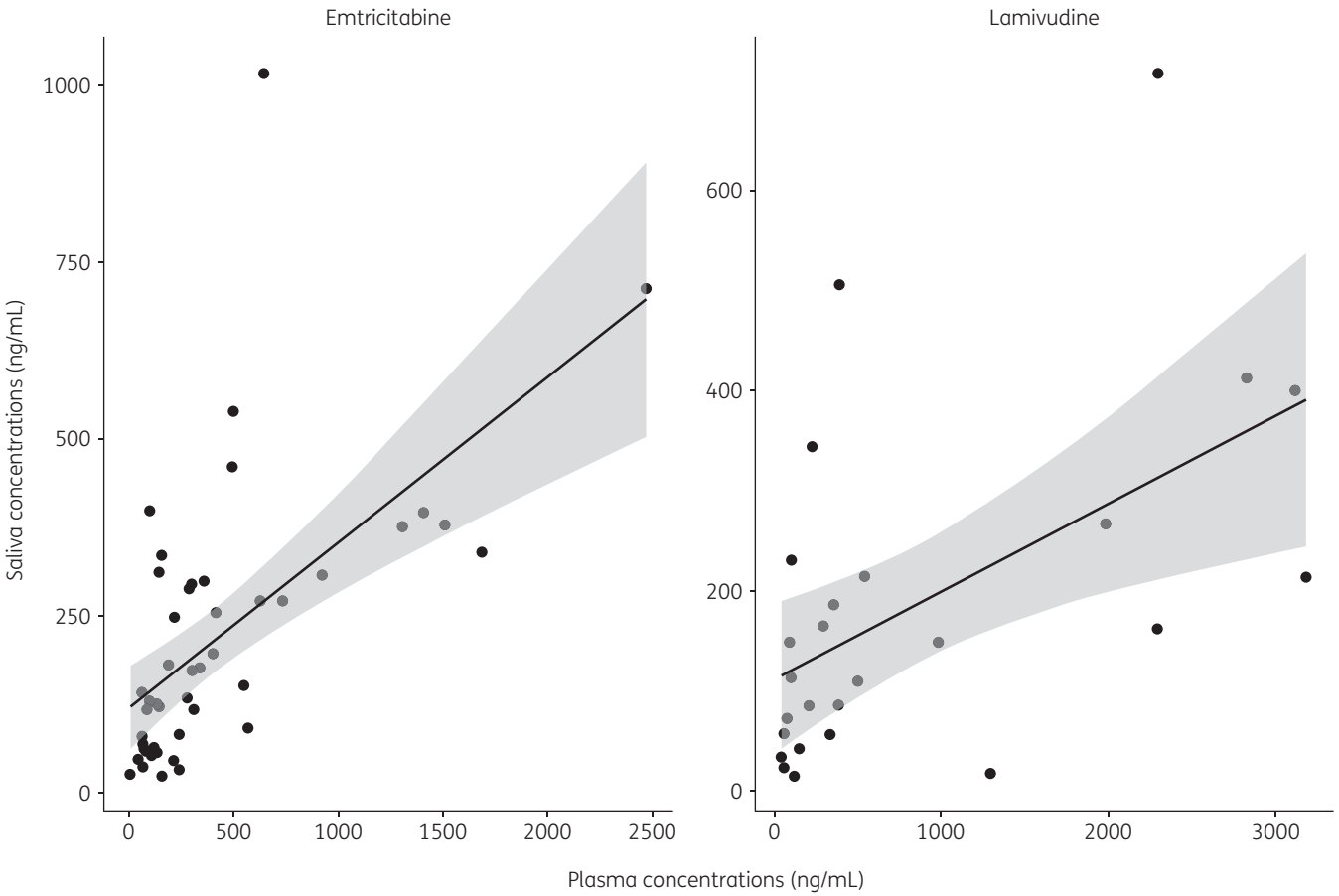

Figure 1. Correlation between saliva and plasma concentrations ($n = 47$ and $n = 26$ for emtricitabine and lamivudine, respectively). Points represent paired plasma and saliva concentrations. The solid line represents the correlation between these concentrations, with its 95% CI shown in grey.

properties (i.e. protein binding, saliva pH, salivary flow rates and degree of ionization) or patient characteristics (i.e. renal clearance or adherence issues).

Importantly, saliva concentrations from individuals not receiving emtricitabine or lamivudine were below the limit of quantification (LOQ) in saliva (<10 ng/mL), confirming the specificity of the method. Assuming that emtricitabine in saliva follows the same kinetics as in plasma, the $t_{1/2}$ of emtricitabine in saliva calculated for each patient using a population pharmacokinetics model of emtricitabine[12] gave a mean $t_{1/2}$ of 9.4 ± 0.88 h (minimum = 8.4 h, maximum = 12.1 h). It can be extrapolated that after 31 h (i.e. $3.3 \times t_{1/2}$) emtricitabine levels in saliva would lie above the assay LOQ of 10 ng/mL in all patients, whereas after 60 h and 72 h (2.5 days and 3 days TAD, respectively) the saliva emtricitabine concentration would fall below the LOQ cut-off of the assay in 91% and 100% of patients, respectively. Thus, knowing the emtricitabine or lamivudine concentrations determined in saliva allows the identification of non-adherent patients who have missed their ART for at least the last 3 days.

In conclusion, despite a limited correlation between plasma and saliva concentrations, saliva constitutes a suitable non-invasive surrogate to identify patients who are non-adherent to ART. These first results have been obtained using an MS assay. However, if developed as a qualitative, immunoassay-based, easy-to-use and non-invasive point-of-care test, this assay would be of invaluable clinical usefulness to reduce the number of unnecessary VL measurements and genotype determinations in cases of proven non-adherence, and may thus prevent unnecessary switches to second-line ART.

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Members of the Swiss HIV Cohort Study

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Efficacy and safety of raltegravir in switch strategies in virologically suppressed patients: long-term data from clinical practice

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Sir,

We appreciated the recently published work by d'Arminio Monforte *et al.*[1] on the durability of integrase strand transfer inhibitors (INSTIs) in a large cohort of treatment-naive HIV-positive patients. Indeed, INSTI-based regimens have become the first choice for initial HIV therapy, but they are also very popular as part of three-drug or two-drug switching strategies. Eleven years have passed since the first-generation INSTI, raltegravir, was introduced. Despite the availability of the new INSTIs, such as dolutegravir (with higher genetic barrier) and elvitegravir (available as a single tablet regimen), raltegravir still plays an important role in combination ART (cART).[2] The major advantages of using raltegravir are the virtual absence of potential interactions with concomitant drugs and its high bioavailability irrespective of food intake.

To investigate the safety and efficacy of raltegravir in the setting of cART optimization, we performed a retrospective study enrolling HIV-1-infected, virologically suppressed (defined as HIV-RNA $<$50 copies/mL) patients switching to a raltegravir-containing dual or triple therapy. The study period ranged from September 2008 to May 2017. We evaluated the percentage of patients free from treatment discontinuation (TD; discontinuation of raltegravir for any reason regardless of whether the remaining antiretroviral drugs used in the combination had been stopped or not) and from virological failure (VF; defined as two consecutive counts of HIV-RNA $\geq$50 copies/mL or one of $\geq$1000 copies/mL) at weeks 48, 96 and 144. Kaplan–Meier curves and Cox regression models were performed to estimate the time to event and the predictors of TD and VF. Data analysed in the present study were selected from an internal observational database, which collects the main clinical and demographic characteristics of every patient who gave informed consent to personal data record since the time of HIV diagnosis.

VI.3. Severe rosuvastatin accumulation with rhabdomyolysis due to drug interactions and low cardiac output syndrome.

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LETTER TO THE EDITOR

Severe rosuvastatin accumulation with rhabdomyolysis due to drug interactions and low cardiac output syndrome

An 85-year-old patient presented to the emergency department because of orthopnoea and peripheral oedema. He was known for diabetes mellitus and coronary artery disease with severe chronic heart failure. Eighteen months earlier, he already showed biventricular dilatation and systolic dysfunction (20-25% left ventricular ejection fraction) with moderate mitral valve regurgitation at transthoracic echocardiography. On current physical examination, he showed moderate oedema of the legs. On chest X-ray, there was upper lobe pulmonary venous congestion and small bilateral pleural effusion. Laboratory testing revealed moderate creatinine elevation (150 μmol L^{-1}; normal <107). Liver enzymes and creatine kinase levels were normal. The patient's daily therapy consisted of acetylsalicylic acid 100 mg, metoprolol 50 mg, sacubitril/valsartan 200 mg (97/103 mg), torasemide 10 mg, spironolactone 12.5 mg, rosuvastatin 10 mg, and sitagliptin 100 mg.

He was admitted to the general internal medicine ward with a diagnosis of subacute biventricular decompensation, but his condition progressively deteriorated with oliguria, worsening dyspnoea, and increasing peripheral oedema despite the administration of loop diuretics. Amiodarone was introduced because of non-sustained ventricular tachycardia at 24-hour continuous ECG monitoring. Five days later, the patient began to feel muscular weakness and to walk unstable. After another week, he showed peripheral vasoconstriction with large pitting oedema, a slight scleral jaundice, and complained of widespread muscular pain. Laboratory tests revealed that creatinine had risen to 513 μmol L^{-1}, GPT (ALT) to 1103 U L^{-1} (normal <51), bilirubin to 41.7 μmol L^{-1} (normal <21.0), and lactate to 4.1 mmol L^{-1} (normal <2.2). Moreover, creatine kinase levels were 6794 U L^{-1} (normal <190), hs troponin T was 204 ng L^{-1} (normal <14), and urine analysis revealed myoglobinuria. He was admitted to the intensive care unit, where severe cardiac dysfunction was confirmed by transthoracic echocardiography and trans-pulmonary thermodilution. A diagnosis of low-cardiac output syndrome with multi-organ dysfunction was made. The origin of the rhabdomyolysis was thoroughly investigated: Infections (bacterial, viral, or parasitic), trauma, thermal injury, or toxins were ruled out or were deemed unlikely. Thyroid function was normal; blood sugar and electrolytes (phosphates, potassium) were within the normal range or only slightly elevated. We hypothesized that rhabdomyolysis was caused by the accumulation of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase-inhibitor rosuvastatin, as statins are known to be one of the top three prescription drugs responsible for elevations of creatine kinase levels.[1]

Sacubitril/valsartan and rosuvastatin were discontinued, and a dobutamine infusion was initiated which led to improvement of the haemodynamic parameters, normalization of lactate levels, rapid decrease of liver enzymes, and recovery of a valid diuresis. Crystalloid and sodium bicarbonate infusions were started, aiming at volume repletion and urine alkalinisation, to limit the renal toxicity of the haem pigments. A water balance adequate to the cardiac condition was maintained by loop diuretics. Five days later, creatine kinase reached a peak value of 18478 U L^{-1} and then began to decrease daily by 30-50%. Creatinine stabilized at around 380 μmol L^{-1} without the need for renal replacement treatment.

Four sequential plasma concentrations of rosuvastatin 38, 61, 72, and 85 hours after the last rosuvastatin intake were analysed by liquid chromatography/mass spectrometry (Figure 1). Concentrations measured more than 36 hours after the last dose were well above the levels usually seen in patients receiving rosuvastatin 10 mg once daily. These concentrations were even remarkably higher than those expected at peak[2] and may be explained by the co-existence of several factors: acute liver injury combined with acute renal failure, and drug-drug interactions. Actually, rosuvastatin plasma concentrations are known to increase about 3-fold in patients with severe renal impairment (CrCl <30 mL $minute^{-1}$).[3] Furthermore, C_{max} and AUC may be increased in patients with Child-Pugh class B hepatic impairment.[4]

Rosuvastatin is a substrate of OATP1B1, and interactions have been described with OATP inhibitors. Notably, the interaction with faldaprevir, an OATP-inhibiting anti-HCV medication, can lead to increases in AUC and Cmax of rosuvastatin of 15- and 33-fold, respectively.[5]

Sacubitril is an OATP inhibitor, and a physiologically based pharmacokinetic model predicted a moderate interaction between sacubitril and atorvastatin, with a 1.7-fold increase of Cmax and a 1.3-fold increased AUC.[6] In fact, one case of severe rhabdomyolysis was reported after 3 weeks of coadministration of sacubitril/valsartan and atorvastatin.[7] However, atorvastatin is largely eliminated by CYP3A4, with little contribution from OATP. Since only approximately 10% of an orally administered rosuvastatin dose is metabolized,[8] its elimination is much more dependent on OATP. Furthermore, rosuvastatin has a slightly higher affinity for OATP1B1 compared to atorvastatin.[9] It seems therefore reasonable to assume that the magnitude of the interaction would be greater with coadministration of sacubitril and rosuvastatin. Actually, in a recent animal study,

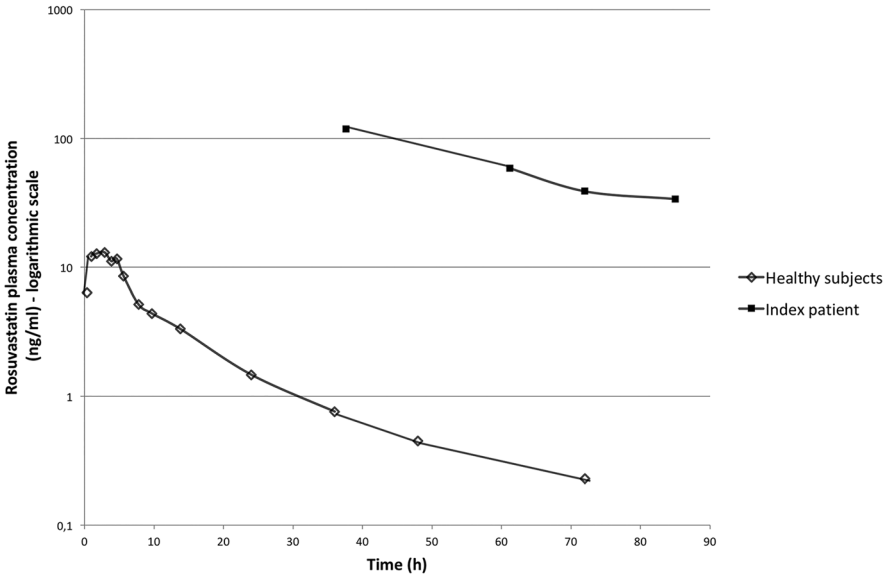

FIGURE 1 Comparison between measured rosuvastatin plasma concentrations in the index patient and mean plasma values found in 10 healthy subjects after a single oral dose of 10 mg. Adapted from J. Gao et al.[2]

rosuvastatin AUC was increased 11-fold by coadministration of sacubitril/valsartan to rats.[10]

Valsartan itself is also an OATP1B1 inhibitor, with IC50 values comparable to sacubitril.[11,12] LBQ657, sacubitril's active metabolite, is only a weak inhibitor of OATP1B1.[12] However, its longer half-life and higher plasma concentrations compared to sacubitril, especially in the presence of renal failure, potentially reinforced this interaction and resulted in a clinically significant effect.[13,14]

Finally, amiodarone could have slightly contributed to increasing rosuvastatin concentrations due to the inhibition of enzymes partially responsible for the metabolism of rosuvastatin, such as CYP2C9. The Naranjo Adverse Drug Reaction Probability Scale score was 6, making it probable that the adverse drug reaction was precipitated by amiodarone, as the last factor added to those already existing.[15]

In conclusion, this case suggests that the interaction between rosuvastatin, sacubitril/valsartan, and amiodarone can significantly increase rosuvastatin plasma concentrations, with subsequent rhabdomyolysis, especially in patients who develop renal and liver failure due to severe heart dysfunction. Sacubitril/valsartan has already been included in the guidelines for the treatment of chronic heart failure; therefore, particular attention must be paid when treating patients who are increasingly receiving this combination of drugs.

COMPETING INTERESTS

There are no competing interests to declare.

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VI.4. Noninferiority of Simplified Dolutegravir Monotherapy Compared to Continued Combination Antiretroviral Therapy That Was Initiated During Primary Human Immunodeficiency Virus Infection: A Randomized, Controlled, Multisite, Open-label, Noninferiority Trial.

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MAJOR ARTICLE

Noninferiority of Simplified Dolutegravir Monotherapy Compared to Continued Combination Antiretroviral Therapy That Was Initiated During Primary Human Immunodeficiency Virus Infection: A Randomized, Controlled, Multisite, Open-label, Noninferiority Trial

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(See the Major article by Hocqueloux et al on pages 1498-505 and Editorial Commentary by Rijnders and Rokx on pages 1506–8.)

Background. Patients who start combination antiretroviral therapy (cART) during primary human immunodeficiency virus type 1 (HIV-1) infection show a smaller HIV-1 latent reservoir, less immune activation, and less viral diversity compared to patients who start cART during chronic infection. We conducted a pilot study to determine whether these properties would allow sustained virological suppression after simplification of cART to dolutegravir monotherapy.

Methods. EARLY-SIMPLIFIED is a randomized, open-label, noninferiority trial. Patients who started cART <180 days after a documented primary HIV-1 infection and had an HIV-1 RNA <50 copies/mL plasma for at least 48 weeks were randomized (2:1) to monotherapy with dolutegravir 50 mg once daily or to continuation of cART. The primary efficacy endpoint was the proportion of patients with <50 HIV-1 RNA copies/mL on or before week 48; noninferiority margin 10%.

Results. Of the 101 patients randomized, 68 were assigned to simplification to dolutegravir monotherapy and 33 to continuation of cART. At week 48 in the per-protocol population, 67/67 (100%) had virological response in the dolutegravir monotherapy group vs 32/32 (100%) in the cART group (difference, 0.00%; 95% confidence interval, −100%, 4.76%). This showed noninferiority of the dolutegravir monotherapy at the prespecified level.

Conclusion. In this pilot study consisting of patients who initiated cART during primary HIV-1 infection and had <50 HIV-1 RNA copies/mL for at least 48 weeks, monotherapy with once-daily dolutegravir was noninferior to cART. Our results suggest that future simplification studies should use a stratification according to time of HIV infection and start of first cART.

Clinical Trials Registration. NCT02551523.

Keywords. primary HIV infection; dolutegravir; monotherapy; simplification; randomized controlled trial.

Long-term toxicity of combination antiretroviral therapy (cART) is a substantial contributor to morbidity in chronically infected human immunodeficiency virus type 1 (HIV-1)–positive individuals [1]. Nucleoside reverse transcriptase inhibitors (NRTIs) are the main reason for ART-related toxicity. Trying to avoid NRTIs, initial randomized studies explored protease inhibitor–based monotherapy strategies. From a virological point of view, protease inhibitor–based monotherapy was clearly inferior to cART and was therefore not introduced into clinical practice [2]. However, at least in the context of more frequent viral load monitoring in a clinical trial, it did not lead to the loss of more treatment options compared to cART [3].

Dolutegravir is a second-generation integrase strand transfer inhibitor (INSTI) used as a component of preferred cART [4]. Several simplification studies were recently performed with dolutegravir as a main active drug [5, 6]. A dolutegravir-based dual-therapy regimen in combination with lamivudine or rilpivirine shows promising results [5, 7]. In contrast, 3 randomized, controlled trials that explored the efficacy of dolutegravir-based monotherapy revealed inferiority compared to cART [6, 8, 9]. Notably, all protease inhibitor and dolutegravir simplification studies were conducted in patients initiating cART during chronic HIV-1 infection. Importantly, patients who initiated cART during the early phase of

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HIV-1 infection harbor a markedly reduced HIV-1 reservoir [10, 11] and show low levels of viral diversity [12, 13].

The Zurich Primary HIV Infection Study (ZPHI) is an ongoing observational study enrolling individuals with documented primary HIV infection, that is, individuals identified within 180 days after estimated date of infection [14]. Since 2002, immediate start of cART is offered to the ZPHI participants. We hypothesized that individuals who start cART early have a smaller HIV-1 reservoir and, therefore, are the best candidates to maintain viral suppression after switching to dolutegravir monotherapy comparable to that seen under continued cART.

We tested our hypothesis in a pilot study consisting of individuals who had been successfully treated with cART for at least 48 weeks since primary HIV-1 infection before being randomized to dolutegravir monotherapy.

METHODS

Study Design and Patients

All patients enrolled in this trial have a documented primary HIV-1 infection, and the majority of them (85%) are enrolled in the ZPHI, an open-label, nonrandomized, observational, multisite study (NCT02551523) [14]. All patients were participating in the Swiss HIV cohort study, a long-term observational study [15]. Primary HIV-1 infection was defined as published elsewhere [16].

In this randomized, open-label, noninferiority trial, we recruited patients aged ≥18 years with a primary HIV-1 infection and no previous antiretroviral treatment failure, no prior treatment interruption, no major resistance mutations to INSTIs according to the Stanford algorithm [17], an HIV-1 RNA of less than 50 copies/mL plasma for 48 weeks or longer, and a negative hepatitis B virus surface antigen. Patients with documented resistance to any NRTI, non-NRTI, or protease inhibitors were allowed to be included. Exclusion criteria were pregnancy or breastfeeding, use of contraindicated drugs to dolutegravir, and previous intolerance to dolutegravir.

We obtained ethics committee approval at all participating centers in accordance with the principles of the 2008 Declaration of Helsinki. All participants gave their written informed consent before undergoing any study procedure.

Study Procedures

Patients in the monotherapy group were simplified to oral dolutegravir 50 mg once daily, and those in the cART group continued their current regimen consisting of an INSTI, a boosted protease inhibitor, or non-NRTI, in combination with 2 NRTIs. We assessed HIV-1 RNA in plasma using the COBAS AmpliPrep/TaqMan HIV-1 Test Vs 2.0 with a limit of detection ≤20 HIV-1 RNA copies/mL plasma. We measured total HIV-1 DNA in peripheral blood mononuclear blood cells (PBMCs) using an in-house digital droplet polymerase chain reaction assay at baseline and week 48. We assessed markers for proximal renal tubulopathy and lipid levels at baseline and at week 48. All patients were asked for a lumbar puncture at baseline and at week 48. ART drug levels were measured in plasma and in cerebrospinal fluid (CSF) at week 0 and week 48. The study procedures are described in detail in the Supplementary Materials.

Outcomes

The primary endpoint was noninferiority of the virological response between treatment groups, defined as the proportion of patients without a virological failure on or before week 48. Virological failure was defined as 2 consecutive viral loads (14 days or longer but not more than 30 days apart) above 50 HIV-1 RNA copies/mL plasma. The window of visit was ±4 weeks, thus an HIV-RNA value obtained between week 44 and week 52 was included in the primary efficacy analysis. If the HIV-1 RNA measurement was missing at week 48, we included the last documented HIV-1 RNA measurement prior to week 48 in the primary efficacy analysis (last observation carried forward principle).

Secondary endpoints were quantification of total HIV-1 DNA in PBMCs, central nervous system (CNS) virological escape (defined as less than 40 HIV-1 RNA copies/mL CSF), frequency of blips (defined as 1 viral load between 50 and 400 followed by a viral load below 50 HIV-1 RNA copies/mL plasma within 30 days), number of adverse events and serious adverse events, changes in CD4 cell count, new onset of proximal tubular renal dysfunction (defined as pathological tubulopathy markers according to Fux et al [18]), changes in lipid profiles, withdrawing consent and lost to follow-up and switching assigned treatment for any cause before and after simplification.

Statistical Analyses

The study was powered to detect noninferiority at 48 weeks, assuming a response of 95% in both groups. Thus, to show noninferiority at a margin of 10% at week 48, with a significance value of 5% and a power of 80%, we estimated the sample size at 138 assessable patients. The final study population consisted of 101 patients because of the lower rate of recruitment as anticipated during the last few months of the study. The decision to stop recruitment was taken by the sponsor (H.F.G.) and was independent of any efficacy analysis. The static unstratified multiblock randomization with multiplier 3 integrated in SecuTrial was used to allocate patients in a 2:1 ratio to monotherapy with dolutegravir or continuation of cART including 3 patients per block with computer-generated random number sequences. More details are provided in the Supplementary Materials.

RESULTS

Of 430 patients assessed for study enrollment, 101 (23.4%) were eligible for the study and agreed to participate (Figure 1). Between 30 November 2015 and 10 March 2017, we randomized 101 patients in a 2:1 ratio, allocating 68 (67%) to dolutegravir

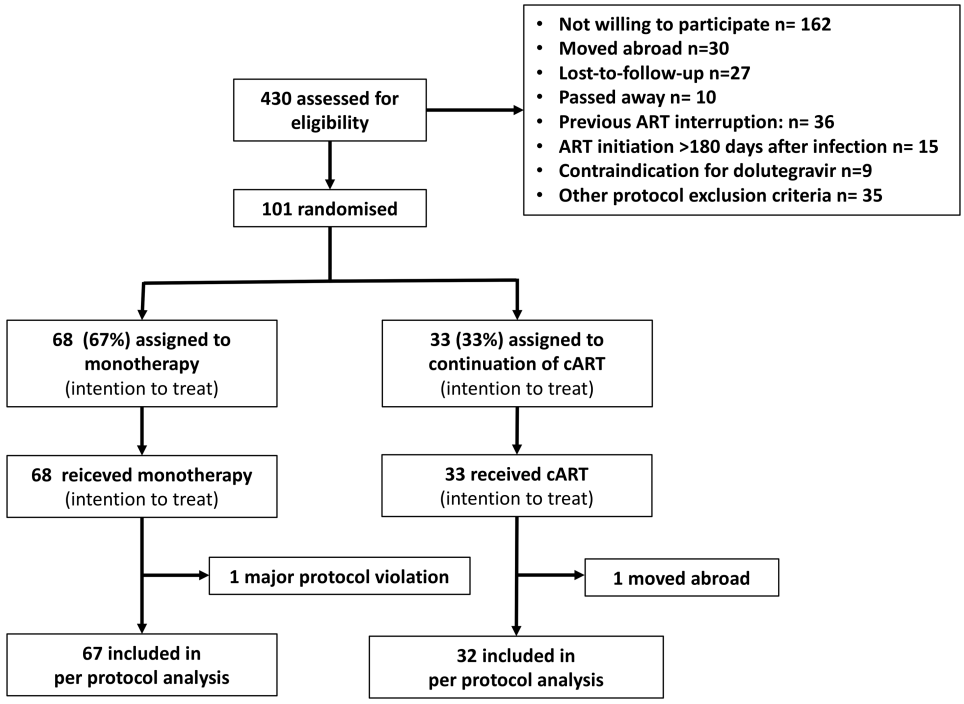

Figure 1. Trial profile. Abbreviation: cART, combination antiretroviral therapy.

monotherapy and 33 (33%) to cART (Figure 1). Baseline characteristics are depicted in Table 1.

Efficacy

In the per-protocol population, all 67 patients in the monotherapy group and all 32 patients in the cART group had a virological response on or before week 48 (Figure 2). One patient in the monotherapy group was excluded during the study due to a major protocol violation. This patient did not fulfill the definition of a primary HIV-1 infection; however, this information was missed at screening, and the patient was therefore incorrectly enrolled into the study. Detailed information on this patient is provided in the Supplementary Materials. In the cART group, 1 patient moved abroad at week 26 (Figure 1). Noninferiority was shown at the prespecified level of 10%, as the difference in efficacy between monotherapy and cART was similar. We also noted noninferiority in the intention-to-treat population (67/68, 98.5% dolutegravir versus 33/33, 100% cART; difference 1.47%, 95% CI [−100%, 6.85%]). The patient in the cART group who moved abroad was counted as success in the intention-to-treat analysis, assuming that this patient maintained viral suppression after study discontinuation. This assumption led to a more conservative approach in interpreting the data.

HIV-1 Reservoir

We measured total HIV-1 DNA in PBMCs at baseline and at week 48. The median $\log_{10}$ total HIV-1 DNA change from baseline to week 48 in the dolutegravir monotherapy arm was −0.16 compared to −0.10 in the cART arm (P = .312; Figure 3). There was a significant decay of the total HIV DNA in the monotherapy arm at week 48 compared to baseline (P = .0004). The HIV DNA decay among the cART group did not reach statistical significance (P = .0982). The total HIV-1 DNA levels for single patients are shown in Supplementary Figure 1. We recorded viral load blips that did not lead to treatment interruption in 1 (1.5%) of 68 patients in the monotherapy group (607 visits) and 1 (3.0%) of 33 patients (124 visits) in the cART group (P < .001 from the Poisson regression for rate of blips; Figure 4). The median change in CD4 cell count from baseline was 74 cells/μL blood (interquartile range [IQR], −32 to 198) for monotherapy and 24 cells/μL blood (IQR, −144 to 116) for cART (P = .124) (Supplementary Figure 2). All patients in both study groups were adherent to study drugs for the 48 weeks of study.

Plasma and CSF Dolutegravir Levels Assessment

At baseline, 23 (33.8%) patients in the monotherapy group and 14 (42.4%) in the cART group agreed to have a lumbar puncture. At week 48, 10 (14.9%) patients in the monotherapy group and 2 (6.2%) in the cART group agreed to have a second lumbar puncture. The CSF viral load was undetectable in all sampled patients at all times tested (Figure 5). Plasma and CNS concentration of dolutegravir measurements at weeks 0, 4, and 48 are depicted in Figure 6. According to the percentile curves of Aouri et al [19], 3.7% (n = 6) of dolutegravir concentrations in

Table 1. Baseline Characteristics of Study Participants

Characteristic	Overall (N = 101)	Monotherapy (n = 68)	Combination ART (n = 33)
Age (y)	42 (33–47)	42 (33–47)	43 (35–46)
Male (%)	97 (96.0)	65 (95.6)	32 (97.0)
Ethnicity (%)			
White	93 (92.1)	62 (91.2)	31 (93.9)
Black	5 (5.0)	4 (5.9)	1 (3.0)
Asian	2 (2.0)	2 (2.9)	0 (0.0)
Hispanic	1 (1.0)	0 (0.0)	1 (3.0)
HIV transmission risk (%)			
Men who have sex with men	84 (83.2)	56 (82.4)	28 (84.8)
Heterosexual	15 (14.9)	10 (14.7)	5 (15.2)
Other	2 (2.0)	2 (2.9)	0 (0.0)
HIV-1 subtype (%)			
B	63 (62.4)	44 (64.7)	19 (57.6)
CRF01_AE	8 (7.9)	5 (7.4)	3 (9.1)
CRF02_AG	5 (5.0)	1 (1.5)	4 (12.1)
Other	17 (17.0)	11 (10.0)	6 (18.0)
NA	8 (7.9)	7 (10.3)	1 (3.0)
Body mass index (kg/m^2)	23.8 (22.4–26.6)	23.7 (22.1–26.2)	24.2 (22.5–27.4)]
Fiebig stage (%)			
I–II	2 (2.0)	1 (1.5)	1 (3.0)
II–III	21 (20.8)	16 (23.5)	5 (15.2)
IV–VI	58 (57.4)	36 (52.9)	22 (66.7)
NA	20 (20.0)	15 (22.0)	5 (15.0)
Days from infection until ART start	38 (28–77)	39 (27–73)	36 (29–113)
Years on ART before study entry	3.6 (2.0–6.0)	3.8 (1.9–6.1)	3.3 (2.0–5.5)
Nadir CD4 cell count (cells/µL)	358 (265–486)	376 (263–496)	329 (269–442)
CD4 cell count (cells/µL)	716 (584–918)	730 (610–920)	669 (545–881)
Integrase strand transfer inhibitor–based regimen (%)	55 (54.5)	40 (58.8)	15 (45.5)
Dolutegravir-based regimen (%)	46 (45.5)	33 (48.5)	13 (39.4)
Backbone regimen (%)			
Abacavir based	28 (27.7)	22 (32.4)	6 (18.2)
Tenofovir based	69 (68.3)	46 (67.6)	23 (69.7)
Other	4 (4.0)	0 (0.0)	4 (12.1)
Number of VL measurements during 48 weeks before study	3 (3–4)	3 (3–4)	4 (3–4)
Rate of VL monitoring under ART before study (values/48 weeks)	4.1 (3.6–4.8)	4.1 (3.5–4.7)	4.2 (3.8–5.0)

Data are median (interquartile range) or n (%) and assessed at baseline (day of randomization). Characteristics with significant difference (P value from Mann-Whitney or Fisher exact test < 0.05) between the study groups: HIV subtype (P = .033) and backbone regimen (P = .009). The remaining characteristics were balanced between the groups.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; NA, not available; VL, viral load.

the plasma were below the 10th percentile. All dolutegravir concentrations in CSF exceeded the in vitro 50% inhibitory concentrations (IC 50) for wild-type HIV (0.2 ng/mL) [20].

Safety

Eight (7.9%) of 101 patients in the safety population developed serious adverse events (monotherapy 6 [8.8%] of 68; cART 2 [6.1%] of 33), none of which were deemed related to the study drug (Table 2). Adverse events related to the study drug occurred in 15 (14.9%) participants (monotherapy 9 [13.2%]; cART 6 [18.2%]). ART switch due to adverse events was less frequent in the monotherapy group (0%) than in the cART group (3 patients, 9.1%; P = .033). We noted no significant differences in change from baseline in renal function, proximal renal tubulopathy markers, total cholesterol, triglycerides, and low-density lipoprotein between groups at week 48 (Supplementary Figure 3, panels A and B; Supplementary Figure 4, panels A–D; Supplementary Figure 5, panels A–D).

DISCUSSION

Our early simplified randomized clinical trial shows noninferiority of dolutegravir monotherapy compared to cART in patients who initiated cART during primary HIV-1 infection and showed viral suppression for at least 48 weeks prior to switching to dolutegravir monotherapy. No virological failure occurred in the per-protocol population. This is in sharp contrast to previous randomized trials that revealed inferiority of

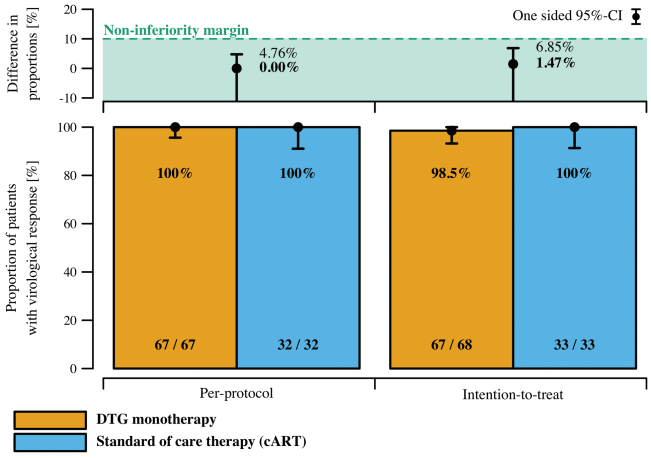

Figure 2. Virological response at week 48 defined as absence of virological failure in first 48 weeks for the per-protocol and intention-to-treat populations among the dolutegravir (DTG) monotherapy group (orange bars) and the standard-of-care combination antiretroviral therapy (cART) group (light blue bars). Virological failure is defined as 2 consecutive human immunodeficiency virus type 1 RNA >50 copies/mL in plasma. The proportion of patients with virological response in each group is depicted in the lower plot, while the upper plot summarizes the difference in proportions of patients with virological response between the study arms. The black symbols depict the proportions and the respective differences, and the error bars indicate the 95% exact confidence intervals (CIs). The light green shading represents the area of 95% CI upper bound for which the noninferiority is concluded. The CIs for the single proportions are the Clopper-Pearson (exact) CIs.

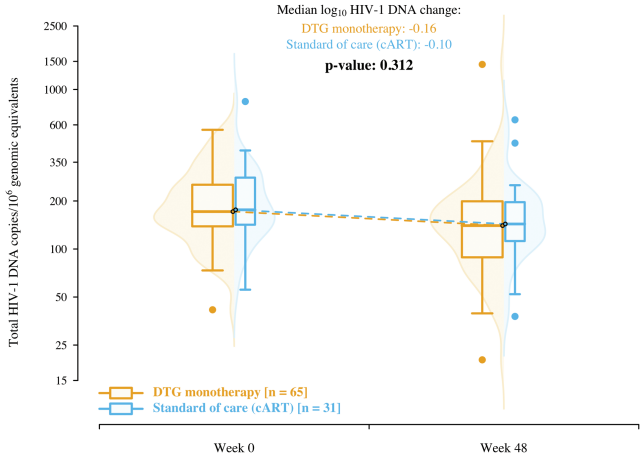

Figure 3. Total human immunodeficiency virus type 1 (HIV-1) DNA quantification from baseline compared to week 48 stratified by DTG monotherapy (orange) vs cART (blue). The shaded areas depict the distribution of the HIV-1 DNA values in both groups at baseline and at week 48. The dashed lines connect the medians of $\log_{10}$ HIV-1 DNA values at baseline and week 48. Abbreviations: cART, combination antiretroviral therapy; DTG, dolutegravir.

dolutegravir monotherapy compared to cART [6, 8, 9]; the virological failure rate was so high, that all studies were prematurely discontinued. Furthermore, emergence of drug resistance to currently licensed INSTIs occurred among patients failing on dolutegravir monotherapy [6, 8, 9]. Data from nonrandomized, partly retrospective clinical studies are less clear [21, 22]. Some studies show maintained viral suppression after switch from cART to dolutegravir monotherapy, others do not.

Notably, all prior dolutegravir monotherapy studies were conducted in patients who started their first cART during chronic HIV-1 infection. In contrast, we conducted our simplification study in patients who initiated cART during primary HIV-1 infection and were virologically suppressed for at least 48 weeks prior to entering the study. We chose this strategy because patients with early ART initiation have an approximately 10-fold lower latent HIV-1 reservoir [10, 11], less immune activation [23], and less viral diversity [12, 13] compared to patients who initiated cART during chronic infection. Our a priori hypothesis was that these properties would enable sustained virological suppression after simplification to dolutegravir monotherapy. With our novel approach to stratify patients for dolutegravir monotherapy according to their time of HIV-1 infection at the start of first cART, all patients maintained viral suppression. A potential explanation is that among patients with a small HIV-1 latent reservoir, the stochastic chance of activation of latently HIV-1–infected cells is less likely compared to patients with a large reservoir. Supporting this, the HIV-1 latent reservoir size predicted virological failures in the randomized controlled dolutegravir monotherapy trial conducted by Wijting el al [6, 24]. Moreover, the large variation in time to virological failure in this study suggests that stochastic reactivation could be the mechanism for failure [25]. In addition, a low nadir CD4 cell count and thus a surrogate for length of untreated HIV infection and a big reservoir size were predictive for virological failure in the MONCAY trial [9]. In addition, it has been shown that patients treated early with cART have less immune activation compared to those treated during chronic HIV-1 infection [23]. This may translate to a lower extent of activation of latently HIV-1–infected cells. Finally, patients who are treated early maintain a low level of viral diversity in blood as well as sanctuary sites, such as gut-associated tissue and the CNS [12, 26]; therefore, viral escape is less likely to occur.

To assess potential changes in the HIV-1 latent reservoir while on dolutegravir monotherapy, we measured total HIV-1 DNA levels in PBMCs from patients at baseline and at week 48 and found that there was a comparable slight decay in the total HIV DNA load in both groups. Our finding that the total HIV DNA load in the monotherapy group did not increase over time suggests that the HIV-1 latent reservoir was not replenished on dolutegravir monotherapy. Supporting this, the frequency of viral blips was very low in both treatment groups but even lower in the dolutegravir monotherapy group despite a much higher sampling frequency.

To investigate whether patients on dolutegravir monotherapy are at increased risk for CNS escape, that is, ongoing viral replication in the CNS in the presence of suppressed viremia in the plasma, we performed longitudinal lumbar punctures and

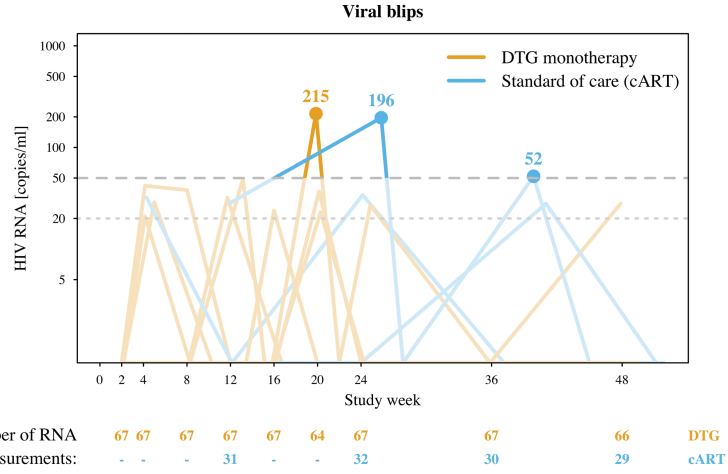

Figure 4. Viral blips stratified by dolutegravir (DTG) monotherapy vs standard-of-care combination antiretroviral therapy (cART). One patient in the DTG monotherapy group experienced a single blip at week 20, and 1 patient in the standard-of-care cART group had 2 blips. The light colors show the human immunodeficiency virus type 1 (HIV-1) RNA levels above the limit of detection (20 copies/mL; dotted light gray line), which were not classified as viral blips (>50 copies/mL; depicted by dashed gray line). Among the 13 patients with at least 1 HIV-1 RNA measurement above 20 copies/mL, four patients were from the cART group (3 participants with a single level >20 copies/mL and 1 with 3 HIV-1 RNA levels >20 copies/mL). In the DTG monotherapy group, one patient had 3 HIV-1 RNA levels >20 copies/mL, one patient had 2 HIV-1 RNA levels >20 copies/mL, and seven patients had a single HIV-1 RNA level above 20 copies/mL. The failing patient was excluded from this analysis.

measured the HIV-1 RNA in the CSF in a subset of patients at baseline and at week 48. In all CSF samples, HIV-1 RNA was not detected above the limit of quantification of 40 HIV-1 RNA copies/mL CSF at baseline (n = 37) and at week 48 (n = 12). We also measured dolutegravir drug levels in the CSF as a proxy for CNS penetration. In line with previous work [20], we found that in all patients on dolutegravir monotherapy or on dolutegravir-containing cART, the concentrations in CSF exceeded the in vitro IC 50 for wild-type HIV-1. This finding suggests that in our specific patient population, dolutegravir monotherapy is able to achieve therapeutic concentrations in the CNS and to prevent CNS escape at this sanctuary site.

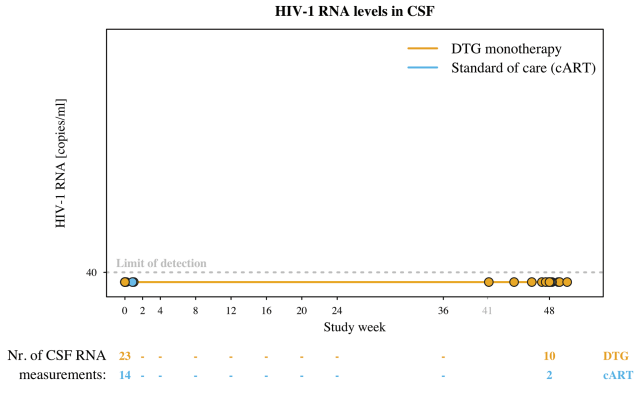

Figure 5. Human immunodeficiency virus type 1 (HIV-1) RNA measurements in the cerebrospinal fluid (CSF). Patients were asked to have a lumbar puncture performed at baseline and at week 48. The patient with virological failure at week 36 was the only patient who received an additional lumbar puncture at week 41. Orange and blue circles depict the single HIV-1 RNA measurements in CSF for the dolutegravir (DTG) monotherapy and standard-of-care combination antiretroviral therapy (cART) groups, respectively. The dotted gray line represents the detection limit.

The main goal of ART simplification is to reduce long-term toxicity associated with ART and to reduce costs. Indeed, almost all currently licensed antiretroviral drugs showed significant toxicity in post-marketing surveillance studies [27]. Thus, simplifying a patient to dolutegravir monotherapy could be a reasonable strategy to reduce long-term toxicity and daily pill counts in some patients. Although we did not find significant changes from baseline in lipid levels and proximal tubulopathy markers, it is possible that the follow-up time of 48 weeks was too short to detect clinically meaningful changes.

The strength of our study is that we tested a new and clear hypothesis for the first time: Do viral and immunological properties that result from early cART initiation translate into successful viral suppression after switch to dolutegravir monotherapy? We selected our patients based on a distinct clinical phenotype, and we combined our treatment intervention with several laboratory measurements to investigate the potential positive and negative impact of a dolutegravir monotherapy on toxicity markers, the HIV-1 reservoir, and potential CNS compartmentalization. A limitation of our study is that we were not able to recruit the targeted number (n = 138) of patients that was calculated for the targeted 80% power. Therefore, the study results rely on a small number of patients. The major reason for the low recruitment was the high frequency of patient visits we requested for safety reasons. Close HIV-1 RNA monitoring was performed in the dolutegravir monotherapy arm during the first 6 months to detect a potential virological failure as early as possible. Even though we enrolled fewer patients than

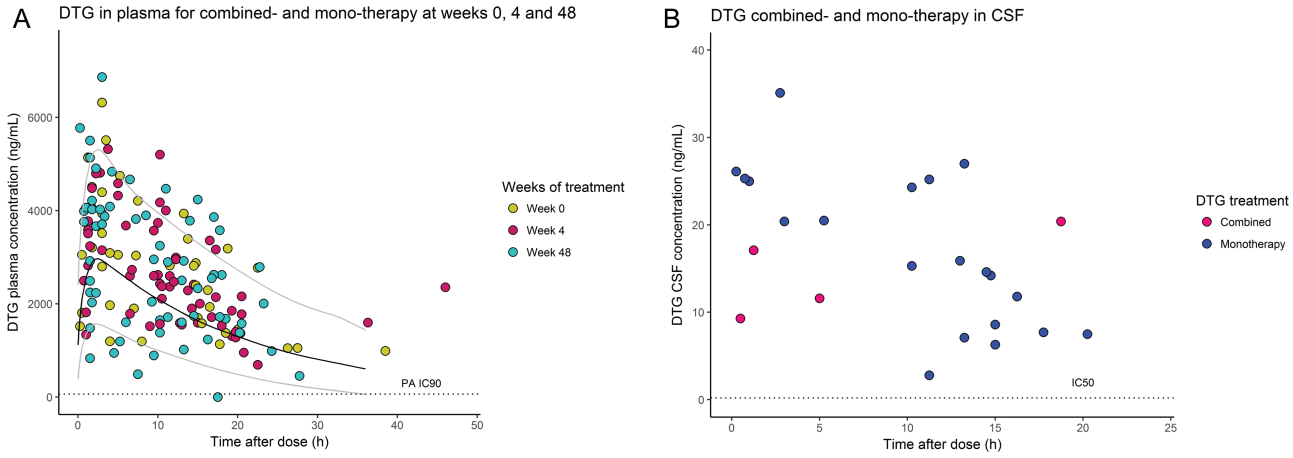

Figure 6. Dolutegravir (DTG) drug level measurements in plasma (*A*) and in CSF (*B*). *A*, Plasma DTG concentrations (yellow circles, week 0; red circles, week 4; green circles, week 48), with the median population predicted concentration (dark gray line) and the 10% and 90% prediction interval (light gray lines). The dashed black line represents the protein-adjusted 90% inhibitory concentration at 64 ng/mL. *B*, Cerebrospinal fluid DTG concentrations (blue circles, DTG monotherapy; purple circles, DTG combined with other antiretrovirals). The in vitro 50% inhibitory concentration of 0.2 ng/mL is represented by the dashed black line. Abbreviations: CSF, cerebrospinal fluid; PA IC90, protein-adjusted 90% inhibitory concentration.

Table 2. Adverse Events Among Study Participants

AE Among Study Participants	Overall	Monotherapy	Combination Antiretroviral Therapy	*P* Value
Number of patients[a]	87 (86.1)	61 (89.7)	26 (78.8)	.217
Study drug related	15 (14.9)	9 (13.2)	6 (18.2)	.557
Serious adverse event	8 (7.9)	6 (8.8)	2 (6.1)	1.000
Antiretroviral therapy switch due to AE	3 (3.0)	0 (0.0)	3 (9.1)	.033
Intensity				
Mild	86 (85.1)	60 (88.2)	26 (78.8)	.240
Moderate	18 (17.8)	10 (14.7)	8 (24.2)	.274
Severe	4 (4.0)	3 (4.4)	1 (3.0)	1.000
Laboratory AE	14 (13.9)	10 (14.7)	4 (12.1)	1.000
Laboratory AE, intensity				
Mild	13 (12.9)	10 (14.7)	3 (9.1)	.538
Moderate	1 (1.0)	0 (0.0)	1 (3.0)	.327
Sexually transmitted infection	30 (29.7)	23 (33.8)	7 (21.2)	.248
Common cold	21 (20.8)	16 (23.5)	5 (15.2)	.436
Other infection	14 (13.9)	13 (19.1)	1 (3.0)	.032
Headache	7 (6.9)	3 (4.4)	4 (12.1)	.212
Liver enzyme elevation	7 (6.9)	6 (8.8)	1 (3.0)	.422
Skin rash	7 (6.9)	4 (5.9)	3 (9.1)	.680
Arthralgia	6 (5.9)	4 (5.9)	2 (6.1)	1.000
Headache after lumbar puncture	7 (6.9)	4 (5.9)	3 (9.1)	.680
Creatinine elevation	5 (5.0)	2 (2.9)	3 (9.1)	.327
Sleeping disorder	5 (5.0)	4 (5.9)	1 (3.0)	1.000
Diarrhea	5 (5.0)	3 (4.4)	2 (6.1)	.661
Fatigue	4 (4.0)	2 (2.9)	2 (6.1)	.595
Back pain	4 (4.0)	4 (5.9)	0 (0.0)	.300
Commotio cerebri	4 (4.0)	3 (4.4)	1 (3.0)	1.000
Depression	3 (3.0)	2 (2.9)	1 (3.0)	1.000
Microhematuria	3 (3.0)	3 (4.4)	0 (0.0)	.549
Viremia	2 (2.0)	0 (0.0)	2 (6.1)	.105

Data are n (%).

Abbreviation: AE, adverse event.

[a]Number represents patients with at least 1 AE.

originally anticipated, noninferiority could be demonstrated. Since the power in noninferiority studies guarantees that the probability of showing noninferiority when the new treatment is indeed noninferior to the control is sufficiently high, the post study power is meaningless in case of significant results. Another possible limitation is the short follow-up period of 48 weeks. To address this limitation, we prolonged the study duration to 4 years at the time when the first studies reported a large number of patients failing on dolutegravir monotherapy. Finally, one potential limitation is that the frequent HIV-1 RNA measurements during the first 24 weeks might have increased adherence in the monotherapy group. However, these patients only came for short additional blood draw visits with the study nurse without counseling, and adherence, as measured by pill counts upon visits, did not differ between groups.

Our pilot study has potential implications for the management of HIV-1–infected patients. Our results suggest that success of simplification strategies using dolutegravir monotherapy is likely governed by early start of treatment with subsequent low latent HIV-1 reservoir size, low level of viral diversity, and low immune activation. It suggests that trials to evaluate simplification strategies should stratify between patients first treated during primary and chronic infection. This is of particular relevance because today all HIV-1–infected patients should be treated immediately regardless of their CD4 count [28]; thus, the fraction of patients treated early after infection most likely will increase. As our data show, these patients may be potentially overtreated for decades if patient population stratification is not performed in future simplification trials. Recent studies show that simplification with boosted protease inhibitors or dolutegravir each combined with lamivudine seem to work [29, 30], supporting the possibility that future ART could be personalized much more than it is today. One can even imagine that in the future, HIV-1 maintenance therapies will be started by measuring the size of the latent reservoir, for example, by proviral HIV-1 DNA or other future assays, and might be predictive of who can receive monotherapy. Therefore, more prospective controlled future simplification studies are needed that use stratification strategies according to the time of HIV infection and start of first cART guided by measurements of the latent reservoir. In addition, longer follow-up is important as for all prospective clinical cART trials because failure was observed after week 48 in several studies [6, 9].

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. The study was designed by D. L. B. and H. F. G. Data acquisition was done by D. L. B., F. T., C. G., B. H., C. D., P. W. S., M. G., C. B., D. S., S. B., K. N., C. P., H. K., M. F., B. B., K. J. M., L. D., J. B., and H. F.G. Statistical analysis was performed by T. T. and R. D. K. H. F. G. supervised the study. D. L. B. wrote the first draft. All investigators contributed to data collection and interpretation of the data, reviewed drafts of the manuscript, and approved the final manuscript.

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Data sharing agreement. The study protocol and individual participant data that underlie the results reported in this article will be available after deidentification following article publication to investigators whose proposed use of the data has been approved by an independent review committee to achieve aims in the approved proposal. Proposals should be directed to huldrych.guenthard@usz.ch to gain access. Data requestors will need to sign a data access agreement.

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CHAPTER VII: DISCUSSION AND PERSPECTIVES

The widespread use of ARV has dramatically changed the prognostic and care of PLWH. PLWH live longer, develop therefore age-related comorbidities, and consequently receive significant polymedication in addition to their ARV regimens. Pharmacokinetic data of ARVs and comedications in aging PLWH were limited leading to uncertainty on the actual dose adjustment, if any, necessitated when aging. The objective of this work was therefore not only focused solely on DDIs, but also aimed at providing new insights about the importance of such DDIs in an aging PLWH population.

Our first study showed that cardiovascular drugs were highly prescribed in PLWH despite their high risk for DDIs. 73% of elderly PLWH received at least one cardiovascular drug and 11% of these drugs were involved in potential DDIs with ARV drugs. The assessment of the magnitude of DDIs between cardiovascular drugs and ARVs requires the development and validation of accurate LC-MS/MS bioassays. One of the developed bioanalytical method aimed at quantifying plasma levels of commonly prescribed cardiovascular comedications in PLWH. This bioassay was developed mostly for research purposes and was successfully applied to the two observational studies conducted during this work, and was also found useful for clarifying a case report a deleterious DDIs occurring outside the present clinical study. For the first time overall, the magnitude of DDIs between cardiovascular drugs and ARV drugs has been evaluated in a real clinical setting, outside the stringent context of clinical studies.

Multiple approaches have been used to quantify the magnitude of DDIs between ARV and comedications, and to evaluate the impact of aging on drug PK. Apart from the presented popPK models and NCA, real-life clinical data collected during our observational studies have been integrated into PBPK models developed by Prof Catia Marzolini at the University of Basel for the definitive validation of their *in silico* approaches. Results from these comparative studies -not part of the present thesis- will be published shortly.

First, the impact of aging on the magnitude of DDIs was evaluated using these three (*i.e.* popPK, NCA and PBPK) approaches. We have formally demonstrated that overall, age has a limited impact on the importance of DDI. Indeed, age was not retained as a significant covariate in any of the developed popPK models for amlodipine, atorvastatin and rosuvastatin, suggesting that age does not impact drug PK, and thereby does not influence the magnitude of DDIs. NCA conducted in elderly PLWH demonstrated that DDI magnitudes between amlodipine, atorvastatin, rosuvastatin and ARV drugs were comparable to those reported in young individuals. In the same way, clinical data combined with PBPK modelling for amlodipine, atorvastatin, rosuvastatin and other comedications demonstrated that aging does not influence DDI magnitude regardless of the DDI mechanism, the DDI mediators (enzymes, transporters) or the involved drugs. Combined together, these results support that the clinical management of DDIs can be similar in aging and younger PLWH.

Secondly, different PK approaches allowed the evaluation of the impact of aging on ARVs PK. For example, older age was unexpectedly associated with higher dolutegravir elimination in the popPK analysis. In addition, dolutegravir exposure was shown to be 5% and 35% higher in elderly PLWH compared to the younger group, using NCA and PBPK modelling, respectively. Taken together, these studies elucidated a limited effect of age on dolutegravir pharmacokinetics suggesting no dose adjustment in elderly PLWH. More generally, the PBPK model predicted a 1.75-fold decrease in ARV clearance across adulthood, while C_{max}, T_{max} and V_d remained unaltered by aging. These results demonstrated a marginal impact of aging on ARV PK and thus, dose adjustment based on age is a priori not necessary in the absence of severe comorbidities.

In addition to the evaluation of DDIs, the popPK models developed for amlodipine, atorvastatin and rosuvastatin allowed to identify factors influencing he concentration-time profiles of the drugs. This might help clinicians to identify patients at risk of either being overexposed or presenting suboptimal exposure of the evaluated comedication, and who may benefit from individualised clinical interventions.

Considering the high inter-individual variability for each evaluated drug, the potential for DDIs and the associated clinical consequences, the popPK models could be implemented in a Bayesian tool for dosage adjustment developed by the Service of clinical Pharmacology at CHUV and the School of Management and Engineering Vaud, in collaboration with EPFL. This software, Tucuxi®, is a user-friendly solution for the optimization of drug treatments following concentration monitoring. Based on one or several plasma concentration measurements observed in a patient, the software applies Bayesian calculations to find the most likely concentration-time profile for this patient. Afterwards, the software suggests dosage adjustment to ensure optimal drug exposure. Nevertheless, the strength of such an approach relies in the availability of therapeutic targets, which are unfortunately either lacking or remain so far undefined for the evaluated cardiovascular drugs. Dosage adjustment should be firstly based on the monitoring of clinical response such as blood pressure (for amlodipine) or lipids profile (for statins). Tolerability should also be considered to avoid adverse events such as muscular or hepatic side effects associated with statin treatments.

In conclusion, this work aimed at expanding our knowledge on treatment optimization in PLWH. The analytical bioassays and popPK models presented in this thesis may guide the individualization of both ARV treatments and comedications, in the purpose of maximizing efficacy and safety. The developed pharmacokinetic models support findings from previously published DDI studies when available, and

strengthen the dosage recommendations based either on theoretical considerations or on DDI studies with other interacting drugs.

Although efficacy and tolerability of ARV drugs improved significantly, long-term adherence remains the most important determinant for sustained viral suppression. Medication adherence to ARVs is affected by multiple factors such as treatment-related adverse events or complexity and social stigma associated with the daily ARV drug intake. A promising approach to overcome the adherence issue is the development of long-acting injectable formulations allowing once-monthly or every two months intramuscular administrations. Although this approach has been successfully applied to antipsychotics and hormonal contraceptives for more than 30 years, the PK of such long acting ARV drugs and the associated inter-individual variability in real life patients outside the strict frame of clinical studies remains to be explored. Such PK studies to come will therefore benefit from the availability of the third LC-MS/MS methodology described in the present manuscript, to secure the prescription of long-acting injectable ARVs and newer oral ARV drugs, with regard not only to efficacy but also tolerability and long-term safety.

CHAPTER VIII: APPENDICES

VIII.1. Proposal of our project to the SHCS scientific board

Model-Based Simulation and Clinical Validation of Drug-Drug Interactions in the Swiss HIV Cohort Study

Authors: Laurent Decosterd, Perrine Courlet, Chantal Csajka, Mathias Cavassini, Catia Marzolini and Thierry Buclin

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1. Administrative Information

Principal investigator: Laurent Decosterd

Responsible investigator: Catia Marzolini

Coordinating centre: Lausanne

Study sites: Lausanne, Basel

Beginning of the study: 01.01.2017

Study duration: 3 years

Budget requested from the SHCS: 0 CHF

Date of first submission: 01.11.2016

If the current version is a revision of a previously submitted proposal:

Date of resubmission:

Give a point-by-point reply to the comments received from the Scientific Board and highlight the resulting relevant changes in this proposal with yellow.

Point-by-point reply to the comments from the Scientific Board:

-

_LD___**I confirm**, that I have read the Guidelines for Project Submission and that the project fulfils all criteria (http://www.shcs.ch/40-project-submission-guidelines)
(Any issue that might interfere with the guidelines should be discussed in a cover letter)

_LD____I confirm that all co-authors have seen the current version of the project and agreed with the submission of the current version of the protocol

2. Summary

Background:

Thanks to highly active antiretroviral therapies (ARTs), HIV-infected patients live longer and older, with common age-related health issues requiring numerous co-medications. HIV-HCV co-infected patients (30% of SHCS) are also likely to receive new anti-HCV drugs. At present, drug-drug interactions (DDIs) between antiretroviral drugs (ARVs) and co-medications are mostly predicted based on current knowledge of metabolic pathways.

Study Aims:

- quantification of magnitude of DDIs between ARVs, anti-HCV drugs and common co-medications.
- analysis using population pharmacokinetics (POP PK)
- comparison of DDIs simulations using POP PK versus physiologically based pharmacokinetic (PBPK) modeling.

Study Design:

Prospective observational study implying systematic PK records at each SHCS visit and determination of plasma levels of ARVs, anti-HCV drugs, AND relevant co-medications measured by multiplex mass spectrometry.

POP PK models of co-medications will allow: i) comparison with available PK data from reference individuals, ii) dissecting the influence of covariates (age, etc…).

3. Background

Highly active antiretroviral therapies (ARTs) have definitively transformed HIV infection from a deadly disease into a manageable chronic condition. Consequently, HIV-infected patients live older, with common age-related health issues, and are likely to receive numerous additional co-medications. The high potential of reciprocal drug-drug interactions (DDIs) between antiretroviral drugs (ARVs) and the various co-medications, not to mention the influence of chronic exposure of these drugs associations on their long-term tolerance profiles remains incompletely characterized (1).

The incorporation of ARVs TDM data into the SHCS database provides pharmacokinetic and clinical variables together with efficacy endpoints, which offers a unique resource for research in the constant evolving field of HIV therapy.

DDIs with ARVs: because of their pharmacokinetic properties, these therapeutic agents have a high potential for DDIs. Protease Inhibitors (PIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Integrase Inhibitors (INIs) are metabolized by cytochromes P450 (CYPs), and/or uridine 5'-diphospho-glucuronosyltransferase (UGTs) and some of these agents can inhibit and/or induce different CYP and UGTs isoforms (2).

DDIs with ARVs can also occur via other mechanisms including drug transporters (3) (4), complexation with divalent cations (5) and pH-dependent drug absorption (6). The prevalence of DDIs with HIV therapy has been assessed in several studies (7-13), with potential interactions found in about one third of patients receiving antiretroviral therapy. The issue of DDIs is likely to worsen with the aging HIV population encountering age-related morbidities (14, 15) susceptible to increase the burden of poly-medication.

DDIs lead to substantial risks of either toxicity or decreased efficacy and subsequent emergence of drug resistance, with potential HIV transmission to seronegative partners (16). All the above elements constitute therefore strong arguments to prevent, identify and manage DDIs.

Although two publicly available websites (www.hiv-druginteractions.org; www.hep-druginteractions.org) are valuable resources for addressing the problems of DDIs, notably in the setting of HIV-HCV infections, 90% of the reported DDIs are only based on the knowledge of metabolic pathways of drugs. There is currently a major lack of real-life clinical data on the magnitude and management of DDIs, especially in the context of poly-morbid, poly-medicated patients poorly accounted for in treatment guidelines largely elaborated for *single* diseases (17).

DDIs with anti-HCV drugs: similarly to ARVs, new anti-HCV drugs are revolutionizing the treatment of chronic hepatitis C. In a context of HCV-HIV co-infection treatment (about 30% of all HIV patients in Switzerland are co-infected with HCV), information on DDIs with the new anti-HCV drugs is limited. Available information derives from the knowledge of their metabolism pathways. To the best of our knowledge, while studies on the DDIs potential of paritaprevir, ombitasvir, dasabuvir, daclatasvir, simeprevir, ledipasvir and sofosbuvir have been reported (18-20), there is at present limited published information on DDIs between ARVs and the latest generation anti-HCV agents ledipasvir, grazoprevir/elbasvir and velpatasvir, not to mention sofosbuvir.

Therapeutic Drug Monitoring (TDM) of antiretroviral drugs is a powerful tool to identify and manage DDIs. Despite limited clinical validation, TDM of ARVs is now commonly considered in case of virological failure, adverse drug reactions, in special clinical conditions (pregnancy, paediatrics, liver failure...), and when DDIs are suspected (21). For the latest anti-HIV drugs (i.e. raltegravir,

elvitegravir/cobicistat, rilpivirine, dolutegravir, etc), the availability of concentration exposure monitoring is certainly an important component of patient's follow-up.

For the new anti-HCV drugs, as the duration of treatment for hepatitis C is shortening, the role of TDM seems limited. Yet, TDM is still likely to provide useful information in specific cases such as suspected DDIs, problematic patho-physiological conditions (i.e. altered renal function and sofosbuvir treatment (22)). To that endeavour, a multiplex mass spectrometry method is currently being developed at the Lausanne Center and will be soon available for clinicians for monitoring plasma levels of sofosbuvir (+ its metabolite GS331007), daclatasvir, paritaprevir, ombitasvir, dasabuvir, ledipasvir, grazoprevir, elbasvir and velpatasvir.

Population kinetics/dynamics of antiviral drugs and interactions: population-based approaches seem to be a best way to characterize the pharmacokinetic profile of a drug of interest and to capture the contribution of multiple genetic and non-genetic factors affecting drug levels. Population pharmacokinetic analyses of the earlier NNRTIs (efavirenz (23-25), nevirapine (26), etravirine (27) and the more recent rilpivirine ((28)); the former PIs lopinavir (29-31), atazanavir (32)), indinavir (33) and the latest darunavir (34); for the integrase inhibitors (INIs) raltegravir (35) elvitegravir /cobicistat (36), and dolutegravir ((37)) and for maraviroc (38), tenofovir (39) and enfuvirtide,. have been able to quantify variability and to assess relevant sources of variations in drug levels, including drug-drug interactions.

4. Own Research in the Field

The Lausanne Center has a long-standing expertise in the clinical pharmacology and pharmacokinetics of antiviral drugs, testified by a number of publications in the field. Over the last four years, the SNF has supported our research program on the "clinical pharmacology and pharmacogenetics of HIV and HIV/HCV treatments in the complex care co-infected individuals" (SNF N° 324730-141234). SNF has renewed its support in 2016 for the present research project focused on DDIs, in conjunction with the SHCS (SNF N° 324730-165956).

A tandem mass spectrometry platform, an integrated pharmacology and genetics database for the SHCS, a center of excellence in population pharmacokinetic modeling and a contribution to a pharmacogenetics platform are available for further coordinated research projects.

Laurent Decosterd, the head of the Laboratory of Clinical Pharmacology, is known to the SHCS for being involved in numerous research projects requiring bioanalytical developments by mass spectrometry.

Perrine Courlet, Pharmacist, PhD student, will perform the analytical development, and will be in charge, with technical guidance and under medical supervision, to organize and regulate logistic aspects for data collection, mass spectrometry analysis and population PK analysis.

Chantal Csajka is an expert in population pharmacokinetics notably focused on antiviral therapy. She has participated to a large number of translational studies within the SHCS cohort.

Matthias Cavassini is a member of SHCS Scientific Board, he is in charge of the HIV outpatient clinics in Lausanne, has a particular interest in HIV care, resistance and adherence and has carried out several studies within the SHCS. PK data and samples collection at the Lausanne Center for this study will be carried out under his supervision. He is the PI for the NAMACO project, related (albeit distinct) from the present project.

Catia Marzolini, is a member of SHCS Scientific Board, based at the Basel Center. Over the last decades she has gained international recognition for various landmark works on the clinical pharmacology of ARVs. She is currently also in charge of maintaining and continuously improving the publicly available www.hiv-druginteractions.org. She will supervise the PK data and samples collection at the Basel Center. She is the PI for the SNF-supported *PBPK* analysis, linked to the project.

Thierry Buclin, Head of the Division of Pharmacology at CHUV has co-authored multiple publications with the SHCS. He provides his expertise in statistical analysis notably via the development of mathematical tools for the aggregation of population pharmacokinetic data from relevant co-medications using meta-analyses techniques and the program EzeCHiel/Tucuxi developed thanks to the SNF-supported NanoTera ISyPeM2 project.

5. Study Hypotheses

Antiretroviral treatments have a high potential for DDIs with co-medications, not only for an increasingly aging population receiving numerous drugs for common age-related health issues, but also in the context of HIV-HCV co-infection treatment. Some of these DDIs may be of clinical significance.

The framework provided by SHCS represents a unique setting to investigate at a population level those DDIs in the real-life conditions.

6. Study Aims and Objectives

- Analytical development by multiplex mass spectrometry of commonly used co-medications
- Analytical development for latest generation anti-HCV drugs
- Population pharmacokinetics analysis of ARV and relevant common co-medications (and anti-HCV drugs) using sparse sampling approach: i) comparison with available PK data from reference individuals, ii) dissecting the influence of other covariates (age, etc…).

- Model-based simulation of DDIs and comparison with simulations obtained using the PBPK modeling approach

7. Study Design and Plan

Monitoring drug levels of ARVs and co-medications

For budget reasons, the present study on DDIs will be first limited to two centers: Basel and Lausanne. After one year, the progress of the project will be re-evaluated before possible deployment to other SHCS centers. The PK data (time/date of last drug intake and blood collection, for ARVs and co-medications) at each SHCS visit will be entered in the TDM database (Lausanne) and then be exported into the SHCS database so that these data will be available to everyone for other SHCS projects.

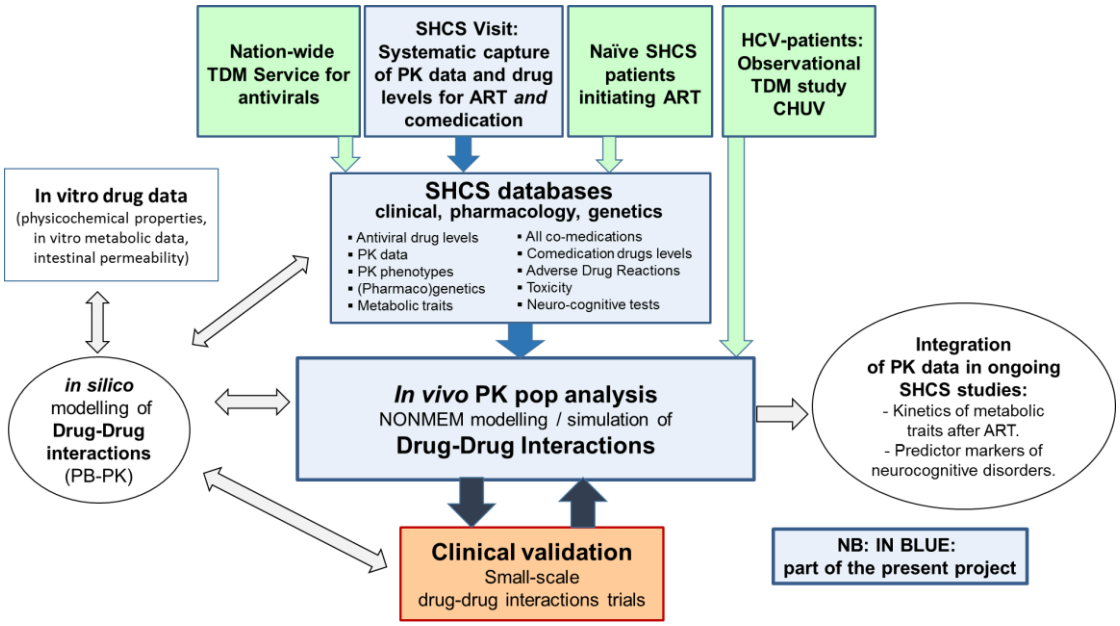

Figure 1: Working frame for population-PK analysis and model-based simulation of drug-drug interactions. In blue, the frame of the present proposal.

SHCS Pharmacology database

The current procedure for feeding the SHCS pharmacology database is the following: Information regarding drugs pharmacokinetics (drug, dosage, time of drug administration and concentrations, time/date of blood collection) as well as comedications (ARVs and other drugs) are provided by the routine TDM-ARV Access database of the Division of clinical pharmacology (PCL) at Lausanne. All other information (demographics, physio-pathological, environmental and others) is emanating directly from the SHCS database. PK data extraction is undertaken regularly (≈ every 1 to 4 months) from PCL database to the SHCS data center. Securitized data exportation is been implemented at CHUV-Lausanne to take into account the new location of the SHCS database at Zurich.

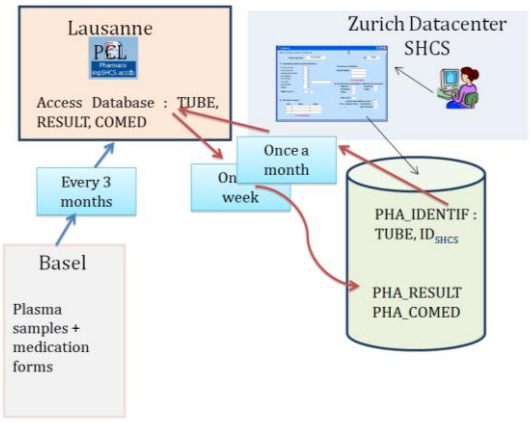

Figure 2: Overview of the PK data form routine TDM merging procedure between the Division of clinical pharmacology at Lausanne and the SHCS database at Zurich

Systematic capture of PK data at SHCS visits, for ARVs AND co-medications

Pharmacokinetics analyses are critically dependent on the access to data on drug dosage, time of last drug intake and time of blood sampling.

A pilot study carried-out at the Basel Center has examined the optimal procedure to capture these critical information: Two alternative options have been tested: i) either the patients received at home a letter to remind their SHCS appointment for the blood intake, **together with the medication form (see figure 4 below) to fill in at home and bring to the nurse**, or conversely, ii) the patients have filled the form on the spot at their arrival at the SHCS visit. The first procedure was clearly found to be the best approach not only in terms of effectiveness but also with regard to patients' and healthcare providers' convenience, routine logistics and general workload. The PK data from ARVs and co-medications will be recorded by the laboratory staff into the TDM database (Lausanne) before their exportation in the SHCS database at Zurich so that these data will be available for all SHCS scientists.

Practically, the patients coming for a SHCS visit are identified and they receive the medication form one week before the SHCS visit. This administrative procedure will be performed either by the study nurse or the administration personnel based on the center organisation (Figures 3 and 4).

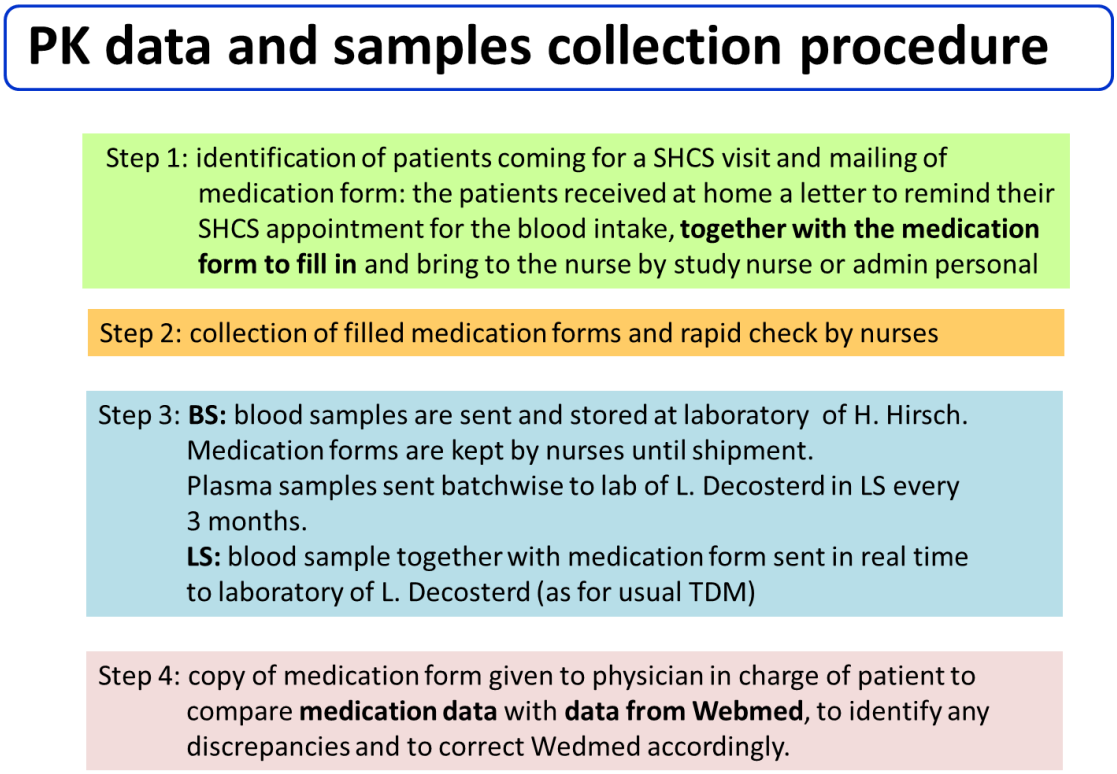

Figure 3: The four subsequent steps for PK data and plasma samples collection procedure

Patientenfragebogen: Medikamentenliste

Graue Felder bitte nicht ausfüllen

Kohorten-ID:	Datum der Blutprobe:	Uhrzeit der Blutprobe:

Information für Patienten: Im Rahmen Ihrer Behandlung nehmen wir regelmässig Blutproben (BP). Diese Proben können dazu verwendet werden, um die Medikamentenspiegel im Zusammenhang mit Medikamenteninteraktionen, Medikamententoxizitäten oder anderen pharmakologischen Belangen zu messen. Um die Medikamentenspiegel korrekt interpretieren zu können, sind wir auf Ihre umfassenden Informationen bezüglich aller Ihrer zurzeit eingenommenen Medikamente angewiesen. Bitte füllen Sie aufgrund dessen diese Liste mit den Namen und Dosierungen aller von Ihnen zurzeit eingenommener Medikamente aus (auch Medikament, die nicht in Zusammenhang mit Ihrer HIV-Infektion stehen). Wichtig: Bitte geben Sie das Datum und die Zeit der letzten Medikamenteneinnahme vor dem Blutprobentermin an. Vielen Dank für Ihre Unterstützung.

Medikamentenliste

Medikament	Dosis	Anzahl pro Tag	Datum und Zeit der letzten Einnahme vor Blutprobe	
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
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	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:
	mg	x/Tag	Datum:	Zeit:

Bitte hier das Etikett der Blutproben aufkleben

Figure 4: Example of medication form (available in German and French) received by the patient before the SHCS visit. Medications are filled in by the patient at home, and the top selection is filled in by the nurse at the time of blood intake.

Of note: this form as well as the data collection procedure have been presented at the last CLC meeting (31.8.2016) and have been formally approved.

The medication forms are collected, and briefly checked by nurses. **In Basel**, these forms are kept by nurses until shipment. The blood sample taken within the SHCS visit is sent for centrifugation to H. Hirsch's laboratory: the collected plasma sample is then stored at -80° C. An aliquot (0.5 ml) of plasma is sent batch-wise to the Lausanne laboratory every 3 months. **In Lausanne**, the PhD student in charge, or the laboratory staff, will come to the Infectious Diseases Outpatient Clinic to collect the blood sample and medication forms, similarly to what is organised for the standard routine TDM service.

Finally, a copy of medication forms is given to the physician in charge of the patient follow-up to compare medication data recorded in Webmed, to identify any discrepancies and to correct Webmed accordingly. Divergence on the list of treatment drugs may occur when patients consult other specialists that may prescribe drugs without notifying the infectious diseases specialists. The checking/update of the co-medication list in the Webmed database is a valuable consequence of this research project on daily patients' follow-up.

Selection of co-medications under study

Since a considerable number of co-medications are given to SHCS patients, it is necessary to select the drugs to study in priority. The choice is based on:

- the frequency of use in the patients of the SHCS.
- the theoretical risk of potential DDIs the absence of real-life data for novel, or possibly less frequently used drugs which are characterized by a narrow therapeutic index (new anti-HCV agents, cytostatic agents, novel targeted anticancer drugs, immuno-suppressants, etc...)

Table 1 is a non-exclusive list of co-medications likely to be analysed for the above reasons.

Initially, we consider to concentrate on a first selection of 15 to 20 drugs, that will be analysed by multiplex liquid chromatography coupled to tandem mass spectrometry.

For the sake of internal control validation, the study of a positive control of DDIs is considered in the project. Candidate drugs association could be those well known to interact *via* CYP3A4 metabolism (for example, some statins /ritonavir, etc.)

Table 1: Selected examples of therapeutic classes and comedications likely to be analyzed (not exclusive list)

Therapeutic Class	**Drugs**
statins	atorvastatin, pravastatin, rosuvastatin
beta-blockers	metoprolol, propranolol
calcium channel inhibitors	amlodipine, verapamil
antiplatelets/anticoagulants	clopidogrel, phenprocoumon
anxiolytics	diazepam
antidepressants	escitalopram, mirtazapine, venlafaxine
targeted therapy of cancer	imatinib, nilotinib, dasatinib, erlotinib, etc
cytostatic drugs	cyclophosphamide, dacarbazine, docetaxel, irinotecan, paclitaxel, vincristine, etc

7.1. Patients

All patients from Lausanne and Basel coming for a SHCS visit will be enrolled in this study.

In 2014, a total of **9198 SHCS patients** have had *at least* one cohort visit.

As previously stated, the implementation of PK data record and samples collection is planned to be deployed initially in only two SHCS centers:

- Basel: ≈ 1000 patients, 2000 cohort visits/year
- Lausanne: ≈ 1000 patients, 2000 cohort visit/year

It is estimated that a total of ≈ 3000 filled medication forms will be obtained (i.e. 1500 per Center).

7.2. Intervention

No intervention is planned.

7.3. Study Evaluations

The implementation of PK data record and samples collection is planned to be deployed initially only to Basel and Lausanne. After one year, the progress of the project will be evaluated to decide whether it should be extended to other SHCS centers.

7.4. Data Analysis

Population pharmacokinetics modelling and simulation: for the antiviral drugs, we will take advantage of TDM data for HIV and HCV drugs currently in the pharmacology SHCS database:

- to perform population pharmacokinetic analyses of new anti-HIV drugs and last generation anti-HCV drugs
- to characterize the influence of different covariates (age, drug of the co-medication etc..)
- to apply the developed models to simulation studies of drug-drug interactions or in special situations identified .

For the other therapeutic classes, the sparse sampling approach implies that drug levels in samples taken at unselected times after drug intake must be interpreted with respect to dosing interval and dosage to yield PK data that can be exploited. This can be done through Bayesian maximum *a posteriori* approaches, using NONMEM® (Non-linear Mixed Effect Modeling). Comparisons between SHCS and control patients can be made with the help of the program EzeCHiel/Tucuxi that allows the aggregation of population pharmacokinetic data from relevant co-medications using meta-analyses techniques. This program is being developed at CHUV thanks to the SNF-supported NanoTera ISyPeM2 project.

Identification of DDIs from prospective TDM service and SHCS study: We will take advantage of data captured during our routine TDM Service for HIV and HCV drugs and also from the present SHCS study on DDIs. Starting from the "normality" or "expectedness" of a drug measurement result under a given dosage regimen with reference to population analyses, we can suspect the presence of a drug interaction (beside compliance problems or pharmacogenetics issues). The quantification of variability and the assessment of relevant sources of variations in drug levels, including drug-drug interactions, will be performed using NONMEM, as successfully applied in our previous studies for the analysis and simulation of the interactions of raltegravir-atazanavir (40), darunavir-ritonavir (34) and more recently, elvitegravir-cobicistat ((36))

Modeling and simulation of drug-drug interactions: In parallel to *in vivo* data analysed by NONMEM, *in silico* PBPK modeling approaches currently being developed by Catia Marzolini at the Basel Center will be applied to simulate a virtual clinical study scenario, notably in aging patients. For the clinical validation of such *in silico* predictions, pharmacokinetic data obtained from the systematic capture of TDM samples in SHCS patients will be of invaluable importance.

PBPK models will be developed for drug combinations frequently prescribed in the SHCS patients and for which clinical data on DDIs are lacking. In addition, such models will be developed for drug combinations less frequently used but difficult to manage due to narrow therapeutic index. For each of these drug combinations, clinical data obtained from the SHCS observational study will be used to validate the PBPK models. When applicable, results of POP PK analyses including interacting drug as covariates will also be confronted to PBPK predictions.

7.5. Time Frame

The link between TDM database and the SHCS database was critical for being able to start the collection of samples, and the record of PK data (drug dosage, time of last drug intake, time of blood collection, for both ARVs and co-medications). The first tests for the transfer of TDM data from Lausanne and Zurich Centers have been performed in October, and the process has been reported to be fully operational by the end of the year.The entire research program on DDIs is scheduled to start in January 2017, for a total duration of three years

7.6. Status of Ethical Approval

Separate ethics approval not required, covered by the general SHCS approval.

8. Study Budget

- No support is asked from SHCS for the initial one-year project.

Item	Center	Unit cost CHF	Number	Total CHF
• **Step 1** Identification of patients coming for a SHCS visit and mailing of medication forms by study nurse or admin personnel • **Step 2** Collection of filled medication forms and rapid check by nurses	**Basel**	15	1500	22500
	Lausanne	15	1500	22500
Step 3 Sending and storage of blood samples	**Basel**	5	1500	7500
Step 3 Sending and storage of blood samples (insured by PhD student and laboratory staff at Lausanne Center)	**Lausanne**		1500	0
TOTAL (covered by SNF grant to Decosterd)				**52500**

- The financial compensation for the initial one-year project limited to Basel and Lausanne Centers will be insured by SNF N° 324730-165956.

9. Other Information

9.1. Specification of samples and data to be used in this project

☒ SHCS main database
☐ Resistance database
☒ Genetic database
☐ Mochiv database
☒ Plasma drug concentration
☐ Cell samples
☒ Plasma samples
☒ Sampling pool

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VIII.2. Letter and medication form sent to patients for their participation in the study SHCS #815

Patient
Adresse

Lausanne, le date

Objet : Formulaire médicament/prise de sang

Madame, Monsieur,

Lors de votre prochaine prise de sang, nous allons mesurer les concentrations de médicaments dans votre sang.
Afin de bien interpréter le dosage des médicaments, nous avons besoin de connaitre la liste complète de **tous les médicaments** que vous prenez actuellement.
Nous vous prions donc de bien vouloir indiquer dans le formulaire ci-joint le **nom**, la **dose** ainsi que l'**heure** à laquelle vous avez pris la **dernière prise** de vos médicaments **avant votre rendez-vous pour la prise de sang** (remarque : si vous prenez plusieurs médicaments et à des moments différents, merci d'indiquer les heures de dernière prise pour chacun des médicaments).

Ce formulaire rempli par vos soins est à rapporter lors de votre prochain rendez-vous pour une prise de sang. Ce rendez-vous est prévu le date à heure.

Si vous avez des questions concernant **cette étude**, n'hésitez pas à contacter Perrine Courlet, pharmacienne responsable de cette étude, au 0795563270.

Cordialement,

Consultation ambulatoire de maladies infectieuses

BH 07-872
1011 Lausanne-CHUV
Tél. secrétariat +41 21 314 10 22
Fax +41 21 314 10 08

SHCS #815 : fenêtres grises à remplir par l'infirmière lors de la prise de sang, fenêtres jaunes à remplir par le laboratoire

Formulaire pour les patients: liste des médicaments actuels

- Compléter le formulaire avec la liste complète de vos médicaments
- Indiquer le nom, la dose, et l'heure de la dernière prise
- Ne pas prendre vos médicaments le matin du jour de la visite
- Formulaire rempli à remettre à l'infirmière lors de la visite

N° de Cohorte :	Date de la prise de sang:	Heure de la prise de sang :

Liste des médicaments

Nom du médicament	Dose journalière	Date et heure de la dernière prise de médicament
_____	mg x/j	Date: Heure:
_____	mg x/j	Date: Heure:
_____	mg x/j	Date: Heure:
_____	mg x/j	Date: Heure:
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_____	mg x/j	Date: Heure:
_____	mg x/j	Date: Heure:

Numéro du tube

815_

BIOTHEQUE PLASMA

1 monovette EDTA 2.7 ml

Nombre d'aliquot : 2

Quantité/aliquot : 300 µL et 700 µL

Date et heure de congélation : ……..

VIII.3. Protocol submitted to the ethics committee for the PK study with rich sampling

Drug-drug interactions between antiretroviral drugs and cardiovascular drugs in elderly patients from the Swiss HIV Cohort Study.

Research legislation:	Ordinance on human research with the exception of Clinical trials
Type of Research Project:	Research project involving human subjects
Risk Categorisation:	A
Project Leader:	Lausanne Center :

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PROTOCOL SIGNATURE FORM

The project leader (main center) and the investigator (at the local center/site) have approved the protocol version 2 ***(dated 23.03.2018)***, and confirm hereby to conduct the project according to the protocol, the Swiss legal requirements (1, 2), the current version of the World Medical Association Declaration of Helsinki (3) and the principles of Good Clinical Practice.

Project leader (Service of Clinical Pharmacology, Lausanne)

Site *[Service of Clinical Pharmacology, Bugnon 17, 1011 Lausanne]*

Name: Buclin Thierry

Date:
23.03.2018 Signature:

If applicable and not identical with project leader:

Sponsor 1: Laurent Arthur Decosterd (Swiss National Science Foundation, grant N° 324730-165956)

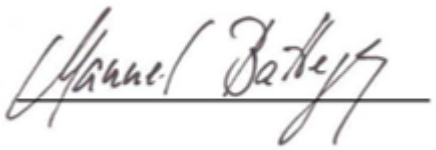
Name: Laurent Arthur Decosterd

Date:
23.03.2018 Signature:

Sponsor 2: Manuel Battegay/Catia Marzolini (Swiss National Science Foundation, grant N° 324730_166204)

Name: Manuel Battegay

Date: 23.03.2018 Signature:



Name: Catia Marzolini

Date: 23.03.2018 Signature:

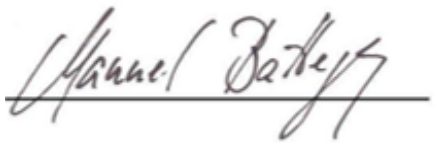
Local Project Leader at local center/site:

This page must individually be signed by all participating local Project Leaders.

University Hospital Basel, Petersgraben 4, 4031 Basel

Name of Local Project Leader: Battegay Manuel

Date: 23.03.2018 Signature:



1. GLOSSARY OF ABBREVATIONS

ARV	*Antiretroviral*
AUC	*Area Under the Curve*
BASEC	*Business Administration System for Ethical Committees*
CHUV	*Centre Hospitalier Universitaire Vaudois*
CRF	*Case Report Form*
CYP	*Cytochrome P450*
Darunavir/r	*Ritonavir-boosted darunavir*
DDI	*Drug-Drug Interaction*
FOPH	*Federal Office of Public Health*
HAART	*Highly active antiretroviral treatments*
HIV	*Human Immunodeficiency Virus*
HRA	*Human Research Act*
HRO	*Ordinance on Human*
NNRTI	*Non-nucleoside Reverse Transcriptase Inhibitor*
P-gp	*P-glycoprotein*
PI	*Protease Inhibitor*
USB	*Universitätsspital Basel*

2. BACKGROUND AND PROJECT RATIONALE

Highly active antiretroviral treatments (HAART) have transformed HIV infection from a deadly disease into a manageable chronic condition. As a consequence, HIV-infected individuals live longer and the proportion of older individuals within the HIV infected population is constantly growing (5). Thus, the management of HIV infection is becoming more complex as patients encounter more age related chronic but also severe morbidities such as hypertension, diabetes, tumors or acute diseases leading to polypharmacy and consequently to potential drug-drug interactions (DDI). In addition, aging is characterized by the decline in the function of organs which may impact the pharmacokinetics of drugs and thereby the magnitude of DDI.

Antiretrovirals are amongst the therapeutic agents with the highest potential for DDIs. Protease inhibitors (PIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are extensively metabolized by CYPs, and can inhibit and/or induce different CYP isoforms (4). DDIs with ARV agents can also occur at the level of drug transporters (5), glucuronidation enzymes (7), complexation with divalent cations (6) and pH-dependent drug absorption (8). The prevalence of DDIs with HIV therapy has been assessed in several studies performed in USA, the Netherlands, UK, Switzerland, and in resource-limited countries (9-15). These studies indicate that DDIs with HIV therapy are common and affect 19-41% of prescriptions, while clinically significant interactions are found in 1:3 patients receiving antiretroviral therapy. The issue of DDIs is likely to worsen with the aging HIV population where multiple treatments for co-morbidities may interact with HIV therapy (16). Importantly, DDIs can lead to substantial risks of either toxicity or decreased efficacy and subsequent emergence of drug resistance and potential HIV transmission to seronegative partners. For these reasons, the prevention, identification, and management of DDIs are crucial for patient care. However, one of the current issues is that there is a limited number of drug combinations evaluated in clinical studies. Therefore, the management of DDI is challenging in clinical practice, particularly in elderly individuals considering that available data on drug-drug interactions are mostly conducted in healthy volunteers and therefore might not reflect the situation of elderly individuals.

We aim to determine the magnitude of DDIs between antiretroviral drugs and commonly prescribed co-medications (e.g. amlodipine, rosuvastatin and atorvastatin) in HIV-infected patients of the SHCS. We will focus on patients over 60 years old, without excluding younger patients. For that purpose, we plan to conduct pharmacokinetic investigations initially in patients treated with a darunavir/ritonavir- or a dolutegravir-containing regimen and who receive one of the cardiovascular drug of interest. Eligible patients will undergo a pharmacokinetic investigation while treated with darunavir/ritonavir (strong inhibitor of drug metabolizing enzymes) and dolutegravir (no inhibitory effect on drug

metabolizing enzymes) in order to determine the magnitude of the interaction with the co-medication.

In addition, the same study framework will serve to examine other drug combinations which are susceptible to interact and whose exploration could be of interest from a clinical point a view to stimulate further confirmatory research, including associations of other cardiovascular agents with other antiretroviral drugs. These investigations are called “exploratory investigations” throughout the document.

Besides their common use in elderly HIV-infected patients, amlodipine, atorvastatin and rosuvastatin were selected due to their predisposition to be victim of drug-drug interactions.

Amlodipine is a calcium channel blocker predominantly metabolized by CYP3A4/5 (17, 18) therefore there is a potential for interaction with strong CYP3A4 inhibitors such as darunavir/ritonavir, or with inducers such as efavirenz. The product information label mentions an increase in amlodipine exposure (+60%) in presence of diltiazem, a CYP3A4 inhibitor. The label indicates that a more pronounced increase is expected with other strong inhibitors like ritonavir however without further detail on the magnitude of the interaction and guidance on how to adjust amlodipine dosage.

Atorvastatin is a lipid-lowering agent predominantly metabolized by CYP3A4 into two major active metabolites: *ortho*-hydroxy atorvastatin and *para*-hydroxy atorvastatin which have a similar activity as the parent drug (19) (20). Importantly, the entry of atorvastatin in the liver, the site of action and metabolic biotransformation and elimination, is dependent on the organic anion transporter protein (OATP1B1/1B3) (21). PIs inhibits this hepatic uptake transporter and therefore have are expected to substantially increase atorvastatin exposure by both inhibiting the entry of the statin in the liver and by further inhibiting its biostransformation. This interaction can lead to serious side effects such as rhabdomyolysis (22). The current recommendations indicate to initiate atorvastatin at a lower dosage in presence of darunavir although formal DDI studies have not been performed particularly in elderly individuals.

Rosuvastatin is also a lipid-lowering agent. Contrary to atorvastatin, rosuvastatin undergoes minor metabolism. However, similarly to atorvastatin, rosuvastatin is actively transported in the liver by OATP1B1/3. Rosuvastatin is also a substrate of BCRP, a transporter present in the intestine and the liver where it limits the absorption and the biliaire elimination of drugs substrates. (23). PIs are also inhibitors of BCRP and therefore are expected to increase rosuvastatin exposure however no guidance is available on how to adjust rosuvastatin dosage in elderly.

Considering the actual paucity of data, this study is timely in view of an aging HIV population and will provide guidance on how to best manage DDI in this fragile population. In addition, these full

pharmacokinetic investigations will allow to consolidate and to refine previous population pharmacokinetic models developed using single point drug measurements through our TDM service (24, 25).

The study falls in the category A as the planned measures for sampling biological material or collecting personal data entail only minimal risks and burdens (clinical observations and peripheral venous blood sampling).

3. PROJECT OBJECTIVES AND DESIGN

3.1 Hypothesis and primary objective (and if applicable also secondary objectives)

Aging is characterized by physiological changes which can impact drug pharmacokinetics and thereby the magnitude of drug-drug interactions. We aim to assess the pharmacokinetics of amlodipine, atorvastatin and rosuvastatin in presence of darunavir/ritonavir (inhibitor of drug metabolizing enzymes and drug transporters) and in presence of dolutegravir (no inhibitory effects on cytochromes or transporters involved in the disposition of the evaluated co-medications) in order to characterize the magnitude of drug-drug interactions in elderly individuals. The first condition will be called the “interaction” condition, and the second condition the “control condition”.

3.2 Primary and secondary endpoints

Primary endpoint:

- Quantification of concentrations of ARV and cardiovascular drugs in plasma and characterization of the change in the area under the curve (AUC) of the cardiovascular drug, namely amlodipine, atorvastatin and rosuvastatin in the presence of darunavir/ritonavir (inhibitory effect) or dolutegravir (no inhibitory effect).

Secondary endpoint:

- Exploration of further associations of any other ARV and cardiovascular drugs to detect whether the area under the curve (AUC) of the cardiovascular drug reveals a clinically significant change in case of modification of the ARV treatment.

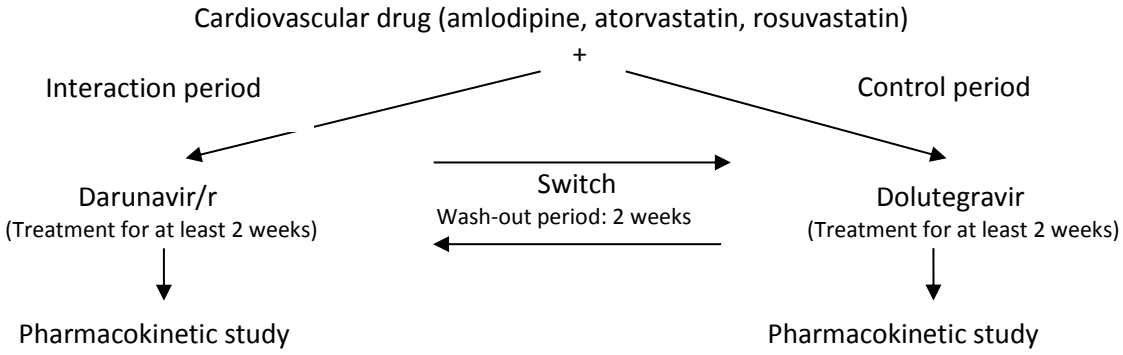
The characterization of the interaction between ARV and cardiovascular comedications will be performed using non-compartmental methods. In addition, we will develop population pharmacokinetic models for individual cardiovascular drugs and assess the effect of the coadministered antiretroviral drug (this project is part of a separate SNF funded project).

3.3 Project design

A multi-center, open, observational design will be used to assess the pharmacokinetic interaction between selected ARV and cardiovascular drugs. The patients will be recruited in parallel from two centers: HIV clinic at the University Hospital of Lausanne (head of the clinic: PD Dr. M. Cavassini) and HIV clinic at the University Hospital of Basel (head of the clinic: Dr M. Stoeckle). The pharmacokinetic investigations will be performed in the clinical investigations units of each respective hospital following the same standardized investigation protocol. Importantly, the measurement of drug levels will be performed by a single laboratory (laboratory of Clinical Pharmacology at the University Hospital of Lausanne, head: Prof. L. Decosterd) to eliminate inter-laboratory analytical biases.
The patients will be eligible if they receive a once daily ritonavir-boosted darunavir- or a dolutegravir-containing regimen. These regimens, as well as the comedications of interest will be given for at least 2 weeks prior to the blood intake in order to measure steady-state levels of drugs.

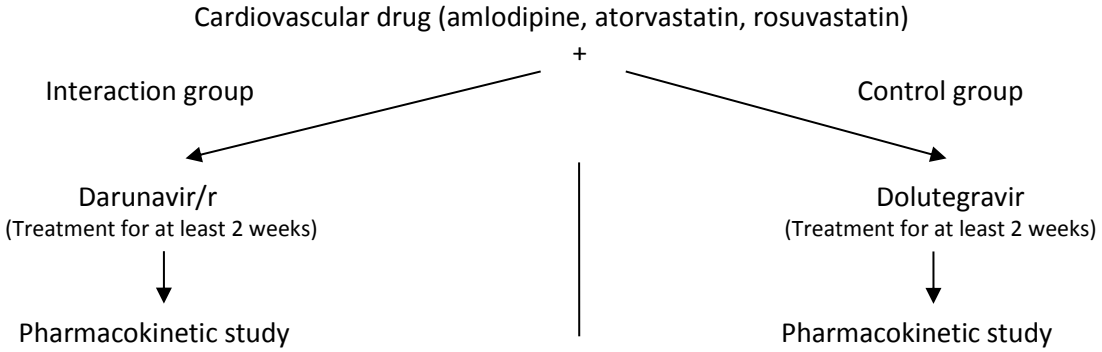
The following scenarios will be considered for inclusion:

a) patients already treated with one of the cardiovascular drugs of interest (*i.e.* amlodipine, atorvastatin or rosuvastatin) and requiring a change of ARV for medical reasons. A change from darunavir/r to dolutegravir may occur for metabolic side effects or treatment simplification (single pill regimen). Although less likely to be observed, a change from dolutegravir to darunavir/r could be motivated in patients intolerant to dolutegravir (e.g. CNS side effects) or infected with a virus resistant to integrase inhibitors precluding the use of raltegravir or elvitegravir/c. These patients will be offered to participate twice in the study: once before and once after changing ARV. Such patients will therefore constitute their own control. The second investigation will be performed at least 2 weeks after initiating the new ARV treatment and adjusting accordingly the dose of the comedication (if necessary), in order to rule out any carryover effect and to measure steady state plasma concentrations.

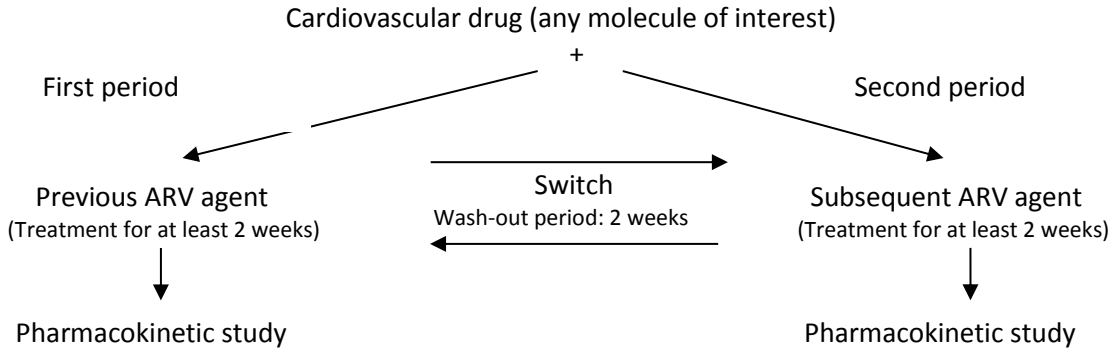


The proportion of patients in this scenario is expected to be small compared to the total of patients included in the study. Yet they will be particularly informative. Importantly, this study will have no influence on medical decisions to modify the antiretroviral treatment.

b) patient treated with one of the cardiovascular drug of interest together with one of the ARV of interest (*i.e.* darunavir/r or dolutegravir). A single pharmacokinetic investigation per patient will be performed in this scenario and two distinct groups (interaction group and control group) will be considered for the pharmacokinetic analysis.



c) patient treated with any cardiovascular drug of exploratory interest (*i.e.* antihypertensive, cardiotropic agent, anticoagulant or antiagregant, agent acting on metabolic risk factors) and requiring a change of ARV treatment for clinical reasons, considered at risk of modifying the disposition of this cardiovascular drug. The patient will be offered to participate twice to the study: before and after changing ARV, so to constitute an own control. The second investigation will be performed at least 2 weeks after initiating the new ARV treatment and adjusting accordingly the dose of the comedication in order to rule out any carryover effect and to measure steady state plasma concentrations:



4. PROJECT POPULATION AND STUDY PROCEDURES

4.1 Project population, inclusion and exclusion criteria

The patients fulfilling the study inclusion criteria described thereafter will be asked to participate in the study. As described in the power calculation, we are aiming to enrol 10 patients for each drug combination which represents a total of 60 PK studies (i.e. 2 antiretroviral drugs each combined with 3 comedications). This number can be reduced if we enrol patients receiving simultaneously two comedications of interest in which case both pharmacokinetic profiles could be measured within the same investigation.

Extra patients included in the exploratory investigations will not be included in the main analysis, but their results will be presented separately.

Inclusion criteria:

- documented HIV-infection
- informed consent as documented by signature (Appendix Informed Consent Form)
- included in the SHCS and followed-up in the HIV Clinic in Lausanne or in Basel
- treatment with a HIV therapy including either once-daily ritonavir-boosted darunavir or dolutegravir (or others ARV drugs for the exploratory investigations)
- treatment with one or eventually 2 of the comedications of interest, *i.e.* amlodipine, atorvastatin or rosuvastatin (or any drug potentially involved in clinically relevant DDI for the exploratory investigations).
- Ability to comply with the study requirements

The presence of any one of the following exclusion criteria will lead to exclusion of the participant:

- Presence of severe comorbidities (i.e. cirrhosis (Child-Pugh score C), heart failure (NYHA 3-4), advanced kidney impairment (KDOQI 4-5)) which can substantially impact the pharmacokinetic of drugs and significantly confound the study results.

- Presence of interacting non HIV comedications (*i.e* comedications with known, strong inhibitory or inducing effects on drug metabolizing cytochromes and drug transporters, which might significantly confound the study results)
- Participants incapable of jugement or participants under tutelage
- Known or suspected non-compliance, drug or alcohol abuse considered at risk to significantly confound the study results
- Inability to follow the procedures of the study, *e.g.* due to language problems, psychological disorders, dementia of the participant,
- Enrolment of the investigator, his/her family members, employees and other dependent persons.
- Women who are pregnant or breastfeeding

The primary outcome of the study is the change in the AUC of the cardiovascular drugs amlodipine, atorvastatin and rosuvastatin in the presence of darunavir/ritonavir (inhibitory effect presumed) or dolutegravir (no inhibitory effect presumed).

The study size is calculated using a Z-test taking into account a significance level of 0.05 and a power of 80% and the standard deviation. The coadministration of amlodipine and indinavir/ritonavir has been studied in young HIV-infected subjects (17). The median, minimum and maximum amlodipine AUC, when administered alone, were shown to be 122, 75 and 234 ng*h/mL respectively. This corresponds to a mean and standard deviation of 138 ± 47 ng*h/mL (conversion is done using the method of Hozo *et al.* (26). The coadministration of amlodipine together with indinavir/ritonavir increased the median, minimum and maximum amlodipine AUC to 230, 99 and 312 ng*h/mL respectively. This corresponds to a mean and standard deviation of 218 ± 62 ng*h/mL. Based on these data, the calculated sample size per drug pair is:

$$n = \frac{\left(Z_{\alpha} + Z_{1-\beta}\right)^2 \times 2 \times \sigma^2}{(\Delta)^2} = \frac{(1.96 + 0.8416)^2 \times 2 \times 62^2}{(218 - 138)^2} = 9.4 \approx \mathbf{10}$$

where *n* is the calculated sample size, Z_{α} is a constant depending on the significance level and whether it is a one-sided or two-sided effect, $Z_{1-\beta}$ is a constant set by convention according to the power of a study, σ^2 is the variance between people and Δ is the effect difference as detected in the reported clinical study. In this particular case, since the variance between people is unknown, we used the highest reported standard deviation.

This power analysis does not include extra patients recruited for exploratory investigations.

4.2 Recruitment, screening and informed consent procedure

The physicians of the HIV Clinics in Basel and Lausanne will be asked to identify potential eligible patients from their list of patients. As mentioned in the exclusion criteria, the selection of eligible patients will also take into account the current comorbidities and comedications. Thus, patients with severe comorbidities or interfering comedications will not be contacted for the study. Eligible patients will be asked to come to a study information meeting where they will receive details about the study goal and procedure from the study coordinator of the corresponding study site. The patients will have the opportunity to ask questions and will be free to accept or decline their participation in the study. If they accept to be included, they will be asked to sign the informed consent (see annexes) and an appointment for the pharmacokinetic investigation will be scheduled.

Patients will be offered a gift card of 100 CHF per investigation day to reward them for the time dedicated to the study. In addition, the study budget will cover the costs related to the transportation (for patients living outside of Lausanne) and the meals.

Note: the patients will be identified by their SHCS number (i.e. 5 digits number) throughout the study in order to maintain the patient's confidentiality.

4.3 Study procedures

The overall study duration including the recruitment of patients and the pharmacokinetic investigations (i.e. 60 clinical investigation days) is anticipated to last 18 months. The study duration for an individual patient will last either two weeks (patient undergoing antiretroviral treatment change in which case a full day pharmacokinetic investigation will be performed before and again two weeks after the antiretroviral treatment change) or one day (patient remaining on the same antiretroviral treatment in which case only one full day pharmacokinetic investigation will be performed). The pharmacokinetic investigations will be done in the HIV outpatient clinic at CHUV for the patients recruited in Lausanne and in the Clinical Investigation Unit (CTU) at USB for the patients recruited in Basel. The standardized study procedure is provided thereafter.

Procedure for the pharmacokinetic investigation:

On the morning of the investigation, the patient will take the antiretroviral medications together with the comedication of interest with a standard breakfast. Serial blood samples will be collected into EDTA-K monovettes (2.7 ml) from a catheter (Venflon) positioned in the forearm at the following time-points: t = 0 (just before the drug intake) and 30 minutes, 1, 2, 3, 4, 6, 8, 10, and 12 hours after the drug intake. A certain flexibility in sampling times is allowed, provided that dosing and sampling times are carefully recorded. The patient will then spend the night at home and return the following morning to provide the last sample of blood 24 hours after the drugs intake. In total, 30 ml of blood will be required for a full pharmacokinetic investigation). A second full pharmacokinetic investigation will be

performed for patients undergoing antiretroviral treatment change for clinical reasons. The investigation will be scheduled two weeks after switching treatment in order to be at steady-state and will be performed as described above.

The time of the drugs intake and blood samples will be documented in the patient case report form (CRF). Specific information will also be collected: time of the last drugs intake before the clinical investigation, complete list of comedications and any side effect reported by the patient during the investigation. All other relevant clinical information (CD4, viral load, chemistry laboratory parameters), co-morbidities will be extracted from the SHCS database.

Sample treatment procedure:

For the investigations performed in Lausanne, the blood samples together with the CRF will be shipped at room temperature on the same day to the laboratory of clinical pharmacology at CHUV. Upon arrival, the blood samples will be centrifuged and the separated plasma will be frozen at -80°C until analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS).

For the investigations performed in Basel: the blood samples will be centrifuged and stored at -80°C at the CTU unit and sent every 3 months on dry ice to the laboratory of clinical pharmacology at CHUV. The tubes will be identified with the patient's SHCS number, the date, time of the blood sampling and the name of the study "DDI study SHCS_815".

Drug levels measurements:

Drug levels of ARV will be quantified by LC-MS/MS using the methods developed in the framework of our routine TDM service (27-29). In addition, LC-MS/MS methods will be newly developed for the quantification of the cardiovascular drugs (amlodipine, atorvastatin, rosuvastatin and the active metabolites, *i.e.* ortho-, para-hydroxy atorvastatin, and atorvastatin lactone).

Pharmacokinetic analyses:

The pharmacokinetics parameters will be first estimated by classical non-compartmental approaches:

- Maximal concentration (C_{max})
- Minimal concentration (C_{min})
- Area under the Curve (AUC)
- Slope of the terminal phase (λ_z)
- Clearance (Cl)
- Half-life ($t_{1/2}$)

For the cardiovascular drugs, these parameters will be compared when combined with darunavir/ritonavir or dolutegravir using a variance analysis from log-transformed values. The analysis will accommodate the partial pairing of parameter values obtained in patients investigated in cross-

over, through the inclusion of a random patient factor assumed to take independent values only between patients. PK parameters of ARV drugs will be simply described.

Thereafter, population analyses will be performed by non-linear mixed effect modelling using the software program NONMEM®. This approach allows the characterization of the average pharmacokinetic profile of cardiovascular drugs from data pooled over all sampled individuals and quantify inter- and intra-individual variability. The advantage of this approach is that it allows to analyse sparse (few drug levels per patients) and unbalanced data. The models will integrate active metabolite when appropriate.

The pharmacokinetic variables quantifying cardiovascular drugs exposure will be calculated from the individual Bayesian pharmacokinetic parameters and will be presented with area under the curve (AUC), maximal (C_{max}) and minimal (C_{min}) drug concentrations and drug clearance (Cl).

For extra pharmaceutical agents included in the exploratory investigations, only the classical non-compartmental calculations will be performed, and the results will be simply described without formal statistical testing.

4.4 Withdrawal and discontinuation

The patients have the right to withdraw from the study at any time and for any reasons. All data previously collected for these subjects will be anonymized, kept and drug concentrations in the plasma will be analyzed.

5. STATISTICS AND METHODOLOGY

5.1. Statistical analysis plan

The calculation of the sample size is provided under 3.3.

The Wilcoxon signed rank test be used to compare cardiovascular drug concentrations when coadministered with darunavir/ritonavir and dolutegravir. Significance will be defined as a p value less than 0.05.

5.2. Handling of missing data

Participants dropping out of the study will not be retained in the final analysis. These participants will be replaced until achievement of the desired number of study participants.

6. REGULATORY ASPECTS AND SAFETY

6.1 Local regulations / Declaration of Helsinki

This research project will be conducted in accordance with the protocol, the Declaration of Helsinki [3], the principles of Good Clinical Practice, the Human Research Act (HRA) (2) and the Human Research Ordinance (HRO) (1) as well as other locally relevant regulations. The Project Leader acknowledges his responsibilities as both the Project Leader and the Sponsor.

6.2 Notification of safety and protective measures (HRO Art. 20)

The project leader is promptly notified (within 24 hours) if immediate safety and protective measures have to be taken during the conduct of the research project. The Ethics Committee will be notified via BASEC of these measures and of the circumstances necessitating them within 7 days.

6.3 Serious events (HRO Art. 21)

If a serious event occurs, the research project will be interrupted and the Ethics Committee notified on the circumstances via BASEC within 7 days according to HRO Art. 21 (A serious event is defined as any adverse event where it cannot be excluded, that the event is attributable to the sampling of biological material or the collection of health-related personal data, and which:
a. requires inpatient treatment not envisaged in the protocol or extends a current hospital stay;
b. results in permanent or significant incapacity or disability; or c. is life-threatening or results in death.)

6.4 Radiation

Not applicable.

6.5 Amendments

Substantial changes to the project set-up, the protocol and relevant project documents will be submitted to the Ethics Committee for approval according to HRO Art. 18 before implementation. Exceptions are measures that have to be taken immediately in order to protect the participants.

6.6 End of project

Upon project termination, the Ethics Committee is notified within 90 days.
The samples' and patients' data will be registered in an excel file, identifying patients with their SHCS number. The patient's blood samples and health data will be identifiable only with the SHCS number of patients, and kept for a maximum time of 15 years after the termination of the study and then destroyed.

6.7 Insurance

In the event of project-related damage or injuries, the liability of the CHUV and USB provides compensation, except for claims that arise from misconduct or gross negligence.

7. FURTHER ASPECTS

7.1 Overall ethical considerations

The management of DDI remains a key aspect of the care of HIV infected patients. One of the current issues is the limited number of drug combinations evaluated in clinical studies which makes the management of drug-drug interactions difficult. Also, available studies are mostly conducted in healthy volunteers and therefore do not reflect the situation of elderly patients. The present project aims to characterize the magnitude of drug-drug interactions between commonly prescribed antiretroviral drugs and cardiovascular drugs in elderly HIV-infected individuals. The results of this investigation will allow to personalize drug treatments and dosages while addressing the problem of interactions with co-medications, and thereby provide the most efficient and safest patient's care. More generally, this proposal aims to bridge a large translational research gap starting from mechanistic pharmacokinetics, going through population modeling, pharmacodynamics and ending up at the level of pharmaco-epidemiology, in an endeavour to bring significant contributions to current knowledge in the field of drug-drug interactions.

The participation to this clinical investigation will essentially require extra blood sampling (11 x 2.7 blood samples; 22 samples in patients investigated in cross-over). To avoid multiple punctures, blood samples will be collected from a catheter positioned in the forearm. This procedure is very common and is usually devoid of risks or complications. Finally, the patients will be informed that the participation in the study is voluntary and that withdrawal from the study will not affect their subsequent medical assistance and treatment.

7.2 Risk-Benefit Assessment

As this project corresponds to an observational study of category A, it does not expose to specific risks for patients outside the usual risks associated with repeated blood sampling through venous catheter over one or two days. Furthermore, this study does not expose participants to a novel treatment but rather allows for a closer monitoring of their current treatment.

All data gathered will be treated confidentially according to the law of data protection. The participants will be informed that some personal data will be accessible to the study investigators but will be kept confidential. During the data analysis, the investigators will assure that the participant's anonymity will be maintained (the participants will not be identified by their names but by an identification number on all source documents) and will guarantee that data are not accessible to unauthorized persons.

The participation to this study has little chance to produce a direct benefit for the patient through an adjustment of the comedication based on the drug measurements in the patient, which will be available only late. Still this project might indirectly improve the management of DDI in HIV treatment.

8. QUALITY CONTROL AND DATA PROTECTION

8.1 Quality measures

The study will be performed in accordance with International Conference on Harmonization (ICH GCP). All investigators involved in this study will have GCP training. Data files will be protected. The investigators will maintain adequate records documenting the conduct of the study. Copies of protocols, CRF, measurements results, analyses, correspondence, records of informed consent and all other documents relevant to the study will be kept on file by the principal investigator or his designee for a period of time as defined by local law for the preservation of hospital patient documents but at least up to 10 years after study completion.

In the laboratory, quality controls will be performed for drug levels quantification. The quality controls will be analyzed throughout the analytical run. The criteria of acceptance for an analytical run will include that 67% of all quality controls are within ± 15% of the theoretical values otherwise the run will be re-analyzed. The lab head will review the results of the drug quantification. The overall data collection and data entry will be reviewed by the investigator coordinating the study.

8.2 Data recording and source data

Once the paper CRF is completed, the data will be entered in an Excel file. To minimize errors, only one person will be allowed to enter the data in the file. Moreover, a regular backup of the database (every 10 patients) will be performed to ensure the traceability of the data. Before any pharmacokinetic or statistical analysis, the data entry will be double checked by the coordinator of the study and the corresponding computer file will be “frozen” i.e. protected as read-only immediately thereafter. All data management will be made on computers from the CHUV network, ensuring proper safety, backups, protection against unauthorized access and firewall.

Source documents include: signed informed consent, CRF (including data on last dose intake, time of blood sampling, comedications), and observations. These source data will be available at the site to document the existence of the study participants.

8.3 Confidentiality and coding

The **project data** will be handled with uttermost discretion and will be only accessible to authorized personnel who require the data to fulfil their duties within the scope of the research project. On the CRFs and other project specific documents, participants are only identified by a unique participant number.

Direct access to source documents will be permitted for purposes of monitoring, audits and inspections (ICHE6, 6.10). During the study, access to dataset will be authorized only for the person entering the data and for the investigator double checking the database. Importantly, data will be coded from the initial screening and at every subsequent steps of the study.

These source data will be kept all together in a binder which will be locked in a secure location in the division of clinical pharmacology. The source data and all other results will be entered in the study database. Access to the database will be allowed to the person entering the data and to the investigator supervising the clinical study. The electronic database will be regularly backed-up.

The **biological material** in this project is not identified by the participant name but by a unique participant number (*i.e* SHCS number of the patient). The biological material is appropriately stored in a restricted area only accessible to the authorized personnel. The biological material will be stored at -80°C at the laboratory of clinical pharmacology. An alarm will start if the temperature exceeds ±5°C. Moreover, the freezer is connected to the Evisense labguard ® system, which ensures the continuous control of temperature, preventing accidental degradation of samples. It also ensures the complete traceability of the temperature in the equipment.

The biological samples collected at the USB study site will be sent on dry ice every 3 months to Perrine Courlet, laboratory of clinical pharmacology, CHUV, Bugnon 19, 1011 Lausanne. The corresponding CRF will be sent by separate mail.

8.4 Retention and destruction of study data and biological material

The patient's blood samples and health data will be kept for a maximum time of 15 years after the completion of the study and then destroyed.

9. FUNDING / PUBLICATION / DECLARATION OF INTEREST

Thus study is performed in the framework of a SNF funding.

The results of the study will be submitted to local, national and/or international congresses and to peer reviewed journals for publication. The principal investigator and the supervising investigator will be senior and corresponding authors on the publication. All other investigators contributing to the study will be on the publication. Importantly, the study participants will be informed about the results of the study by their HIV physician.

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VIII.4. Participant information sheet for the PK study with rich sampling

Interactions médicamenteuses entre la thérapie antirétrovirale et les médicaments cardio-vasculaires chez les patients âgés de la Swiss HIV Cohort Study (SHCS)

Cette étude est organisée par la Division de Pharmacologie et Toxicologie clinique du CHUV, en collaboration avec les services de maladies infectieuses du CHUV et de l'Hôpital Universitaire de Bâle.

Madame, Monsieur,
Nous vous proposons de participer à notre étude. Cette feuille d'information décrit le projet de recherche dans une version longue (version détaillée).

Information détaillée

1. Objectifs du projet de recherche

Dans le cadre de votre suivi pour votre infection à VIH, vous recevez un traitement antirétroviral. Le succès de ce traitement va de pair avec une exposition constante du virus à l'action des médicaments. Grâce aux antirétroviraux, l'infection par le VIH est devenue une maladie que l'on peut stabiliser sur le long terme. Par conséquent, les patients infectés par le VIH vivent de plus en plus longtemps et sont confrontés à des problèmes de santé liés à l'âge, telles que les maladies cardio-vasculaires. Ceci conduit à des associations médicamenteuses complexes, avec un risque d'interactions médicamenteuses. A ce jour, très peu d'études ont mesuré l'exposition à ces médicaments chez les personnes âgées recevant des antirétroviraux.
Nous aimerions quantifier l'effet de votre traitement antirétroviral sur les autres médicaments que vous prenez. Nous nous intéressons plus particulièrement à trois médicaments, qui sont l'amlodipine (anti-hypertenseur), l'atorvastatine et la rosuvastatine (hypo-cholestérolémiant). Mais nous sommes également ouverts à explorer d'autres médicaments.

2. Sélection des personnes pouvant participer au projet

La participation est ouverte à toutes les personnes séropositives, faisant partie de la Swiss HIV Cohort Study (SHCS) et qui, en outre, reçoivent à la fois un traitement antirétroviral (darunavir-ritonavir ou

dolutégravir, ou éventuellement d'autres antirétroviraux), et au moins une des 3 comédications cardio-vasculaires citées (amlodipine, atorvastatine, rosuvastatine, ou autres).
Cette étude est en revanche fermée aux personnes incapables de discernement ou sous tutelle.

3. Informations générales sur le projet

- Il s'agit d'une étude nationale, qui recrute des patients suivis à Lausanne et à Bâle.
- Tous les patients de la SHCS ayant une thérapie antirétrovirale contenant du darunavir-ritonavir ou du dolutégravir (ou autres), et un des 3 médicaments d'intérêt (amlodipine, atorvastatine, rosuvastatine, ou autres) peuvent participer à cette étude.
- Il s'agit de prélever une série d'échantillons de sang sur une journée afin de suivre heure après heure les concentrations sanguines (taux sanguins) de vos médicaments (antirétroviral et comédication). En pratique, les participants demeurent 12 heures dans notre centre d'investigation clinique, passent la nuit à la maison et reviennent le lendemain matin pour la dernière prise de sang prélevée 24 heures après le début de l'investigation.
  Cette investigation pourra être répétée une deuxième fois si votre traitement antirétroviral doit être modifié pour des raisons médicales et que vous devez recevoir un autre médicament antirétroviral qui fait aussi l'objet de cette investigation.
- Les médicaments sont administrés à la dose prescrite par votre médecin.
- L'étude sera terminée pour vous après la prise de sang de 24 heures ou, le cas échéant, de la deuxième investigation. Les résultats de l'étude vous seront communiqués par votre infectiologue.
- Au total, nous prévoyons d'inclure 60 patients pour cette étude (10 patients pour chaque comédication lorsque administrée en présence de darunavir/ritonavir ou dolutégravir).

Nous effectuons ce projet dans le respect des prescriptions de la législation suisse. La commission cantonale d'éthique compétente a contrôlé et autorisé le projet.

4. Déroulement pour les participants

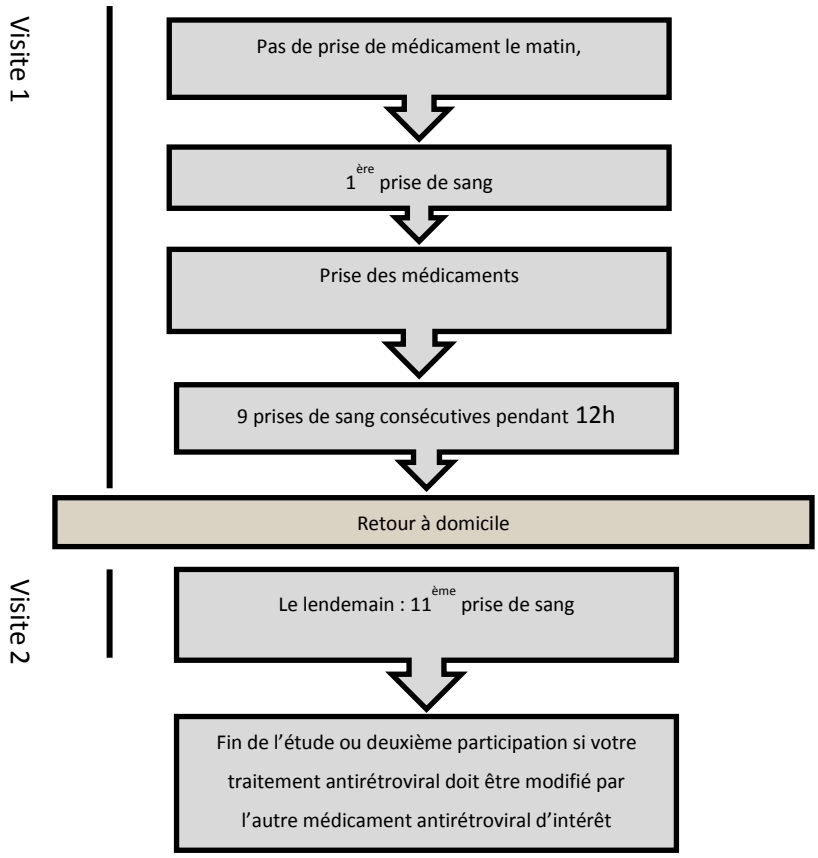
En pratique (voir schéma ci-dessous), vous serez convoqué à 2 reprises au service de maladies infectieuses du CHUV afin de prélever des échantillons sanguins. Ces échantillons seront utilisés pour mesurer les concentrations de vos médicaments (antirétroviraux et médicaments cardio-vasculaire). Ces échantillons seront également associés à vos données personnelles liées à votre santé qui seront récoltées dans votre dossier médical.

Avant votre visite à la consultation médicale prévue pour l'investigation, notez très précisément sur votre feuille de rendez-vous l'heure à laquelle vous avez pris la dernière dose de vos médicaments avant ce rendez-vous. Ces informations sont essentielles à l'étude. Ne prenez pas les médicaments antirétroviraux avant la consultation, mais emmenez votre traitement habituel avec vous.

Lors de la consultation pour l'investigation, un cathéter veineux (Venflon) sera inséré dans une veine de votre avant-bras par une personne qualifiée. Le but ici est de permettre des prises de sang répétées sans devoir vous piquer à chaque fois.

Une prise de sang initiale vous sera faite pour déterminer les concentrations de médicaments restant suite à la dernière dose. Après cette première prise de sang, vous prendrez sur place la dose prescrite de vos médicaments antirétroviraux et de votre comédication (amlodipine, atorvastatine ou rosuvastatine), au cours d'un petit déjeuner offert par le CHUV.

À environ 30 minutes, 1, 2, 3, 4, 6, 8, 10 et 12h après la dose, des prélèvements de sang seront pratiqués pour déterminer les concentrations sanguines. Vous devrez rester à l'hôpital pendant ces 12h. Vous rentrerez ensuite à votre domicile et vous reviendrez à l'hôpital le lendemain afin de réaliser une dernière prise de sang correspondant aux concentrations de médicaments 24h après la dernière prise. Au total, 30 ml (11 tubes de sang de 2.7 mL) vous seront prélevés pour cette étude.



La durée de l'étude est de 18 mois, mais dépendra de la capacité de recrutement des patients. Les résultats finaux de l'étude seront disponibles lorsque le nombre minimum requis de patients aura été atteint (au plus tôt mi 2019).

5. Bénéfices pour les participants

La participation à ce projet pourra être bénéfique pour vous puisqu'elle pourra permettre un ajustement du dosage de vos médicaments, selon le degré de l'interaction observée. De plus, les résultats du projet contribueront à mieux adapter les doses de médicaments pour d'autres patients qui reçoivent le même traitement que vous..

6. Droits des participants

Vous êtes libre d'accepter ou de refuser de participer au projet. Si vous choisissez de ne pas participer ou si vous choisissez de participer et revenez sur votre décision pendant le déroulement du projet, vous n'aurez pas à vous justifier. Cela ne changera rien à votre prise en charge médicale habituelle. Vous pouvez à tout moment poser toutes les questions nécessaires au sujet de l'étude. Veuillez-vous adresser pour ce faire à la personne indiquée à la fin de la présente feuille d'information.

7. Obligations des participants

En tant que participant au projet, vous serez tenu :

- de suivre les instructions médicales de votre médecin investigateur, et de vous conformer au plan de l'étude (notamment vous rendre aux visites prévues par l'étude). Nous vous rappelons qu'il est demandé de ne pas pendre son traitement avant de venir à la visite pour l'investigation, mais de l'amener avec vous au rendez-vous.
- d'informer votre médecin-investigateur / direction du projet de tous les médicaments que vous pourriez prendre, même les médicaments de médecine complémentaire ou toute forme en vente libre (plantes médicinales, remèdes homéopathiques, essences spagyriques, remèdes asiatiques, et préparations vitaminiques).

8. Risques

En participation au projet, vous serez exposé à des risques mineurs tels que la pose d'un cathéter et les prises de sang. Des réactions locales et transitoires pourraient apparaître au site de prélèvement (rougeur, induration, œdème, hématome, saignement).

9. Découvertes pendant le projet

Le médecin-investigateur vous avisera pendant l'étude de toute nouvelle découverte susceptible d'influer sur les bénéfices de l'étude ou votre sécurité, et donc sur votre consentement à y participer. En cas de découvertes fortuites qui, chez vous, pourraient contribuer à la prévention, au diagnostic et au traitement de maladies existantes ou probables dans le futur, le médecin investigateur vous en informera.

10. Confidentialité des données

Pour les besoins de l'étude, nous enregistrerons vos données personnelles et médicales. Seul un nombre limité de personne peut consulter vos données sous une forme non codée, et exclusivement afin de pouvoir accomplir des tâches nécessaires pour le déroulement du projet. Les autres personnes participant au projet ne peuvent consulter vos données que sous une forme codée. Le codage signifie que toutes les données permettant de vous identifier (p. ex. le nom, la date de naissance, etc.) sont remplacées par un code, de sorte que les personnes ne connaissant pas ce code ne peuvent pas lier ces données à votre personne. Toutes les personnes impliquées dans l'étude de quelque manière que ce soit sont tenues au secret professionnel. Vous avez à tout moment le droit de consulter vos données.

Durant son déroulement, le projet peut faire l'objet d'inspections. Celles-ci peuvent être effectuées par la commission d'éthique qui s'est chargée de son contrôle initial et l'a autorisé, mais aussi être mandatées par l'organisme qui l'a initié. Il se peut que la direction du projet doive communiquer vos données personnelles et médicales pour les besoins de ces inspections.

11. Retrait du projet

Vous pouvez à tout moment vous retirer de l'étude si vous le souhaitez.

Après l'analyse nous rendrons vos données et votre matériel biologique anonymes, en effaçant définitivement le code les reliant à votre personne (ou le cas échéant : et nous détruirons entièrement votre matériel biologique après conservation pendant 10 ans). Après cela, plus personne ne pourra savoir que ces données et ce matériel sont les vôtres.

12 Rémunération des participants

Si vous participez à ce projet, vous ne recevrez pour cela aucune rémunération. Les investigateurs ont toutefois prévu de vous offrir un bon d'achat de 100 CHF pour vous remercier du temps consacré. Les dépenses, telles que les frais de transport, qui découlent directement de la participation à l'étude vous seront remboursées (selon tarif des transports publics 2ème classe)

13 Réparation des dommages subis

Les dommages de santé que vous pourriez subir du fait de cette étude relèvent de la responsabilité de l'organisme ou de l'entreprise qui l'a initiée et est en charge de sa réalisation. En cas de dommages causés dans le cadre de l'étude, le CHUV répondra de ces derniers en sa qualité de promoteur conformément aux dispositions légales applicables. Les conditions et la procédure sont fixées par la loi. Si vous avez subi un dommage, veuillez-vous adresser au médecin responsable du projet.

14 Financement du projet

L'étude est intégralement financée par le FNS.

15 Interlocuteur(s)

En cas de doute, de craintes ou d'urgences pendant ou après l'étude, vous pouvez vous adresser à tout moment à l'un des interlocuteurs suivants :

- Médecin pharmacologue responsable du projet :

Prof Dr med Thierry Buclin

Service de pharmacologie clinique

Bugnon 17

CHUV. 1011 Lausanne – CH
Téléphone : +41 79 556 68 58
e-mail : Thierry.Buclin@chuv.ch

- Médecin infectiologue responsable, site de Lausanne :

PD MER Dr Matthias Cavassini
Division des maladies infectieuses
Rue du Bugnon 46, BH07/866
CHUV. 1011 Lausanne - CH
Téléphone : +41 21 314 1022
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Collaborateurs

- Pharmacienne responsable, site de Bâle :

PD Dr PhD Catia Marzolini
Infectiologie et hygiène hospitalière
Universitätsspital Basel
Téléphone: 077 454 92 28
e-mail: Catia.Marzolini@usb.ch

- Pharmacienne responsable du site de Lausanne :

Perrine Courlet
Service de pharmacologie clinique
Bugnon 17
CHUV. 1011 Lausanne – CH
Téléphone : +41 79 556 32 70
e-mail : Perrine.Courlet@chuv.ch

Déclaration de consentement

Déclaration de consentement écrite pour la participation à un projet de recherche

- Veuillez lire attentivement ce formulaire.
- N'hésitez pas à poser des questions lorsque vous ne comprenez pas quelque chose ou que vous souhaitez avoir des précisions.

Numéro BASEC du projet : **(après soumission à la commission d'éthique compétente) :**	
Titre de l'étude : (titre scientifique et titre usuel)	Interactions médicamenteuses entre la thérapie antirétrovirale et les médicaments cardio-vasculaires chez les patients âgés de la Swiss HIV Cohort Study (SHCS)
Institution responsable : (adresse complète) :	Division de pharmacologie clinique du CHUV Bugnon 17 CHUV 1011 Lausanne – CH
Lieu de réalisation du projet :	Lausanne et Bâle
Directeur / directrice du projet sur le site : (nom et prénom en caractères d'imprimerie) :	Lausanne : Prof. Thierry Buclin Bâle: Prof. Manuel Battegay
Participant / participante : (nom et prénom en caractères d'imprimerie) : Date de naissance :	☐ femme ☐ homme

- Je déclare avoir été informé, par le médecin investigateur /par la personne assurant l'information soussigné(e), oralement et par écrit, des objectifs et du déroulement du projet ainsi que des effets présumés, des avantages, des inconvénients possibles et des risques éventuels.
- Je prends part à cette étude de façon volontaire et j'accepte le contenu de la feuille d'information qui m'a été remise sur le projet précité. J'ai eu suffisamment de temps pour prendre ma décision.
- J'ai reçu des réponses satisfaisantes aux questions que j'ai posées en relation avec ma participation au projet. Je conserve la feuille d'information et reçois une copie de ma déclaration de consentement écrite.
- J'accepte que mon médecin traitant soit informé de ma participation au projet.
- J'accepte que les spécialistes compétents de l'institution, du mandataire du projet, de la Commission d'éthique compétente pour cette étude, puissent consulter mes données brutes afin

de procéder à des contrôles, à condition toutefois que la confidentialité de ces données soit strictement assurée.

- Je serai informé des découvertes (fortuites) ayant une incidence directe sur ma santé.
- Je sais que mes données personnelles (et échantillons biologiques) peuvent être transmises / transmis à des fins de recherche **dans le cadre de ce projet uniquement** et sous une forme codée.
- Si je bénéficie / devais bénéficier d'un traitement médical en dehors de l'institution responsable de ce projet, j'accepte que le médecin responsable du projet du projet contacte les médecins traitants afin d'obtenir mes données médicales pertinentes pour ce projet.
- Je peux, à tout moment et sans avoir à me justifier, révoquer mon consentement à participer à l'étude, sans que cela n'ait de répercussion défavorable sur la suite de ma prise en charge médicale usuelle. Je sais que les données médicales et le matériel biologique (échantillons de sang) qui ont été recueillis jusque-là seront cependant analysés.
- Je suis informé qu'en cas de dommages éventuels dans le cadre de l'étude, le CHUV répondra de ces derniers en sa qualité de promoteur conformément aux dispositions légales applicables.

Lieu, date	Signature du participant / de la participante

Attestation du médecin investigateur /de la personne assurant l'information : Par la présente, j'atteste avoir expliqué au participant / à la participante la nature, l'importance et la portée du projet. Je déclare satisfaire à toutes les obligations en relation avec ce projet conformément au droit en vigueur. Si je devais prendre connaissance, à quelque moment que ce soit durant la réalisation du projet, d'éléments susceptibles d'influer sur le consentement du participant / de la participante à prendre part au projet, je m'engage à l'en informer immédiatement.

Lieu, date	Nom et prénom du médecin investigateur / de la personne assurant l'information aux participants en caractères d'imprimerie. Signature du médecin investigateur / de la personne assurant l'information

VIII.5. Poster presented at the EACS congress 2019

Central nervous system penetration of antiretroviral drugs in HIV-positive patients with neurocognitive impairment, assessed from paired plasma-CSF concentrations

Perrine Courlet[1], Phanie Bidlingmeyer[2], Isaure Nadin[3], Marc Sanchez[4], Thierry Buclin[1], Laurent Decosterd[1], Katharine Darling[2], Renaud Du Pasquier[3], Matthias Cavassini[2]
[1]Service of clinical pharmacology ; [2]Service of Infectious Diseases ; [3]Department of clinical neurosciences ; [4]Service of psychiatry
all from Lausanne University Hospital and University of Lausanne, Switzerland

SNF 324730-165956 to LAD

Introduction and objectives

- Antiretroviral (ARV) drug penetration into cerebrospinal fluid (CSF) has been incompletely examined.
- High central nervous system (CNS) penetration is associated with a better control of HIV replication in the CSF.
- However, the association between CSF ARV penetration and neurocognitive impairment is disputed.
- **We aimed to evaluate factors associated with ARV CSF passage**

Methods

- Patients seen at the Lausanne University Hospital Neuro-HIV Platform since 2013.
- Multidisciplinary clinical examination.
- NCI classified according to Frascati criteria.
- Therapeutic drug monitoring in paired plasma and CSF samples (liquid chromatography coupled with tandem mass spectrometry).
- Free fraction retrieved from the summary of products characteristics.
- Linear mixed-effects models (R) with log-transformed CSF/plasma ratios.

Results

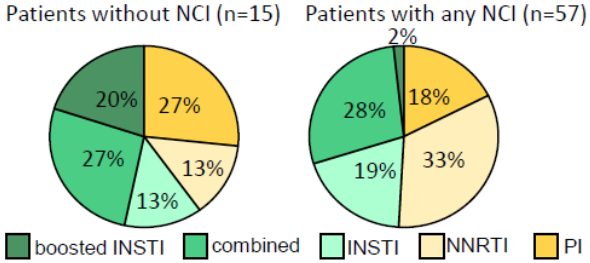
- 210 paired plasma/CSF were obtained in 72 patients.

Table 1: Patients' characteristics

Characteristics (N=72)	Median [IQR] or n (%)
Age (years)	48 [42-56]
Men	39 (54)
Body weight (24 missing values)	69 [63-77]
CD4T-cell count	581 [437-787]
Plasma HIV RNA< 50 copies/mL	68 (94)
CSF HIV RNA< 20 copies/mL	66 (92)
NCI	
Asymptomatic NCI	12 (17)
Mild NCI	4 (6)
HIV-associated dementia	1 (1)
Non-HIV associated NCI	40 (56)

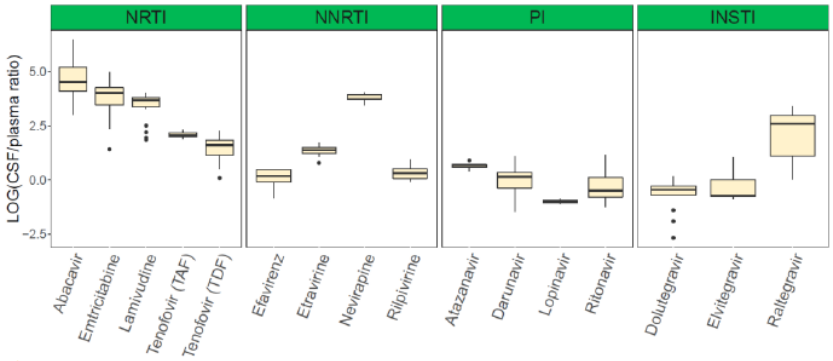
Abbreviations: IQR: interquartile range

Figure 1: Type of ARV regimens by neurocognitive status



- NNRTIs are more represented in patients with any NCI compared to patients without NCI.

Figure 2: CSF/plasma ratio of ARV drugs stratified by therapeutic classes



- CSF to plasma ratios were highly variable (Coefficient of variation 668%).

Table 2: Factors associated with log(CSF/plasma) ratio in univariate analyses

	Estimate	*Confidence interval (CI)*
Free fraction	0.032	0.027-0.037
Time after dose	0.047	0.018-0.075
Age	-0.0034	-0.028 to 0.021
Gender	-0.0615	-0.59 to 0.47
Body weight	-0.0005	-0.03 to 0.03
CSF albumin level	-0.0017	-0.005 to 0.001

- Log(CSF/plasma) ratio slightly increases along dosing interval by 5% per hour
- Compared to dolutegravir, abacavir, emtricitabine, nevirapine and lamivudine increased log(CSF/plasma) ratio by 4-5.5 fold.

Conclusion

- **CSF/plasma ratio markedly varies between ARVs.**
- **Free fraction is an imperfect proxy for CSF concentration.**
- **CSF penetration needs to be considered according to specific ARV molecule rather than ARV class.**

Abbreviations: INSTI: integrase inhibitor; NNRTI: non nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; combined: PI+INSTI, PI+NNRTI, PI+INSTI+NNRTI

VIII.6. Posters presented at the CROI conferences 2019 and 2020

0466

POLYPHARMACY, INAPPROPRIATE DRUGS & DRUG-DRUG INTERACTIONS IN HIV-INFECTED ELDERLY

Perrine Courlet[1*], Catia Marzolini[2,3*], Matthias Cavassini[4], Manuel Battegay[2], Monia Guidi[1,5], Susana Alves Saldanha[1], Deolinda Alves[4], Vreneli Waelti Da Costa[4], Chantal Csajka[1,5], Laurent Decosterd[1], and the Swiss HIV Cohort Study.

*Equal contribution to the work.

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Background

- Antiretroviral therapy (ART) has transformed HIV infection from a deadly disease to a manageable chronic condition.
- People living with HIV (PLWH) are aging, experience age-related physiological changes and comorbidities, predisposing to the risk of polypharmacy, drug-drug interactions (DDIs) and inappropriate medication use.

Aims

- To assess the prevalence of polypharmacy and cardiovascular/central nervous system (CNS) drug use across age groups.
- To determine the type of ART as well as the prevalence of potential DDIs (PDDIs) and inappropriate drugs in < 65 vs ≥ 65 years-old PLWH.
- To assess the prevalence of deleterious DDIs by looking at comedications dosages.

Methods

Study design	Multicentre (Lausanne & Basel) prospective study within the Swiss HIV Cohort Study. PLWH were contacted before their bi-annual follow-up visit to fill in a form recording all their current medications (including over-the-counter drugs).
Data	1675 collected forms from 1034 PLWH (among which 131 (13%) were ≥ 65 years-old).
Polypharmacy	≥ 5 non-HIV drugs.
Inappropriate medications	Anticholinergic drugs (anticholinergic risk scale, ARS ≥ 3) and benzodiazepines.
Screening of potential DDIs	Between ART and cardiovascular or CNS drugs using the Liverpool drug interaction database (https://www.hiv-druginteractions.org).

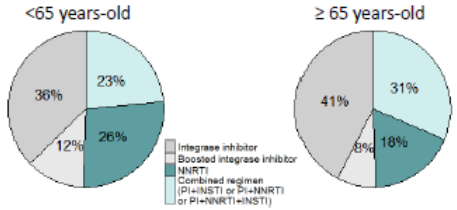
Results

Patients characteristics

Total number of participants	1034 (100%)
Median age, [IQR]	51 [42-58]
Male gender, n (%)	707 (68)
Date of HIV diagnosis, n (%)	
Before 1990	124 (12)
1990-2000	253 (25)
2001-2010	393 (38)
≥ 2011	228 (22)
Transmission risk, n (%)	
Homosexual contacts	385 (37)
Heterosexual contacts	488 (47)
Intravenous drug use	70 (7)
HIV RNA < 50 copies/mL, n (%)	998 (96)
Median CD4 T-cell count, [IQR]	683 [524-904]
Polypharmacy, n(%)	167 (16)

IQR=interquartile range

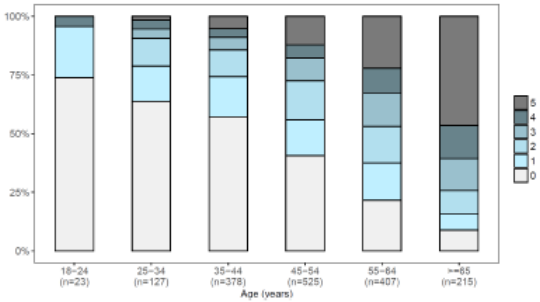
Type of ART by age group



PI = protease inhibitor / INSTI = integrase inhibitor / NRTI=non-nucleoside reverse transcriptase inhibitor

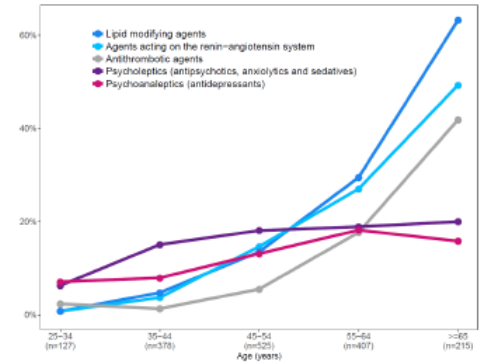
- While INSTIs are highly prescribed in both age groups, elderly PLWH have more complex ART characterized by a higher DDI potential.

Number of comedications by age group



- Polypharmacy is highly prevalent in PLWH ≥ 65 years (46%) compared to other age groups.

Type of comedications by age group

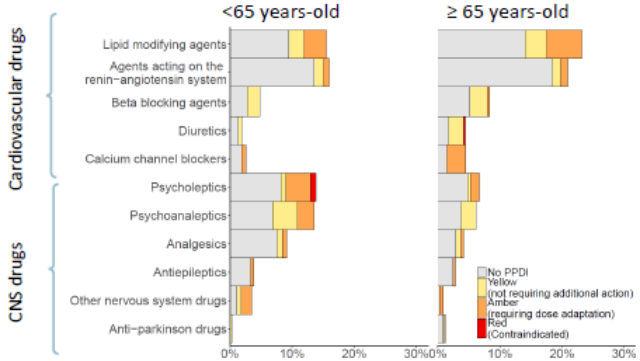


- Prescription of cardiovascular drugs increases with age whereas use of CNS drugs stays stable.

Inappropriate medications in elderly

- Inappropriate medications were found in 14% of elderly PLWH and included mostly benzodiazepines, not recommended as elderly are more sensitive to their effects.
- Considering all patients, the most frequently prescribed drug with ARS ≥ 3 was hydroxyzine, representing 33% of drugs with ARS ≥ 3.

Potential DDIs by age group



- Although PDDIs remain common, their rate is decreasing compared to a previous analysis of the SHCS (Marzolini *et al.* AVT 2010) due to the increasing use of unboosted INSTIs.
- PDDIs with cardiovascular drugs were more common in elderly PLWH whilst CNS drugs were mainly involved in PDDIs in younger PLWH.
- Of interest, no deleterious DDIs were detected as physicians have appropriately adjusted drug dosage in problematic orange and red DDIs. For instance, quetiapine dosage was reduced to 1/6 in presence of boosted protease inhibitors.

Conclusion

- In our Swiss population, use of unboosted INSTIs is growing thus decreasing the rate of PDDIs. However, 1/3 of elderly had complex ART regimens acting as perpetrator of DDIs. Given the higher prevalence of polypharmacy and the additional risk of inappropriate drug use, caution is needed when prescribing in elderly.
- Education on geriatric medicine principles and periodic medications review is warranted to limit the risk of prescribing errors in this vulnerable, growing population.

This work was supported by the Swiss National Science Foundation grant SNF 324730-165956 to LAD

REAL-LIFE MANAGEMENT OF DRUG-DRUG INTERACTIONS BETWEEN ANTIRETROVIRALS AND STATINS

0447

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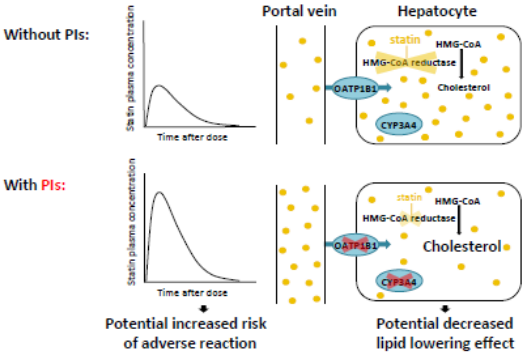
Introduction

- The management of dyslipidemia in people living with HIV (PLWH) is challenging:
  - Some antiretrovirals (ARVs) like protease inhibitors (PIs) and efavirenz can cause dyslipidemia.
  - Statins, the first-line drugs for the treatment of dyslipidemia, are subject to drug-drug interactions (DDIs).
- Statins enter the liver, the site of action and subsequent metabolic elimination, via active transport by OATP1B1. PIs inhibit OATP1B1, resulting in:
  - Higher systemic exposure of statins and related potential increased risk of adverse drug reactions
  - Potential Impaired pharmacodynamic effect by not entering the liver.



- It is currently unknown:
  - How DDIs between ARVs and statins are apprehended and managed in clinical practice.
  - Whether a better management of dyslipidemia could be achieved when using non-interacting ARVs.

Methods

- Study design: PLWH from Lausanne and Basel enrolled in the SHCS were eligible if they received one commonly prescribed statin (*i.e.* rosuvastatin, atorvastatin, pravastatin, pitavastatin) concomitantly to ARVs.
- Calculation of individual cholesterol targets:
  - Total cholesterol (TC): ≤ 5 mmol/L (if absence of cardiovascular risks) and ≤ 4 (if presence of cardiovascular risks) in agreement with the 2018 European AIDS Clinical Society guidelines[1]
  - Low-density-lipoprotein (LDL): Framingham score
  - Non-HDL (high-density lipoprotein) = TC-HDL : individual LDL target + 0.8 mmol/L
- Plasma concentration of statins: quantified using liquid chromatography coupled with tandem mass spectrometry[2]. Measured concentrations compared to published pharmacokinetic profiles.

[1]EACS Guidelines version 9.1. October 2018. (Available from: http://www.eacsociety.org/files/2018_guidelines-9.1-english.pdf
[2]Courlet P, et al. Journal of chromatography B. 2019;1125:121733.

Table 1: Statin maximal recommended daily doses according to coadministered ARV

	Rosuvastatin	Atorvastatin	Pravastatin	Pitavastatin
Boosted[a] atazanavir	10 mg	10 mg	40 mg	4 mg
Boosted[a] darunavir	20 mg	40 mg	40 mg	4 mg
Boosted elvitegravir	20 mg	40 mg	40 mg	4 mg
Non-interacting ARV drugs	20 mg	80 mg	40 mg	4 mg

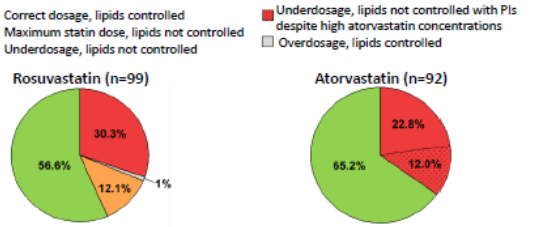
[a]Boosting either with ritonavir or cobicistat

Table 2: Evaluation of the management of DDIs

Lipid targets	Dosing recommendation	Statin plasma concentration	Management of DDIs
Both achieved	→		Correct
At least one not achieved	√	< lower limit of quantification	*a priori* correct, suggestive of non-adherence
	√	√	*a priori* correct, suggestive of a problem of statin efficacy
	X	→	Incorrect

Results

- Response to statin treatment and management of DDIs:

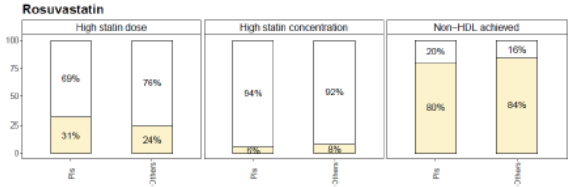


Underdosing with non-achievement of lipid targets represented 1/3 of rosuvastatin and atorvastatin prescriptions. Insufficient lipid control was observed despite high atorvastatin concentrations suggestive of DDI impairing statin entry in the liver.

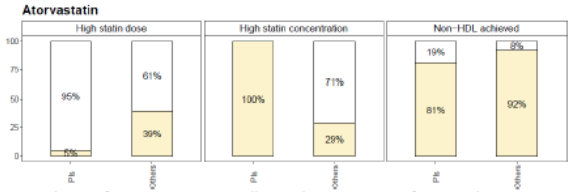
Insufficient lipid control common despite maximal pravastatin or pitavastatin dosage, suggestive of lower statin efficacy.

- Statin treatment and response in presence of PIs versus non-interacting and lipid neutral ARV drugs

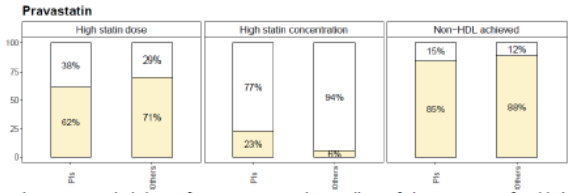
Yellow bars = % statin prescriptions with high statin dose, high statin plasma concentration and achievement of non-HDL-cholesterol targets. Others = dolutegravir, raltegravir or rilpivirine. High statin dose: 20 mg for rosuvastatin; 40 mg for atorvastatin and pravastatin.



High recommended doses of rosuvastatin used regardless of the presence of PIs. Proportion of high rosuvastatin concentrations is low and comparable with PIs vs non-interacting ARVs.



Lower doses of atorvastatin generally used in presence of PIs. High atorvastatin concentrations in presence of PIs. Despite high concentrations, non-HDL targets were less often achieved in presence of PIs likely due to both their inhibitory effect on OATP1B1 and their effect on lipids.



High recommended dose of pravastatin used regardless of the presence of PI likely due to its known weak DDI risk.

Conclusion

- **Suboptimal management of dyslipidemia is common in PLWH due to statin underdosing or use of low intensity statins leading overall to 41% of statin prescriptions with suboptimal response.**
- **Unboosted INIs based regimens and/or treatment with rosuvastatin or atorvastatin should be favoured in patients with refractory dyslipidemia.**