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Programmatic assessment of a third-line regimen implementation for adult HIVinfected in Cambodia

Phearavin Pheng

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Programmatic assessment of a third-line regimen implementation for adult HIV- infected in Cambodia

*Evaluation de l'implantation d'un programme de 3ème ligne pour des
sujets infectés par le VIH au Cambodge*

Thèse de doctorat de l'université Paris-Saclay

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
ANC	antenatal care
ANRS	ANRS I Emerging infectious diseases
ANRS	Agence Nationale de Recherches sur le Sida et les hépatites virales
Anti-HCV	HCV antibody
aOR	Adjusted odd ratio
ART	antiretroviral therapy
ART	antiretroviral treatment
ART	Antiretroviral therapy
ARV	Antiretroviral drugs
AST	Aspartate aminotransferase
ATV	Atazanavir
AUClast	areas under the plasma concentration time curve from 0 to the last time sampling time
AUCtau	areas under the plasma concentration time curve during a dosing interval
BMI	Body mass index
CBC	Complete blood count
CDC	Communicable Disease Control
CHAI	Clinton Health Access Initiative
CIs	confidence intervals
Cmax	maximum concentration
Co	observed predose concentration
CRAs	clinical research assistants
CRF	Case report form
CV	coefficient of variation
DAA	Direct-Acting Agents
DRV	Darunavir
DSC	Decision Support Committee
DSMB	Data safety & monitoring board
DTG	Dolutegravir
EAC	Enhanced Adherence Counseling
ELISA	Enzyme-linked immunosorbent assay
ERHZ	Ethambutol rifampicin isoniazid pyrazinamide
GART	genotypic antiretroviral resistance testing
GCLP	Good clinical laboratory practice
GCP	Good clinical practice
HCC	hepatocellular carcinoma
HCV	hepatitis C virus

HICs	high-income countries
HIV	Human Immuno-Deficiency Virus
INSTI	Integrase Strand Transfer Inhibitor
IPC	Institute Pasteur in Cambodia
IQR	interquartile range
IRIS	Immune reconstitution inflammatory syndrome
LMICs	low-and middle-income countries
MoH	Ministry of Health
MSF	Médecins Sans Frontières
MSM	men who have sex with men
MTCT	Mother to child transmission
NCHADS	National Center for HIV/AIDS, dermatology and STD
NECHR	National Ethic Committee for Health Research
NMCH	National maternal and child health program
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OR	odd ratio
PI	Protease Inhibitor
PK	Pharmacokinetic
PLWHIV	people living with HIV
PWIDs	people who inject drugs
PWUDs	people who use drugs
RAMs	resistance-associated mutations
SAE	Serious adverse event
SCO	a signal-to-cutoff
SD	standard deviation
SUSAR	suspected unexpected serious adverse reaction
Tmax	time of maximum concentration
TWG	technical working group
UHS	University of Health Sciences
UNAIDS	United Nations Programme on HIV and AIDS
VCCT	Voluntary Confidential Counseling and Testing
VL	Viral load
WHO	World Health Organization

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1. Introduction

Selon les dernières estimations sur l'épidémie mondiale de VIH en 2021, le VIH reste un problème majeur de santé publique dans le monde, avec environ 38,4 millions de personnes vivant avec le VIH (PVVIH), 1,5 million de personnes nouvellement infectées par le VIH et 650 000 personnes décédées de causes liées au VIH. Au niveau mondial, la couverture du traitement antirétroviral s'améliore, puisque 75% des personnes vivant avec le VIH avaient accès à ce type de traitement à la fin de 2021, contre 25% en 2010.

La disponibilité d'un nombre limité de médicaments de première, deuxième et troisième ligne est un problème dans les pays à ressources limitées pour une gestion adéquate du traitement des patients afin d'obtenir de meilleurs résultats. De nombreux pays ont été réticents à mettre en œuvre des traitements de troisième ligne au niveau national en raison de la disponibilité limitée et des prix élevés des médicaments. Le faible volume du marché et la nécessité de nouveaux tests de laboratoire, tels que les tests de résistance génotypiques, constituent également des obstacles nationaux importants pour le développement de nouvelles politiques. Pourtant, l'OMS a émis l'hypothèse que le nombre de personnes sous traitement de troisième ligne augmentera pour atteindre plus d'un demi-million d'ici 2025.

L'OMS encourage les programmes nationaux sur le VIH à développer des stratégies qui tiennent compte du financement, de la durabilité et de la fourniture d'un accès équitable aux antirétroviraux pour première, deuxième et troisième lignes. Cependant, les données probantes pour guider les stratégies de troisième ligne dans les pays à ressources limitées, dont le Cambodge, sont rares : il existe peu d'études sur les nouveaux agents et aucune étude sur ces nouveaux traitements n'a encore été menée au Cambodge.

Depuis la mise à disposition des traitements antirétroviraux dans le programme national cambodgien en 2002, la cohorte des patients arrive à maturité : le programme national est ainsi confronté à la gestion des patients qui ne répondent pas aux traitements de première et de deuxième lignes. Dans le cadre de l'amélioration des résultats du traitement du programme afin de maintenir le 3ème objectif de 95% de charge virale indétectable chez les patients pris en charge dans le système de soins, il était nécessaire que le programme national élabore une politique pour les stratégies de troisième ligne pour la gestion des patients en échec de première et de deuxième ligne au Cambodge.

En ce qui concerne l'infection par le VHC, malgré les progrès réalisés dans la mise en place d'un programme public de lutte contre le VHC, les ressources nécessaires à l'élimination complète du virus doivent être mobilisées pour atteindre les objectifs de l'OMS visant à éliminer le VHC en tant que menace pour la santé publique d'ici 2030 au Cambodge. L'évaluation de la prévalence de l'infection par le VHC et l'identification des groupes les plus à risque d'infection contribuent à prioriser les interventions requises et à renforcer le niveau de preuve pour le développement d'une politique adéquate et la mobilisation de ressources pour lutter contre cette infection. Afin de contribuer aux efforts déployés au Cambodge pour éliminer l'infection par le VHC, l'Université des sciences de la santé (UHS) et ses partenaires ont mené en 2016 une étude visant à évaluer l'ampleur de l'infection par le VHC chez les femmes enceintes et chez les personnes vivant avec le VIH/SIDA (PVVIH) au Cambodge.

Cette thèse se situe dans un cadre général de programme de recherches visant à améliorer le contrôle des infections VIH et VHC au Cambodge. Elle avait pour but d'évaluer, d'une part, une stratégie de troisième ligne antirétrovirale contre le VIH et, d'autre part, de décrire la prévalence de l'infection par le VHC au Cambodge. La thèse s'appuie sur deux études que j'ai mises en place, coordonnées et analysées : 1) l'étude ANRS 12374 3DICAM conçue pour évaluer l'efficacité et la pharmacocinétique d'un

régime de troisième ligne associant dolutégravir, darunavir boosté par le ritonavir et un inhibiteur nucléosidique (INTI) optimisé chez des adultes infectés par le VIH en échec de deuxième ligne, et 2) une étude de prévalence du VHC menée pour évaluer la prévalence de l'infection par le VHC au Cambodge.

Le manuscrit de thèse est structuré en 4 chapitres qui comprennent l'introduction, Matériaux et Méthodes, Résultats, Discussion et perspectives. Le premier chapitre de l'Introduction est consacré à une présentation générale des contextes du VIH et du VHC au Cambodge, à la justification de la thèse et aux objectifs généraux de la thèse. Le deuxième chapitre présente les matériel et les méthodes utilisés pour les deux études. Comme mon projet de thèse s'appuie sur deux études, dans cette section, j'ai organisé la présentation des méthodes utilisées par étude : d'abord, les matériel et les méthodes de l'étude de séroprévalence VHC au Cambodge, puis l'étude ANRS 12374 3DICAM. Ce chapitre se concentre également sur le développement et la mise en œuvre de l'étude ANRS 12374 3DICAM. Ensuite, le troisième chapitre expose les résultats des deux études. Je termine par une discussion générale des résultats des 2 études dans le quatrième chapitre. Dans ce chapitre, je présente également les défis/problèmes et les leçons apprises concernant la mise en œuvre du programme de troisième ligne conçu comme l'étude ANRS 12374 3DICAM et des perspectives de recherches et d'interventions pour améliorer le dépistage, la prise en charge et le contrôle des infections VIH et VHC au Cambodge et des patients VIH traités avec un traitement de troisième ligne au Cambodge.

2. Etude de la séroprévalence de l'infection VHC au Cambodge

Contexte : Au Cambodge, les connaissances sur l'épidémiologie de l'hépatite C sont essentielles pour prioriser les interventions requises afin d'atteindre l'objectif d'élimination de l'Organisation mondiale de la santé d'ici 2030. Afin de comprendre pleinement l'ampleur de l'hépatite virale et de renforcer les preuves pour l'élaboration de politiques appropriées et adéquates ainsi que la mobilisation des ressources pour lutter contre cette infection, nous avons fait une étude pour estimer la prévalence du

VHC et les facteurs de risque de transmission dans quatre provinces parmi les femmes enceintes qui venaient pour les soins prénatals et parmi les personnes vivant avec le VIH au Cambodge.

Méthodes : Entre mars et avril 2016, nous avons mené une enquête transversale dans quatre zones géographiques diverses : la capitale Phnom Penh et trois provinces : à l'ouest (Battambang), au sud-ouest (Preah Sihanouk) et au nord-ouest (Siem Reap) du Cambodge. Nous avons recueilli des informations sur les caractéristiques démographiques et les comportements à risque et effectué des tests de dépistage des anticorps anti-VHC (Anti-HCV) parmi les femmes enceintes venant pour la suivie périnatale dans les centres de santé et parmi les patients recevant des soins pour le VIH dans les hôpitaux. Nous avons calculé la prévalence du VHC parmi les deux populations et effectué des analyses de régression logistique pour identifier les facteurs de risque associés à la positivité des anticorps anti-VHC.

Résultats : Sur les 935 participants, 510 (54,6%) étaient des femmes enceintes et 425 (45,4%) des PVVIH. La prévalence des anticorps anti-VHC était significativement plus élevée chez les PVVIH que chez les femmes enceintes (29/425, 6,8% contre 5/510, 0,9%, $P < 0,001$). Parmi les régions géographiques, la province de Preah Sihanouk (Sud-Ouest) présentait la prévalence anti-VHC la plus élevée parmi les PVVIH (12,0 %, $P = 0,031$). Il n'y avait pas de différence géographique significative de la prévalence anti-VHC chez les femmes enceintes. Dans les analyses multivariées, l'infection par le VHC était significativement associée au fait d'avoir un membre de la famille positif pour le VHC (OR=7,6 [95%CI: 1,01-57,84], $P = 0,048$) et à des antécédents d'injection de médicaments par voie intraveineuse au cours des cinq dernières années (OR=7,1 [95%CI : 2,79-18,10], $P < 0,001$). Les facteurs de risque d'infection par le VHC n'ont pas pu être analysés chez les femmes enceintes en raison du faible nombre de femmes infectées. Il convient toutefois de noter que les cinq femmes enceintes infectées par le VHC ont toutes rapporté des antécédents d'injection de médicaments par voie

intraveineuse dans les cinq années précédentes. Globalement, une histoire d'injection de médicaments par voie intraveineuse était un facteur de risque majeur, rapporté par 33 des 34 personnes trouvées infectées par le VHC (contre 665 des 901 personnes non infectées).

Conclusions : L'infection par le VHC est fréquente chez les PVVIH cambodgiennes, avec une prévalence de l'ordre de 7%, en partie liée à une pratique d'injection de médicaments par voie intraveineuse et à une transmission intrafamiliale du virus. Le dépistage et la prise en charge systématiques du VHC chez les PVVIH - et le dépistage des membres de leur famille - sont nécessaires.

3. Conception et mise en place d'une évaluation programmatique d'un régime de troisième ligne combinant darunavir/r, dolutegravir et inhibiteur nucléosidique optimisé au Cambodge, basée sur le design de l'étude ANRS 12374 3DICAM

Contexte: L'OMS encourage les programmes nationaux à développer les stratégies pour le traitement antirétroviral de troisième ligne, avec des schémas thérapeutiques comprenant de nouveaux médicaments présentant un risque minimal de résistance croisée par rapport aux schémas précédemment utilisés ; pour les patients en échec de première ligne avec l'efavirenz et de deuxième ligne avec un inhibiteur de protéase, l'efficacité des combinaisons comprenant darunavir et dolutegravir semble être comparable que le backbone comprenne des inhibiteurs nucléosidiques ou non nucléosidiques en termes de suppression virologique mais les données sont encore limitées. L'étude 2PICAM, menée au Cambodge en 2013 pour évaluer l'efficacité virologique d'un traitement de deuxième ligne à base d'inhibiteur de protéase (PR), a montré un taux très élevé de succès virologique (90%) ; de plus, une période de 3 mois de counseling renforcé pour augmenter l'adhésion aux traitements a pu remettre en succès virologique la moitié des patients en échec. Pour autant, un traitement de troisième ligne s'avérait nécessaire pour les patients ayant un échec confirmé du

traitement de deuxième ligne, notamment ceux avec une résistance génotypique à l'inhibiteur de protéase contenu dans le régime de deuxième ligne.

Cependant, un traitement standard de troisième ligne n'était pas encore disponible au Cambodge en raison de données limitées sur l'efficacité de l'association des nouveaux traitements recommandés par l'OMS. Pour cela, le programme national a conçu l'étude ANRS 12374 3DICAM pour évaluer l'efficacité et la pharmacocinétique d'un traitement de troisième ligne associant le dolutégravir, le darunavir boosté par le ritonavir et un inhibiteur nucléosidique INTI optimisé chez des adultes infectés par le VIH en échec confirmé de deuxième ligne.

Méthodes : l'étude 3DICAM était un essai de 48 semaines multicentrique ouvert à un bras. L'essai a inclus entre octobre 2018 et avril 2021 dans 13 cliniques ART au Cambodge 49 patients avec un niveau plasmatiques d'ARN-VIH >1000 copies/mL (CV) et une résistance génotypique à l'atazanavir et/ou à tous les NRTI. Le critère principal était la proportion de patients avec une CV<40 copies/mL à W24. La pharmacocinétique du DRV (600/100mg) deux fois par jour et du DTG (50mg) une fois par jour à l'état d'équilibre a été évaluée pour les 20 premiers participants dans l'étude.

Résultats : Sur les 49 participants, à W24, 59% des participants (IC95% : 44-73%) avaient une CV<40 copies/mL et 81,6% (IC95% : 68-91%) <1000 copies/mL. Les taux de réponse étaient similaires à W48, 55,1% (40,2-69,3%) et 79,5% (65,6-89,7%), respectivement. Aucun patient n'a arrêté le traitement de l'essai. Les concentrations minimales médianes de DRV et de DTG étaient proches de celles précédemment rapportées dans d'autres populations. Les facteurs de risque d'échec viral à W48 comprenaient un taux de CD4 bas, <200 cellules/ μ L et une infection transmise par voie mère-enfant.

Conclusions : Un traitement de troisième ligne combinant DRV/r, DTG et un NRTI optimisé apparaît efficace pour obtenir chez des patients multi-prétraités une

diminution significative de la charge virale dans la première année suivant la modification du traitement, et est associé à une réponse immunologique. Nous n'avons pas trouvé de rôle du nombre de mutations de résistance, ni de la charge virale plasmatique à l'initiation de la 3^{ème} ligne. Les données pharmacocinétiques ont montré des niveaux comparables à ceux d'autres populations non cambodgiennes. Au total, l'ensemble de ces données plaide en faveur d'une mauvaise observance persistante pour expliquer l'échec de la 3^{ème} ligne de certains de ces patients. Les stratégies d'aide à l'observance lors de l'initiation du traitement antirétroviral, et au cours de la prise en charge, en particulier chez les patients présentant une réponse virologique sous-optimale, restent un élément clé d'une thérapie VIH au long cours, même dans le contexte de sauvetage récent de médicaments antirétroviraux puissants.

4. Perspectives et conclusions

Plusieurs questions restent sans réponse autour de la mise en œuvre d'un programme de troisième ligne conçu selon l'étude 3DICAM et d'autres analyses sont nécessaires pour fournir des informations au programme national qui lui permettent de développer une stratégie de traitement de troisième ligne qui prenne en compte le financement, la durabilité et d'un accès équitable au traitement antirétroviral au Cambodge. Les projets suivants seront envisagés suite aux résultats de l'étude 3DICAM :

1. Réaliser une analyse des données sur les résultats des 32 patients qui n'ont pas été inclus dans l'étude parce qu'ils n'étaient pas résistants au traitement de deuxième ligne lors de la pré-inclusion et auraient dû changer pour un traitement de troisième ligne si nous n'avions pas effectué de tests génotypiques lors de la pré-sélection. Il sera essentiel d'analyser des résultats de ces patients pour évaluer l'efficacité de la stratégie et garantir une utilisation optimale des ressources au Cambodge.
2. Évaluer le profil de résistance aux médicaments chez les adolescents ou les

jeunes adultes (<25 ans) infectés par transmission mère-enfant dans la cohorte nationale et revoir les recommandations de surveillance, en particulier pour ce groupe de patients, afin de diagnostiquer précocement un échec de traitement et changer rapidement pour un traitement plus efficace pour optimiser le succès du traitement à long terme dans ce groupe de population difficile à traiter.

3. Évaluer les stratégies existantes pour l'amélioration de l'observance actuellement mises en œuvre dans le programme national et explorer une stratégie d'intervention dynamique d'observance pour aider les adolescents et les jeunes adultes (<25 ans) à maintenir réponse virologique à long terme.

4. Réaliser une analyse des coûts de la mise en œuvre de la troisième ligne au Cambodge en se basant sur la conception de l'étude 3DICAM.

En conclusion, les deux études de mon projet de thèse ont contribué en termes de connaissances scientifiques et ont fourni des preuves pour informer la mise en œuvre des stratégies d'intervention pour l'élimination de l'hépatite C d'ici 2030 au Cambodge ainsi que pour guider les stratégies de troisième ligne pour le programme national au Cambodge de lutte contre le VIH afin de maintenir les objectifs 95-95-95 de l'ONUSIDA d'ici 2025. Les résultats de l'étude de prévalence VHC ont mis en lumière la nécessité d'un dépistage systématique des membres de la famille vivant dans le même foyer que la personne infectée par le VHC et ce groupe devrait être la prochaine population cible pour le dépistage et l'accès aux soins. Les résultats de l'étude de 3^{ème} ligne de traitement VIH suggèrent un bon taux de réponse à la stratégie de troisième ligne utilisant le DRV et le DTG chez des patients infectés par le VIH multi résistants au Cambodge, mais la stratégie doit être accompagnée d'une stratégie innovante pour les jeunes patients infectés par voie mère-enfant, même dans le contexte d'un sauvetage récent avec des médicaments antirétroviraux puissants.

Articles published, submitted and in preparation

Published:

- **Pheng P**, Meyer L, Ségéral O, Chea P, Yi S, Tuot S, et al. **Hepatitis C seroprevalence among people living with HIV and pregnant women in four provinces in Cambodia: an integrated bio-behavioral survey.** BMC Infect Dis. 2022;22(1):177. Published: <https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-022-07163-2>

Submitted:

Phearavin PHENG, Olivier SEGERAL, Sovannarith SAMRETH, Monidarin CHOU, Chanroth CHHOUNG, Sovatha MAM, Chandara MOM, Chan Leakhena PHOEUNG, Bora NGAUV, Kimhuy LIP, Remonie SENG, Anne-Marie TABURET, Laurence MEYER, Vichea OUK, Penhsun LY*, Cecile GOUJARD*, the ANRS 12374 3DICAM study Group. **Effectiveness and Pharmacokinetics of a third-line cART regimen combining dolutegravir, ritonavir-boosted darunavir and optimized NRTI in HIV-infected treatment-experienced adults in Cambodia: the ANRS 12374 3DICAM study.**

Conferences, Scientific Communications

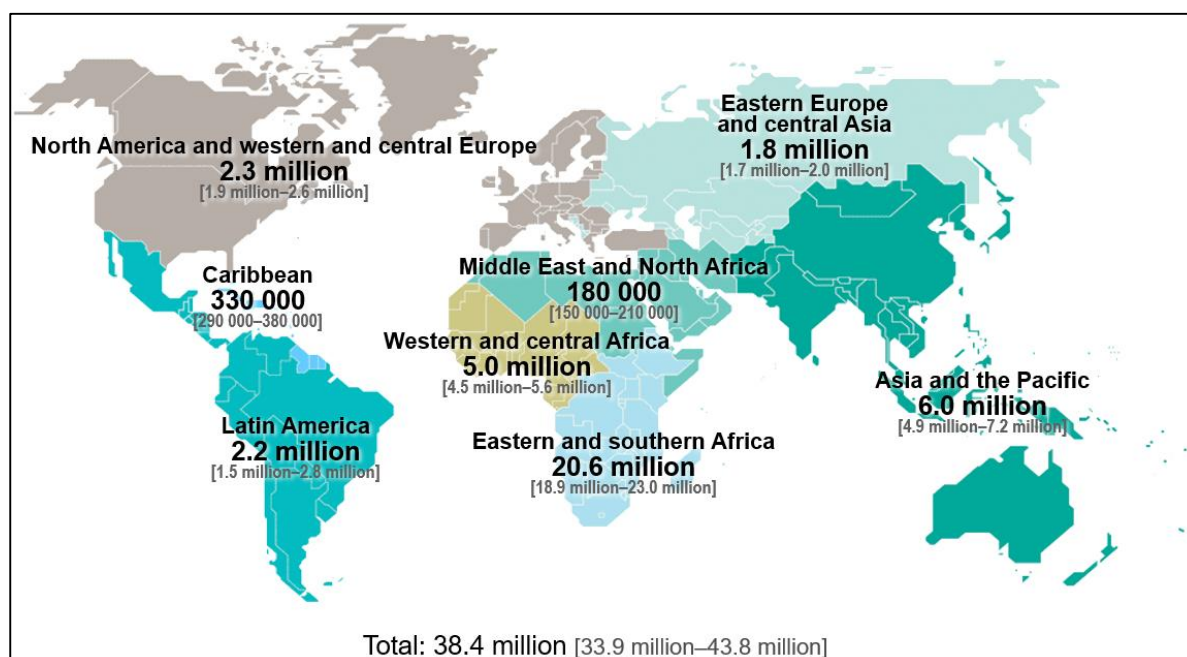
- The international conference on HIV/AIDS and infectious diseases CAMBODIA ANRS Scientific Days 2021, Phnom Penh, Cambodia (oral presentation).
 - *“Dolutegravir, darunavir/ritonavir and optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia - ANRS 12374 3DICAM study, Preliminary results and pharmacokinetic sub-study”*

1 INTRODUCTION

1.1 Global epidemiology of HIV virus infection

Despite major progress in the global HIV response, the latest estimates on the global HIV epidemic in 2021 reported that HIV continues to be a major global public health issue, having claimed approximately 38.4 million people living with HIV (PLHIV), with 1.5 million people becoming newly infected with HIV and 650 000 people dying from HIV-related causes despite effective HIV treatment and tools to prevent, detect and treat opportunistic infections (1). The Asia and Pacific region has the second-highest number of people living with HIV in the world with an estimate of 6.0 million people living with HIV (2) (Figure 1) and 240 000 of new HIV infections was estimated in this region (3).

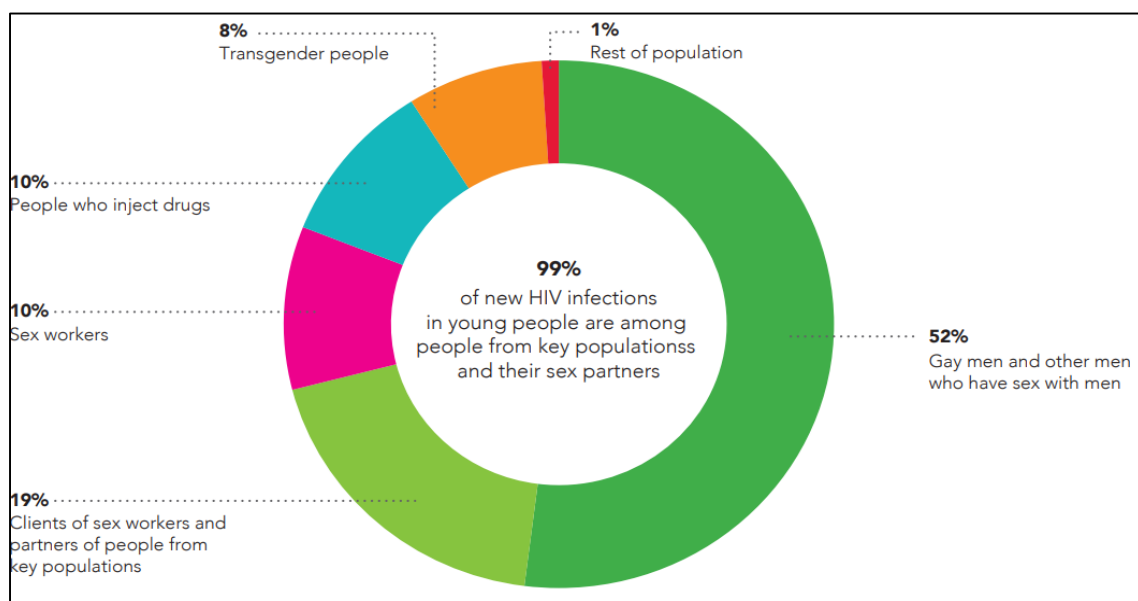
Figure 1: global estimation of adults and children living with HIV, 2021(2)



While HIV incidence is declining overall, it is increasing in some countries and regions. Globally, in 2021, it was estimated about 4 000 of adults and children were newly infected a day and about 3 600 were among adults aged 15 years and older, of

whom almost 48% were pregnant women, about 31% were among young people aged 15-24 years (2). In Asia and the Pacific region, UNAIDS data showed that new HIV infections was rising where they had been falling over the past 10 years (4). In some countries of this region, almost half of new infections are among young people (3). In the region overall, the HIV epidemic is even more concentrated among people from key populations and their sex partners, who account for 99% of new HIV infections in people aged 15–24 years. More than half of new HIV infections (52%) in young people were among gay men and other men who have sex with men, while people who inject drugs, female sex workers and transgender people together accounted for almost 30% of new infections. Clients of sex workers and sex partners of people from key populations accounted for almost 20% of new infections (3) (Figure 2).

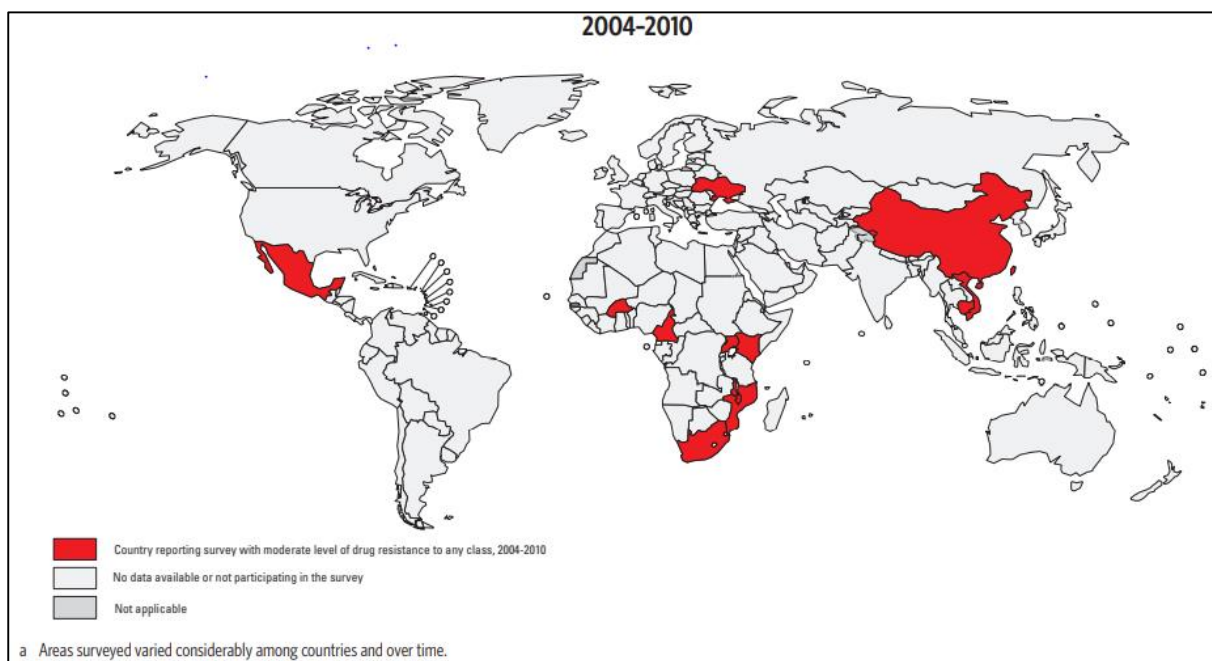
Figure 2: Distribution of new HIV infections among young people in the Asia and Pacific region by key population, 2020 (3).



Since a WHO-recommended public health approach to antiretroviral therapy (ART) has been widely implemented in resource-limited countries to response to the global HIV epidemic, globally, the antiretroviral therapy coverage was improving as 75% of people living with HIV were accessing ART at the end of 2021, up from 25% in

2010 (5). Despite the increased access to effective ART, the emergence and transmission of HIV drug resistance is inevitable among people on treatment even when appropriate antiretroviral therapy was provided and high levels of adherence were achieved (6). According to the 2012 WHO HIV drug resistance report (7), of 72 WHO surveys of transmitted drug resistance conducted between 2004 and 2010, 20 (28%) were classified as having moderate (between 5% and 15%) prevalence of resistance (Figure 3) (8). The proportion of surveyed areas reporting moderate levels of transmitted drug resistance increased from 18% in 2004-2006 to 32% in 2007-2010 and an increasing trend in prevalence of nonnucleoside reverse transcriptase inhibitor (NNRTIs) and protease inhibitor (PI) resistance were observed ranging from 5% in 2004-2006 to 22% in 2007-2010 and 0% in 2004-2006 to 4% in 2007-2010, respectively.

Figure 3: Geographical distribution of WHO surveys with moderate (between 5% and 15%) levels of drug resistance to any drug class^a (8)



1.2 Determinants of HIV drug resistance

Bertagnolio et al reported that there were four potential categories of factors contributing to the selection of HIV drug resistance: regimen and drug-specific, virus-

related, patient-specific and programmatic (9).

- Regimen and drug-specific factors included the choice of regimen used (NNRTI-based regimens vs regimens using boosted protease inhibitors (bPI) in place of non-nucleosides), suboptimal regimens such as single-dose nevirapine for preventing mother-to-child transmission and inappropriate prescribing practices, interactions between drugs and the use of complex regimens with a high pill burden were increased the risk of developing HIV drug emergence (7).
- Virus factors: findings from previous studies showed that pre-treatment HIV drug resistance was strongly associated with virological failure and further acquisition of resistance after the first year of NNRTI-based first-line antiretroviral therapy and individuals with transmitted HIV drug resistance were expected to accumulate more NRTI resistance at the time of virological failure, leading to a growing number being treated with a boosted PI and two NRTI with partial or no activity at the time of switch to second-line therapy (7).
- Patient-specific: it was well recognized that adherence to antiretroviral therapy was an essential component of individual and programmatic treatment success. Poor adherence to antiretroviral therapy was found as a predictor of virological failure emergence of HIV drug resistance, disease progression and death (7).
- Programmatic factors: programme-level factors, such as limited human resources, inadequate infrastructure and weak supply management systems, was also negatively affect treatment adherence and retention in care and facilitate the emergence of population-level HIV drug resistance (7).

It is expected that the number of patients failing to respond to current first- and second-line treatment has also increased worldwide, most frequently in the low- and middle-income countries (LMICs) where the access to new effective ART is not available yet. However, in these countries, access to viral load monitoring and the ability to detect treatment failure accurately is limited which may challenge these countries to

make decisions on treatment modification. In addition, due to restricted guideline for the use of genotyping test in these countries, there was limited data on HIV drug resistance using as the basis for selecting future first-line treatment regimens and identifying the most effective second- and third-line therapies for patients failing first- and second-line combinations. All these limitations had challenged the national programmes to perform routine surveillance of transmitted and acquired HIV drug resistance to optimize programme planning and management and to inform antiretroviral therapy policy as recommended WHO.

The availability of a limited number of first-, second- and third-line drugs is also an issue for those countries for proper treatment management of the patients to achieve a better treatment outcome. Many countries were reluctant to implement third-line regimens at the national level because of limited availability and high prices of the drugs. Low market volume and the need for new lab tests, such as genotyping tests and new policies, were also significant national barriers. However, the WHO made the assumption that the number of people on third-line treatment will increase to more than half a million by 2025 (10).

In the countries where there was a need for third-line treatment while expanding access to first-line therapy, WHO suggested that the national programmes should develop policies for third-line therapy that consider funding, sustainability and the provision of equitable access to ART (11). However, there was limited evidence to guide third-line strategies in resource-limited settings, where there were few studies of newer agents (11); no study on these new treatments has been conducted yet in Cambodia.

Since the availability of ART in the public programme in 2002, the Cambodian HIV cohort is maturing, and the national programme has been facing the management of patients failing to respond to first- and second-line treatment. As part of improvement

of the treatment outcome of the program to maintain the 3rd 95s target¹, there was a need for the Cambodian national program to develop a policy for third-line strategies for the management of patients failing to respond to first- and second-line regimens in Cambodia.

1.3 Global epidemiology of hepatitis C virus infection

Regarding HCV infection, Hepatitis C virus (HCV) infection is a major public health concern, with an estimated 130–150 million chronic hepatitis C virus infection worldwide (12) and 350 000 deaths each year due to hepatitis C-related causes, mostly cirrhosis and hepatocellular carcinoma (HCC) (13). It is estimated that there were 3.2 million adolescents and children with chronic hepatitis C infection (14).

About 30% of infected persons spontaneously clear the virus within 6 months of infection without any treatment and the remaining 70% of persons will develop chronic HCV infection (14). Of those with chronic HCV infection, the risk of cirrhosis ranges from 15% to 30% within 20 years (14). The prevalence of HCV varies substantially around the world. WHO reported that HCV occurs in all regions and the distributions of the infection varied across the geographical regions. The estimated global prevalence of HCV is 2-3% (15) and approximately 8% of pregnant women have HCV infection (12), 6.2% of people living with HIV have serological evidence of past or present HCV infection (14), with the highest prevalence in low- and middle-income countries (LMICs) (16). The highest burden of disease is in the Eastern Mediterranean Region and European Region, with 12 million people chronically infected in each region. In the South-East Asia Region and the Western Pacific Region, an estimated 10 million people in each region are chronically infected. Nine million people are chronically infected in the African Region and 5 million the Region of the Americas

¹ 95% of people living with HIV who are on treatment with suppressed viral loads.

(14). Globally, about 2.9 million people living with HIV/AIDS (PLHIV) are co-infected with hepatitis C virus (12). The major cause of morbidity and mortality among people co-infected with HIV and viral hepatitis is potentially related to liver diseases (17). It is recommended that this population should be prioritized for screening and providing with an appropriate and effective treatment for both HIV and hepatitis.

In 2016, the World Health Organization (WHO) Global Health Sector Strategy for Viral Hepatitis calls for the elimination of viral hepatitis by 2030, aiming at a 90% reduction in incidence and a 65% reduction in mortality. To reach this target, 90% of chronic hepatitis cases need to be diagnosed, and 80% of eligible cases treated (12).

HCV treatment with direct-acting antivirals (DAA) can cure more than 95% of persons with hepatitis C infection, but access to diagnosis and treatment is low. Data show that 9.4 million people are receiving treatment to cure chronic hepatitis C virus infection, an almost 10-fold increase from the baseline of 1 million at the end of 2015 (18). Progress in scaling up the treatment has been sufficient to reverse the trend of increasing mortality from hepatitis C for the first time. However, globally, the number of people dying from hepatitis C still remains daunting at 1.1 million per year (18). The global response to hepatitis C virus was gaining momentum, but access to testing and treatment remained far below needs, and mortality was increasing.

Although several countries have been made good progress in moving towards reaching the 2030 90% testing and 80% treatment coverage targets for HCV elimination, there remains a major testing and treatment gap. In 2019, still only 21% of the 58 million persons with chronic HCV infection had been diagnosed, and 13% treated (18). Achieving the global hepatitis goals for HCV elimination by 2030 will require a radical simplification of care pathways to overcome barriers in access to HCV testing and treatment.

The presentation of this thesis project is structured into 4 chapters following the

structure of the scientific report, which include the introduction, Materials and Methods, Results, Discussion and perspectives. The first chapter of the introduction will be dedicated to a general presentation of HIV and HCV in the global contexts and in Cambodian contexts, the thesis justification and the objectives of the thesis. The second chapter will present the material and methods used for the proposed studies of the thesis. As my thesis project consisted of two studies, in this section, I organized the presentation of the methods used by studies: first, I started with the methods of study 1 on the burden of HCV infection in Cambodia, followed by the methods of study 2: the ANRS 12374 3DICAM study. Then, the third chapter will be the section results. In this section, I follow the same organization flow as in the methods section, in which I will present the results of study 1 on the burden of HCV infection in Cambodia and then the results of the ANRS 12374 3DICAM study. I will finish by a general discussion of the findings of the 2 studies presented in the thesis in the fourth chapter and perspectives for future research on the management of HCV infection and on the management of HIV patients treated with a third-line regimen in Cambodia.

1.4 HIV and HCV context in Cambodia

1.4.1 HIV Epidemiological data

In Cambodia, the first case of HIV was reported in 1991 (19). In the 1990s, transmission of HIV substantially occurred among the general population (20) and was rapidly spreading, reaching the highest HIV prevalence rate (2.8%) among the adult population (15–49 years) in the Asian region in 2000 (19). The sharp increase in infection rates in the 1990s was largely due to a blooming sex industry between 1995 and 1998 (21). In Cambodia, heterosexual intercourse was the predominant means of transmission among other principal means of HIV transmission, such as sexual intercourse between persons of same sex, blood transfusion, reuse of needles for injections, and parent-to-child transmission (19).

Cambodia has successfully decreased HIV incidence and prevalence among the general population. As a result of an effective preventive program consisting of STI care services and the 100% condom use program, a multisector approach that involved local authorities, health workers, police, brothel owners, sex workers and outreach and peer educators, as well as a rapid increase in access to antiretroviral treatment (ART) since 2003, the prevalence of HIV infection among the general population aged 15–49 years declined from 2.8% in 2000 (19) to 0.6% in 2015 (22) and the annual number of new infections has dropped 20-fold (23). However, the prevalence remains high among key populations. Based on the latest Integrated Biological and Behavioral Survey, the prevalence was 3.2% in 2016 among entertainment workers (24), 5.7% in 2017 among people who use drugs (PWUDs) (25), 15.2% in 2017 among people who inject drugs (PWIDs) (25), 4.0% in 2019 among men who have sex with men (MSM) (26).

1.4.2 HIV testing and treatment cascade

In 2014, an ambitious treatment “90-90-90” target to fast-track the global fight against HIV/AIDS by 2020 was announced by the Joint United Nations Program on HIV/AIDS. Of these three targets, 90% of all people living with HIV (PLHIV) should know

their HIV status, 90% of all people diagnosed with HIV should receive sustained antiretroviral therapy (ART), and 90% of all people on ART should achieve viral suppression (27). Due to the political leadership and commitment of the government of Cambodia which has made ending AIDS a top public health priority, the HIV services were decentralized and were available even in remote rural areas as since 2016 and the country adopted a strategy to treat everyone as soon as they are diagnosed with HIV. As a result, in 2017, Cambodia was reported as the country which had the highest HIV treatment coverage in Asia and the Pacific. Cambodia reached this global 90-90-90 target three years before the world's deadline and was reported by the UNAIDS in 2017 as one of only seven countries worldwide to have already achieved the 90-90-90 targets (28). It was a huge achievement for a country which had one of the fastest-growing AIDS epidemics in the region 20 years ago.

One of key successful elements of HIV response, Cambodia has developed and published the first HIV clinical guidelines during the launch of Continuum of Care in 2003 to ensure high quality HIV/AIDS care and treatment. The guidelines were revised in November 2007, 2012, 2015 and 2019 as the Anti-retroviral therapy sites expanded and ensure the clinical knowledge and quality treatment are up to date to the intentional scientific standard align with 2019 WHO recommendations and other resources on the use of Antiretroviral Drugs for Treating and Preventing HIV Infection.

As of end 2020, a new ambitious targets were set by UNAIDS calling for 95% of all people living with HIV to know their HIV status, 95% of all people with diagnosed HIV infection to receive sustained antiretroviral therapy, and 95% of all people receiving antiretroviral therapy to have viral suppression by 2025.

After achieved the 90-90-90 targets in 2017, Cambodia announced its intent to further control the HIV epidemic by achieving the UNAIDS 95-95-95 targets (95% of those infected know their status; 95% of those HIV positive enrolled in ART; 95% of those on treatment are virally suppressed at 12 months) and moving towards the

elimination of new HIV infection by 2025 (29). These targets, combined with ambitious primary prevention targets and focused attention to supporting enablers, aim to bridge inequalities in treatment coverage and outcomes and accelerate HIV incidence reductions by focusing on progress in all sub-populations, age groups and geographic settings.

1.4.3 Evaluation of first, second-line and third-line regimen in Cambodia

In Cambodia, the first-line regimen was available in late 2002 through Médecins Sans Frontières (MSF) HIV program activities located in the Capital city of Phnom Penh. Access to ART treatment was progressively scaled up, and the Voluntary Confidential Counselling and Testing (VCCT) and ART site were extended to other provinces across the country. As of the end of 2020, there were 71 VCCT sites and 71 ART sites (Pediatric = 41) across Cambodia's 25 provinces (30).

The national guidelines were revised in October 2015, and treatment was recommended for all patients regardless of the CD4 cell count. The recommended first line was TDF/3TC/EFV, and the recommended second line was AZT/3TC/ATVr. A first viral load is performed at 6 months, 12 months and then yearly. If CD4 > 350/mm³, national guidelines recommend using only viral load monitoring with no need for ongoing routine CD4 assays. In patients with virological failure, an adherence boost for 3 months and viral load control at 3 months must be performed before switching to a second-line regimen. A viral load scale-up strategy was also defined in 2015, including dissemination of the new guidelines, strengthening the capacities of the laboratories and implementation of a standard operating procedure. For patients with virological failure, genotypic antiretroviral resistance testing (GART) is performed at the Institute Pasteur of Cambodia in partnership with the National Center for HIV/AIDS, Dermatology and STD (NCHADS) laboratory.

In 2020, there were an estimated 75 000 people living with HIV in Cambodia; 63 000 (84%) knew their status, 62 000 (83%) received ART, and 60 000 (81%) had viral

load suppression (31). Despite high coverage of ART in Cambodia, the number of patients with treatment failures and drug resistance has increased due to several factors, such as long-term exposure to suboptimal treatment and suboptimal adherence. Identifying the patients in failure of the treatment and immediately switching to the most effective treatment is necessary for ART program success.

Several studies have been conducted in Cambodia to assess the effectiveness of WHO-recommended first line regimen. The results reported good outcomes in adults (1-3) and children (32) and a high level of adherence (33, 34). In 2013, the national program conducted a nationwide cross-sectional study (the ANRS 12276 2PICAM study) to assess the virological effectiveness of PI-based second line regimen in Cambodia as well as the impact of adherence boosting in patients experiencing virological escape. The study enrolled 1317 adults on PI-based second-line regimen for ≥ 6 months at 13 ART sites. In this study, a genotype resistance test was used to confirm virological failure. This study reported a high rate of virological suppression with 90% (1182/1317) of patients exhibiting virological success (< 250 copies/mL) at inclusion. The findings from this study also showed that 3 months of boosted adherence counselling among the patients with detectable VL improved the treatment outcome as almost half of these patients could be rescued (50%, 63/135) of those with a detectable VL could be rescued). In this study, 5.5% (72/1317) of patients on the PI-based regimen had confirmed virological failure after 3 months of boosted adherence and 22.2% (12/54) of patients with confirmed virological failure had resistance to ATV/r and needed to switch to third-line regimen. The findings from this study have been used by the national program as evidence-based information to adapt HIV management guidelines. In 2016, a technical working group was developed to help clinicians to manage patients on PI-based second-line regimens with a viral load above 1000 copies/mL and to improve the quality of adherence support. In 2017, a framework for adherence counselling was finalized. Guidance to enhance ART adherence was developed. This guidance note details the process of each counselling session for

patients suspected of clinical and/or immunological failure (detectable viral load \geq 40 copies/mL). Sessions of 30 minutes of counselling must be done one to one (patient and counsellor) every month for three consecutive months to explore the barriers to adherence, to find solutions to improve adherence, and to monitor the adherence progress of each enhanced adherence counselling session using the Enhanced Adherence Counselling Form. The study also suggested that HIV genotyping resistance tests appeared necessary to guide decisions for switching to a third-line regimen. The findings from the 2PICAM study forecasted the need for alternative second- and third-line regimens. However, there were limited available evidence to guide third-line strategies in resource-limited settings, including Cambodia. The WHO recommends that national programs should develop their policies for third-line therapy that consider funding, sustainability and provision of equitable access to ART.

1.4.4 Adherence counselling and resistance-associated mutations (RAMs) to second-line regimen

The control of adherence is a critical factor of ART success and WHO recommends adherence support intervention prior to deciding any change of ART regimen. In addition to 2PICAM study, similar results of adherence boosting strategy were reported in India where 34% of PI-based regimen patients with virological failure had a suppressed VL after 6 months (35) and in South Africa where 64% of PI-based regimen patients were rescued (36). Of note, in the 2PICAM study, among the 72 patients who could not be rescued by Enhanced Adherence Counselling (EAC) and had confirmed VL failure, 21 patients presented no resistance to PI or NRTI drugs and should have been rescued if adherence counselling was fully effective. This suggests that adherence-counselling interventions still have room for improvement to be fully effective. This study also suggested that genotypic antiretroviral resistance testing seems necessary to detect the patients with second-line treatment failure and in need to switch to the third-line. The main observed RAMs in the 2PICAM study were M184I/V, T215F/Y, M41L and D67N for RT and M46I/L and V82A for protease. These

protease mutations were similar to those reported in Nigeria (36) and Vietnam (37) which confirmed high level of resistance to LPV and ATV with a risk of resistance increase overtime and worsening outcomes. Cross-resistance between ATV/r and LPV/r was reported in Cambodia (38) , Vietnam (37) and India (39). LPV/r is currently recommended as an alternative option in the national guidelines. Prevalence of dyslipidemia was reported to be high in LPV-treated Cambodian patients with a 10-year risk of coronary heart disease ranging from 8% to 24% according to the score used (40). For these reasons, DRV/r, which presents a favourable lipid profile in clinical studies, seems to be the best option for patients with HIV-1 resistant to ATV/r whatever the sensitivity to LPV/r. Among the 72 patients with virological failure in the 2PICAM study, 12 were sensitive to ATV/r but resistant to all NRTIs. For these patients, a PI/r monotherapy could not be recommended as the viral load may not be fully suppressed; furthermore, this strategy showed a high risk of viral load elevation even for patients suppressed at baseline (41). Association of PI/r and integrase inhibitor seems, in that case, an interesting option (11) as reported by WHO even if data are still missing to conclude to a recommendation.

1.4.5 DRV/r and integrase inhibitor combination

New agent belonging to the integrase strand transfer inhibitors [INSTI] class such as dolutegravir (DTG) and darunavir (DRV/r) in combination with one or two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) were recently recommend as first- and second-line ART for PLHIV.

In the SPRING-2 study, once-daily dolutegravir was non-inferior to twice-daily raltegravir in treatment-naive, HIV-infected patients after 96 weeks (49). Effectiveness and safety seem similar but DTG has a higher genetic barrier to resistance compared to the others INSTIs (50). Its pharmacokinetic characteristics allow once-daily administration, without boosting and with a low grade of drug–drug interactions. For all these reasons, DTG was recommended by WHO as an alternative for first-line

regimen.

DRV/r was mainly evaluated in combination with raltegravir (RAL). This combination, using as a first-line regimen in the NEAT 001/ANRS 143 trial, was reported as non-inferior to a standard tenofovir/emtricitabine plus DRV/r regimen (10) but the cumulative risk of selection of integrase mutations was higher after 96 weeks in the DRV/r + RAL arm compared to the standard arm (42). The same combination, used as a simplification among patients on suppressive ART, was proved to be effective to maintain virological suppression (43).

For patients infected with multidrug-resistant virus with few remaining treatment options, the TRIO study showed high rates of virological suppression (90%) and favorable immunological outcomes at 48 weeks with a regimen containing RAL, etravirine, and DRV/r (44). The resistance-associated mutations were low for the 14 patients experiencing virological failure (45). In India, MSF reported favorable outcomes with the combination DRV/r + RAL in a small group of patients failing a second-line regimen after boosted-adherence counselling (46).

The DRV/r + DTG combination has been less studied. Effectiveness of a salvage therapy with DRV/r and DTG in highly ART-experienced subjects was reported in Italy (47) with a proportion of viremic patients declining from baseline to week 24 from 43.4% to 6.2% and in France in a retrospective(48). While developing the 3DICAM study, evaluation of this combination for a second-line regimen compared to current recommendation is ongoing within an international randomized-controlled study conducted by the Kirby Institute in Australia (NCT03017872), to our knowledge, no study evaluates this combination for a third-line regimen.

The place of NRTI in third-line regimen continued to be discussed. NRTI-sparing and NRTI-containing regimens seem to be comparable in terms of virological suppression. Adverse events and treatment discontinuation seem to be lower with

NRTI-sparing regimen but the difference was not statistically significant and WHO conclude to « prefer NRTI-containing regimen for HBsAg positive patients and discuss case by case for other patients according to GART and comorbidities ». The MOBIDIP study conducted in sub-Saharan Africa reported that maintenance therapy with boosted protease inhibitor plus lamivudine was associated with a high rate of success than boosted protease inhibitor monotherapy, despite the presence of M184V mutations at first-line treatment failure (51).

1.4.6 Pharmacokinetic (PK) characteristics of DRV/r and DTG

PK characteristics of both drugs administered with 2 nucleos(t)ide analogs are well established either in healthy volunteers or HIV-infected patients. Main PK parameters assessed in HIV infected patients are summarized in the table 1 (52-54).

Table 1: Pharmacokinetic Parameter of darunavir and dolutegravir in HIV infected patients

Pharmacokinetic Parameter	Ritonavir boosted darunavir (DRV/r)		Dolutegravir (DTG)
	600/100 mg BID	800/100 mg QD	50 mg QD
C _{max} – µg/mL	8.14	5.47	3.34
T _{max} - h	2.5 - 4		2
Food effect	Yes ↗absorption by 40%	Yes ↗absorption by 50%	
C _{min} or C _{trough} – µg/mL	3.2	2.04	0.83
AUC – µg.h/mL	54	88	43
T _{1/2} - h	15-17		11-12
Protein binding-%	92% (albumin and 1glycoprotein)		>99% (albumin and 1glycoprotein)
Elimination	Mainly biotransformation		
Metabolic pathways	CYP3A		UGT1A1 (main) CYP3A
Transporters	DRV substrate of Pgp;		Substrate of Pgp, BCRP

In brief, darunavir trough concentration when administered on a BID dosing was several folds higher than the protein binding-corrected EC₅₀ value for wild-type virus (0.055µg/mL). As expected C_{trough} was lower on QD dosing than on BID dosing but the average concentration, was high enough for wild-type virus suppression and for

sustained efficacy for patients with undetectable viral load. Interestingly, some PK parameters of DRV/r and DTG in HIV-infected patients were close supporting coadministration of both drugs: rate of absorption was rapid with a time to C_{max} (T_{max}) around 2h and half-lives in the same range. Although based on different metabolic pathways potent drug-drug interaction was not expected, ritonavir was known to induce UGT1A1 and could lower DTG concentrations. There was a study conducted in healthy volunteers which assessed the effect of DRV/RTV 600/100 mg BID on DTG concentrations when administered at a 30 mg QD dosing for 5 days (55). A decrease in DTG concentrations was observed as indicated by the geometric mean ratio of AUC_t, C_{max} and C_{trough} of DTG administered with DRV/r versus alone and (90% confidence interval) which were 0.78 (0.72, 0.85), 0.89 (0.83, 0.97), 0.62 (0.56,0.69), respectively. Although there was a 40% decrease in DTG trough concentrations, the geometric mean concentration of DTG when coadministered with DRV/r was 0.45 µg/mL, which were higher than the protein adjusted 90% inhibitory concentration (0.064 µg/mL). Indeed, this study was conducted with a 30 mg DTG dosing, lower than the 50 mg recommended dosing which was used in the proposed 3DICAM study. Although these data were reassuring, there was no data in “real life” situation neither in Caucasian nor Asian HIV-infected patients where the potency of the drug-drug interaction could be altered.

1.5 HCV situation in Cambodia: epidemiology, prevention, treatment and care of viral hepatitis C in the health care system in Cambodia

In Cambodia, no national program for hepatitis has been implemented to date, however, several measures contributing to mitigate the transmission of hepatitis have been implemented by the Cambodian government since the 1990s, such as a systematic screening of blood donors for HCV testing in 1996, generalization of single-use syringes (public and private sectors) in 2000 and the infection control policy/Hand Hygiene MOH-WHO in 2010. Nevertheless, apart from small cohorts, the screening of hepatitis is mainly an individual initiative. Still in 2022, systematic screening of HCV is

not performed, even for pregnant women, and few patients with chronic HCV infections are aware of their status.

In 2016, at the beginning of the thesis project, there were no available data on the burden and epidemiologic characteristics of hepatitis C at a national level. The available HCV prevalence data were limited and restricted to selective areas (the northwestern Cambodia and a hospital-based in the Capital City) and the epidemiology of HCV in Cambodia remained uncertain. The prevalence data among people living with HIV/AIDS were reported with a wide variation estimation ranging from 7.6% (56) to 78.5% (57). In Cambodia, the HCV prevalence was high in people who inject drugs (30.6%) (58) while a low prevalence was found among young adults aged less than 45 years old (0.6%) (59). However, this data is restricted to Battambang area. No data on the HCV prevalence among pregnant women was available in Cambodia. Due to the lack of appropriate laboratories and high cost of HCV testing and treatment, screening for HCV has not been routinely provided in health care settings including ANC clinics to prevent vertical transmission.

As of 2016, viral hepatitis management and care was considered a priority in Cambodia. Access to diagnostic tool-adapted and effective therapeutic protocols has become a reality in the public and private sectors. As HCV treatment with direct-acting antivirals (DAA) is generally curative and success rates in LMICs are similar to those in high-income countries (HICs), identifying people who are in need for treatment and providing access to effective treatment and care for HCV infection was a crucial step toward HCV elimination in Cambodia. In this situation, the need for epidemiology and disease burden information was highlighted as the first step to promote future analysis and to guide future guidelines. In particular, serological surveys could improve the understanding of the distribution of HCV infection in the general population and identify specific high-risk groups in need of treatments.

In the same year (in 2016), free access to direct-acting antiviral agents (DAA) for

HCV monoinfected patients and HIV/HCV coinfecting patients was initiated by Médecins sans Frontières (MSF) in the 3 main hospitals of Phnom-Penh (Calmette, Khmer-Soviet friendship and Kossamak hospitals, then expanded to Northwest Cambodia in 2018) (59). Following the initiation of MSF France program activities, in 2018, the first working group for the management of HCV infection was developed by the Ministry of Health (MoH) in collaboration with MSF France and other collaborative partners in the country to work on the development of clinical management guidelines and the development of the national strategic plan for HCV elimination in Cambodia by 2030. This technical working group was coordinated by the department of Communicable Disease Control (CDC) in Cambodia under the Ministry of Health.

In Cambodia, addressing HCV infection in PLHIV could possibly be done through expanding access to treatment for hepatitis C for people with HIV who are co-infected with HCV in the existing National HIV program. However, the epidemiology of hepatitis C remains uncertain, and prevalence data are limited with a wide variation in reported estimations among this population ranging from 7.6% (56) to 78.5% (57) and the data restricted in selective areas. The most at-risk population seems to be patients who have household member with liver disease, aged over 40 years old, possibly because of historical exposures to unsafe medical injections or transfusions (56, 57, 60)

Previous data showed that in Cambodia, the HCV prevalence was high in people who inject drugs (30.6%) (58) and low prevalence were found among young adult aged less than 45 years old (0.6%) (59). However, no data on the HCV prevalence among pregnant women are available in Cambodia. Due to the lack of appropriate laboratories and high cost of HCV testing and treatment, screening for HCV has not been routinely provided in health care settings including ANC clinics to prevent vertical transmission.

1.6 Thesis justification

In Cambodia, the knowledge on epidemiology of hepatitis C is essential for prioritizing the required intervention to achieve the World Health Organization goal

for elimination by 2030. Despite the progress toward establishing a public program for HCV, resources for full elimination are yet to be materialized to meet WHO targets of eliminating HCV as a public health threat by 2030 in Cambodia. Although several studies on HCV burden in Cambodia had been conducted in the past, there was at the beginning of this thesis project a paucity of evidence among pregnant women and among people living with HIV, when available, the epidemiological data were restricted to specific regions of Cambodia (North-western and Capital city). The hepatitis C burden may vary geographically across regions in the country and among subgroups of population. To fully understand the extent of viral hepatitis burden and to strengthen the evidence for proper and adequate policy development as well as resource mobilization to address this infection, we undertook a study to estimate HCV burden and transmission risk factors in four provinces among pregnant women (considered as a proxy for general population) attending antenatal care (ANC) and among people living with HIV in Cambodia. These four provinces were selected for the present study for assessing the HCV prevalence rates as they, together, were considered a good proxy for the general population of Cambodia. This HCV prevalence study was implemented in 2016 when epidemiological data of HCV infection in Cambodia was not available yet for general population as well as for people living with HIV.

Regarding HIV, WHO recommends the development of policies by national programs for third-line ART that should include new drugs with minimal risk of cross-resistance to previous used regimens such as integrase inhibitors or second generation non-nucleotide reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). The available data from high-income countries support the use of ritonavir-boosted darunavir, etravirine, dolutegravir and raltegravir containing regimens in treatment-experienced adults. However, to date, there was no clear recommendation on the third-line therapy yet due to limited available data on the effectiveness of the combination of these new drugs using as third-line treatment. Cambodia needs the data on

the effectiveness of the combination of these new ART treatments to guide the development of guideline for third-line program in Cambodia. In this context, the ANRS 12384 3DICAM study was implemented to evaluate the efficacy and safety of a third-line cART regimen combining dolutegravir (DTG), ritonavir-boosted darunavir (DRV/r) and optimised NRTI in Cambodian HIV-infected treatment-experienced volunteers with genotypic resistance to ATV and/or all NRTIs.

1.7 General objectives of the thesis

The initial thesis project has been developed with the objective to conduct a programmatic evaluation of a third-line regimen implementation containing darunavir/r, dolutegravir and optimized NRTI in Cambodia based on the design of the ANRS 12374 3DICAM study which included two additional sub-studies: The Pharmacokinetic (PK) study of DRV/r and DTG and the Cost analysis on third-line implementation in Cambodia (detail protocol in annex 1). Due to different delays, including the ongoing COVID-19 pandemic which has impacted on the progress of this thesis project, the costing analysis of the ANRS 12374 3DICAM study will not be presented in this thesis project as the data is not available yet. Therefore, the aims of my thesis project were revised and the current thesis project objectives are as follows:

1. To assess the prevalence of HCV infection in four provinces in pregnant women and in HIV-infected adults in Cambodia.
2. To implement and to conduct a programmatic evaluation of a third-line regimen containing darunavir/r, dolutegravir and optimized NRTI in Cambodia based on the design of the ANRS 12374 3DICAM study.
 - 2.1. To assess the 6-month and 12-month clinical and virological outcomes of a third-line regimen combining DRV/r, DTG and optimized NRTI in Cambodian HIV-infected adults.
 - 2.2. To study pharmacokinetic (PK) parameters of DRV/r and DTG.

2 MATERIALS & METHODS

This thesis is part of a general research program aimed at improving the control of HIV and HCV infections in Cambodia. My thesis project aims to assess the magnitude of HCV infection in Cambodia and to implement and assess a third-line program in Cambodia, for better management of both HIV and HCV infections.

Data used for the analysis and publications in this thesis consisted of two studies: the data of an HCV prevalence study funded by the University of Health Sciences and its partner to assess the burden of HCV infection in Cambodia (Objective 1 of the thesis) and the ANRS 12374 3DICAM study (Objective 2.1 of the thesis) and its sub-study pharmacokinetic (PK) parameters of DRV/r and DTG that has been funded by the National Agency for HIV/AIDS Research (ANRS) (Objective 2.2 of the thesis).

2.1 Study 1: the Burden of HCV infection in Cambodia

2.1.1 Study design

This study was an integrated bio-behavioral (cross-sectional) survey undertaken among PLHIV receiving HIV care at the ART clinics and pregnant women attending public ANC clinics in four diverse geographical areas the Cambodia. The study objectives were:

1. To assess the prevalence of hepatitis C and its subtypes pregnant women and PLHIV
2. To determine the knowledge on risk factors, transmission and attitude differences between these two groups
3. To identify risk factors associated with hepatitis C infection in these populations.
4. To document the different practices on care and treatment of hepatitis C in Cambodia

2.1.2 Study settings

This study was carried out between March and April 2016 in antiretroviral therapy (ART) sites for PLHIV and in ANC clinics for pregnant women, located in four diverse geographical areas, including the capital city of Phnom Penh and three provinces in the West (Battambang), the Northwest (Siem Reap) and the Southwest regions (Preah Sihanouk) of Cambodia.

2.1.3 Eligibility criteria

Individuals were eligible for the survey if they were at least 18 years old and either living with HIV/AIDS and had visited the ART clinics or pregnant women who visited the public ANC clinics during the study period.

2.1.4 Sample size, sampling and recruitment

We used Open Epi software version 3 to calculate the sample size. Assuming the prevalence of HCV is 5% among pregnant women and 10% among PLHIV, to achieve a 2% margin of error for the pregnant women and 3% for PLHIV, power fixed at 80%, at 5% alpha-level, a design effect of 1, and a non-response rate of 10%, 502 participants were required for pregnant women and 423 for PLHIV.

A two-stage cluster sampling method was employed to select the eligible participants. Firstly, the probability-proportional-to-size-sampling method was used to select 51 clusters (34/153 clusters for pregnant women and 17/21 clusters for PLHIV) from the selected geographical regions. A cluster was a health center for pregnant women and an ART clinic for PLHIV. Secondly, a consecutive sampling strategy was used to invite 15 pregnant women and 25 PLHIV from each cluster. Each participant was informed individually by the research team of the purposes and scope of the study, procedures involved, the expected duration, the potential risks and benefits involved, and any potential discomfort it may entail and signed the consent form if they agreed to participate in the study. The participants underwent face-to-face interviews using a

structured questionnaire, following by a blood sample collection for HCV testing. Prior to data collection, all interviewers attended a training course to ensure they understood and followed standard procedures. The participants were only identified by a unique anonymous code (no identifiable information was collected). The code was used to link each participant to their sociodemographic information, their HCV results and their appointment time when they were back to the clinics to get their HCV results. The questionnaire was checked for completeness, accuracy and legibility, and validated questionnaires were sent to the University of Health Sciences (UHS) for data entry.

2.1.5 Behavioral questionnaire

The questionnaire was used to collect information on socio-demographic characteristics of participants such as age, sex, marital status, occupation and education. Multiple-choice questions were used to assess participants' knowledge, attitudes and practices related to HCV infection. The knowledge questions explored four broad dimensions: basic information about HCV infection, mode of transmission, vaccination and treatment availability. Information on potential risk factors of HCV infection included history of intramuscular and intravenous injections, blood transfusion, and cases of HCV in the household. For each question, participants were offered three response options: "No", "Yes" and "Don't know".

2.1.6 Laboratory testing

A 5 ml blood sample was drawn from participants for HCV testing. Samples were kept at room temperature (18°C – 25°C) for a maximum of 6 hours and then sent to the UHS laboratory in Phnom Penh. After centrifugation, plasma specimens were stored at -80°C for analysis. HCV antibody testing was done with a third-generation (G3) HCV ELISA assay (MONOLISA ANTI HCV PLUS version 3, Bio-Rad), according to the manufacturer's instructions. Specimens showing a signal-to-cut-off (SCO) ≥ 1 were considered as positive for the anti-HCV antibody. Those with a SCO value < 1 were considered negative. All samples with a SCO ≥ 1 were further tested to confirm chronic

HCV infection with a commercial HCV RNA viral load (VL) assay (OMUNIS, Clapiers, France). All runs were done on Bio-Rad CFX96 real-time PCR machines (Bio-Rad). HCV genotyping was performed in NS5B gene, using an in-house semi-nested RT-PCR and ANRS protocol.

2.1.7 Statistical analyses

The prevalence of HCV infection among pregnant women and among PLHIV were presented as percentages and 95% confidence intervals (CIs), computed using exact methods (for binomial distribution). Categorical variables were presented as frequencies and percentages, while continuous variables were described with means and standard deviation (SD). Comparisons of categorical variables between HCV+ and HCV- participants across different socio-demographic and behavioral risk factors were performed using chi-squared or Fisher's exact tests accordingly. Multivariable logistic regression analysis was performed to identify risk factors associated with HCV antibody positivity. All non-collinear variables with a $P \leq 0.2$ in bivariate analyses or considered to be potential confounders based on previous studies were included in the models. Data were managed and analyzed using STATA 16 (StataCorp, USA), and p -values less than 0.05 were considered statistically significant.

2.1.8 Ethics approval and consent to participate

This study was approved by the National Ethics Committee for Health Research in Cambodia on 22 /09/2015 (338 NECHR) and in compliance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before the interview and the blood sample collection.

2.1.9 Personal contribution to study 1 on the Burden of HCV infection in Cambodia

Briefly, my contribution to this study included proposal development, study implementation as a project manager, data analysis and manuscript writing for the presented article.

2.2 Study 2: The ANRS 12374 3DICAM study

2.2.1 Development and implementation of the ANRS 12374 3DICAM study

WHO recommends that national programs should develop policies for third-line ART and that third-line regimens should include new drugs with minimal risk of cross-resistance to previously used regimens; for patients failing first-line with efavirenz and second-line with protease inhibitor: DRV/r + DTG (or RAL) ± 1-2 NRTIs and NRTI-sparing and NRTI-containing regimen seem to be comparable in term of virologic suppression but data are still limited (11).

The results of the 2PICAM study showed that there was a need for third-line regimen for the patients with confirmed second-line treatment failure in Cambodia (61). However, the standard third-line treatment was not yet available due to limited data on the efficacy of the combination of the new treatments recommended by WHO and pharmacokinetic characteristics of DRV and TDG drugs administered with two nucleoside analogs are well established either in healthy volunteers or HIV-infected volunteers but pharmacokinetic data on both drugs were missing for Asian population.

For that, the national program designed the ANRS 12374 3DICAM study to assess the effectiveness and pharmacokinetics of a third-line regimen combining dolutegravir, ritonavir-boosted darunavir and optimized NRTI in HIV-infected treatment-experienced adults in Cambodia. The results of the ANRS 12374 3DICAM study will be used to adapt HIV management guidelines for third-line treatment in Cambodia.

In 2017, the ANRS 12374 3DICAM study was accepted for financial contribution in the framework of ANRS's 2nd call for 2017. The coordinating investigators of the study were Pr Cécile GOUJARD and Dr Ly Penhsun, a former director of the HIV national program. The list of the study teams will be presented in the annex 3.

2.2.2 Study main hypothesis

- A genotypic resistance test (GART) is warranted for all patients not rescued after enhanced adherence counselling (EAC).
- In the case of intermediate or full resistance to ATV/r, DRV/r is the best option, but pharmacokinetic data are missing for the Asian population
- For patients without HIV-1 resistance to ATV/r but with extensive resistance to all NRTIs, a third-line regimen must be considered.
- DTG is the best option among integrase inhibitors
- Optimized NRTI recycling must be performed with at least lamivudine and one other NRTI if sensitive.
- Switching from DRV 600/100 twice daily to DRV/r 800/100 QD when VL is controlled needs to be evaluated to simplify treatment and improve adherence.

2.2.3 Study objectives

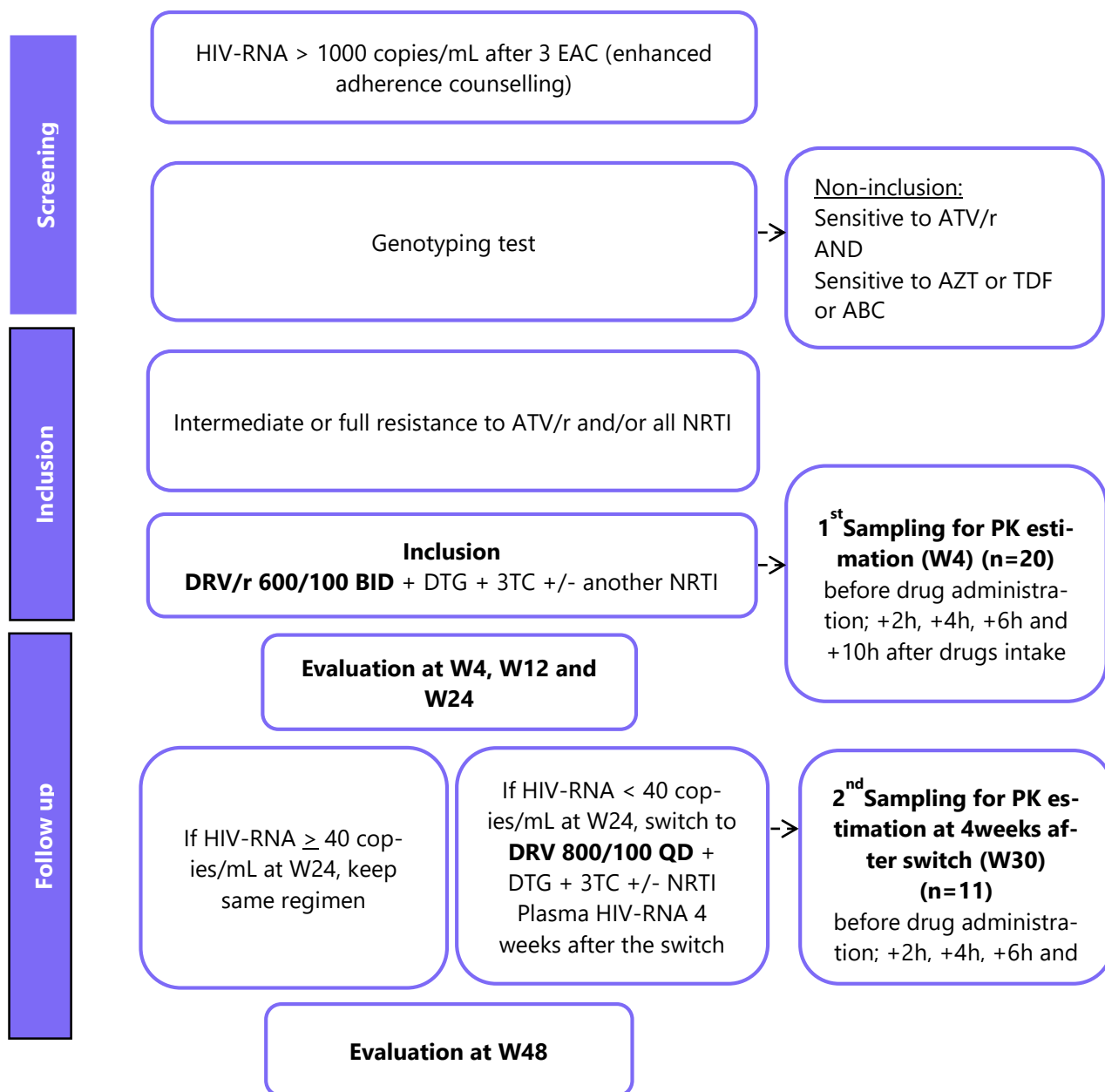
- **Principal objective:** to assess the virological effectiveness of a third-line regimen combining dolutegravir (DTG), ritonavir-boosted darunavir (DRV/r) and optimized NRTI after 6 months of treatment in Cambodian HIV-infected adults who failed a protease inhibitor (PI)-based second-line regimen despite 3 months of enhanced adherence counselling (EAC) with evidence of intermediate or full resistance to atazanavir (ATV) or sensitivity to ATV but resistance to all NRTIs.
- **Secondary objectives**
 - Evaluate the virological effectiveness of a third-line regimen combination at 12 months
 - Evaluate the safety of the third-line regimen combination
 - Evaluate the adherence of third-line regimen combination
 - Evaluate the immune restoration (increase in CD4 cell count) and the frequency of IRIS

- Estimate plasma exposure and pharmacokinetic parameters of DRV/r 600/100 twice daily and DTG 50 mg once daily
- Estimate the pharmacokinetic parameters of DRV/r 800/100 once daily vs DRV/r 600/100 bid in the patients who switched
- Assess the effectiveness of switching DRV/r from 600/100 twice daily to 800/100 once daily for patients with plasma HIV1 RNA < 40 copies/mL at 6 months

2.2.4 Study design

The ANRS 12374 3DICAM study was a one-arm, multicentre, phase II prospective study to conduct a programmatic evaluation of third-line antiretroviral therapy implementation in Cambodia. Cambodian adult HIV-infected treatment-experienced volunteers with treatment failure on second-line ART in 13 ART sites were recruited. A third-line cART regimen combining dolutegravir (DTG), ritonavir-boosted darunavir (DRV/r) and optimised NRTI was proposed to participants and they were followed up for 48 weeks. Figure 4 summarises the study design.

Figure 4: the study design.



2.2.5 Roles and responsibilities of the different collaborative partners for the implementation of study

2.2.5.1 Study coordination

The ANRS 12374 3DICAM study was developed and implemented within the national program in collaboration between several institutions, including the methodology and management center and Laboratory at the faculty of Pharmacy of

the University of Health Sciences (UHS), the HIV/AIDS care unit and Laboratory of the HIV national program, the provincial Referral Hospitals and ART clinic sites, the national maternal and child health program (NMCH) and the Institute Pasteur in Cambodia (IPC). The study was implemented under the coordination of the methodology and management center at UHS in Cambodia.

The ANRS 12374 3DICAM study was designed as a program-integrated study involving different actors within the health care system in Cambodia. The section below details the role of different collaborative institutions in the study.

2.2.5.2 Methodology and Data Management Center at UHS

The Methodology and Data Management Centre, based at the Grant Management Unit of the UHS, was responsible for overall operational coordination, budget management, study procedures development, methodology, SAE reporting to the ANRS Pharmacovigilance Department, data entry, management and analysis and scientific report writing. This team consisted of 4 people, including 1 project manager, 2 clinical research assistants and 1 data manager.

2.2.5.3 INSERM U1018, CESP, le Kremlin-Bicêtre, Villejuif, France

The INSERM U1018 research team included Professor Laurence Meyer and Dr Remonie Seng, the latter being responsible for statistical method analysis in collaboration with the Methodology and Data Management Centre at UHS and contributed to scientific report writing for this study.

Professor Cécile GOJJARD, which is also the coordinating investigator of this study provided support in clinical management of the patients to all site investigators during the study period and also contributed in scientific writing of this study.

Doctor Anne-Marie TABURET contributed to the design of the Pharmacokinetic sub-study of darunavir and dolutegravir and she also contributed and provided

technical support to the Laboratory of the Faculty of Pharmacy at UHS for pharmacokinetic analysis of this sub-study.

2.2.5.4 Laboratory of the Faculty of Pharmacy at UHS

The laboratory, located at the faculty of pharmacy of the UHS, was responsible for the pharmacokinetic substudy and the biobank. Two people were involved in this laboratory work. One is a pharmacokinetic analysis coordinator, and the other is a laboratory assistant.

2.2.5.5 HIV/AIDS care unit at the HIV National Program

This team was responsible for coordinating all clinical activities for the study including recruitment, training and management of the site investigators, organising and supporting clinical patient follow-up on site, supervision of study drug management, coordinating and organizing a monthly meeting of the technical working group and decision support committee for making decisions on patient enrolment and the selection of third-line treatment for patients in collaboration with the Methodology and Data Management Centre of the UHS as well as the Institute Pasteur laboratory, national programme laboratory and ART clinics/sites.

2.2.5.6 Laboratory of the HIV National Program

The HIV National Laboratory was responsible for blood sample management and transportation from ART clinics/sites to referring laboratories. This central laboratory received samples from the study sites and dispatched them to the laboratory of the Faculty of Pharmacy at the UHS for biobanking and the Institute

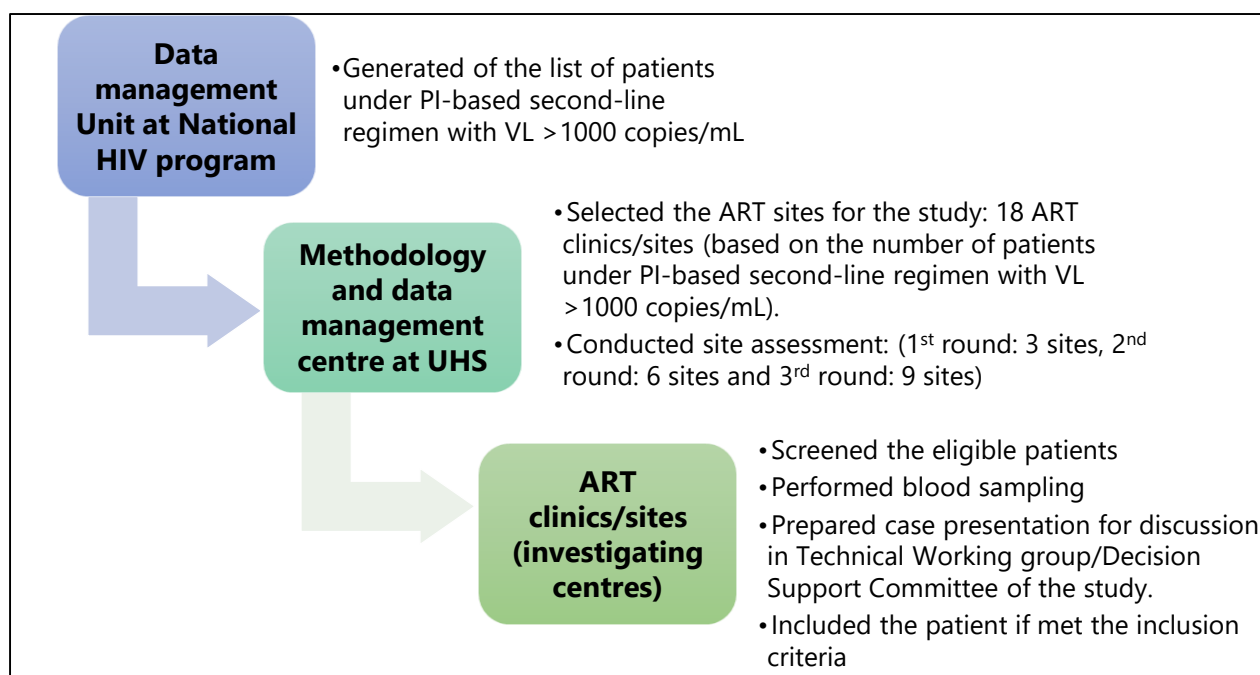
Pasteur for biological testing except CD4-T-cell count and HIV-1 RNA tests, which were performed in the national laboratory.

2.2.5.7 ART clinics (Investigating centres)

For pragmatic reasons, screening and enrolment was performed in the largest ART sites first and then progressively expanded to the smallest ART sites (Figure 5). The selection of ART clinics/sites was based on the number of eligible patients to be screened for the study (number of patients with last viral load >1000 copies/mL after 3 sessions of enhance-based counselling). The site selection process is detailed as follows:

- First, I made a request to the data management unit of the national program to provide the list of all patients under PI-based second-line regimen with last viral load > 1000 copies/mL from all ART sites in Cambodia. Based on this list, I selected the ART clinics/sites with the largest number of patients with last viral load >1000 copies/mL.
- Second, for administrative and logistic matter, I decided to open the ART sites for the study with several rounds: starting the first round with the 3 oldest and largest cohorts in Phnom Penh (Khmer Soviet Friendship Hospital, Sihanouk Hospital Center Hope and Social Health Clinic), then the second round with 6 ART sites (National Pediatric Hospital, Calmette Hospital, Takeo Provincial Hospital, Siem Reap Provincial Hospital, Battamabang Provincial Hospital and Kampong Cham Provincial Hospital) and the last round with 9 ART sites (Sihanouk Ville Provincial Hospital, Kampot Provincial Hospital, Sothnikum Referral Hospital, Maung Reussey Referral Hospital, Thmorkol Referral Hospital, Sampov Loun Referral Hospital, Prey Veng Provincial Hospital, Kampong Chhnang Provincial Hospital and Pursat Provincial Hospital).

Figure 5: Study site selection diagram



The study conducted 18 ART clinics/sites assessments. Among the 18 assessed sites, 15 ART clinics/sites (5 in Phnom Penh and 10 in provinces that were based in each provincial referral hospital) were selected for study. (list of ART clinic/sites in Table 1).

Table 2: list of ART clinic/sites selected for patients screening in 3DICAM study

No.	Provinces/Cities	ART clinics/sites	Code Site
1	Battambang	Maung Reussey Referral Hospital	0201
2	Battambang	Battambang Provincial Hospital	0202
3	Battambang	Thmor Kol Referral Hospital	0204
4	Battambang	Sampov Loun Referral Hospital	0203
5	Kampong Cham	Kampong Cham Provincial Hospital	0301
6	Kampong Chhnang	Kampong Chhnang Provincial Hospital	0401
7	Kampot	Kampot Provincial Hospital	0701
8	Phnom Penh	Khmer-Soviet Friendship Hospital	1201
9	Phnom Penh	Calmette Hospital	1202
10	Phnom Penh	National Pediatric Hospital	1204
11	Phnom Penh	Sihanouk Hospital Center of Hope	1205
12	Phnom Penh	Social Health Clinic	1208
13	Prey Veng	Prey Veng Provincial Hospital	1402
14	Pursat	Pursat Provincial Hospital	1501
15	Siem Reap	Siem Reap Provincial Hospital	1702
16	Siem Reap	Sothnikum Referral Hospital	1704
17	Sihanouk Ville	Sihanouk Ville Provincial Hospital	1801
18	Takeo	Takeo Provincial Hospital	2101

The study team in each ART clinic/site comprised 2 investigators (medical doctors), 1 counsellor, and 1 nurse who was nominated by the ART site coordination to participate in the study. The site investigator was responsible for screening and performing medical follow-up of the included patients. Counsellor was responsible for providing patients with counselling sessions and assessing their adherence. Nurses who were involved in the study were assigned to perform blood sampling as per the protocol visits. Therefore, he/she was also responsible for preparing and transferring the samples to the laboratory of the national HIV program.

2.2.5.8 National maternal and child health program

The National maternal and child health program was responsible for consulting women of childbearing age who participated in the study on pregnancy and contraception methods. Prior to the inclusion visit, all childbearing age patients were scheduled for a consultation visit with a gynaecologist at the NMCH for contraceptive services.

2.2.5.9 Institute Pasteur in Cambodia

All biological testing required by the study protocol of the patients enrolled in the study was performed at the Institute Pasteur in Cambodia, except CD4-T-cell count and HIV-1 RNA testing, which were performed at the laboratory of the HIV national program.

2.2.6 Patient screening and recruitment

Once the site was selected, the investigators identified the eligible patients

to be screened for the study. Then, the identified patient was asked to participate in the study. If informed consent was granted, blood sampling was performed, and samples were sent to the laboratory of the national HIV program. The results of the screening visit were sent to the investigator at the ART sites. As soon as the investigators received the results of the screening visit, they prepared the case presentation and submitted it to the technical working group. At that time, a face to face meeting of the technical working group was organized to discuss the proposed case and decide to include the patient in the study if the patient met the inclusion criteria.

2.2.7 Decision Support Committee of the study (DSC) and the organization of Committee Meeting

The Decision Support Committee of the study was set up not only to provide advice and recommendations to site investigators regarding the occurrence of tuberculosis or pregnancy during the study or any morbidity event occurring during the study but also to make decisions with investigators on ART management about any important clinical issues for where investigators wish to share their experience before making a decision. The composition of the DSC consisted of 1/Two physicians specialized in HIV infection, 2/One pharmacist specialized in PK and 3/Two principal investigators of the study. Other specialists could be called at DSC's request. The DSC meeting was organized regularly on a monthly basis or upon request through phone calls, e-mails, online forums, or any other means in case of a morbidity event. The investigators of each site were responsible for preparing the documents for the

meeting and sending them by e-mail to the Methodology and Management Center at UHS before the meeting. The Methodology and Management Center at UHS was responsible for scheduling and inviting the investigators to the DSC meeting, and the AIDS care unit of the National program was responsible for inviting other members who were also members of the Technical Working Group of the national program to attend the meeting. After the meeting, one of the members of the DSC provided a recommendation on a written report. Then, the report was sent to the methodological center at UHS for inclusion in the study files.

2.2.8 Training of the study team

The training sessions were organized for all study team members by the Methodology and Management Center at UHS in collaboration with the AIDS Care Unit of the National HIV Program according to the rhythm of research sites opening for the study. In total, seven training sessions (1 refreshment training at Siem Reap Site) were organized during the study period for each selected ART clinic/site for the study. All study members, such as investigators, counsellors, nurses, laboratory staff, CRAs, and data managers, were invited to join the training. Each training session covered the presentation of the study protocol, patient informed consent process, case report form filling, SAE management and reporting and good clinical practice as well as other relevant study procedures. The AIDS Care Unit of the National HIV Program was responsible for providing the session on the protocol presentation, and the remaining sessions were presented by the Methodology and Management Center at UHS.

2.2.9 Study population

All the patients who met the following criteria were enrolled in the study.

2.2.9.1 Inclusion criteria

- Age \geq 15 years old the day of inclusion
- Documented HIV-1 infection
- Failing an NNRTI-based first-line regimen
- Failing a PI-based second-line regimen after 3 months of adherence boosting
- HIV strain intermediate or fully resistant to ATV/r or sensitive to ATV but both resistant to AZT, ABC and TDF
- For women of childbearing age (15-44 years old): acceptance to use effective contraceptive methods
- Informed consent was obtained with an information sheet given and explained before the inclusion visit and the consent form.

2.2.9.2 Non-inclusion criteria

- History of antiretroviral treatment including darunavir and integrase inhibitor
- Active pregnancy < 12 weeks of amenorrhea or desire of pregnancy during the duration of the study
- Opportunistic infection in the acute phase at inclusion, including tuberculosis treated for less than one month and/or with no stable clinical condition
- Advanced cirrhosis (Child–Pugh score B or C)
- Creatinine clearance < 50 ml/mn
- Any concomitant medical condition that, according to the clinical site investigator, would contraindicate participation in the study.
- Concurrent participation in any other clinical study without written agreement of the two study teams.

2.2.10 Outcomes

- **Primary outcome:** Proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 6 months
- **Secondary outcomes**
 - Proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 12 months
 - CD4 cell count at 6 and 12 months
 - Proportion of patients with IRIS
 - Proportion of patients with permanent study treatment discontinuation during the first year
 - Incidence of grade 3-4 adverse events (ANRS grading table)
 - Proportion of patients with adherence > 90% during one year
 - Proportion of patients who switched from 600/100 twice daily to 800/100 once daily at 6 months
 - Plasma darunavir concentration of 600/100 twice daily and 800/100 once daily
 - Plasma dolutegravir concentration.
 - Proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 12 months among those switched from 600/100 twice daily to 800/100 once daily

2.2.11 Strategies and treatment

Each patient was informed of the objectives and the total duration of the study as well as the benefits and risks to participate. An information sheet in Khmer was given to each patient. After signature of the consent form, Genotyping Resistance Test (GART) and pre-inclusion samples were done for all patients failing a PI-based second-line regimen according to national guidelines after three months of Enhanced adherence counseling (EAC).

After receiving the results of the GART and the preinclusion sample, the technical working group (TWG) of NCHADS made the decision to switch the patient to the third-line regimen or to continue the second-line regimen with the new EAC.

- **Continuation of second-line regimen**

The PI-based second-line regimen continued if 1/GART showed sensitivity to ATV 2/GART showed sensitivity to at least one of the three NRTIs recommended in Cambodia (AZT, ABC and TDF). In that case, adherence counseling was boosted according to the new national guidance, and the patient was not enrolled in the study. A new viral load assessment was repeated three months after. Clinical, immunological and virological of these patients were evaluated by the technical working group of the national program.

- **Initiation of third-line regimen**

In case of intermediate and/or full resistance to ATV/r or sensitivity to ATV but both resistance to AZT, ABC and TDF and after confirmation of eligibility criteria, patients could be enrolled in the study. A third-line regimen was initiated including DRV/r 600/100 twice daily + DTG 50 mg once daily + 3TC 300 mg once daily +/- one fully or intermediately sensitive NRTI among TDF, ABC and AZT. The choice of the last NRTI was discussed and decided by the TWG according to the HBsAg status, the result of the GART and the medical history of the patient. For safety concerns regarding risk of neural tube defects in children born to women living with HIV who were taking dolutegravir, women of childbearing age (15-44 years old) agreed to participate in this

study were referred to national reproductive health services for effective contraceptive methods before the initiation of third-line treatment.

At 6 months, plasma HIV-1 RNA was measured. The decision of switching from DRV/r 600/100 twice daily to DRV/r 800/100 once daily was made according to the HIV-1 RNA result and if the results showed:

- HIV1-RNA < 40 copies/mL and no resistance to DRV at inclusion: a switch to DRV/r 800/100 mg once daily was performed, and adherence counseling was provided to explain about the new dosing with the patients.
- HIV1-RNA > 40 copies/mL and/or intermediate or full resistance to DRV at inclusion: the same regimen was continued, adherence counseling was provided for all patients, and a new virological assessment was performed at 9 and 12 months.

- **Tuberculosis occurrence before and during the study**

Screening of tuberculosis were made at pre-inclusion visit using the WHO 4 questions about TB symptoms and a systematic chest X-ray. In case of suspicion of tuberculosis, Xpert MTB/RIF were performed on sputum sample. If criteria were in favor of a probable or confirmed TB, patients were addressed to ART sites to receive TB treatment immediately and were not enrolled in the study in a first step. It could be possible to enroll the patient after one month of TB treatment if the patient presents a stable clinical condition.

Screening of tuberculosis were also made at each follow-up's consultation using the WHO 4 questions about TB symptoms. In case of suspicion of TB, Xpert MTB/RIF were performed on sputum sample as well as chest X-ray. Depending on clinical

presentation, Xpert MTB/RIF were performed on any relevant extra-pulmonary specimen.

If criteria in favor for probable or confirmed TB:

- Tuberculosis treatment could be provided using the standard WHO-recommended regimen (2RHZE/4RH).

- Dolutegravir could be switched from 50 mg once daily to 50 mg twice daily and residual concentration of DTG could be performed two weeks after rifampicin initiation.

- The dosage of patients receiving DRV/r 800/100 qd must be increased to the 600/100 mg bid dosage. Residual concentration of DRV could be performed two weeks after rifampicin initiation.

- **Pregnancy occurrence during the study**

The strategy proposed in the 3DICAM study was revised since July 2019 as WHO recommend to continue DTG if pregnancy is identified after the first trimester. Before the first trimester, WHO reports that risk–benefit models suggested the benefits of DTG were likely to outweigh the risks, which was the case in our population of women treated in the context of third-line regimen. The following recommendations were reported in the procedures with the possibility to adjust according to the situation:

- In all cases, DRV/r 600/100 twice daily must be prescribed
- DTG could be continued
- Ultrasound at first trimester (12 SA) and second trimester (22SA) was recommended to detect any neural tube defect with an experimented radiologist

- **Treatments used in the study**

None of the drugs used in the study is experimental. Antiretroviral drugs were

delivered free of charge by NCHADS. The same supply chain was used as for all ARVs, with a 6-month delivery in the clinical site and a monthly dispensation for each patient.

The study covered the costs of common non-antiretroviral drugs including TB treatment, complementary exams and hospitalizations occurring from inclusion to month 12, if these tests, care and treatment have been justified by the study investigators.

- **ART regimen**

- Darunavir 600 mg tablet: one tablet twice daily
- Darunavir 800 mg tablet: one tablet once daily
- Ritonavir 100 mg tablet: one tablet twice or twice daily
- Darunavir/ritonavir 400 mg/50 mg : two tablets once daily
- Dolutegravir 50 mg: one tablet once daily
- Optimized NRTI treatment: could include tenofovir, zidovudine, abacavir and lamivudine

2.2.12 Study schedule

Once the patient signed the written informed consent, 13 visits were scheduled for all patients during the 48 weeks of study period: at screening (-8 weeks), weeks 0,2,4, 8, 12, 16, 20,24, 30 (for patients switch to DRV once daily), 36 and 48.

- **Pre inclusion visit (V0)**

During the pre-inclusion visit, the medical investigator:

- Provided oral information, information sheet
- Checked eligibility criteria
- Collected consent form signed
- Collected socio-demographic data, medical history including past OI and treatment received
- Performed a clinical exam including BMI and Karnofsky score

- Asked the 4 questions about TB symptoms and performed specific exam (Xpert MTB/RIF, urine LAM) if one question at least is positive
- Performed biological sample including: complete blood count, HBsAg, HCV Ab, serum transaminases, serum creatinine, GGT, bilirubine, coagulation profile, albumin, CD4 T-cell count, and GART, urine pregnancy test (women of childbearing age)
- Performed chest X-ray
- Plan the inclusion visit (W0)

Between pre-inclusion visit and inclusion visit, biological samples including GART and request form were sent to NCHADS laboratory and UHS for biochemistry and GART. The TWG was in charge to collect the results and to take a therapeutic decision. This decision was communicated to the investigators on ART site in a delay of around 8 weeks.

- **Inclusion visit (V1)**

The inclusion-visit occurred within around 8 weeks after the pre-inclusion visit for some cases it took more than 8 weeks due to availability of the GART results. During the inclusion visit, the medical investigator checked the results of the pre-inclusion visit tests and verified that the patient met all inclusion criteria and exhibited none of the non-inclusion criteria.

Patients who did not meet all inclusion criteria, or met at least one non-inclusion criterion, could not be enrolled in the study. They benefited from the routine care provided in the country, according to the NCHADS. The TWG was in charge to follow up the outcomes of these patients, as some of them could be enrolled later in the study

(active opportunistic infection cured, pregnant women after delivery, accumulation of resistance mutations).

Patients who met all inclusion criteria and did not meet any non-inclusion criterion were enrolled on the same day.

For these patients, the medical investigator:

- Collected the decision of the TWG for ART regimen
- Informed the patient of the therapeutic decision
- Checked eligibility criteria
- Performed a clinical exam including BMI, Karnofsky score and hip and waist size and collect possible events occurred during the last month including concurrent medications
- Asked the 4 questions about TB symptoms and perform specific exam (Xpert MTB/RIF, urine LAM) if one question at least is positive
- Performed biological sample including: complete blood count, serum transaminases, serum creatinine, glycemia and lipids evaluation, urine pregnancy test (women of childbearing age)
- Gave prescription of drugs to the patient with the list of contraindicated drugs
- Addressed the patient to the counselor for ART-related adherence counseling
- Planned the visit W2

- **Follow up visits**

Beside the full clinical examination, the adherence counseling and pregnancy test for women in childbearing age were also scheduled at every visit for the patient.

The CD4 counts were measured at W24 and W48. Plasma HIV-1 RNA was assessed at W4, W12, W24, W30 (if switched to darunavir once daily), W36 and W48. Serum transaminases, complete blood count and serum creatinine were assessed every visit after initiating the third-line treatment up to W12, then repeated at W24, W36 and W48.

2.2.13 Monitoring visits

Monitoring activities were coordinated by the Methodology and Data Management Center at the University of Health Sciences. In this study, we had 2 clinical research assistants to perform the monitoring visit for all over 13 ART sites participating in the study across Cambodia. In total we conducted 159 visits during the study period.

2.2.14 Sample size calculation and statistical analysis

By using the Fleming method with one stage, with a sample size of 45 patients we could conclude that the success rate is at least 60% if the total number of failures does not exceed 12 (alpha at 0.05, power at 0.90, expected success rate of 80%). Based on previous data from the 2PICAM study (61), we expected 60 patients to require third-line treatment over 30 months, which would lead to an expected number of patients to be recruited of 54 if the refusal rate was 10%.

We summarized baseline characteristics such as sociodemographic characteristics, medical history, CD4 cell count and plasma viral load by their median and interquartile range (IQR), or mean and standard deviation, or frequencies with %

and 95% confidence intervals (CI), as appropriate.

The primary outcome of this study was the percentage of participants with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at week 24, presented with its 95% confidence interval. The participants who died, were lost to follow-up or had a missing viral load value at week 24 were considered to have a failure of treatment for the primary outcome. The comparison of participants with plasma HIV-1 RNA < 40 copies/mL and \geq 40 copies/mL across different baseline categorical characteristics was performed using chi-squared or Fisher's exact tests accordingly.

The percentage of participants with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 48-week, the median change in CD4 cell count, the incidence of grade 3-4 adverse events (ANRS grading table), discontinuation of study treatment, adherence to treatment strategy (proportion of patients with adherence > 90%), the percentage of participants who switched from DRV/r 600/100 twice daily to DRV/r 800/100 once daily at week 24, plasma drug concentrations (darunavir and dolutegravir) were assessed as secondary outcomes.

Data management and data analysis were performed using STATA 15 (StataCorp, USA); p-values of less than 0.05 were considered statistically significant. 95% confidence intervals for percentages were computed using a binomial distribution.

2.2.15 Ethical consideration

This study was conducted with regard to fundamental ethical principles described in the Declaration of Helsinki and in the ANRS ethics charter for research in developing countries. This study was also approved by the National Ethic Committee for Health Research in Cambodia on 02/07/2018 (159NECHR). The study was registered at ClinicalTrial.gov (NCT03602690). Written informed consent was obtained from all participant prior to screening procedures.

2.3 Pharmacokinetic sub-study

Pharmacokinetic characteristics of DRV and TDG drugs administered with two nucleoside analogs are well established either in healthy volunteers or HIV-infected volunteers but pharmacokinetic data on both drugs were missing for Asian population.

We conducted a pharmacokinetic study within a sub-study of the ANRS13374 3DICAM study to assess DRV and DTG exposure and pharmacokinetic parameters at steady state 4 weeks after administration of DRV/r 600/100mg twice daily (period 1) or DRV/r 800/100mg once daily (period 2) and DTG 50 mg once daily in HIV-infected treatment-experienced adults in Cambodia.

The first 20 patients enrolled in the 3DICAM study who were on a stable regimen were invited to participate in this pharmacokinetic sub-study. All patients who agreed to participate to the study gave their written informed consent prior to blood sampling.

The dosage of DRV/r600/100mg twice daily or DRV/r 800/100mg once daily and

DTG50mg once daily was administered and plasma level of DRV and TDG were measured at steady-state (four weeks of starting treatment or four weeks after treatment switch at Week 24 if HIV-1 RNA<40Log10cp/mL). Blood samples were collected, in each study site, on Week 4 and Week 30 (for the patients switched to DRV/r 800/100mg once daily) prior to and after dosing at times +2h, +4h, +6h and +10h. Then the blood samples were transferred to the laboratory of the Faculty of Pharmacy at UHS for preparation of plasma samples. All plasma samples were kept at -80 °C for centralized analysis in this laboratory at the end of the sub-study.

Drug levels were assayed by liquid chromatography in the laboratory of faculty of pharmacy at University of Health Sciences, Phnom Penh, using slight modifications of previously published methods (Wang et al.,2016 and Hirano et al., 2010). The lower limit of quantification was 500 ng/mL and standard curves were linear up to 10000 ng/mL for both drugs. All analytical runs were validated by external quality control (Coefficient of Variation CV<5%).

Pharmacokinetic parameters were estimated by a model independent method. The areas under the plasma concentration time curve from 0 to the last sampling time, 10 h postdosing (AUClast), were determined at steady state and calculated according to the linear up/log down trapezoidal rule. AUC during a dosing interval (AUCtau) could not be estimated as the slope of concentrations of both darunavir and dolutegravir versus time decline was erratic. Therefore, only the observed maximum concentration (Cmax), the observed predose concentration (Co), and the time of Cmax

(Tmax) obtained from the plasma concentration-time curve were reported. Results were expressed as median and interquartile range (IQR).

2.4 Personal involvement in the ANRS 12374 3DICAM study

My thesis project has been accepted for financial support from the National Agency for HIV/AIDS Research (ANRS) through “allocation doctorale” in 2018. The project aimed to conduct a programmatic assessment of a third line regimen implementation including darunavir/ritonavir, dolutegravir and optimized NRTI for HIV-infected subjects in Cambodia based on the design of the ANRS 12374 3DICAM study that has been already accepted for funding by ANRS at the time I developed this thesis project.

I was a project manager of the ANRS 12374 3DICAM study while I enrolled to my PhD program in 2019. As a project manager and as a PhD student during this past three years, I actively participated to all steps of the project implementation since the beginning, including overall operational coordination as project manager of the study, participation to protocol and procedures writing, case report form (CRF) design, database design and development, training of clinical research assistants (CRAs), data management and cleaning, data analysis and interpretation and manuscript writing. We faced to many challenges to implement the study due to the nature of the project itself and the COVID-19 pandemic while implementing the study.

The implementation of ANRS 12374 3DICAM study started in July 2018 and the

first inclusions started in October 2018 and ended in April 2021. The duration of inclusions was initially planned to be 12 months to include 54 patients in the study but actually it was extended to 30 months due to low recruitment rates and the impact of COVID 19 pandemic. The last visit of the last patient was in April 2022. During that time, I also worked on data management and data cleaning and I performed the statistical analysis and writing up the manuscript presented in this thesis.

3 RESULTS

3.1 Study 1: the Burden of HCV infection in Cambodia

3.1.1 Characteristics of study participants

The characteristics of the 935 study participants (510 pregnant women and 425 PLHIV) are summarized in Table 3. The mean age of the participants was 42.9 years (SD, 9.3) for PLHIV and 27.6 years (SD, 5.3) for pregnant women. Approximately half of participants in both groups reported not having formal schooling or having Less than or primary school (46.6% in PLHIV and 45.3% in pregnant women), about 98.0% of pregnant women and only 64.0% of PLHIV were married. More than half of participants in both groups were farmers/self-employed and around 40% reported ever migrated to another country or to other places.

Table 3: Characteristics of study participants by population group (n=935), Cambodia, 2016

Characteristics	PLWHA (N=425)		Pregnant Women (N=510)	
	n	%	n	%
Mean age in years (±SD)	42.95 (±9.37)		27.68 (±5.33)	
Age group, years				
≤30	28	6.59	363	71.18
31-40	137	32.24	142	27.84
>40	260	61.18	5	0.98
Sex				
Male	189	44.47	-	-
Female	236	55.53	510	100
Education				
No formal schooling/Less than or primary school	198	46.59	231	45.29
Secondary school	100	23.53	148	29.02
High school/University or higher	127	29.88	131	25.69
Marital status				
Never married	37	8.71	10	1.96
Currently married	272	64.00	499	97.84
Separated/divorced/Widow	116	27.29	1	0.20
Main occupation				
Employee (Government/ Non-government)	111	26.12	110	21.57
Retired/Unemployed/home duties	76	17.88	129	25.29
Farmer/Self- employed/ Others	238	56.00	271	53.14
Residential mobility				
Always live here	240	56.47	328	64.31
Ever migrated (to another country/to other places)	184	43.29	181	35.49
Geographic Area				
Battambang (West)	75	17.65	165	32.35
Siem Reap (Northwest)	75	17.65	135	26.47
Preah Sihanouk (Southwest)	25	5.88	30	5.88
Phnom Penh (Central)	250	58.82	180	35.29

3.1.2 Knowledge/attitudes on hepatitis C prevention and care

Participants indicated high levels of willingness to get tested for HCV and to go for further investigations or treatment if HCV test positive. Correct answers to questions about whether blood should be screened for hepatitis C before transfusion, and barbers should use new blades or safe equipment for ear and nose piecing were reported by more than 90% of participants in both groups (Table 4).

Table 4: Knowledge/Attitudes on hepatitis prevention and care

Variables	PLWHA (N=425)		Pregnant Women (N=510)	
	n	%	n	%
Willing to get tested for hepatitis C	420	98.82	509	99.80
Willing to go for further investigations or treatment if HCV test positive	423	99.53	509	99.80
Asking for or use a new/sterilized syringe or needle	344	80.94	396	77.65
Getting the blood screened for hepatitis C before transfusion	389	91.53	394	77.25
Asking barber to use new blades or for safe equipment for ear and nose piecing	394	92.71	399	78.24

3.1.3 Knowledge on basic information about hepatitis C

Table 5 shows the knowledge items on basic information about the disease. Almost all participants in both groups (98.82% in PLHIV vs 97.45% in pregnant women) chose the correct answer to question whether people living with hepatitis C damage their liver when they drink alcohol. Lower proportion (less than 70% in both group) of participants reported the correct answer to the question if hepatitis C infected person should get vaccinated against hepatitis A and B. The proportion of correct answer to Knowledge about symptoms of being infected with hepatitis C was low in pregnant

women (15.49%) compared to PLHIV (41.18%).

Table 5: Knowledge of participants about basic information about hepatitis C

Variables	PLHIV (N=425)		Pregnant Women (N=510)	
	Correct answer			
	n	%	n	%
Successful hepatitis C treatments can result in the hepatitis C virus being completely removed or cleared from one's blood	420	98.82	497	97.45
Hepatitis C infected person should get vaccinated against hepatitis A and B	290	68.24	327	64.12
Person can live with hepatitis C for many years without knowing the he/she has been infected with the virus	274	64.47	270	52.94
Knowledge about any symptoms of being infected with hepatitis C	175	41.18	79	15.49
Successful hepatitis C treatments can result in the hepatitis C virus being completely removed or cleared from one's blood	270	63.53	291	57.06
A person with hepatitis C can be identified by physical appearance	225	52.94	231	45.29

3.1.4 Knowledge of participants about hepatitis C mode of transmission

As seen in Table 6, more than half participant (60%) in both groups chose the correct answers to 7 knowledge items on mode of transmission. In contrast, there were a high proportion of respondents in both groups who answered incorrectly to 4 items. Many of respondents supported that *Snorting cocaine with shared straws etc. increase risk of hepatitis C transmission* (PLHIV: 76.94% vs pregnant women: 76.08%). The respondents also incorrectly agreed that *Coughing and sneezing can spread hepatitis C* (PLHIV: 68.47% vs pregnant women: 70.20%). Most of respondents incorrectly

supported the statement that *Hepatitis C can be spread from shared kitchen cups, plates or utensils* (PLHIV: 83.76% vs pregnant women: 74.51%). Less than one-third of PLHIV (30.59%) and 47.06% of pregnant women incorrectly agreed with a statement that *Once someone's hepatitis C virus has been completely and cleared, he/she can get re-infected with hepatitis C.*

Table 6: Knowledge of participants about hepatitis C mode of transmission

Variables	PLHIV (N=425)		Pregnant Women (N=510)	
	Correct answer			
	n	%	n	%
People with hepatitis C can safely share their toothbrush and razor with others	309	72.71	410	80.39
Blood transfusion can transmit hepatitis C	355	83.53	382	74.90
People can get infected with hepatitis C from tatoos and body piercing	350	82.35	336	65.88
Peple who inject drugs with used needles are at high risk of hepatitis C infection	386	90.82	435	85.29
Snorting cocaine with shared straws etc. increase risk of hepatitis C transmission	52	12.24	59	11.57
Babies born to hepatitis C pregnant women can be infected with hepatitis C	311	73.18	368	72.16
Hepatitis C can be transmitted to someone during sexual intercourse	258	60.71	309	60.59
Coughing and sneezing can spread hepatitis C	96	22.59	106	20.78
Hepatitis C can be spread from shared kitchen cups, plates or utensils	59	13.88	95	18.63
Hepatitis C can be transmitted by hugs or handshakes	353	83.06	450	88.24
Once someone's hepatitis C virus has been completely and cleared, he/she can get re-infected with hepatitis C	218	51.29	181	35.49

3.1.5 Knowledge of participants about hepatitis C vaccine and treatment availability

Table 7 shows the knowledge on vaccine and treatment availability. Four questions were asked to identify the knowledge on vaccine and treatment of the participants. Very low proportion of participants (about only 10%) in both group reported correct answer to the question about knowledge on vaccine availability. Similar proportion of incorrect answer were found in both groups of participants for the statement that there exists hepatitis C vaccine to prevent people from getting infection (PLHIV: 64.24% vs. pregnant women: 63.33%). Both groups of participants incorrectly supported that there is effective vaccination for hepatitis C (PLHIV: 64.47% vs pregnant women: 63.73%). Regarding the knowledge on treatment availability, only about half of participants in both groups knew that there is any treatment for hepatitis C (PLHIV: 49.18% vs pregnant women: 57.84%).

Table 7: Knowledge of participants about vaccine and treatment availability

Variables	PLHIV (N=425)		Pregnant Women (N=510)	
	Correct answer			
	n	%	n	%
Vaccine availability				
There exist hepatitis C vaccine to prevent people from getting infection	55	12.94	71	13.92
There is effective vaccination for hepatitis C	274	12.00	325	11.76
Treatment availability				
people with hepatitis C Can be cured with any herbal medicine	312	73.41	329	64.51
Know if there is any treatment for hepatitis C	209	49.18	295	57.84

3.1.6 Attitude toward hepatitis C infection, care and treatment for hepatitis C

3.1.6.1 Endorsement of undesired responses on attitude items related to indifference

Overall, the proportion of individuals who endorsed undesired responses on attitude items related to indifference by study population groups was low. A majority of participants reported undesired response to attitude items with less than 20%. However, there were 2 specific items on HCV treatment of undesired responses on attitude related to indifference were reported by more than 20% participants. A low proportion of participants agreed that *Treatment for hepatitis C is not necessary if there is no symptom* (21.65% in PLHIV compared to 35.88% in pregnant women). Similar proportion of participants in both groups agreed that *if I found that I have hepatitis C, I would not seek for treatment* (PLHIV: 37.88% vs pregnant women 34.51%) (Table 8)

Table 8: Endorsement of undesired responses on attitude items related to indifference

Variables	PLHIV (N=425)		Pregnant Women (N=510)	
	Endorsed answer			
	n	%	n	%
Compared to other diseases such as diarrhea, malaria, TB, hepatitis C doesn't need any treatment like those diseases	41	9.65	77	15.10
Hepatitis C patient can live in regular good health without any medications	36	8.47	52	10.2
Hepatitis is not fatal	5	1.18	27	5.29
Hepatitis C treatment does not improve the patient's health	28	6.59	50	9.8
Treatment for hepatitis C is not necessary if there is no symptom	92	21.7	183	35.88
I don't think I can get hepatitis c	68	16.00	113	22.16
If I found that I have hepatitis C, I would not seek for treatment	161	37.9	176	34.51

3.1.6.2 Endorsement of undesired responses on attitude items related to shame, stigma and fear

Table 9 shows proportion of individuals who endorsed undesired responses on attitude items related to shame, stigma and fear by study population groups. A high proportion of participants in both groups endorsed undesired response as that *If a seller infected with hepatitis C, I will not buy his/her foods* (PLHIV: 71.53% vs Pregnant Women: 78.63%).

Table 9: Endorsement of undesired responses on attitude items related to shame, stigma and fear

Variables	PLHIV (N=425)		Pregnant Women (N=510)	
	Endorsed answer			
	n	%	n	%
Feeling ashamed to seek care and treatment, if I am infected with hepatitis C	18	4.24	35	6.86
If I found that I have hepatitis C, I would feel ashamed to get infection	6	1.41	29	5.69
If a seller infected with hepatitis C, I will not buy his/her foods	304	71.5	401	78.63
I have ever seen patient infected with hepatitis C being stigmatized	205	48.2	165	32.35
If I am diagnosed with hepatitis C, the most worry I have would be the fear of death	144	26.8	141	27.65
If I am diagnosed with hepatitis C, the most worry I have would be to spread disease to the family	142	33.4	233	45.69
If I am diagnosed with hepatitis C, the most worry I have would be the cost of treatment	129	30.4	91	17.84
If I am diagnosed with hepatitis C, the most worry I have would be the isolation from family	5	1.18	1	0.2
I avoid meeting hepatitis C patients	89	20.9	96	18.82
If I am diagnosed with hepatitis C, my reaction would be a fear	145	34.1	291	57.06
If I am diagnosed with hepatitis C, my reaction would be a sadness	128	30.1	158	30.98

3.1.7 Healthcare and behavioral factors potentially related to hepatitis C infection

Within the past 5 years, high proportions of participants had received intravenous medication (77.6% in PLHIV, 72.1% in pregnant women) or intramuscular injection (46.1% in PLHIV, 82.1% in pregnant women), or visited the dentist (47.7% in PLHIV, 40.5% in pregnant women (Table 10).

Table 10: Healthcare and behavioral factors potentially related to hepatitis C infection

Variables	PLWHA (N=425)		Pregnant Women (N=510)	
	n	%	n	%
Healthcare and behavioral factors^a				
Ever been admitted to a hospital or clinic	115	27.06	167	32.75
Ever received intra-muscular medication injection	196	46.12	419	82.16
Ever received intravenous medication injection	330	77.65	368	72.16
Ever received blood transfusion	12	2.82	4	0.78
Ever undergone any plastic surgery procedure	12	2.82	7	1.37
Ear/nose piercing or tattoo	10	2.35	21	4.12
Ever visited dental clinic for dental care	203	47.76	207	40.59
Ever used any illicit drug injection	2	0.47	0	0.00
Having a family member positive for HCV	31	7.29	20	3.92
HCV testing				
Ever tested for viral hepatitis infection	181	42.59	169	33.14
Ever tested for hepatitis C	85	20.00	27	5.29

^a Healthcare and behavioral factors reported over the past five years

3.1.8 Prevalence of HCV infection

HCV antibody prevalence was as high as (29/425) 6.8% [95% CI: 4.6-9.6] in PLHIV and was low (5/510) 0.9% [95% CI: 0.3-2.7]) in pregnant women. Of the 34 anti-HCV-positive individuals, 24 (70.6%) had detectable HCV RNA, with substantially higher proportions of chronic infection in PLHIV compared to pregnant women (23/29) 79.3% [95% CI: 60.2-92.0] vs. (1/5) 20.0% [95% CI: 0.5-71.6], $P=0.007$. A phylogenetic analysis of 24 samples with a detectable HCV RNA showed that (11/24) 45.8% were genotype 1b; (1/24) 4.1% were genotype 2a and (8/24) 33.3% were genotype 6 (6:4.1%, 6a:4.1%,

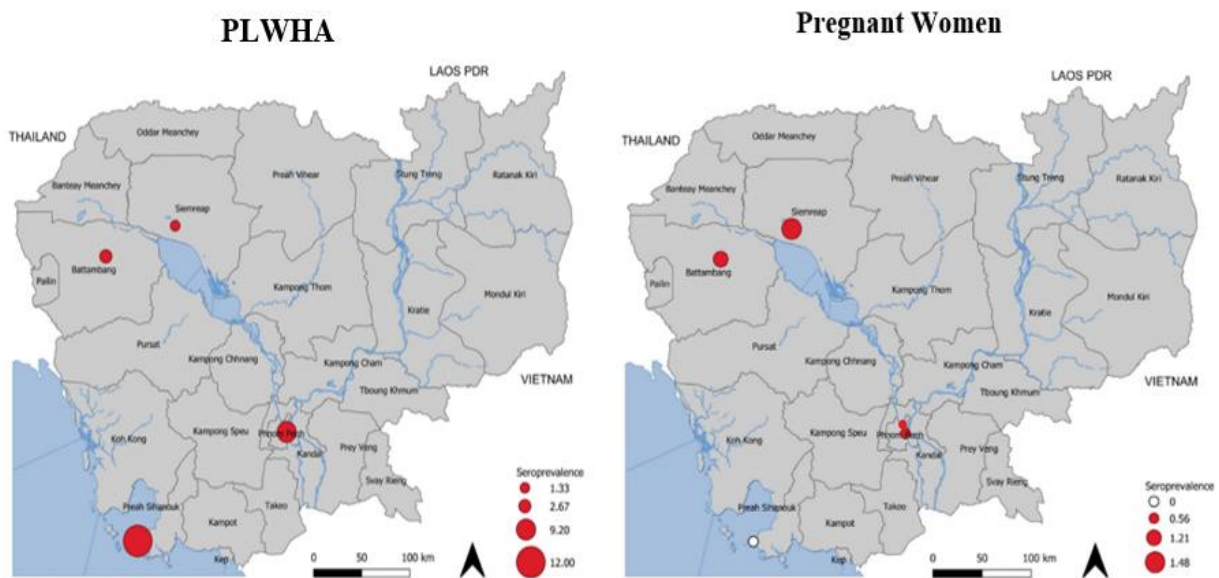
6e:12.5%, 6q:8.3%, 6r:4.1%) and 16.6% failed to amplify. In PLHIV, anti-HCV prevalence generally increased with age, from (1/28) 3.5% [95% CI: 0.0-18.3] in the youngest group (18-30 years) to (21/260) 8.0% [95% CI: 5.0-12.0] in the oldest group (>40 years old), but the difference was not statistically significant ($P=0.51$). In pregnant women, the anti-HCV prevalence was (4/363) 1.1% [95% CI:0.3-2.7] in the 18-30 years age group and (1/142) 0.7% [95% CI: 0.0-3.8] in the 31-40 years age group (Table 11).

Table 11: seroprevalence and viremic prevalence per sex and per age category (n=935), Cambodia, 2016

Characteristics	PLHIV (N=425)				Pregnant Women (N=510)			
	Serology		Viraemia		Serology		Viraemia	
	n/N	Prevalence 95%CI	n/N	Prevalence 95%CI	n/N	Prevalence 95%CI	n/N	Prevalence 95%CI
Overall	29/425	6.82 [4.61-9.65]	23/425	5.41 [3.46-8.00]	5/510	0.98 [0.31-2.27]	1/510	0.20 [0.01-1.08]
Age group, years								
18-30	1/28	3.57 [0.09-18.34]	1/28	3.57 [0.09-18.34]	4/363	1.10 [0.30-2.79]	1/363	0.28 [0.00-1.52]
31-40	7/137	5.11 [2.07-10.24]	6/137	4.38 [1.62-9.28]	1/142	0.70 [0.01-3.86]	0/142	0.00 [0.00-2.56]
>40	21/260	8.08 [5.06-12.08]	16/260	6.15 [3.55-9.80]	0	0.00 [0.0-52.18]	0/5	0.00 [0.00-52.18]
Sex					-	-	-	-
Male	13/189	6.88 [3.71-11.47]	11/189	5.82 [2.94-10.17]	-	-	-	-
Female	16/236	6.78 [3.92-10.77]	12/236	5.08 [2.65-8.71]	-	-	-	-

We observed that in PLHIV group, Preah Sihanouk province (Southwest) had the highest anti-HCV prevalence among the four studied geographic areas: Preah Sihanouk province (3/21) 12.0% [95% CI: 2.5-31.2], Battambang (West) (2/75) 2.6% [95% CI: 0.3-.9.3], Siem Reap (Northwest) (1/75) 1.3% [95% CI: 0.0-7.2], Phnom Penh (Central) (23/250) 9.2% [95% CI: 5.9-13.4] (Figure 6). There was no significant difference in anti-HCV prevalence across the four regions in pregnant women.

Figure 6: Geographical distribution of HCV prevalence among PLHIV (n=425) AND pregnant women (n=510) in Cambodia, 2016



In PLHIV population, anti-HCV prevalence was significantly higher among those who had a history of intravenous medication injection in the past five years, at (28/330) 8.4% [95% CI: 5.7-12.0] compared to (1/95) 1.0% [95% CI: 0.0-5.7]) in those who had not. The same was observed in pregnant women, where all five HCV-infected women reported intravenous medication injection in the past five years (5/368) 1.3% [95% CI:0.4-3.1] in those with this history vs. (0/142) 0.0% [95% CI: 0.0-.2.5] in those without. In PLHIV, the anti-HCV prevalence was significantly higher in those who had a family member positive for HCV than in those who did not (9/31) 29.0% [95% CI: 14.2-48.0] vs. (20/394) 5.0% [95% CI: 3.1-7.7] (Table 12).

Table 12: Prevalence of Hepatitis C virus antibody per demographic characteristics of participants (n=935), Cambodia, 2016

Characteristics	PLWHA (N=425)		P	Pregnant Women (N=510)		^a P
	Anti HCV Positive			Anti HCV Positive		
	n/N	%	n/N	%		
Education						
No formal schooling/Less than or primary school	15/198	7.58	0.85	4/231	1.73	0.39
Secondary school	6/100	6.00		1/148	0.68	
High school/University or higher	8/127	6.30		0	0.00	
Marital status						
Never married	2/37	5.41	0.84	0	0.00	1.00
Currently married	20/272	7.35		5/499	1.00	
Separated/divorced/Widow	7/116	6.03		0	0.00	
Main occupation						
Employee (Government/ Non-government)	8/111	7.21	0.59	0/110	0.00	0.71
Retired/Unemployed/ home duties	7/76	9.21		1/129	0.78	
Farmer/Self- employed/ Others	14/238	5.88		4/271	1.48	
Ever received intra-muscular injection in the past five years						
No	12/229	5.24	0.16	0	0.00	0.59
Yes	17/196	8.67		5/419	1.19	
Ever received blood transfusion in the past five years						
No	28/413	6.78	0.83	5/506	0.99	1.00
Yes	1/12	8.33		0	0.00	
Ever visited dental clinic in the past five years						
No	12/222	5.41	0.23	4/303	1.32	0.65
Yes	17/203	8.37		1/207	0.48	
Ever received intravenous medication injection in the past five years						
No	1/95	1.05	0.01	0	0.00	0.33
Yes	28/330	8.48		5/368	1.36	
Having a family member positive for HCV						
No	20/394	5.08	<0.001	5/490	1.02	1.00
Yes	9/31	29.03		0	0.00	
Geographic area						
Battambang (West)	2/75	2.67	0.031	2/165	1.21	0.810
Siem Reap (Northwest)	1/75	1.33		2/135	1.48	
Phreah Sihanouk (Southwest)	3/25	12.00		0	0.00	
Phnom Penh (Central)	23/250	9.20		1/180	0.56	

^aFisher's exact test

3.1.9 Risk factors for HCV

Table 13 shows the results of crude and adjusted logistic regression on factors associated with HCV serological status among PLHIV. In bivariate analysis, participants who reported ever received intravenous medication injection in the last five years (OR= 8.7, 95% CI: 1.1-64.9, $P=0.04$) and having a family member positive for HCV (OR=7.6, 95%CI: 3.1-18.7, $P<0.001$) were associated with an increased risk of HCV infection. In a multivariable model (adjusted for age and sex), HCV infection remained significantly associated with having a family member positive for HCV (OR=7.6, 95%CI: 1.0-57.8, $P=0.048$) and a history of intravenous medication injection in the last five years OR=7.1 (95%CI: 2.7-18.1, $P<0.001$). A multivariable analysis could not be performed among pregnant women due to the small number of HCV-infected women.

Table 13: univariate and multivariate analysis of HCV risk factors for people living with HIV, Cambodia, 2016

Characteristics	Anti HCV positive		Unadjusted			Adjusted ^a		
	n=29	%	OR (95% CI)	P	OR (95% CI)	P		
Age group, years								
18-30	1	3.57	Ref		Ref			
31-40	7	5.11	1.45	[0.17-12.30]	1.42	[0.15-12.77]		
>40	21	8.00	2.37	[0.30-18.33]	0.20	2.52 [0.30-20.88]	0.28	
Sex								
Male	13	6.88	Ref		Ref			
Female	16	6.78	.98	[0.46-2.10]	0.97	.89 [0.38-2.03]	0.78	
Ever received intravenous medication in the past five year								
No	1	1.05	Ref		Ref			
Yes	28	8.48	8.71	[1.17-64.91]	0.04	7.67 [1.01-57.84]	0.048	
Having a family member positive for HCV								
No	20	5.08	Ref		Ref			
Yes	9	29.03	7.65	[3.12-18.75]	<0.001	7.10 [2.79-18.10]	<0.001	

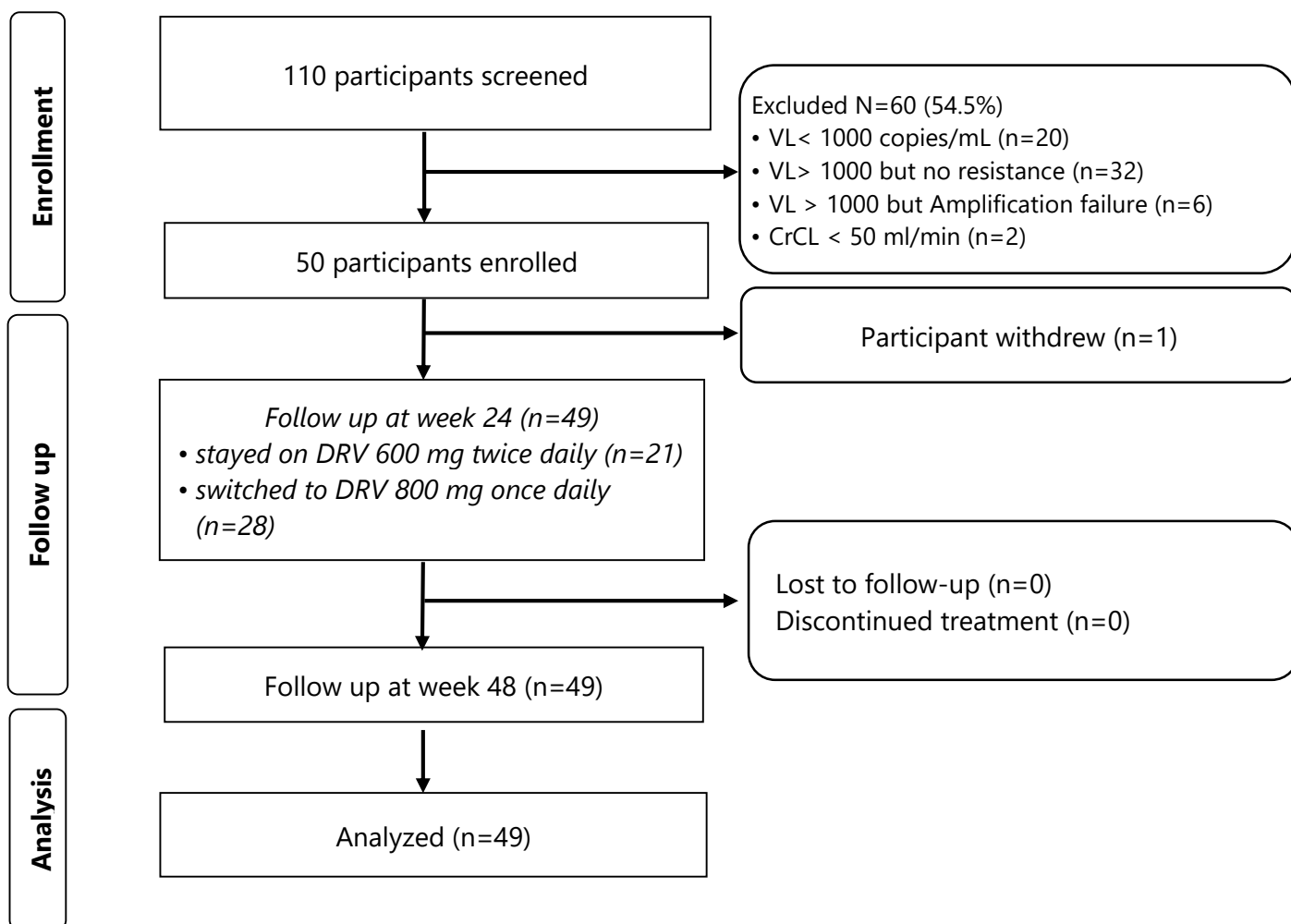
^aVariables included in the multivariate model were: age, gender, history of intravenous medication injection, having a family member positive for HCV.

3.2 Study 2: the ANRS 12374 3DICAM study

3.2.1 Baseline characteristics

Between October 2018 and April 2020, 110 patients were screened and 50 participants were enrolled in the study in 13 clinics. The reasons for non-enrolment were reported in figure 7. One participant withdrew from the study at inclusion before any uptake, leading to a total of 49 participants included in the analysis (Figure 7).

Figure 7: patient flow



The baseline characteristics of study participants are summarised in Table 14. Of the 49 participants, 61% (30) were male. The median age was 40 years (IQR: 24-46). The median CD4 at baseline was 195 cells/ μ L (36-353). The median plasma HIV RNA level was 4.4 log₁₀ copies/mL (3.6-5.2). The median previous time on cART was 4 years of first-line (3-7) and 5 years of second-line (3-7). Thirteen patients (26.5%) had been infected via mother-to-child transmission. All participants started third-line treatment with twice-daily DRV, once-daily DTG, in combination with only 3TC for 36.8% (n=18), or 3TC plus TDF for 57.1% (n=28) or AZT for 6.1% (n=3).

Table 14: Characteristics of participants enrolled in the Study (n=49)

Characteristic	All (n=49)	
General and demographic data		
Gender, Male, n (%)	30	(61.2)
Age (years), median (IQR)	40	(24-46)
Body Mass index, Kg/m ² , Median (IQR)	21	(19-24)
HBsAg Positive, n (%)	2	(4.0)
Anti-HCV Positive, n (%)	2	(4.0)
Adherence assessment, n (%)		
Good	36	(73.4)
Moderate	10	(20.4)
Poor	3	(6.1)
Route of infection,n (%)		
Mother to child	13	(26.5)
Heterosexual	33	(67.3)
MSM	1	(2.0)
Other/unknown	2	(4.1)
Duration of cART(years), Median (IQR)		
First-line ARV regimen	4	(3-7)
Second-line ARV regimen	5	(3-8)
overall	13	(8-14)
History of opportunistic infection (clinical stage), n(%)		
B	4	(8.2)
C	20	(40.8)
Prescribed third-line regimen, n (%)		
DRV/r / DTG / 3TC / TDF	28	(57.1)
DRV/r / DTG / 3TC/AZT	3	(6.1)
DRV/r / DTG / 3TC	18	(36.8)
Laboratory data, Median (IQR)		
CD4 cell-count (cells per μ L)	195	(36-353)
HIV RNA Viral load (Log ₁₀ copies/mL)	4.4	(3.6-5.1)
Hemoglobin level, g/dL	13.5	(11.9-15.2)
AST level, U/L	36	(28-44)
ALT level, U/L	31	(22-42)
CrCl, mL/min	104.5	(85.4-123.2)
Glycemia,mmol/L	5.15	(4.68-5.7)
Total cholesterol,mmol/L	4.14	(3.08-4.8)
Triglycerides,mmol/L	1.91	(1.43-2.8)

3.2.2 Antiretroviral drugs resistance pattern of study participants at inclusion

Table 15 shows the antiretroviral drugs resistance pattern of study participants at inclusion. The median number of baseline resistant associated mutations (RAMs) in nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI) and protease inhibitors (PI) was 4 (2-5.5), 3 (2-4) and 8 (6-9), respectively. The majority of the participants (81.6%, n=40) had full or intermediate resistance to 3TC, 53% (n=26) to AZT, 47% (n=23) to TDF and 67.4% (n=33) to ABC. Thirty-seven participants (75.5%) had full (n=18) or intermediate (n=19) resistance to ATVr. Only one patient had full resistance to DRVr. In total, 21 (43%) participants had full (n=16) or intermediate (n=5) resistance to AZT/TDF/ABC.

Table 15: Antiretroviral drugs resistance pattern of study participants at inclusion (n=49)

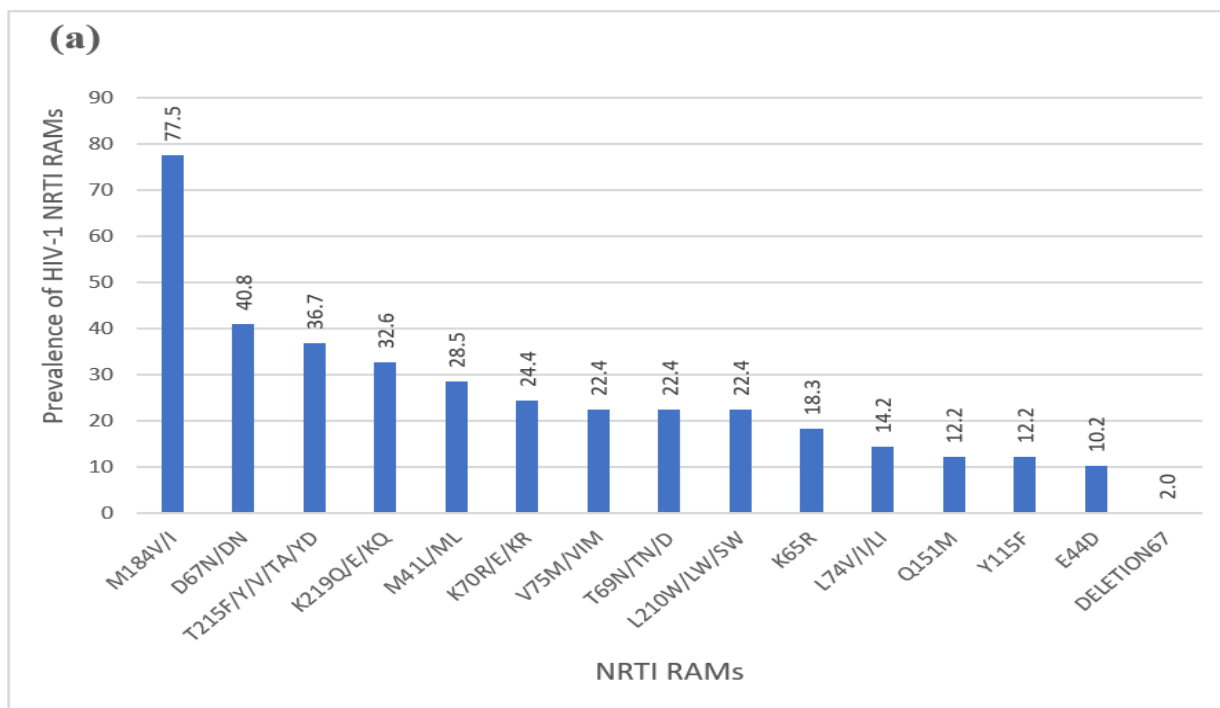
Number of resistance associated mutations (RAMs), Median (IQR)	
NRTI RAMs	4 (2-5.5)
NNRTI RAMs	3 (2-4)
PI RAMs	8 (6-9)
Antiretroviral drugs resistance pattern, n (%)	
NRTI	
AZT	26 (53.1)
D4T	33 (67.4)
DDI	22 (44.9)
3TC	40 (81.6)
TDF	23 (46.9)
ABC	33 (67.4)
NNRTI	
EFV	42 (85.7)
NVP	42 (85.7)
PI	
LPVr	17 (34.7)
ATVr	18 (36.7)
DRVr	1 (2.0)
NRTI: Nucleoside Reverse Transcriptase Inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; PI: Protease Inhibitor; RAM: resistance associated mutations; AZT: Zidovudine; D4T: Stavudine; DDI: Didanosine; 3TC: Lamivudine; TDF: Tenofovir disoproxil fumarate; ABC: Abacavir; EFV: Efavirenz; NVP: Nevirapine; LPV/r: Lopinavir/ritonavir; ATV/r: Atazanavir/ritonavir; DRV/r : Darunavir/ritonavir	

3.2.3 Genotypic resistance analysis for HIV drug resistant mutations with NRTI, NNRTI and PI at inclusion

Genotypic resistance analysis for HIV drug resistant mutations with NRTI, NNRTI and PI was done for all patients enrolled in the 3DICAM study. In our study, we found that the M184V/I mutation (77.5%) was the most prevalent NRTI-related-related HIV-1 resistance mutations, followed by the thymidine analogue mutations D67N/DN (40.8%), T215F/Y/V/TA/YD (36.7%) and K219Q/E/KQ (32.6%). The mutation K65R, which can be

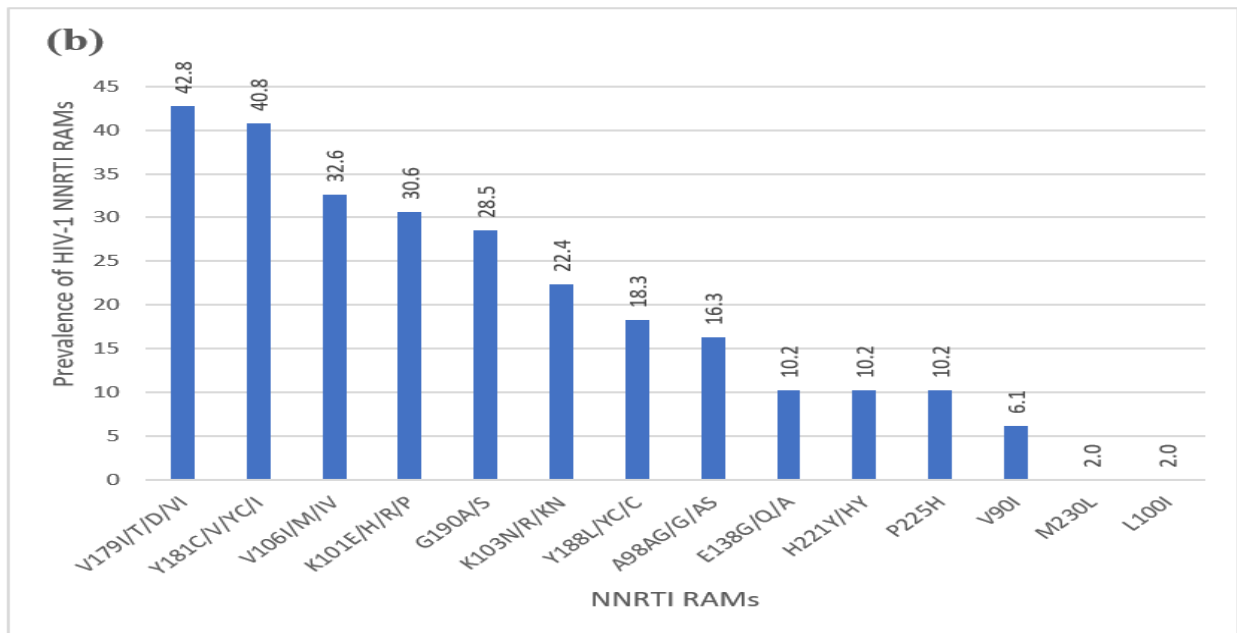
selected by TDF, found in 18.3% of the genotypes HIV strains (Figure 8).

Figure 8: Prevalence of different nucleoside reverse transcriptase inhibitor (NRTI)-related HIV-1 resistance mutations (n=49). RAMs: resistance associated mutation



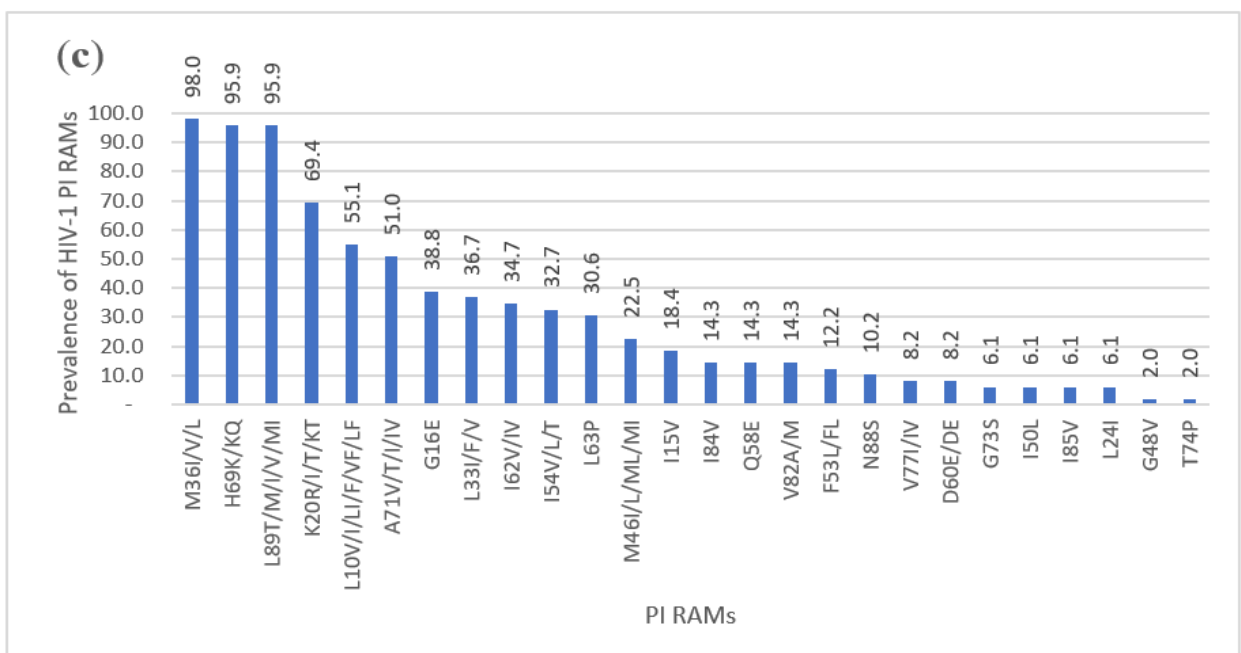
Regarding the non-NRTI (NNRTI)-related resistance mutations, the V179I/T/D/VI was the predominantly detected mutation accounted for 42.8%, followed by the Y181C/V/YC/I (40.8%), the V106I/M/IV (32.6%) and the K101E/H/R/P (30.6%) (Figure 9).

Figure 9: Prevalence of different non-NRTI related HIV-1 resistance mutation (n=49). RAM: resistance associated mutations



The most common PI-related HIV drug resistance mutations were the M36I/V/L (98.0%), the H69K/KQ and the L89T/M/I/V/MI (95.9% each), followed by the K20R/I/T/KT (69.4%), the L10V/I/LI/F/VF/LF (55.1%) and the A71V/T/I/IV (51.0%) (Figure 10).

Figure 10: Prevalence of different protease inhibitor (PI)-related HIV-1 resistance mutation (n=49). RAM: resistance associated mutations.



3.2.4 Outcomes at Week 24 and Week 48 in 3DICAM study

Table 16 presents the outcomes at week 24 and week 48 in 3DICAM study.

At week 24, 29 out of the 49 patients, 59.1% [95% CI: 44.2-73.0] had an HIV-1 RNA <40 copies/mL and 40 out of the 49 patients, 81.6% [95% CI: 67.9-91.2] had an HIV-1 RNA <1000 copies/mL. No patient died, lost-to-follow up or stopped the therapeutic regimen at week 24. About half of patients, 46.9% [95% CI: 32.5-61.7] reached CD4 cell-count \geq 350 cells per μ L. Almost all of patients, 93.8% [95%CI:83.1-98.7] reported with adherence >90% during the 24 weeks of the followed up. Out of the 49 patients, 29 had a viral load below 40 copies/ml at week 24 and 28 subsequently switched to once-daily DRV 800 mg according to the protocol (the 29th did not switch because of genotypic resistance to DRV at baseline. About one-fourth of the patients, 18.4% [95% CI: 08.7-32.0] experienced at least once grade 3-4 adverse events or Serious Advous Event during 24 weeks of the follow-up.

At week 48, all the 49 patients were still in the study. Overall, 27/49 patients, 55.1% [95% CI: 40.2-69.3] had an HIV-1 RNA < 40 copies/mL and 39/49 patients, 79.5% [95% CI: 58.9-85.0] had an HIV-1 RNA < 1000 copies/mL. CD4 cell count had increased above 350 cells per μ L in 38.8% [95%CI: 25.1-53.7] patients and above 500 cells per μ L in 22.4% [95%CI: 11.7-36.6]. The median CD4 change was higher in those who reached a viral load below 40 at week 48 than in the others (140 vs. 55/ μ L, $p < 10^{-4}$). One patient who had genotypic resistance to DRV at baseline remained below 40 copies/mL at W48. Of the 21 patients who did not have a viral load below 40 copies/mL at week 24, 7/21,

33.3% [95%CI: 14.5-56.9] reached a viral load below 40 copies/mL at week 48. Out of the 28 patients who switched to once-daily DRV 800 mg at week 24, 20 patients, 71.4% [95% CI: 51.3-86.7] remained below 40 copies/mL at week 48. Eight patients experienced a viral increase (6 of them increased between 48 and 122 copies/mL and up to 404 623 and 63 495 copies/mL for the other 2 patients). Of note, 50% of these 8 patients had been infected via mother-to-child route, vs. 9.5% (2/21) of the remainders, $p=0.033$ (Table 16).

Table 16: Outcomes at Week 24 and Week 48 in 3DICAM study (n=49)

Characteristic	Week 24 (N=49)			Week 48 (N=49)		
	n/N	%	95% CI	n/N	%	95% CI
Primary outcome: virological response						
HIV RNA Viral load (copies/mL)						
< 40	29	59	[44.2-73.0]	27	55.1	[40.2-69.3]
< 200	37	76	[61.1-86.6]	36	73.4	[58.9-85.0]
< 1000	40	82	[67.9-91.2]	39	79.5	[65.6-89.7]
Other secondary outcomes						
CD4 >350 cell-count (cells per µL)	23	47	[32.5-61.7]	19	38.8	[25.1-53.7]
CD4 >500 cell-count (cells per µL)	9	18	[08.7-32.0]	11	22.4	[11.7-36.6]
Adherence > 90% during study period	46	94	[83.1-98.7]	44	89.8	[77.7-96.6]
Switch from DRV/r 600/100mg twice daily to DRV/r 800/100mg once daily	28	57	[42.2-71.1]	-	-	-
HIV RNA <40copies/mL in patient staying with DRV/r600/100mg twice daily	-	-	-	7/21	33.3	[14.5-56.9]
HIV RNA <40copies/mL in patient switched to DRV/r800/100mg once daily	-	-	-	20/28	71.4	[51.3-86.7]
Patient presented at least once grade 3-4 adverse events, SAE	9	18.4	[08.7-32.0]	13	26.5	[14.9-41.0]

3.2.5 Adverse Event (AE) grade 3-4 or Serious Adversous Events (SAE) reported during study period through Week 48

Over the study period until week 48, 26.5% [95%CI: 14.9-41.0] of the patients (n=13) presented at least one grade 3-4 adverse event or a Serious Adverse Event (SAE) (Table 17). One participant who had been experienced a cryptococcal meningitis at week 4 (CD4: 85 cells per μ L and viral load 3.03 Log10 copies/mL) and one participant had a zona grade 1 at week 12 (baseline CD4: 199 cells per μ L and viral load 4.93 Log10 copies/mL). Three participants stopped TDF due to a decrease in creatinine clearance and two participants were referred for diabetes treatment. No patient stopped the trial treatment (DRV/DTG) due to an adverse event.

Table 17: Adverse Event (AE) grade 3-4 or Serious Advous Events (SAE) reported during study period through Week 48

Adverse Events	Patients with (nonserious or serious) adverse events (n=49)	Patients with AEs Grade 3-4 or SAEs
Infections and infestations		
Cryptococcal meningitis	1	1/1
Zona*	1	1/1
Investigations		
Alanine aminotransferase increased	22	3/22
Aspartate aminotransferase increased	29	2/29
Creatinine clearance decreased	38	5/38
Fasting blood glucose increased	15	2/15
LDL cholesterol increased	2	1/2
Platelet count decreased	5	1/5

*it was not a Grade 3-4 AE but it is an event of interest related to HIV infection

3.2.6 Factors associated with virological success below 40 copies/mL

At week 24, Female patients had a similar virological response 60.0% [[95%CI: 36.0-80.8] to men, 58.6%. About 66% [95%CI: 47.7-80.8] of patients aged \geq 25 years had

an HIV-1 RNA <40 copies/mL while they were only 42.8% [95%CI: 17.6-71.1] in patients <25 years, but the difference was not statistically significant ($P=0.15$). The proportion of patients with HIV-1 RNA <40 copies/mL tended to be greater in patients with baseline CD4 cell-count ≥ 200 cells per μL than in patients below, 69.5% [95%CI: 47.0-86.7] vs 50.0% [95%CI: 29.9-70.0], but the difference was not statistically significant, ($P=0.15$). Virological response below 40 copies/mL did not differ according to baseline HIV-1 RNA <5 or $\geq 5 \log_{10}$ copies/mL, 63.6% [95%CI: 45.1-79.5] vs 50.0% [95%CI: 24.6-75.3], ($P=0.36$). Virological response below 40 copies/mL in patients with adherence assessment of at least 90% at week 24 did was higher than patients with adherence assessment <90%, 62.2% [95%CI: 46.5-76.2] vs 25.0% [95%CI: 0.63-80.5], ($p= 0.291$). Duration of previous first and second line regimens and type of NRTI given in the third-line regime were not associated with virological success at week 24. Resistance to at least one NRTI, ABC, AZT or TDF was not either associated with virological success. In a multivariate analysis comprising gender, age and CD4 counts, both an age superior or equal to 25 years and baseline CD4 counts superior or equal to 200 cells/ μL tended to be associated with a higher probability of virological success, although it was not statistically significant ($p=0.13$ and $p=0.15$, respectively) (Table 18).

Table 18: Factors associated with virological success below 40 copies/ml at Week 24 (n=49)

Characteristic	Week 24							
	<40 copies/mL		Univariate analysis			^a Multivariate analysis		
	n/N	%	OR	95%CI	P	AOR	95%CI	P
Gender,								
Female	12/20	60.0	Ref			Ref		
Male	17/29	58.6	0.94	[0.29-3.01]	0.92	0.95	[0.27-3.26]	0.93
Age (years)								
<25	6/14	42.8	Ref			Ref		
≥25	23/35	65.7	2.55	[0.71-9.08]	0.15	2.81	[0.75-10.53]	0.13
CD4 cell-count (cells per µL)								
<200	13/26	50	Ref			Ref		
≥200	16/23	69.5	2.28	[0.70-7.39]	0.17	2.47	[0.72-8.41]	0.15
HIV RNA Viral load (Log ₁₀ copies/mL)								
≥ 5 Log ₁₀ copies/mL	8/16	50.0	Ref					
< 5 Log ₁₀ copies/mL	21/33	63.6	1.75	[0.52-5.86]	0.37			
Infected through mother-to-child route								
no	23/36	63.9	Ref					
yes	6/13	46.1	0.49	[0.13-1.75]	0.27			
First-line ARV regimen Duration (years)								
≥4 years	12/18	66.7	Ref					
<4years	17/31	54.8	1.64	[0.50-5.51]	0.42			
Second-line ARV regimen Duration (years)								
≥5 years	16/28	57.1	Ref					
<5 years	13/21	61.9	1.21	[0.38-3.87]	0.74			
Third-line regimen								
DRV/r / DTG / 3TC	9/18	50.0	Ref					
DRV/r / DTG / 3TC / TDF or AZT	20/31	64.5	1.81	[0.55-5.92]	0.32			
Adherence assessment at W24								
<90%	1/4	25.0	Ref					
>=90%	28/45	62.2	4.9	[0.47-51.4]	0.18			
Full resistance to ABC/AZT/TDF								
Yes	9/16	56.2	Ref					
No	20/33	60.6	1.19	[0.35-4.01]	0.77			

OR: Odds-Ratio ^aadjusted; ORs were obtained from multiple logistic regression models including all variables in the column

At week 48, we found that as for week 24, patients with baseline CD4 counts $>200/\mu\text{L}$ had a better virological response than those below 200, the difference being of borderline significance (69.5% vs 42.3%, $p=0.06$) (Table 19). Notably, the youngest individuals (<25 years) again tended to less often have an HIV-1 RNA < 40 copies/mL compared to the older ones (35.7 % vs 62.8%, $p=0.09$). Subjects infected through mother-to-child transmission were significantly less likely to be virological responders at week 48 than the others (23.1% vs. 66.7%, $p=0.01$). In multivariate analysis, the role of young age below 25 years disappeared after adjustment for route of HIV acquisition (Table 19). Baseline CD4 cell counts $>200/\mu\text{L}$ remained borderline significant associated with a better virological response. Duration of previous lines of treatment was neither associated with virological success at week 48, nor at week 24.

Table 19: Factors associated with virological success below 40 copies/mL at Week 48 (n=49)

Characteristic	Week 48							
	<40 copies/mL		Univariate			Multivariate ^a		
	n/N	%	OR	95%CI	P	AOR	95%CI	P
Gender								
Female	13/20	65.0	Ref			Ref		
Male	14/29	48.2	0.50	[0.15-1.62]	0.25	0.42	[0.11-1.68]	0.22
Age (years)								
<25	5/14	35.7	Ref			Ref		
≥25	22/35	62.8	3.04	[0.83-11.07]	0.09	0.67	[0.06-7.82]	0.75
CD4 cell-count (cells per µL)								
<200	11/26	42.3	Ref			Ref		
≥200	16/23	69.5	3.11	[0.95-10.15]	0.06	3.64	[0.93-14.2]	0.06
HIV RNA Viral load (Log ₁₀ copies/mL)								
≥ 5 Log ₁₀ copies/mL	7/16	43.7	Ref					
< 5 Log ₁₀ copies/mL	20/33	60.6	1.97	[0.59-6.63]	0.27			
Infected through mother-to-child route								
no	24/36	66.7	Ref			Ref		
yes	3/13	23.1	0.15	[0.04-0.65]	0.01	0.07	[0.005-1.09]	0.06
First-line ARV regimen Duration (years)								
≥4 years	10/18	55.6	Ref					
<4years	17/31	54.8	1.02	[0.32-3.31]	0.961			
Second-line ARV regimen Duration (years)								
≥5 years	16/28	57.1	Ref					
<5 years	11/21	52.3	0.82	[0.26-2.57]	0.74			
Third-line regimen								
DRV/r / DTG / 3TC	12/18	66.6	Ref					
DRV/r / DTG / 3TC / TDF or AZT	15/31	48.3	0.46	[0.14-1.56]	0.219			
Adherence assessment atW48								
<90%	0/5	0	Ref					
>=90%	27/44	61.3	NC					
Full resistance to ABC/AZT/TDF								
Yes	10/16	51.52	Ref					
No	17/33	62.5	0.63	[0.18-2.16]	0.47			

NC: Not Computable OR: Odds-Ratio ^aadjusted ORs were obtained from multiple logistic regression models including all variables in the column

When considering a threshold of 1000 copies/mL instead of 40 copies/mL, at week 24, we observed similar trends. The association with baseline CD4 cell counts (≥ 200 vs. < 200 cells per μL) was statistically significant (95.6% vs. 69.2%, $p=0.026$) (Table 20). Among the 20/49 patients who had plasma HIV-1 RNA ≥ 40 copies/mL at week 24, 11 had a viral load below 1000 copies/ml, and 10 out of these 11 patients remained below 1000 copies/mL at week 48. Two out of the 9 patients who had HIV-RNA above 1000 copies/mL at week 24 decreased below 1000 copies/mL at week 48. There was a predominance of subjects below 25 years of age in patients > 1000 copies/mL compared to the patients aged ≥ 25 years (5/9, 56% vs. 9/40 23%, $p=0.047$).

Table 20: Comparisons of the percentage of patients with virological threshold below 1 000 cpies/mL at Week 24 (n=49)

Characteristic	W24			
	<1000 copies/mL			P
	n/N	%	95%CI	
Gender,				
Female	16/20	80.0	[56.3- 94.2]	1.00
Male	24/29	82.7	[64.2- 94.1]	
Age (years)				
<25	9/14	64.2	[35.1- 87.2]	0.154
25-50	27/31	87.1	[70.1- 96.3]	
>50	4/4	100.0	[39.7- 100]	
CD4 cell-count (cells per µL)				
<200	18/26	69.2	[48.2- 85.6]	0.026
≥200	22/23	95.6	[78.0- 99.8]	
HIV RNA Viral load (Log ₁₀ copies/mL)				
< 5 Log ₁₀ copies/mL	29/33	87.8	[71.7- 96.5]	0.130
≥ 5 Log ₁₀ copies/mL	11/16	68.7	[41.3- 88.9]	
First-line ARV regimen Duration (years)				
<12 years	19/23	82.6	[61.2- 95.0]	1.00
≥12 years	21/26	80.7	[60.6- 93.4]	
Second-line ARV regimen Duration (years)				
<5 years	16/21	76.1	[52.8- 91.7]	0.470
≥5 years	24/28	85.7	[67.3- 95.9]	
Third-line regimen				
DRV/r / DTG / 3TC / TDF or AZT	22/31	70.9	[51.9- 85.7]	0.018
DRV/r / DTG / 3TC	18/18	100	- -	
adherence assessment atW24				
<90%	2/3	66.6	[9.4- 99.1]	0.464
>=90%	38/46	82.6	[68.5- 92.1]	

3.2.7 Pharmacokinetic parameters

The characteristics of the 20 patients included in the PK sub-study were similar to the characteristics of the overall 49 patients. Briefly, 50% were female, median age was 39.5 years (IQR:26-45.5), median weight 57 Kg (45.5-63.5), median HIV-1 RNA and CD4 were 4.4 Log₁₀ cp/mL (3.9-5.2) and 192 cells/ μ L (58-354), respectively. Participants received DRV/r600/100mg BID plus 3TC/TDF (50%), 3TC (45%), AZT/3TC (5%) (Table 21).

Table 21: Characteristics of participants enrolled in the Pharmacokinetic substudy (n=20)

Characteristic	PK sub-study (n=20)	
General and demographic data		
Gender, Male, n (%)	10	(50.0)
Age (years), median (IQR)	39	(26-45)
Weight, kg, Median (IQR)	57	(45.5-63.5)
Body Mass index, Kg/m ² , Median (IQR)	22	(19.1-25.1)
HBsAg Positive, n (%)	1	(5.0)
Anti-HCV Positive, n (%)	-	-
Adherence assessment, n (%)		
Good	16	(80.0)
Moderate	3	(15.0)
Poor	1	(5.0)
Mode of infection, n (%)		
Heterosexual	13	(65.0)
Homosexual	0	0
PWID	0	0
Mother to child transmission	5	(25.0)
Unknown	2	(10.0)
Duration of cART(years), Median (IQR)		
First-line ARV regimen	4.5	(2.5-8.5)
Second-line ARV regimen	5	(4-8.5)
overall	13	(9-14)
History of opportunistic infection (Clinical stage), n(%)		
B	2	(10.0)
C	9	(45.0)
No history	9	(45.0)
Prescribed third-line regimen, n(%)		
DRV/r / DTG / 3TC / TDF	10	(50.0)
DRV/r / DTG / 3TC/AZT	1	(5.0)
DRV/r / DTG / 3TC	9	(45.0)
Laboratory data, Median (IQR)		
CD4 cell-count (cells per μ L)	192.5	(58.5-354.5)
HIV RNA Viral load (Log ₁₀ copies/mL)	4.40	(3.82-5.2)
Hemoglobin level, g/dL	13.2	(12.2-15.0)
AST level, U/L	31.5	(27-45)
ALT level, U/L	35	(25.5-46)
CrCl, mL/min	104.5	(75.8-120.3)
Glycemia,mmol/L	5.23	(4.73-5.8)
Total cholesterol,mmol/L	4.93	(3.26-6.0)
Triglycerides,mmol/L	2.13	(1.80-3.2)

Plasma drug concentrations of DRV and DTG are presented Table 22. Steady-state plasma exposures of DRV and DTG during a dosing interval showed wide interpatient variability. Median darunavir C₀ at week-4 and at week-30 (after switch) were 2483 µg/L (1344-4836) and 2660 µg/L (574-35490), respectively. Median dolutegravir C₀ at week-4 was 865 µg/L (500-1051). 55% of the PK sub-study patients (n=11) switched after week 24 to DRV/r800/100mg QD since they had reached a viral load below 40 copies/mL. There was no difference between the 2 dosing regimens (DRV/r600/100mg BID vs DRV/r 800/100mg QD), notably in the median C₀ pre-dosing concentration.

Table 22: Pharmacokinetic parameters of darunavir/ritonavir 600mg twice daily (Period 1, n=20) and darunavir/ritonavir 800mg once daily (Period 2, n=11) and dolutegravir 50mg once daily (n=20)

Pharmacokinetic Parameter ^a	Median (IQR) Period 1: DRV/r 600/100 mg BID ^b (n=20)	Median (IQR) Period 2: DRV/r 800/100 mg QD ^c (n=11)	Median (IQR) DTG 50mg QD (n=20)
C _{max} , µg/L	4434 (3694-6658)	4697(3366-7523)	2682 (1922-3444)
T _{max} , h	2 (2-4)	2 (2-4)	2 (2-4)
C ₀ , µg/L	2483(1344-4836)	2660(574-3549)	865(500-1051)

^aC_{max}: maximum concentration; T_{max}: time to achieve maximum concentration; C₀: concentration at time 0 pre-dosing; ^b: BID: twice daily-dosing, ^cQD: once daily-dosing

4 DISCUSSION AND PERSPECTIVES

4.1 General discussion of the studies

4.1.1 Study 1: the Burden of HCV infection in Cambodia

To our knowledge, this study represents the first HCV seroprevalence survey conducted in four different provinces of Cambodia evaluating the burden of HCV infection and its risk factors in these two different groups of population (PLHIV and pregnant women).

For PLHIV, HCV seroprevalence was similar to that found in a hospital-based HIV cohort in Phnom Penh at the same calendar period (7.6%) (56). The anti-HCV prevalence was slightly higher than that reported in Cambodia in 2014 (62) and in neighbouring countries in a regional HIV cohort (63), excluding countries with a high proportion of people who reported that they injected drugs. Chronic HCV infection prevalence among HCV antibody positive was 79% among PLHIV our study, in line with the rates generally found in newly diagnosed HCV patients (64). These results might be useful to guide the local strategy development in prioritizing the routine screening plan focusing on most at risk population for HCV case detection and identifying those eligible for treatment.

Our study showed that HCV seroprevalence among pregnant women was 0.9% [95%CI: 0.3-2.2]. This study could not provide the evidence of different distribution across the regions due to small number of positive cases. In a recent seroprevalence study conducted in Battambang province among the general population, anti-HCV prevalence was 0.6% [95% CI: 0.3–0.9] in adults 18–44 years old (65). These results confirm the low anti-HCV prevalence (below 1%) in people under 40. The currently

available literature shows that HCV seroprevalence in the general population reported in previous studies conducted in Cambodia varied over time with a decreasing trend in the most recent years, from 6.5% in 1993 (65) to 5.8% in 2012 (66) and 0.6% in 2018 (59). The decrease in prevalence of HCV over time may be explained by a variety of reasons. Since 2000, there has been major improvements in clinical practice in the country, with emphasis on sterilization of medical equipment, use of disposable needles and systematic screening of donated blood, all of which contribute to the reduction of blood borne agents such as HCV (60). It should also be noted that the availability of HCV testing and treatment, both in private sectors and public hospitals with the implementation of HCV program of Médecins Sans Frontières, could also have contributed to the decreasing prevalence of HCV by reducing the pool of infectious persons (59, 67). In addition to that, the low anti-HCV prevalence among young age-group could be explained by the age cohort effect for which people in the old-age group were exposed to HCV longer than those in the young-age group. The decreased prevalence in younger population was also observed in Thailand (68, 69).

In multivariate analyses, having ever received intravenous medication injection in the past five year and having a family member positive for HCV were strong, independent risk factors for HCV infection among Cambodian PLHIV, findings that are consistent with previous studies (56, 59, 60). A history of intravenous therapeutic injection was a major risk factor since 33 out of the 34 anti HCV positive participants reported this practice in the past five years, as compared to 665/901 among the

negatives. Therapeutic injections are a frequent practice in Cambodia as evidenced by our results showing that three-quarters of the participants, with both PLHIV and pregnant women reporting having received intravenous medication injection over the past five years. The overall treatment injection usage rate in Cambodia was 5.9 per person-year (women had a higher rate than men (7.5 vs. 4.3 per person-year), reported as one of the highest overall treatment injection usage rates worldwide (70). Moreover, having an HCV-positive family member was an independent risk factor for HCV infection. HCV familial clustering and intra-familial viral transmission have been demonstrated previously in a study by Indolfi et al (71) and in a Chinese study (72). In Cambodia, sharing hygienic products, such as shaving razors, toothbrushes and nail clippers or ear/nose piercing or tattoo are common due to ignorance about sanitation and lack of resources. Our results argue in favor of the need of HCV screening for the whole family if one of member is positive and more particularly for children who are currently mostly unaware of access to HCV care programs. The risk factors of HCV infection could not be analysed in pregnant women due to sample size issue. Of note, all five HCV-infected pregnant women also reported a history of intravenous medication injection in the previous five years.

HCV genotype 1b, 2a and 6 variants were identified. HCV genotype 1b (47.8%) and 6 (30.4%) were predominant in the current study. This finding is consistent with several previous studies in Cambodia, which reported HCV genotype 1b and 6 as the common genotypes observed in Cambodia (56, 66, 67, 73, 74). The genotype 6 variant

was also found as the most common in Vietnam and Laos (73), in blood donors in some regions of Thailand (16) and in intravenous drugs users in China (68, 75, 76).

This study has several limitations. Our study attempted to assess the anti-HCV prevalence rates in different regions across the country to have representative anti-HCV prevalence rates for the overall population in Cambodia. However, our small sample size in Preah Sihanouk (South-western region) may limit the generalizability of the results for this region. Our findings may have been subject to recall bias or social desirability bias resulting in the underreporting of some risky behaviors and practices over the last five years such as the experience of injecting illicit drugs. Last, due to the small number of HCV positive in pregnant women, the risk factors of HCV infection in this group could not be assessed. However, in this study, we can rule out differential misclassification for assessing the risk factors of HCV infection linked to the history of risky behaviors and practices, since interviews were done before the result of HCV serology was known.

In conclusion, we report a high HCV seroprevalence in PLHIV in Cambodia, likely related to past intravenous medication injection and/or intra-familial viral transmission. Our results led the national Cambodia program to offer systematic HCV testing in PLHIV and underline the necessity of care of HCV positive populations, measures which had already been initiated by National Program for adults. Children of HCV-infected parents living in the same household are likely the next target for testing and envisaging care access.

The low prevalence of HCV infection in the population under 40 years old seems to be confirmed but the anti-HCV prevalence varied across geographical area and specific populations, suggesting the need to set up a surveillance system to assess the anti-HCV prevalence across different population groups.

4.1.2 Study 2: the ANRS 12374 3DICAM study

This study reports the results of a programmatic evaluation of third-line regimen strategy implemented within the framework of the Cambodian HIV national program. In this heavily pre-treated, multidrug resistant, population of patients aged over 15 years, a genotypic-driven third-line regimen led to a virological response below 40 copies/mL in 59% and 55% at 24 weeks and 48 weeks of treatment, respectively. Worth noting, 80% of the patients were below <1000 copies at week 24 and week 48. No patient stopped DRV/DTG combination due to an adverse event.

Patients recruited in the 3 DICAM study had experienced 3 months of boosted adherence counselling before pre-enrolment. Adherence was pointed as a major factor of switching from second to third line therapies, and of virological response to third line therapies (61, 77-79). In Cambodia, 47% of patients with second-line therapeutic failure (> 1000 copies/mL) were rescued with adherence boosting and, among them, 29% had no RAM on genotyping test (61). In Western Africa, Eholie et al. reported that adherence reinforcement resulted in viral resuppression in 67% who therefore continued with second line ART (78). However, in the 3DICAM study, among the 110 patients pre-enrolled, only 50 get the inclusion criteria and 32 still get HIV RNA > 1000 copies/ml while the

GART reported sensitivity to the current therapeutic regimen. This result highlights the difficulty to fully support adherence for some of these patients and the possible necessity to evaluate alternative strategies as therapeutic simplification.

In the 3DICAM study, the ART median duration was 13 years, including five years with second line regimens. These patients exhibited high rates of RAMs to NRTI, NNRTI and PI. All patients were naïve of Integrase Strand Transfer Inhibitor (ISTI). All but one patient had a viral strain sensitive to DRV/r, even after exposition to several PI, mostly LPV/r and ATV/r, in their second line regimen. Optimized NRTI were chosen to be combined to DRV/r and DTG, taking into account RAMs; lamivudine was prescribed in all even when the genotypic resistance test did not show sensitivity, as proposed in previous studies (80, 81). The virological response observed at 24 and 48 weeks, for a threshold of 40 copies/ml, is lower than those observed in intensified regimens assessed in highly experienced patients in high income countries (45). Patients experienced different virological outcomes, few of them having no decline in viral load, or a rapid viral rebound after ART initiation, indicating that adherence may not be optimal. Even with at least two drugs (DRV and DTG) fully active on their viral strains, lack of adherence converted in intermediate virological response. However, the rates of virological response at week 48 were of 73% and 79%, according to the thresholds of 200 and 1000 copies/mL respectively. In low- and middle-income countries, studies have shown contrasted results according to patient profiles and salvage drugs prescribed.

In published studies of third-line therapy, modification of ART using DRV/r and/or

INSTI (dolutegravir or raltegravir) and/or etravirine led to very similar results (78, 82-87). Around 80% of patients with HIV RNA viral loads > 1000 copies/mL at third line initiation achieved viral loads < 1000 copies/ml between 24 and 48 weeks, according to the studies. In the multisite ACTG A5288, viral suppression to < 200 copies/mL were observed in 74 to 100% of patients according to their initial profile and drugs prescribed (84). In a prospective study in Western Africa, 41% of patients achieved < 50 copies/mL at week 24 and 59% at week 48(78). In South Africa, 58% of patients suppressed their viral load to < 50 copies/mL at least 6 months after third line initiation approval (86). A majority of our patients were included or followed in the 3DICAM trial during the worldwide SARS-CoV-2 pandemics. Even if ARV delivery was preserved, individual socio-economic factors may have interfered with their daily life and adherence to ART. Unexpectedly, adherence measurements (pill count and visual analogic scale) failed to detect low levels of adherence in failing patients as a white coat effect cannot be excluded. These patients evolving from persistent virological failure on second line ART to ineffective third line ART may represent the patients with more preminent adherence difficulties.

The observed concentrations of darunavir and dolutegravir in this Cambodian population are in the same range as those previously reported in other population of PLHIV, although there is a trend for lower C_{max} of DRV and DTG, likely for slower absorption. Trough DRV concentrations at the 600/100 BID dosing were reported to be 2241 ng/mL and 2900 ng/mL (88, 89). Interestingly, switching to OD dosing did not

impair exposure in our study, although the small number of patients who switched does not allow bioavailability comparison. Trough concentrations were higher than those previously reported in Caucasian population which were in the 1000 and 1800 ng/mL range (90-92). Such discrepancy could be explained by a food effect and highlight the complexity of darunavir pharmacokinetics and its nonlinear dose-concentration relationship (91). C_{max} and trough concentrations of dolutegravir are close to previously reported studies where dolutegravir was combined to a ritonavir boosted darunavir regimen (90, 93).

Among the 28 patients who switched to DRV 800 mg at 24 weeks, 8 had a virological rebound after the switch. Even if for 6 of them, the viral load remained below 200 copies/mL, the relevance of such a switch with the aim of lightening and maintaining adherence remains questionable. The continuation of a twice-daily regimen could be the most effective option for this population of heavily pre-treated patients, even if once daily PK parameters were reassuring.

A part from low baseline CD4 counts, the only risk factor for virological failure at week 48 was the fact to be mother-to-child infected, which is associated with the age ranging 15 – 25 yo. We did not find a role of the number of resistance mutations at baseline, nor of baseline plasma viral load. The PK data showed levels comparable to those of other non-Cambodian populations (88-90, 93). Taken together, this argues for persistent poor adherence in some of these patients. This poor adherence in the younger ones appeared attributable to their history of mother-to-child HIV transmission, and for

some to family issues incidentally reported by the caregivers.

A European study already reported that the risk of triple-class virologic failure was higher in perinatally infected children and adolescents than in a distinct group of heterosexually infected adults (94). A French study also found them at higher risk of having viral failure than persons infected during adulthood with the same duration of HIV infection, even after adjusting for therapeutic history. This difference was even more obvious in teenagers and young adults (13-24 years) and was likely due to problems of adherence specific to these periods of life (95). Transitioning from paediatric to adult care has been shown to make HIV-infected adolescents and young adults be more vulnerable to virological failure (96). In Cambodia, HIV-infected adolescents go to adult consultations from 15 years of age, and this may have been too early for some of them (97).

The ANRS 12374 3DICAM study is a programmatic evaluation of a third-line strategy conducted within the national HIV program. This strategy allowed us to achieve undetectability at the threshold of 1000 copies/mL for 80% of patients at 24 and 48 weeks, the threshold used by the national program to define therapeutic failure. These results are comparable to those reported by the South African national public sector HIV program (86, 98) and confirmed the evidence of the effectiveness of third-line ART regimen provided in a public sector programmatic setting. Some lessons can nevertheless be learned. First, it took more than two years (30 months) to include 50 patients in 13 ART sites across Cambodia, confirming the low number of patients with

PI-based second-line treatment failure who are in need of third-line regimen, as previously reported by the 2PICAM study. The small number of eligible patients in each ART site represents a real challenge for the national program in terms of training health care workers in the use of these new drugs. A dedicated committee has been created within the national program and a decision to switch can only be made after the agreement of this committee, which can also serve as a clinical support to the physicians in charge of the patients. The management of drug procurement and distribution for such small quantities is also challenging, especially for darunavir which is still only used in Cambodia in this indication. The logistical and financial impact of such a third-line strategy for the national HIV program deserves to be further assessed by a cost analysis. Finally, new regimens recommended by the WHO, including integrase inhibitor in first or second line therapies, may impact virological outcome of HIV-infected patients in the future.

In conclusion, third line regimen combining DRV/r, DTG and an optimized background of NRTI appears to be effective to obtain a significant decrease in viral load after one year for the majority of these multi-experienced patients and is associated with immunological response. Adherence strategies at ART initiation, repeated during HIV care, particularly in young patient of mother-to-child infected with suboptimal virological response, remain a key element in life long HIV therapy, even in the context of recent powerful salvage antiretroviral drugs.

4.2 Public health implications of the studies

My thesis project consisted of 2 studies: study 1 aimed at assessing the magnitude of HCV infection in Cambodia, and study 2 was to implement and assess a third-line program in Cambodia. These two studies contribute as evidence for Cambodia to address HCV infection toward the goal set for HCV elimination as a public health threat by 2030 as well as HIV infection to reach the 95-95-95 targets by 2025.

4.2.1 Public health implications of the study 1 on hepatitis C infection

In Cambodia, knowledge of the epidemiology of hepatitis C is essential for prioritizing the required intervention to achieve the World Health Organization goal for elimination by 2030. The overall goal of study 1 was to estimate the HCV prevalence rates among pregnant women and among people living with HIV in four selected provinces across Cambodia.

Although several studies on HCV burden in Cambodia were conducted in the past, there was at the beginning of this thesis project a paucity of evidence among pregnant women and among people living with HIV and, when available, the epidemiological data were restricted to specific regions of Cambodia (northwestern and capital city) which could not be generalized the burden of HCV infection to other geographical regions in Cambodia. The current study contributed to fill the knowledge gap on the burden of HCV among pregnant women (considered as a proxy for general population) and to strengthen the evidence on hepatitis C burden among PLHIV. As presented in article 1, our study found that the HCV burden differed among different population groups:

people living with HIV vs pregnant women; young age group (<40 years) vs old age group (≥ 40 years). This finding has filled the gaps in the HCV burden among pregnant women, which can help to inform future intervention strategies for prioritizing screening and treatment of subgroup populations. Our results also contributed to strengthen the evidence that, in Cambodia, the HCV seroprevalence was high among people living with HIV and among older age groups >40 years old, which confirmed the current national strategy for HCV elimination to prioritize these two subgroup populations for screening and treatment.

Simultaneously, in addition to the past intravenous medication injection, the independent risk factor found in this study was having a family member positive with HCV. The recommended screening strategy should be prioritized for the entire family for those who have member(s) positive for HCV. Currently, the HIV national program offers systematic HCV testing for people living with HIV. Since 2019, 70% of the PLHIV in the national HIV program have been screened and received the HCV treatment under global fund support. However, the activities have been pending for a while due to lack of funding and the proposed new round for financial support from the global fund for scaling up the HCV screening and treatment among PLHIV was just available. Currently, the national program continues to scalp HCV testing for the 30% of PLHIV who remain in the program. Based on evidence from this study, we encourage future implementation programs to consider the entire family, including children of HCV-infected parents living in the same household, as the next target for testing and envisaging care access.

To date, no HCV national program exists in Cambodia for the management of HCV infection, except for the people living with HIV, which has been initiated in the existing health care system by the HIV national program. Since the initialization of the Technical Working Group for viral hepatitis in 2019, progress has been made toward HCV elimination in Cambodia. This Technical Working Group is coordinated by the Department of Communicable Disease Control in Cambodia (CDC) at the Ministry of Health. The treatment guidelines and the national strategic plan for HCV elimination have been developed. Scaling up the hepatitis service delivery has been started within the 12 operational districts where the MSF program activities were implemented, and the service delivery is planned to gradually scale up to 10 additional operational districts per year. However, while progress has been made, Cambodia is behind elimination targets, and HCV services were paused when the MSF France program closed in 2021, as the program implementation relied on MSF program support (technically and financially). The MSF France program was closed and handed over to the Department of Communicable Disease in Cambodia for the implementation of the national strategic plan, while the national allocated resources were not yet available in Cambodia. In this situation, achieving national strategic plan goals for HCV elimination by 2030 is far from reality. There were no clear strategies presented in the national strategic plan on the HCV screening strategy, except the priority to screen and treat the subgroup population of +45 years old. The findings from the current study could be used as strategic information to justify the need for reviewing the screening strategy, for example the next target population for HCV testing and envisaging care access should be prioritized to

the entire family living in the same household of HCV-infected people.

4.2.2 Public health implications of the study 2 on the ANRS 12374 3DICAM study

Prior to the development of the ANRS 12374 3DICAM study, there was no standard recommended third-line regimen available yet. The ANRS 12374 3DICAM study was designed as a programmatic evaluation of a third-line strategy conducted within the national HIV program consisting of a combination of darunavir/r, dolutegravir and optimized NRTI in Cambodia using the genotype testing strategy to make a decision to switch to third-line antiretroviral therapy (ART) in patients with treatment failure on second-line therapy.

The 2PICAM study, which was conducted by the national program in 2013, suggested that genotypic antiretroviral resistance testing (GART) was a necessary option to identify patients who were resistant to PI-based second-line treatment in Cambodia and needed to switch to a third-line regimen. The GART was found to be a cost-effective strategy to identify the patients failing their current treatments as well as to optimize the background treatment and to shorten unnecessary delays in switching the patients to an effective treatment for better outcomes (11, 99, 100). Thus, the GART did form part of the strategy when deciding to switch the patients from second-line to third-line regimens in the design of the ANRS 3DICAM study. In the 3DICAM, at preinclusion, 32/60 patients who were not resistant to a second-line-based regimen would have had to be switched to third-line treatments if we had not performed genotyping tests at prescreening. These 32 patients (continued their second-line regimen with

reinforcement of enhanced adherence counselling. However, in this thesis project, we could not include in the analysis of the data of these patients. Given the high cost of third-line regimens and the challenges in implementing the third-line strategy, having outcome results such what they become for those who were not included in the study will be essential to assess the effectiveness of the strategy to ensure optimal use of resources in Cambodia.

The third-line strategy proposed in the 3DICAM study was the combination of DRV/r 600/100 twice daily and DTG 50 mg once daily. At week 24 after initiation of the third-line regimen, the patients with HIV RNA < 40 copies/mL were switched to DRV/r 800/100 mg once daily. The results of the 3DICAM showed that with this strategy at week 24, 81.6% of patients were virally suppressed at a threshold of 1000 copies/ml, the threshold used by the national program to define therapeutic failure. The proportion of patients reaching the threshold of 1000 copies/ml remained unchanged at week 48 (79.5%). These results are comparable to those reported by other studies in African countries (86, 98) and confirm the evidence of the effectiveness of the third-line ART regimen provided in a public sector programmatic setting. This study provided evidence to guide third-line strategies for Cambodian national HIV programs as well as for resource-limited settings with similar context as Cambodia.

This study also demonstrated that with the proposed strategy of a new treatment, 59% of patients who were heavily pretreated can achieve virological suppression at week 24. The response rates were similar at week 48, with 55% of the patients virally

suppressed. We observed that the only risk factor for virological failure at week 48 was potentially related to the route of HIV infection from mother to child, which was associated with the young age group < 25 years old. This finding provides further evidence of the need for reviewing the current monitoring guidelines for treatment failure such as frequency of viral load measurement among the patients with indicators of possible nonadherence, specifically for the cohort of young adults/adolescents infected through the mother-to-child route of transmission. This would contribute to early diagnosis of treatment failure and timely switching to proper effective treatment to optimize long-term treatment success among this hard-to-treat population group.

In the 3DICAM study, one-third of the patients included were young adults aged < 25 years old. About 43% and 36% of patient aged < 25 years had HIV RNA <40copies/mL at W24 and W48 respectively. At W24, there were 20 patients who were virally nonsuppressed (VL >40 copies/mL), 8 out of these 20 patients being aged <25 years old. Poor adherence is the most common behavioural predictor of treatment failure; however, reliability of the adherence assessment is difficult to measure. In our study, the adherence assessment data collected using the routine adherence assessment tools in the program which included pill-count and visual scale did not reflect the adherence issues of the patients. These data highlight the need for restructured adherence counselling to further improve third-line treatment outcomes and to avoid the development of HIV drug resistance in the long term. The existing strategies for adherence enhancement currently implemented in the national program should be

reviewed, as they might no longer be adapted for adolescents or young adults infected through mother to child transmission. A dynamic adherence intervention strategy to help adolescents and young adults to maintain viral suppression in the long term should be evaluated to improve the adherence treatment outcome for this population group.

Lessons learned through the implementation of the 3DICAM study highlight a real challenge for the national program to contextualize the need for third-line ART in Cambodia. Great efforts have been made to train health care teams for clinical management as well as the management of drug procurement and distribution for a small number of patients. The logistical and financial impact of the proposed third-line strategy for the national HIV program deserves to be further assessed by a cost analysis to provide the national a baseline to ground planning discussions and support strategy development and decision making around resource allocation for an effective third-line program in Cambodia.

4.3 Perspectives

Several questions remain unanswered around the implementation of the third-line program designed as the 3DICAM study and there is a need for further analyses to provide more comprehensive relevant information for the national program to develop policies for third-line therapy that consider funding, sustainability and provision of equitable access to ART in Cambodia. The following analysis and research project will be considered following the findings of the 3DICAM study:

1. Performing analysis of the outcome data of the 32 patients who were not included in the study because they had no genotypic resistance to second-line treatment at preinclusion and would have switched to third-line treatments if we had not had performed genotyping tests at pre-screening. Having outcome results of such patients will be essential to assess the effectiveness of the strategy to ensure optimal use of resources in Cambodia.
2. Assessing drug resistant profile among adolescent or young adults (<25 years old) infected through the mother-to-child route of transmission in the national cohort.
3. Reviewing the current monitoring guidelines for treatment failure, specifically for this group of patients for early diagnosis of treatment failure and timely switching to proper effective treatment to optimize long-term treatment success among this hard-to-treat population group.
4. Evaluating the existing strategies for adherence enhancement currently

implemented in the national program and exploring a dynamic adherence intervention strategy to help adolescents and young adults (<25 years) to maintain long term viral suppression on third-line treatment.

5. Conduct a cost analysis on third-line implementation in Cambodia based on the design of the 3DICAM study (see full substudy protocol in annex 1).

The cost analysis on third-line implementation in Cambodia based on the design of the 3DICAM study will be conducted to answer the following questions:

- What would a third-line program design as the ANRS 12374-3DICAM study cost in Cambodia using DRV and DTG as a backbone?
- And in which proportion of the funds allocated to HIV-related services globally?
- What is the part related to morbidity and toxicity monitoring and management?
- What are the costs of different scenarios: GART with DRV dose reduction and GART without DRV dose reduction?
- How much future funding is needed to expand the program to more people in need?

Cost analysis based on the design of the 3DICAM study is an opportunity to inform national authorities about the relative costs of the third-line implementation and the share of this cost in the funds allocated to HIV-related services globally. A cost analysis could be useful to compare the 3DICAM strategy with and without DRV dose reduction after 6 months of treatment. Impact of extra costs as safety or morbidity

management, out of pocket expense for the patients within the ANRS 12374-3DICAM strategy with and without DRV dose reduction after 6 months of treatment will be estimated within the 2 scenarios. All of information are relevant for government policies. At the international level, cost analysis based on the design of the 3DICAM study could be compared to other third-line programs and can contribute to more accurate international estimates of the resources needed for HIV programs.

Expected outputs

1. Information on costs of a third-line program design as the ANRS 12374-3DICAM study in Cambodia using DRV and DTG as a backbone which will inform national authorities about the relative costs of the third-line implementation and the share of this cost in the funds allocated to HIV-related services globally.
2. Information which is relevant for government policies on the impact of extra costs such as out of pocket expense within the ANRS 12374-3DICAM strategy with and without DRV dose reduction after 6 months of treatment
3. At the international level, information on costs of a third-line program based on the design of the ANRS 12374-3DICAM study could be used to compare with other third-line programs and could be contributed to more accurate international estimates of the resources needed for HIV programs.

4.4 Conclusion

The two studies of my thesis project contributed in term of scientific knowledge and provided evidence to inform the implementation of the intervention strategies for elimination of hepatitis C infection by 2030 in Cambodia as well as the evidence to guide third-line strategies for Cambodian national HIV programs for proper management, treatment and care of HIV/AIDS infection to achieve the 3rd 95 UNAIDS target by 2025. Findings from the HCV seroprevalence study highlighted the priority for systematic screening of family members living in the same household with HCV infected person and this population group should be the next target for testing and envisaging care access. The findings from the ANRS 3DICAM study suggested a good response rate of third-line strategy using DRV and DTG as a backbone in heavily pre-treated multidrug resistant HIV-infected patients in Cambodia, but the strategy must be accompanied by innovative counselling strategy for younger patients infected through MTCT in live long HIV therapy, even in the context of recent powerful antiretroviral drugs.

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6 ANNEXES

Annex 1: Protocol of the ANRS 12374 3DICAM study

Annex 2: The article on Hepatitis C seroprevalence among People living with HIV and pregnant women in four provinces in Cambodia: an integrated bio-behavioral survey.

Annex 3: The list of the ANRS 12374 3DICAM study teams

Annex 4: The manuscript on effectiveness and Pharmacokinetics of a third-line cART regimen combining dolutegravir, ritonavir-boosted darunavir and optimized NRTI in HIV-infected treatment-experienced adults in Cambodia: the ANRS 12374 3DICAM study.

ANRS 12374 3DICAM study

Dolutegravir, Darunavir/ritonavir and Optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia

Protocol Version 5.0- January 27, 2022

Clinicaltrial.gov registration number: NCT03602690

Ethics committee approvals of Protocol version 1.0

Cambodia: Name of the committee: NECHR
Date of approval: 05/12/2017

Ethics committee approvals of Protocol version 2.0

Cambodia: Name of the committee: NECHR
Date of approval: 09/05/2018

Ethics committee approvals of Protocol version 3.0

Cambodia: Name of the committee: NECHR
Date of approval: 02/07/2018

Ethics committee approvals of Protocol version 4.0

Cambodia: Name of the committee: NECHR
Date of approval: 06/11/2020

Sponsor:

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Dolutegravir, Darunavir/ritonavir and Optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia – ANRS 12374 3DICAM study

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STUDY PROTOCOL VERSIONS

Table 1. Version of the ANRS 12374 3-DICAM Study

Version N°	Date	Amendment N°	Main Modifications
1.0	01-Dec-2017	-	-
2.0	09-May-2018	-	<ul style="list-style-type: none"> - Add a criteria restricted to DRV switching at M6 - Clarify of drug dispensation - Add the detail on the specimens in the section "Biobank Management" - Modify CRF: adding genotyping test table and event reporting form table and modifying of timing for HBsAg and HCV Antibody
3.0	02-Jul-2018	-	<ul style="list-style-type: none"> - Modify the eligible criteria follows the recommendation from WHO for women of childbearing age - Add a specific chapter of women of childbearing age. - Add the thresholds of HIV viral load in the statistical analysis (thresholds of 4 and 5 Log copies/mL).
4.0	06-Nov-2020	-	<ul style="list-style-type: none"> - Modify inclusion criteria: remove "VL>1000copies/mL) - Based on new WHO recommendation for pregnancy occurrence in July 2019, modify treatment option for pregnancy (in all cases, DRV/r 600/100 mg twice daily must be prescribed and DTG could be continued). -Add sub study "Costing Analysis"
5.0	-	-	<ul style="list-style-type: none"> -Replace of PI(South) -Revise Consent Form V1.0: offer participants to have voice recorded for Costing Study.

LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ANRS	ANRS Emerging infectious diseases
ALT	Alanine aminotransferase
ANRS	Agence Nationale de Recherches sur le Sida et les hépatites virales
ARV	Antiretroviral drugs
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
ATV	Atazanavir
BAC	Boosted Adherence Counseling
BMI	Body mass index
CBC	Complete blood count
CHAI	Clinton Health Access Initiative
CRF	Case report form
DRV	Darunavir
DSC	Decision Support Committee
DSMB	Data safety & monitoring board
DTG	Dolutegravir
ELISA	Enzyme-linked immunosorbent assay
ERHZ	Ethambutol rifampicin isoniazid pyrazinamide
GCLP	Good clinical laboratory practice
GCP	Good clinical practice
GRT	Genotyping Resistance Test
HCV Ab	Hepatitis C virus antibody
HBs Ag	Hepatitis B virus surface antigen
HIV	Human immunodeficiency virus
IMP	Investigational medicinal product
INH	Isoniazid
IPC	Institut Pasteur du Cambodge
LAM	Lipoarabinomannan
MIC	Minimum inhibitory concentration
MDR	Multi drug resistant
NCHADS	National Center for HIV/AIDS, Dermatology and STD
NRTI	Nucleoside reverse transcriptase inhibitor
PCR	Polymerase chain reaction
PI	Protease Inhibitor
PK	Pharmacokinetic
PMTCT	Prevention of mother-to-child transmission
RH	Rifampicin isoniazid
RNA	Ribonucleic acid
(S)AE	(Serious) Adverse event
(S)AR	(Serious) Adverse reaction
SmPC	Summary of the product characteristics
SOC	System organ class
SOPs	Standardized operating procedures

SUSAR	Suspected unexpected serious adverse reaction
TB	Tuberculosis
TDF	Tenofovir
TMF	Study master file
TWG	Technical Working Group
USAID	United States agency for international development
XTC	Lamivudine (3TC) or emtricitabine (FTC)
WHO	World health organization
ZDV	Zidovudine

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STUDY SUMMARY

Clinicaltrial Id:

Title of Study: Dolutegravir, darunavir/ritonavir and optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia

Short title – Sponsor N°: ANRS 12374 3DICAM study

Sponsor:

Inserm-ANRS

French National Institute of Health and Medical Research (**Inserm**) ANRS I Emerging infectious diseases (**ANRS**)

Coordinating Investigator(s): Pr Cécile GOUJARD / Dr OUK Vichea

Participating countries: CAMBODIA AND FRANCE

Objectives

Principal objective: To assess the virological effectiveness of a third-line regimen combining dolutegravir (DTG), ritonavir-boosted darunavir (DRV/r) and optimized NRTI after 6 months of treatment, in Cambodian HIV-infected adults, who failed a protease inhibitors (PI)-based second-line regimen despite 3 months of boosted adherence counseling (BAC), with evidence of intermediate or fully resistance to atazanavir (ATV) OR sensitivity to ATV but resistance to all NRTI.

Secondary objectives

- Evaluate the safety of third-line regimen combination
 - Evaluate the adherence of third-line regimen combination
 - Evaluate the immune restoration (increase in CD4 cell-count) and the frequency of IRIS
 - Estimate plasma exposure and pharmacokinetic parameters of DRV/r 600/100 twice daily and DTG 50 mg once daily
 - Assess the effectiveness of switching DRV/r from 600/100 twice daily to 800/100 once daily for patients with plasma HIV1 RNA < 40 copies/mL at 6 months
 - Estimate the pharmacokinetic parameters of DRV/r 800/100 once daily vs DRV/r 600/100 bid in the patients who switched
-

Methodology: Non-comparative multicenter pilot prospective study

Expected enrolment

54 patients

Outcomes

Primary outcome: Proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 6 months

Secondary outcomes :

- Proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 12 months
- CD4 cell count at 6 and 12 months
- Proportion of patients with IRIS
- Proportion of patients with permanent study treatment discontinuation during the first year
- Incidence of grade 3-4 adverse events (ANRS grading table)
- Proportion of patients with adherence > 90% during one year
- Proportion of patients switched from 600/100 twice daily to 800/100 once daily at 6 months
- Plasma darunavir concentration with 600/100 twice daily and 800/100 once daily

- Plasma dolutegravir concentration
- Proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 12 months among those switched from 600/100 twice daily to 800/100 once daily

Eligibility	Inclusion criteria
	<ul style="list-style-type: none"> • >= 15 years old the day of inclusion • Documented HIV-1 infection • Failing a NNRTI-based first-line regimen • Failing a PI-based second-line regimen after 3 months of adherence boosting • HIV strain intermediate or fully resistant to ATV/r OR sensitive to ATV but both resistant to AZT, ABC and TDF • For women of childbearing age (15-44 years old): acceptance to use effective contraceptive methods • Informed consent obtained with information sheet given and explained before the inclusion visit and the consent form signed by the participant and the project investigator at the latest the day of the inclusion

Non-inclusion criteria

- History of antiretroviral treatment including darunavir and integrase inhibitor
- Active pregnancy < 12 weeks of amenorrhea and desire of pregnancy during the duration of the study
- Opportunistic infection in acute phase at inclusion including tuberculosis treated since less than one month and/or with no stable clinical condition
- Advanced cirrhosis (Child-Pugh score B or C)
- Creatinine clearance < 50 ml/mn
- Any concomitant medical condition that, according to the clinical site investigator would contraindicate participation in the study
- Concurrent participation in any other clinical study without written agreement of the two study teams

Intervention

Each patient will be informed of the objectives and the total duration of the study as well as the benefits and risks to participate. An information sheet in Khmer will be given to each patient. After signature of consent form, Genotyping Resistance Test (GRT) and pre-inclusion samples will be done for all patients failing a PI-based second-line regimen according to national guidelines after 3 months of boosted adherence counselling (BAC). After receiving results of the GRT and the pre-inclusion sample, the technical working group (TWG) of NCHADS will decide to switch the patient to third-line regimen or to continue second-line regimen with new BAC. PI-based second-line regimen will be continue if 1/ GRT shows sensitivity to ATV 2/ GRT shows sensitivity to at least one of the 3 NRTI recommended in Cambodia (AZT, ABC and TDF). In that case, adherence counselling will be boosted according to the new national guidance and the patient will not be enrolled in the study.

In case of intermediate or fully resistance to ATV/r OR sensitivity to ATV but both resistances to AZT, ABC and TDF and after confirmation of eligibility criteria, patient could be enrolled in the study. A third-line regimen will be started including DRV/r 600/100 twice daily + DTG 50 mg once daily + 3TC 300 mg once daily +/- one fully or intermediate sensitive NRTI among TDF, ABC and AZT. The choice of the last NRTI will be discussed and decided by the TWG according to the HBsAg status, to the result of the GRT and to the medical history of the patient.

At 6 months, plasma HIV-1 RNA will be measured:

- HIV1-RNA < 40 copies/mL and no resistance to DRV at inclusion: a switch to DRV/r 800/100mg once daily will be done and adherence counseling provided to confirm the new dosing with the patient

• HIV1-RNA > 40 copies/mL and/or intermediate or fully resistance to DRV at inclusion: the same regimen will be continued and adherence counseling provided

For all patients, a new virological assessment will be done at 9 and 12 months.

DRV and DTG exposure and pharmacokinetic parameters (Cmax, Cmin and AUC) will be estimated for the 20 first enrolled patients, allowing an intra-patient comparison of the 2 dosing regimens of DRV/r for at least 15 patients.

Statistical methods

According to data available with the ANRS 139 TRIO and ANRS 143 NEAT studies, we could expect that at least 80% of patients will be in virological success after 6 months and we wish to conclude that treatment will be effective for more than 60% of patients

With a sample size of 45 patients, we could conclude that success rate is at least 60% if the total number of failure does not exceed 12 (alpha 0.05, power 90%, Fleming method with one stage).

Among the 3500 patients on PI-based regimen, and according to the results of the 2PICAM study, we could expect that 5% of patients followed in Cambodian sites could be enrolled in the study (175 patients) and one third of them (60 patients) will need third-line regimen at inclusion. With a 10% refusal ratio, we anticipate to enroll 54 patients for third-line regimen while 100 patients will continue second-line regimen.

Estimated planning or study timetable

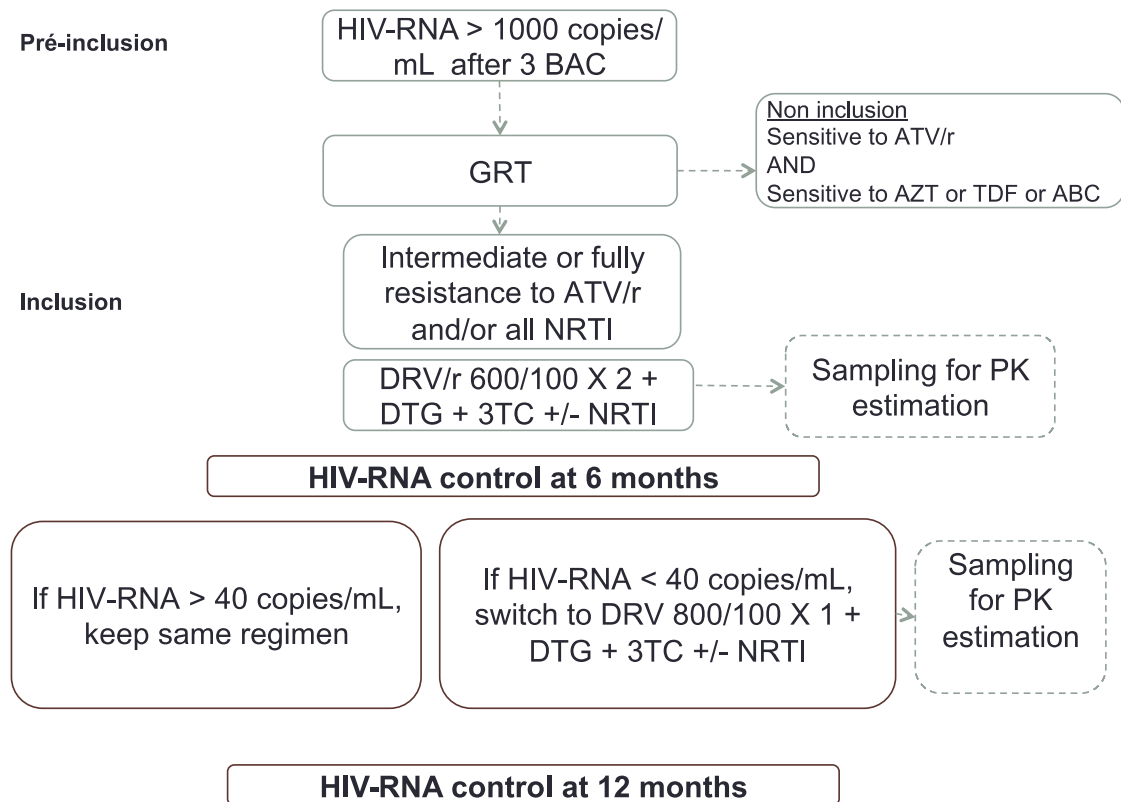
FPFV : first patient first visit : October 2018

LPFV : last patient first visit : May 2021

FPLV : first patient last visit : October 2019

LPLV : last patient last visit : May 2022

1 STUDY SYNOPSIS



2 SCIENTIFIC RATIONALE

2.1.1 HIV situation in Cambodia

The HIV prevalence was further decreased from 0.9% in 2006 to 0.7% in 2012 (HIV projection). At the end of 2015, among the 72,607-estimated number of people living with HIV (PLHIV), 57,651 (79.4%) were diagnosed and know their status, 54,870 (75.6%) were on ART followed in 63 health care sites (36 pediatric), 37,568 were on ART and virologically tested (51.7%) and 35,056 (48.2%) were virologically suppressed.

The national guidelines were revised in October 2015 and treatment was recommended for all patients whatever the CD4 cell-count. The recommended first line is now TDF/3TC/EFV and the second line AZT/3TC/ATVr. A first viral load is performed at 6 months, 12 months and then yearly. If CD4 > 350/mm³, national guidelines recommend to use only viral load monitoring with no need for ongoing routine CD4 assay. In patients with virological failure, an adherence boosting for 3 months and viral load control at 3 months must be done before switching to a second-line regimen.

A viral load scale-up strategy has also been defined in 2015 including dissemination of the new guidelines, strengthening the capacities of the laboratories and implementation of a standard operating procedure. For patients with virological failure, genotyping resistance tests (GRT) are made in Institute Pasteur of Cambodia in partnership with the NCHADS laboratory.

2.1.2 Evaluation of first and second-line regimen in Cambodia

The effectiveness of WHO-recommended first line regimen was assessed in several studies with good outcomes in adults (1)(2)(3) and children (4) related to a high level of adherence (5)(6). In 2011, good outcomes were also reported with lopinavir (LPV/r)-based regimen in a small study of 70 patients after 24 months of follow-up (7). More recently, the TREAT Asia HIV Observational Database reports outcomes of 302 patients under PI-based regimen followed for more than 6 months (8) from different Asian sites, including Cambodia, with a rate of mortality of 1.1 deaths per 100 patients/year and an overall rate of second-line treatment failure of 8.8 failures per 100 patients/year.

The results of the ANRS 12276 2PICAM study were presented at UHS in October 2015 and the article is under process of submission. This study was a nation-wide cross-sectional study aimed to assess the virological effectiveness of PI-based second line regimen in Cambodia as well as the impact of adherence boosting in patients experiencing virological escape. HIV drug resistance mutations were described and an external committee discussed options for alternative second line or third line regimen. Among the 1348 patients receiving PI-based regimen and recruited for the study, 31 were excluded because of non-eligibility criteria and 1317 patients were enrolled for evaluation. The study reports a high rate of virological suppression with 90% of patients exhibiting virological success (< 250 copies/mL) at inclusion. In addition, almost half of those with a detectable VL could be rescued following 3-months of boosted adherence counseling giving a final 94.5% success rate. Higher success rates at baseline were observed in patients with high CD4 cell count, longer duration of PI-based regimen and with ritonavir boosted ATV-containing regimen. Overall, 5.5% of patients on PI-based regimen had confirmed virological failure and 2% were in urgent need of third-line regimen. Such information was of great importance for NCHADS to adapt HIV management guidelines and forecast the needs for alternative second and third line regimen. Following these results, NCHADS has implemented a technical working group (TWG) since September 2016 to help clinicians taking care of patients on 2nd line regimen who had a viral load >1000 copies/mL and to improve quality of adherence support. This TWG is composed of NCHADS staff and different international organizations (CHAI, WHO, FHI, USCDC, ANRS). The first action was to implement a standard adherence tracking form, to be included in all patient charts and to improve adherence support and training of counselors.

2.1.3 Adherence counseling and resistance-associated mutations (RAM) to second-line regimen

The control of adherence is a critical factor of ART success and WHO recommends adherence support intervention prior to deciding any change of ART regimen. Indeed, it was found that a significant number of patients could be rescued after initial identification of treatment failure. In addition to 2PICAM study, similar results of adherence boosting strategy were reported in India where 34% of PI-based regimen patients with virological failure had a suppressed VL after 6 months (9) and more recently in South Africa where 64% of PI-based regimen patients were rescued (10). However in the 2PICAM study, among the 72 patients who could not be rescued by BAC and had confirmed VL failure, 21 patients presented no resistance to PI or NRTI drugs and should have been rescued if adherence counseling was fully effective. This suggests that adherence-counseling interventions still have room for improvement to be fully effective and that GRT seems necessary to detect these patients.

The main observed RAMs in the 2PICAM study were M184I/V, T215F/Y, M41L and D67N for RT and M46I/L and V82A for protease. These protease mutations were similar to those reported in Nigeria (11) and Vietnam (12) which confer high level of resistance to LPV and ATV with a risk of resistance increase overtime and worsening outcomes. Cross-resistance between ATV/r and LPV/r were reported in Cambodia (13), Vietnam (12) and India (14). LPV/r is currently recommended as an alternative option in the national guidelines and prevalence of dyslipidemia was reported to be high in LPV-treated Cambodian patients with a 10-year risk of coronary heart disease ranging from 8% to 24% according to the score used (15). For these reasons, DRV/r, which presents a favorable lipid profile in clinical studies, seems to be the best option for patients with HIV-1 resistant to ATV/r whatever the sensitivity of LPV/r.

Among the 72 patients with virological failure in the 2PICAM study, 12 were sensitive to ATV/r but resistant to all NRTI. For these patients, a PI/r monotherapy could not be recommended as the viral load may not be fully suppressed; furthermore, this strategy showed a high risk of viral load elevation even for patients suppressed at baseline (16). Association of PI/r and integrase inhibitor seems, in that case, an interesting option (17) as reported by WHO even if data still missing to conclude to a

recommendation.

2.1.4 Third-line regimen in LMIC

Third-line treatment in LMIC is still in its infancy as it is estimated that its uptake is limited to 26,000 adult patients. Nevertheless, a recent projected uptake estimates that the total number of people on third-line treatment will increase to half a million by 2025 (26). Current WHO recommendations are:

- National programs should develop policies for third-line ART
- Third-line regimens should include new drugs with minimal risk of cross-resistance to previously used regimens
- WHO recommendations for patients failing first-line with EFV and second-line with PI: DRV/r + DTG (or RAL) ± 1-2 NRTIs
- NRTI-sparing and NRTI-containing regimen seems to be comparable in term of virologic suppression but data are still limited

To our knowledge, only two research studies currently evaluate the effectiveness of third-line regimen in LMIC.

The first one is the ANRS 12269 THILAO study, a multi-country, phase 2b, non-randomized study, in Burkina Faso, Cote d'Ivoire, Mali and Senegal, aiming to assess:

- The efficacy (and associated factors) at 12 weeks of an intensive 3-months adherence reinforcement phase;
- In patients who successfully resuppress at 12 weeks: the percentage of patients with continuing virological suppression on 2nd-line ART at 64 weeks (and factors associated to success);
- In patients with persistent virological failure at 12 weeks: the efficacy (and associated factors) at 64 weeks of a darunavir/r + raltegravir-based 3rd-line regimen.

Data were presented in the IAS congress in Paris in July 2017. Among the 198 patients treated with PI-based regimen and with confirmed plasma HIV-RNA > 1000 copies/mL, 5 die (2%), 130 (66%) resuppress at 12 weeks after adherence reinforcement and 63 (32%) were switched to third-line regimen. At month-16, HIV-RNA was > 400 copies/mL for 17 (13%) patients who continued second-line regimen and for 14 (22%) patients on third-line regimen. Accumulation of resistance mutations between inclusion and Month-16 was 5% on second-line regimen and 3% on third-line regimen.

The second one is an observational cohort of 150 HIV-infected Thai children, conducted by HIVNAT, aiming to assess the virologic and immunologic outcomes after at least 48 weeks of third line antiretroviral therapy combining new drugs such as darunavir, tipranavir, etravirine and raltegravir

2.1.5 DRV/r and integrase inhibitor combination

DRV/r was mainly evaluated combined with raltegravir (RAL). This combination, used as a first-line regimen in the NEAT 001/ANRS 143 trial, was reported as non-inferior to a standard tenofovir/emtricitabine plus DRV/r regimen (18) but the cumulative risk of selection of integrase mutations was higher after 96 weeks in the DRV/r + RAL arm compared to the standard arm (19). The same combination, used as a simplification among patients on suppressive ART, was proved to be effective to maintain virological suppression (20). For patients infected with multidrug-resistant virus with few remaining treatment options, the TRIO study showed high rates of virological suppression (90%) and favorable immunological outcomes at 48 weeks with a regimen containing RAL, etravirine, and DRV/r (21). The resistance-associated mutations were low for the 14 patients experiencing virological failure (22).

In India, MSF reported favorable outcomes with the combination DRV/r + RAL in a small group of patients failing a second-line regimen after boosted-adherence counseling (23).

The DRV/r + DTG combination has been less studied. Effectiveness of a salvage therapy with DRV/r and DTG in highly ART-experienced subjects was reported in Italy (24) with a proportion of viremic patients declining from baseline to week 24 from 43.4% to 6.2% and in France in a retrospective study

(25). Evaluation of this combination for a second-line regimen compared to current recommendation is ongoing within an international randomized-controlled study conducted by the Kirby Institute in Australia (NCT03017872) but, to our knowledge, no study evaluates this combination for a third-line regimen.

In the SPRING-2 study, once-daily dolutegravir was non-inferior to twice-daily raltegravir in treatment-naïve, HIV-infected patients after 96 weeks (27). Effectiveness and safety seem similar but DTG has a higher genetic barrier to resistance compared to the others INSTIs (28). Its pharmacokinetic characteristics allow once-daily administration, without boosting and with a low grade of drug–drug interactions. For all these reasons, DTG is now recommended by WHO as an alternative for first-line regimen.

The place of NRTI in third-line regimen continued to be discussed. NRTI-sparing and NRTI-containing regimens seem to be comparable in terms of virological suppression. Adverse events and treatment discontinuation seem to be lower with NRTI-sparing regimen but the difference is not statistically significant and WHO conclude to « prefer NRTI-containing regimen for HBsAg positive patients and discuss case by case for other patients according to GRT and comorbidities ». Recently, the MOBIDIP study conducted in sub-Saharan Africa reports that maintenance therapy with boosted protease inhibitor plus lamivudine was associated with a high rate of success than boosted protease inhibitor monotherapy, despite the presence of M184V mutations at first-line treatment failure (1).

2.1.6 Pharmacokinetic (PK) characteristics of DRV/r and DTG

PK characteristics of both drugs administered with 2 nucleos(t)ide analogs are well established either in healthy volunteers or HIV-infected patients. Main PK parameters assessed in HIV infected patients are summarized in the table below (29)(30)(31).

	Ritonavir boosted darunavir (DRV/r)		Dolutegravir (DTG)
	600/100 mg BID	800/100 mg QD	50 mg QD
C _{max} – µg/mL	8.14	5.47	3.34
T _{max} - h	2.5 - 4		2
Food effect	Yes ↗absorption by 40%		Yes ↗absorption by 50%
C _{min} or C _{trough} – µg/mL	3.20	2.04	0.83
AUC ₀₋₂₄ – µg.h/mL	54	88	43
T _{1/2} - h	15-17		11-12
Protein binding-%	92% (albumin and 1 glycoprotein)		>99% (albumin and 1 glycoprotein)
Elimination	Mainly biotransformation		
Metabolic pathways	CYP3A		UGT1A1 (main) CYP3A
Transporters	DRV substrate of Pgp;		Substrate of Pgp, BCRP

In brief, darunavir trough concentration when administered on a BID dosing is several folds higher than the protein binding-corrected EC₅₀ value for wild-type virus (0.055µg/mL). As expected C_{trough} will be lower on QD dosing than on BID dosing but the average concentration, will be high enough for wild-type virus suppression and for sustained efficacy for patients with undetectable viral load. Interestingly, some PK parameters of DRV/r and DTG in HIV-infected patients are close supporting coadministration of both drugs: rate of absorption is quite rapid with a time to C_{max} (T_{max}) around 2h and half-lives in the same range.

Although based on different metabolic pathways potent drug-drug interaction is not expected, ritonavir is known to induce UGT1A1 and could lower DTG concentrations. There is only one study conducted in healthy volunteers which assessed the effect of DRV/RTV 600/100 mg BID on DTG concentrations when administered at a 30 mg QD dosing for 5 days (32). A decrease in DTG concentrations was observed as indicated by the geometric mean ratio of AUC_t, C_{max} and C_{trough} of DTG administered with DRV/r versus alone and (90% confidence interval) which are 0.78 (0.72, 0.85), 0.89 (0.83, 0.97), 0.62 (0.56,0.69) respectively. Although there is a 40% decrease in DTG trough concentrations, the geometric mean concentration of DTG when coadministered with DRV/r was 0.45 µg/mL, which are several folds higher than the protein adjusted 90% inhibitory concentration (0.064 µg/mL). Indeed this study was conducted with a 30 mg DTG dosing, lower than the 50 mg recommended dosing which will be used in the proposed study. Although these data are reassuring, there is no data in “real life” situation neither in Caucasian nor Asian HIV-infected patients where the potency of the drug-drug interaction could be altered.

2.1.7 Study main hypothesis

Genotypic resistance test (GRT) is warranted for all patients not rescued after BAC

In case of intermediate or fully resistance to ATV/r, DRV/r is the best option but pharmacokinetic data are missing for Asian population

For patients without HIV-1 resistance to ATV/r but with extensive resistance to all NRTI, a third-line regimen must be considered

DTG is the best option within the integrase inhibitors

Optimized NRTI recycling must be done with at least lamivudine and one other NRTI if sensitive

Switch from DRV 600/100 twice daily to DRV/r 800/100 QD when VL is controlled needs to be evaluated in order to simplify treatment and improve adherence

3 OBJECTIVES OF THE STUDY

3.1.1 Primary objective

The primary objective of the study is to assess the virological effectiveness of a third-line regimen combining dolutegravir (DTG), ritonavir-boosted darunavir (DRV/r) and optimized NRTI after 6 months of treatment, in Cambodian HIV-infected adults, who failed a protease inhibitors (PI)-based second-line regimen despite 3 months of boosted adherence counseling (BAC), with evidence of intermediate or fully resistance to atazanavir (ATV) OR sensitivity to ATV but resistance to all NRTI.

3.1.2 Secondary objectives

The secondary objectives are to:

- Evaluate the safety of third-line regimen combination
- Evaluate the adherence of third-line regimen combination
- Evaluate the immune restoration (increase in CD4 cell-count) and the frequency of IRIS
- Estimate plasma exposure and pharmacokinetic parameters of DRV/r 600/100 twice daily and DTG 50 mg once daily
- Assess the effectiveness of switching DRV/r from 600/100 twice daily to 800/100 once daily for patients with plasma HIV1 RNA < 40 copies/mL at 6 months
- Estimate the pharmacokinetic parameters of DRV/r 800/100 once daily vs DRV/r 600/100 bid in the patients who switched

4 METHODOLOGY

4.1.1 Study design

This is a non-comparative multicenter pilot prospective study to conduct a programmatic evaluation of third line antiretroviral therapy implementation in Cambodia.

4.1.2 Study overview

Patients infected with a viral strain intermediate or fully resistant to ATV/r AND/OR resistant to both zidovudine (AZT), abacavir (ABC) and tenofovir (TDF) will be treated by a combination of DRV/r 600/100 twice daily + DTG 50 mg once daily + 3TC 300 mg once daily +/- one fully or intermediate sensitive

NRTI among TDF, ABC and AZT. The choice of the last NRTI will be discussed and decided by the TWG according to the HBsAg status, to the result of the GRT and to the medical history of the patient.

At 6 months, plasma HIV-1 RNA will be measured: the antiretroviral treatment will be switched from DRV/r 600/100 twice daily to DRV/r 800/100 once daily if plasma HIV-RNA is < 40 copies/mL and in absence of resistance to DRV at inclusion.

For all patients, adverse events occurrence will be recorded very closely.

DRV and DTG exposure and pharmacokinetic parameters (C_{max}, C_{min} and AUC) will be estimated for the 20 first included patients, allowing an intra-patient comparison of the 2 dosing regimens of DRV/r for at least 15 of them.

According to the NCHADS database, the total number of ART sites with at least one patient with possible inclusion criteria is 25. For pragmatic reason, enrollment will be done in the largest ART sites in a first instance and then progressively expanded to smallest ART sites.

4.1.3 Tentative study agenda

First inclusion: October 2018

Inclusion time: 24 months.

Time of follow-up for each participant: 12 months

Last visit for the last patient: October 2021

5 STUDY POPULATION

5.1.1 Inclusion criteria

- Age \geq 15 years old the day of inclusion
- Documented HIV-1 infection
- Failing a NNRTI-based first-line regimen
- Failing a PI-based second-line regimen after 3 months of adherence boosting
- HIV strain intermediate or fully resistant to ATV/r OR sensitive to ATV but both resistant to AZT, ABC and TDF
- For women of childbearing age (15-44 years old): acceptance to use effective contraceptive methods
- Informed consent obtained with information sheet given and explained before the inclusion visit and the consent form signed by the participant and the parents or legal guardians for adolescents at the latest the day of the inclusion

5.1.2 Non-inclusion criteria

- History of antiretroviral treatment including darunavir and integrase inhibitor
- Active pregnancy < 12 weeks of amenorrhea and desire of pregnancy during the duration of the study
- Opportunistic infection in acute phase at inclusion including tuberculosis treated since less than one month and/or with no stable clinical condition
- Advanced cirrhosis (Child-Pugh score B or C)
- Creatinine clearance < 50 ml/mn
- Any concomitant medical condition that, according to the clinical site investigator would contraindicate participation in the study
- Concurrent participation in any other clinical study without written agreement of the two study teams

6 STUDY ENDPOINTS

6.1.1 Primary endpoint

The primary endpoint is the proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 6 months

6.1.2 Secondary endpoints

The following secondary endpoints will be measured :

- Proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 12 months
- CD4 cell count at 6 and 12 months
- Proportion of patients with IRIS
- Proportion of patients with permanent study treatment discontinuation during the first year
- Incidence of grade 3-4 adverse events (ANRS grading table)
- Proportion of patients with adherence > 90% during one year
- Proportion of patients switched from 600/100 twice daily to 800/100 once daily at 6 months
- Plasma darunavir concentration with 600/100 twice daily and 800/100 once daily
- Plasma dolutegravir concentration
- Proportion of patients with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot analysis at 12 months among those switched from 600/100 twice daily to 800/100 once daily

7 STRATEGIES AND TREATMENTS

7.1.1 Strategies

After signature of consent form, Genotyping Resistance Test (GRT) and pre-inclusion samples will be done for all patients failing a PI-based second-line regimen according to national guidelines after 3 months of boosted adherence counselling (BAC), and after discussion in the TWG. Resistance associated viral mutations (RAMs) and drug resistance interpretations are done using GREG2010 software. Drug resistance mutations are defined according to ANRS algorithms (<http://www.hivfrenchresistance.org>). Results are sent to the TWG.

After receiving results of the GRT and of the pre-inclusion samples, the TWG will decide to switch the patient to third-line regimen or to continue second-line regimen with new boosted adherence counseling.

Continuation of second-line regimen

PI-based second-line regimen will be continue if 1/ GRT shows sensitivity to ATV; 2/ GRT shows sensitivity to at least one of the 3 NRTI recommended in Cambodia (AZT, ABC and TDF). In that case, the patient will not be enrolled in the study and adherence counseling will be boosted according to the new national guidance. A new virological assessment will be done after 3 months. Clinical, immunological and virological outcomes of these patients will be evaluated by the TWG.

Initiation of third-line regimen

In case of intermediate or fully resistance to ATV/r OR sensitivity to ATV but both resistances to AZT, ABC and TDF and after confirmation of eligibility criteria, patient will be switched to third-line regimen. **The third line regimen will include DRV/r 600/100 twice daily, DTG 50 mg once daily, 3TC 300 mg once daily and one fully or intermediate sensitive NRTI among TDF, ABC and AZT.** The

choice of the last NRTI will be discussed and decided by the TWG according to the HBsAg status, the result of the GRT and the medical history of the patient.

At 6 months, plasma HIV-1 RNA will be measured:

- If HIV1-RNA is < 40 copies/mL, in absence of resistance to DRV at inclusion: a switch from DRV/r 600/100 twice daily to DRV/r 800/100mg once daily will be done. A specific adherence counseling will be provided to confirm the new dosing with the patient.
- If HIV1-RNA > 40 copies/mL: DRV/r 600/100mg twice daily will be continued and adherence counseling will be reinforced.

For all patients, a new virological assessment will be done at 9 and 12 months.

For patients switched to DRV once daily, HIV RNA will be controlled one month after the switch in addition to 9 and 12 months.

Pharmacokinetic analyses

In the first 20 included patients who agreed and signed the consent form, blood samples will be drawn at steady state one month after treatment initiation (W4). DRV, RTV, DTG and NRTI will be administered in the morning under health care provider supervision and blood samples will be drawn before drug administration and +2h, +4h, +6h and +10h after drugs intake. Samples will be sent twice daily to the pharmacology unit of UHS, centrifuged and the plasma kept frozen until analysis at the end of the study. Participating patients will be hospitalized one day at Social Health Clinic of NCHADS in Phnom Penh. Transportation, food and per diem for participants will be funded by the study.

In some specific cases, as pregnancy or TB co-infection, supplementary PK analyses could be done.

Tuberculosis occurrence before and during the study^[SEP]

Screening of tuberculosis will be made at pre-inclusion visit using the WHO 4 questions about TB symptoms and a systematic chest X-ray. In case of suspicion of tuberculosis, Xpert MTB/RIF will be made on sputum. Depending on clinical presentation, Xpert MTB/RIF could also be performed on any relevant extra-pulmonary specimen. In addition, TB-LAM Ag test will be performed on a single urine specimen for patients with CD4 cell-count < 100/mm³ if the test is available. If criteria are in favor of a probable or confirmed TB, patients will be addressed to ART sites to receive TB treatment immediately and will not be enrolled in the study in a first step. It could be possible to enroll the patient after one month of TB treatment if the patient presents a stable clinical condition.

Screening of tuberculosis will also be made at each follow-up's consultation using the WHO 4 questions about TB symptoms. In case of suspicion of TB, Xpert MTB/RIF will be made on sputum as well as chest X-ray. Depending on clinical presentation, Xpert MTB/RIF could also be performed on any relevant extra-pulmonary specimen. In addition, TB-LAM Ag test will be performed on a single urine specimen for patients with CD4 cell-count < 100/mm³ if the test is available.

If criteria are in favor for probable or confirmed TB:

- Tuberculosis treatment will be provided using the standard WHO-recommended regimen (2RHZE/4RH), consisting of a combination of rifampicin (8-12 mg/kg), isoniazid (4-6 mg/kg), ethambutol (15-20 mg/kg) and pyrazinamide (20-30 mg/kg) administered once a day for 2 months, followed by a combination of rifampicin (8-12 mg/kg) and isoniazid (4-6 mg/kg) administered once a day for the next 4 months. All drugs will be preferentially taken in the early morning before any meal.

- Dolutegravir will be switched to 50 mg once daily to 50 mg twice daily and residual concentration of DTG will be performed two weeks after rifampicin^[SEP] initiation.

- The dosage of patients receiving DRV/r 800/100 qd must be rapidly increased to the 600/100 mg bid dosage. Residual concentration of DRV will be performed two weeks after rifampicin initiation.

DRV dosing could be doubled to overcome the inducing effect of rifampicin, although there is few data to support this (33)

Costing analysis (see detail protocol in Annex 7)

Cost analysis based on the design of the 3DICAM study is an opportunity to inform national authorities about the relative costs of the third-line implementation and the share of this cost in the funds allocated to HIV-related services globally. A cost analysis could be useful to compare the 3DICAM strategy with and without DRV dose reduction after 6 months of treatment. Impact of extra costs as safety or morbidity management, out of pocket expense for the patients within the ANRS 12374-3DICAM strategy with and without DRV dose reduction after 6 months of treatment will be estimated within the 2 scenarios. All these information's are relevant for government policies. At the international level, cost analysis based on the design of the 3DICAM study could be compared to other third-line programs and can contribute to more accurate international estimates of the resources needed for HIV programs.

Costing analysis objectives

- To conduct a cost analysis of the global strategy for third-line program in Cambodia.
- To generate a base of evidence to inform policy around the implementation of national HIV/AIDS third line program.
- To conduct a cost analysis of third-line program according to different strategies
- To assess separately the cost of the training process and its implementation

Costing analysis methods

A cost analysis will be conducted over different scenarios of third-line ART implementation to provide evidence about most advisable strategy. We will estimate, for each scenario, the cost of providing third-line ART to patients in ART sites setting in Cambodia.

Second, we will conduct a qualitative interview with 15 patients among the patients enrolled in the ANRS12374-3DICAM to understand actual and additional spending for seeking care of third-line treatment with the different scenarios. Then information emerged from the qualitative interview will be used to develop a structured questionnaire which will be used to interview face to face with all patients enrolled in the ANRS12374-3DICAM to assess on their additional expense and financial burden related to different scenarios of third-line ART implementation (with and without DRV dose reduction after 6 months of treatment). The information from interview will enable the program national to understand how the third-line treatment impact on the patients, specifically on their expense to seek care at ATR site.

A qualitative interview will be also conducted with health system staffs at national, provincial and district level to identify the information on the resources relevant to third-line treatment implementation. Then all resources and cost items identified from the interview will be listed in structured questionnaire which will be used to collect the costs of third-line treatment implementation.

Women of childbearing age

Women of childbearing age will receive intensive counselling on pregnancy and contraception. They will, in particular, be advised that third-line regimen initiation is not a good time to become pregnant, because: viral suppression may not be reached immediately, with subsequent risks of HIV transmission to the baby; tolerance of DRV and DTG during pregnancy is uncertain.

Recently, the World Health Organization has sent an alert regarding the use of dolutegravir for women of childbearing age. An ongoing study in Botswana found 4 cases of neural tube defects out of 426 women who became pregnant while taking dolutegravir. Neural tube defects are serious birth defects of the brain and spine of the baby and can cause lifelong disabilities (that range from mild to severe) and deaths. They happen very early in pregnancy (first month). That is why active pregnancy < 12 weeks of

amenorrhea and desire of pregnancy during the duration of the study are considered as non-inclusion criteria.

WHO recommends that if other ARVs cannot be used in women of childbearing age, DTG may be considered in cases where consistent contraception can be assured. For this study, we will provide free contraceptive method for all women of childbearing age during all the duration of the study. Contraceptive methods could be condoms, estroprogestative pill or implant and intrauterine device. As AUC of estroprogestative could be decreased of 40% in case of association with ritonavir, we strongly encouraged the use of IDU or the use of condoms for women taking an estroprogestative contraception. Urine pregnancy test will be done every month during the total duration of the study and the women will be informed to come at any moment outside the scheduled consultation in case of delay of menstruation. Folic acid, a B vitamin, is known to reduce the risk of a pregnancy being affected by a neural tube defect when a woman takes 400 micrograms daily before and during early pregnancy. Nevertheless, the intake of folic acid does not prevent entirely the risk of malformations of the neural tube as several etiologies (genetics, chromosomal anomalies, and environmental factors) are considered. It must not be considered as an alternative option to the contraception. We will provide this treatment during all the duration of the study for all women of childbearing age.

Pregnancy occurrence during the study

Since July 2019, WHO recommend to continue DTG if pregnancy is identified after the first trimester. Before the first trimester, WHO reports that risk–benefit models suggest the benefits of DTG are likely to outweigh the risks, which is the case in our population of women treated in the context of third-line regimen. The following recommendations will be reported in the procedures with the possibility to adjust according to the situation:

- In all cases, DRV/r 600/100 twice daily must be prescribed
- DTG could be continued
- Ultrasound at first trimester (12 SA) and second trimester (22SA) is recommended to detect any neural tube defect with an experimented radiologist

7.1.2 Treatments used in the study

None of the drugs used in the study is experimental.

Antiretroviral drugs will be delivered free of charge by NCHADS. The same supply chain will be used as for all ARVs, with a 6-month delivery in the clinical site and a monthly dispensation for each patient. A specific storage for clinical trials will be proposed with 1) temperatures to be recorded; 2) batch numbers and expiration dates of all boxes of ARVs to be recorded, with no specific labelling; Patients will be informed to always take DRV/r with meal, if bid in the morning and in the evening, then if qd in the morning, and to take DTG in the morning;

The study will cover the costs of common non-antiretroviral drugs including TB treatment, complementary exams and hospitalizations occurring from inclusion to month 12, if these tests, care and treatment have been judged justified by the study investigators.

ART regimen

Darunavir 600 mg tablet: one tablet twice daily^[SEP]

Darunavir 800 mg tablet: one tablet once daily^[SEP]

Ritonavir 100 mg tablet: one tablet twice or twice daily

Darunavir/ritonavir 400 mg/50 mg : two tablets once daily (available Q3 2018)

Dolutegravir 50 mg: one tablet once daily^[SEP]

Optimized NRTI treatment: could include tenofovir, zidovudine, abacavir and lamivudine^[SEP]

A fixed-dose combination of DRV 400 mg / RTV 50 mg is expected to come to market in 2018. Patients taking DRV 800 mg and RTV 100 mg singles will be switched onto this FDC (two tablets once daily) when it will become available.

The summary of product characteristic of all drugs used in this study is available at the EMA website http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/epar_search.jsp&mid=WC0b01ac058001d124

TB treatment

TB treatment will be the standard WHO-recommended regimen (2RHZE/4RH), consisting of a combination of rifampicin (8-12 mg/kg), isoniazid (4-6 mg/kg), ethambutol (15-20 mg/kg) and pyrazinamide (20-30 mg/kg) administered once a day for 2 months, followed by a combination of rifampicin (8-12 mg/kg) and isoniazid (4-6 mg/kg) administered once a day for the next 4 months. All drugs will be preferentially taken in the early morning before any meal.

In case of documented resistant TB strain, the 2RHZE/4RH regimen will be modified according to each national guidelines/procedures. In case of multidrug resistant (MDR) TB, second-line TB regimen will be discussed between the country principal investigator and representatives of the National TB program.

Other drugs

All patients with CD4 cell-count < 200/mm³ and no formal contra-indication will be systematically prescribed cotrimoxazole prophylaxis (800/160 mg OD) for the entire study duration. Other chemoprophylaxis (e.g. fluconazole prophylaxis) will be prescribed as per national guidelines.

Patients receiving TB treatment will be systematically prescribed pyridoxine (vitamin B6) for the entire TB treatment duration, unless otherwise recommended by national authorities.

Women of childbearing age will be systematically prescribed folic acid 400 mcg daily during all the study.

Treatments that could not be used because of potential drug-drug interactions are listed in Annex 2

8 STUDY IMPLEMENTATION

8.1.1 Study schedule

Study visit number	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	
	Timing	W - 8	W0	W2	W4	W8	W12	W16	W20	W24	W26	W30 ⁽²⁾	W36	W48
Checking of eligibility criteria	X	X												
Information about the study	X										X			
Signature of consent form	X													
ART-related and adherence counseling		X	X	X	X	X	X	X	X	X		X	X	X
4 questions about TB symptoms	X	X	X	X	X	X	X	X	X	X		X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X		X	X	X
Pregnancy test (women of childbearing age)	X	X ⁴		X	X	X	X	X	X	X			X	X
Complete blood count	X	X	X	X	X	X				X			X	X
Genotyping resistance test	X													
CD4 T-cell count	X									X				X
Serum transaminases	X	X	X	X	X	X				X			X	X
GGT, Bilirubine	X													
Coagulation profile	X													
Albumin	X													
Serum creatinine	X	X	X	X	X	X				X			X	X
Glycemia and lipids evaluation		X												X
RDT HBs antigen	X													
RDT HVC antibodies	X													
Plasma HIV-1 RNA				X		X				X		X	X	X
Chest X-ray	X													
Xpert MTB/RIF, sputum ⁽¹⁾	X	X	X	X	X	X	X	X	X	X		X	X	X
Urine LAM ⁽³⁾	X	X	X	X	X	X	X	X	X	X		X	X	X
Sampling for PK				X								X		
Storage of frozen Plasma		X				X				X			X	X

(1) For patients with at least one positive question among the 4 questions about TB symptoms (2) For patients switched to DRV/r 800/100 once daily

(3) For patients with at least one positive question among the 4 questions about TB symptoms and CD4 < 100/mm³ if the test is available

(4) Pregnancy test will be performed at inclusion and every month for all women of childbearing age and could be repeated during the follow-up in case of period retardations

8.1.2 Patients participation in the study

Information

Patient participation in this clinical study is voluntary. Each potential participant will be informed individually by the investigator of the purpose, scope of the study, procedures involved, duration of follow-up, potential risks and benefits and any discomfort it may entail. In addition to oral explanations, a written information sheet will be systematically provided in Cambodian language (see Appendix A).

Consent

If the patient accepts to be enrolled in the study, he will write his/her full names clearly, and date and sign the consent form, as will do the investigator. Written informed consent must be obtained before any exam related to the study. Before a patient agrees to participate in the pre-inclusion phase and sign the consent form he should be fully aware that the final conclusion regarding eligibility for the study will

be taken within one month, according to the results of the tests done at pre-inclusion (see paragraph 9.3).

The original copy of the form will be stored in a location that is secure and inaccessible to other clinic employees. Participants may be given, if they wish, a copy of the consent form after they sign.

In the case of adolescents (between 15 and 18 years old), the authorization of the person(s) legally responsible is a requirement. All measures must be taken to obtain the accord of both parents when possible, or of the adolescent's legal guardian. For orphans, local regulation must be considered to design the person legally responsible. Researchers must also verify that the adolescent is not opposed and signs the assent.

8.1.3 Pre inclusion visit (V0)

During the pre-inclusion visit, the medical investigator will:

- Provide oral information, information sheet
- Check eligibility criteria
- Collect consent form signed
- Collect socio-demographic data, medical history including past OI and treatment received
- Perform a clinical exam including BMI and Karnofsky score
- Ask the 4 questions about TB symptoms and perform specific exam (Xpert MTB/RIF, urine LAM) if one question at least is positive
- Perform biological sample including: complete blood count, HBsAg, HCV Ab, serum transaminases, serum creatinine, GGT, bilirubine, coagulation profile, albumin, CD4 T-cell count, and GRT, urine pregnancy test (women of childbearing age)
- Perform chest X-ray
- Plan the inclusion visit (W0)

Between pre-inclusion visit and inclusion visit, biological samples including GRT and request form will be sent to NCHADS laboratory and UHS for biochemistry and GRT. The TWG will be in charge to collect the results and to take a therapeutic decision. This decision will be communicate to the investigators on ART site in a maximum delay of 8 weeks.

8.1.4 Inclusion visit (V1)

The inclusion visit will occur within 8 weeks after the pre-inclusion visit.

During the inclusion visit, the medical investigator will check the results of the pre-inclusion visit tests and verify that the patient meets all inclusion criteria and exhibits none of the non-inclusion criteria.

Patients who do not meet all inclusion criteria, or meet at least one non-inclusion criterion, will not be enrolled in the study. They will benefit from the routine care provided in the country, according to the NCHADS. The TWG will be in charge to follow the outcomes of these patients, as some of them could be enrolled later in the study (active opportunistic infection cured, pregnant women after delivery, accumulation of resistance mutations). A specific table sheet will be created for these patients.

Patients who meet all inclusion criteria and do not meet any non-inclusion criterion will be enrolled on the same day.

For these patients, the medical investigator will:

- Collect the decision of the TWG for ART regimen
- Inform the patient of the therapeutic decision
- Check eligibility criteria
- Perform a clinical exam including BMI, Karnofsky score and hip and waist size and collect possible events occurred during the last month including concurrent medications
- Ask the 4 questions about TB symptoms and perform specific exam (Xpert MTB/RIF, urine LAM) if one question at least is positive

- Perform biological sample including: complete blood count, serum transaminases, serum creatinine, glycemia and lipids evaluation, urine pregnancy test (women of childbearing age)
- Give prescription of drugs to the patient with the list of contraindicated drugs
- Address the patient to the counselor for ART-related adherence counseling
- Plan the visit W2

8.1.5 Follow-up visits

Visit week 2 (V2) to week 20 (V7)

During these visits, all patients will be systematically asked about their symptoms and drugs intakes, receive a clinical exam, an ART-related adherence counseling and a complete evaluation of adverse events (graded according to ANRS score).

A special attention to IRIS occurrence will be paid. The 4 questions about TB symptoms will be asked at each consultation and Xpert MTB/RIF on single sputum will be performed for patients with at least one positive question. In addition, TB-LAM Ag test will be performed on a single urine specimen for patients with CD4 cell-count < 100/mm³ if the test is available. Xpert MTB/RIF test could be performed on extra-pulmonary specimen in case of clinical suspicion. In case of RIF+ Xpert MTB/RIF tests, documentation of a possible multidrug-resistant strain will be made using TB culture and/or MTBDR test (Hain test). TB treatment will be adapted accordingly.

Adherence assessment will be done at each consultation using the national standard adherence tracking form available in all ART sites in the country. This assessment will comprise self-reporting questionnaire, pill count and visual analog scale. If adherence level is < 90%, supplementary sessions of adherence counseling could be performed.

Adverse events assessment is described in chapter 11.

A biochemical analysis will be realized at V2, V3, V4, and V5 including complete blood count, serum transaminases and serum creatinine.

Plasma HIV-RNA will be performed at week 4 (V3) and week 12 (V5).

Urine pregnancy test for women of childbearing age will be performed every month and could be repeated during the follow-up in case of period retardations.

Visit week 24 (V8)

This visit is of great importance as the primary endpoint is evaluated during this visit. The investigator:

- Perform a clinical exam including BMI, Karnofsky score and hip and waist size and collect all events occurred during the last 6 months
- Ask the 4 questions about TB symptoms and perform specific exam (Xpert MTB/RIF, urine LAM) if one question at least is positive
- Perform biological sample including: complete blood count, serum transaminases, serum creatinine, CD4 T-cell count, plasma HIV1-RNA viral load and urine pregnancy test (women of childbearing age)
- Collect all adherence and adverse events assessment
- Plan the visit V9 two weeks later to report the results and inform the patient

Visit week 26 (V9) and Visit week 30 (V10)

During the visit week 26, the investigator informs the patient of the results of the week 24 visit.

No serious adverse event were recorded, ART regimen was not stopped, plasma HIV-RNA is < 40 copies/mL and no DRV resistance on GRT at inclusion

The investigator proposes to the patient to switch from DRV/r 600/100 twice daily to DRV/r 800/100 once daily and to continue other drugs as usual. A new ART-related adherence counseling is performed to explain the new dose.

For these patients, a consultation will be planned at week 30 (V10). During this visit, patients will be asked about their symptoms and drugs intakes, receive a clinical exam and an ART-related adherence counseling. Plasma HIV-RNA will be performed to confirm the maintenance of undetectable viral load. If HIV-RNA is > 40 copies/mL on two consecutive samples, DRV will be switched to 600/100 twice daily.

No serious adverse event were recorded, ART regimen was not stopped but plasma HIV-RNA is > 40 copies/mL and/or presence of DRV resistance on GRT at inclusion

The investigator does not change the ART regimen and address the patient for systematic monthly adherence counseling during 3 months.

The next consultation will be planned at week 36 (V11) with a control of plasma HIV RNA.

Serious adverse event were reported and/or ART regimen was stopped

A decision support committee will help investigators to take a decision on ART: discontinuation of some drugs, change in dose, and improvement of side effects...

The management of adverse events is detailed in chapter 11.

Visit week 36 (V11) and week 48 (V12)

All the patients will be systematically asked about their symptoms and drugs intakes, receive a clinical exam, an ART-related adherence counseling and a complete evaluation of adverse events. A biological sample will be collected including complete blood count, biochemical analysis and plasma HIV RNA for V11 and V12 + glycaemia, lipids profile and CD4 cell-count for V12 and urine pregnancy test (women of childbearing age)

For women of childbearing age, urine pregnancy test will be done every month between week 24 and week 48. Women could choose between coming back to consultation or doing at home for the unscheduled visits.

Plasma HIV RNA will be performed:

- For patients switched to DRV once daily, a reversal switch to DRV twice daily will be done if HIV RNA is > 40 copies/mL on two consecutive analyses

- For patients not switched to DRV once daily because of uncontrolled HIV RNA at week 24, a GRT will be performed if HIV RNA is > 1000 copies/mL. If HIV RNA ranges from 40 to 1000 copies/mL, adherence counseling will be done.

8.1.6 End of the study

Study exit preparation

Three months before the end of the study (visit V11, week-36), the medical investigator will explain to the patient, both in verbal and written form, the study exit conditions, the post-study care conditions, and the conditions under which they will be informed about the study results after study completion.

Last study visit

The last visit (V12) will occur 48 weeks after inclusion. Once patients complete 48 weeks of follow-up, they will end their follow-up within the study and will benefit from the regular care provided by the NCHADS.

Post-study care conditions

Once they leave the study, patients will continue to receive care and treatment according to the conditions defined by their country authorities. The country authorities will commit to providing these patients with their ongoing ART regimen at study end.

Communication of study results to the participants

As soon as final results of the study are available, they will be presented, both orally and in writing, to:

- The investigators;
- The national authorities;
- The participants.

A series of documents (written detailed report, and short summary) will be released to help investigators, national authorities and participants to understand the results of the study. Participants may be invited to attend a meeting during which the results will be presented and explained orally.

8.1.7 Withdrawal of consent and loss to follow-up

Consent withdrawal

A patient will be considered to have *withdrawn consent* if s/he no longer wishes to remain in the study. In this case, a “*withdrawal of consent form*” must be filled out.

When a patient who withdraws consent explicitly expresses the will that his/her data be removed from the database and his/her laboratory samples be destroyed, the study team will carry out such will. When a patient who withdraws consent do not express such will, data and samples collected prior to the date of his/her consent withdrawal will be used for the analysis.

Loss to follow-up

When a patient who has not explicitly withdrawn consent does not show up for routine visits, the study team (nurses, counsellors...) will search for him/her by all the way available (phone call, mailing, home-based care team). The study team will contact the patient via phone call or home visit, as long as s/he consented to phone calls and/or home visits in the consent form and provided a phone number and/or home address during the study.

A patient who does not show up for a given scheduled visit will be considered lost-to-follow-up when his/her last contact with the study team (either at the clinic, via telephone, or at home) was recorded prior to the date of his/her last scheduled visit to the clinic as per the study protocol.

9 LABORATORY EVALUATIONS

9.1.1 Blood samples collection and tests

Blood samples will be taken:

- (i) at pre-inclusion, to measure complete blood count, serum transaminases, serum creatinine, GGT, bilirubine, coagulation profile, albumin, HBsAg, HCV Ab, CD4 T-cell count, and GRT
- (ii) at inclusion, to measure complete blood count, serum transaminases, serum creatinine, glycemia and lipids evaluation, urine pregnancy test (women of childbearing age),
- (iii) at week 2 and week 8, to measure complete blood count, serum transaminases and serum creatinine,
- (iv) at week 4 and week 12, to measure complete blood count, serum transaminases, serum creatinine and plasma HIV1-RNA viral load,
- (v) at week 24, to measure complete blood count, serum transaminases, serum creatinine, CD4 T-cell count, plasma HIV1-RNA viral load;

- (vi) at week 36, to measure complete blood count, serum transaminases, serum creatinine and plasma HIV1-RNA viral load,
- (vii) at week 48, to measure complete blood count, serum transaminases, serum creatinine, CD4 T-cell count, plasma HIV1-RNA viral load, glycemia and lipids profile

	V0	V1	V2	V3	V4	V5	V8	V11	V12
Complete blood count: EDTA (mL)	5	5	5	5	5	5	5	5	5
Biochemical analysis: Plasma, heparin lithium (mL)	5	5	5	5	5	5	5	5	5
Coagulation profile: Plasma citrate (mL)	5								
Plasma HIV RNA and GRT: EDTA tube (mL)				5		5	5	5	5
CD4 cell-count: EDTA tube (mL)	5								5
Glycemia and lipids profile: Plasma, heparin lithium (mL)		5							5
Albumin: Plasma, heparin lithium (mL)									
Biothèque : EDTA (mL)		5				5	5	5	5
Total number of tubes at each visit	5	4	2	3	2	4	4	4	6
Quantity of blood at each visit (mL)	25	20	10	15	10	20	20	20	30

9.1.2 Tuberculosis tests

The two TB tests to be employed during the study are the Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) and the Determine® TB-LAM Ag test (Alere, Waltham, MA, USA).

Xpert MTB/RIF test will be performed on single sputum or extra-pulmonary specimen. In case of RIF+ Xpert MTB/RIF tests, documentation of a possible multidrug-resistant strain will be made using TB culture and/or MTBDR test (Hain test). TB treatment will be adapted accordingly.

TB-LAM Ag test will be performed on a single urine specimen in accordance with the manufacturer's instruction if the test is available. For this study, the cut-off point to consider a urine LAM test as positive is grade 2 (visual grade 2 on the Reference Scale Card of the manufacturer).

9.1.3 Pharmacokinetic analyses

For the 20 patients participating in PK analyses, 2 tubes of 5 mL will be collected before the drug intake, then +2h, +4h, +6h and +10h after drugs intake. Samples will be sent twice daily to the pharmacology unit of UHS, centrifuged and the plasma kept frozen until analysis at the end of the study. One tube of 2 mL of plasma will also be kept frozen for further analysis.

9.1.4 Biobank

During the follow-up, blood samples will be collected for all patients and frozen at week 0, 12, 24, 36 and 48. A specific consent form for possible further genetic analyses, including pharmacogenetics, will be provided.

Biobank will be centralized at UHS, in Rodolphe Merieux laboratory and preparation of the aliquots will be done at this location. Each blood sample (5 mL) will be divided in 1 mL of whole blood and 2 mL of plasma frozen in 2 aliquots.

10 ADVERSE EVENTS AND *IN UTERO* EXPOSURE

10.1.1 Definitions

10.1.1.1 Adverse Event (AE)

An adverse event is any untoward medical occurrence in a clinical trial participant, which does not necessarily have a causal relationship with the research or the investigational medicinal product.

10.1.1.2 Adverse Reaction (AR)

An adverse reaction is any untoward and unintended response to an investigational medicinal product related to any dose administered.

10.1.1.3 Severity

The severity of an AE/AR refers to its intensity, or grade. All AEs/ARs should be graded for severity, using the ANRS scale for adverse events

(www.anrs.fr/content/download/2242/12805/file/ANRS-GradeEI-V1-En-2008.pdf)

10.1.1.4 Seriousness

The seriousness of an AE/AR refers to whether or not it should be reported immediately to the sponsor and regulatory authorities.

The judgment as to whether an AE/AR is serious or not is made by the investigator, based on the criteria listed in section 11.1.1.5.

10.1.1.5 Serious Adverse Event (SAE)

“Serious adverse event” (SAE) refers to any untoward medical occurrence or reaction that, at any dose:

- Results in death;
- Is life-threatening (means that the subject was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- Requires hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect;
- Is a grade 4 clinical or biological event;
- Is a grade 3 “special interest” event. For this study, the special interest events are: psychiatric disorders, central neurological disorders, hepatic disorders, renal disorders, mucocutaneous disorders, and all invasive infections;
- Is an “important medical event” (medical events, based upon appropriate medical judgment, which may jeopardize the subject’s health or may require medical or surgical intervention to prevent one of the above characteristics/consequences; e.g.: *allergic bronchospasm requiring intensive treatment at an emergency unit or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization, etc.*).

The following events are not SAEs:

- Hospitalizations with no untoward medical occurrence, such as hospitalization for elective surgery if known prior to start study, social and/or convenience admissions, pre-specified study hospitalizations for observation.
- Medical or surgical procedures (e.g.: *surgery, endoscopy, tooth extraction, transfusion*); only the condition that leads to the procedure should be notified if the condition is referring to a seriousness criteria described above.

The following events are SAE, but do not require reporting to the sponsor:

- SAEs related to pre-existing diseases or prevalent conditions that do not worsen (this includes pre-existing grade 3 and 4 biological events);
- Grade 4 biological adverse events detected between the screening visit and the start of study drug administration.

Deaths must be reported as the outcome of a SAE, not as a SAE itself if the cause is known. If the cause of death is unknown, the death should be reported as “unknown cause of death”.

10.1.1.6 Causality

“Causality” refers to causal relationship between a given AE/AR, the study intervention/medication, and any other concomitant intervention/medication (e.g. in case of drug-drug interaction). A drug can be associated to a given SAE even if the subject is not receiving the drug at the time of the event.

All SAE for which the investigator or the sponsor considers that a causal relationship is a reasonable possibility are considered as suspected *serious adverse reaction* (SAR).

10.1.1.7 Expectedness

An unexpected adverse reaction is an adverse reaction, the nature, the outcome or severity of which is not consistent with the applicable *Reference Safety Information* (RSI). The RSI is the *Summary of the Product Characteristics* (SmPC) for authorized products, and the Investigator's Brochure for unauthorized investigational products or authorized products used in unapproved indication.

All unexpected SAE for which the investigator or the sponsor considers that a causal relationship is a reasonable possibility are considered as *Unexpected Serious Adverse Reaction* (SUSAR).

10.1.1.8 New fact

A new fact is any safety data that could modify significantly the evaluation of the benefit/risk ratio of the investigational medicinal product or the clinical study, likely to affect the safety of participants or that could modify the investigational medicinal product administration, the trial documentation or the conduct of the trial, or to suspend or interrupt or modify the protocol or similar trials (e.g.: *serious adverse event which could be associated with the study procedures and which could modify the conduct of the study; lack of efficacy of an investigational medicinal product used for the treatment of a life-threatening disease, etc.*).

10.1.2 Responsibilities of the investigators

10.1.2.1 Assessment of severity, seriousness, causality

The investigators are responsible for:

- Grading the severity of all AEs (serious and non-serious)
- Reporting all SAEs to the sponsor and to the NECHR, according to the procedures described in section 11.1.2.2.
- Assessing the causality of all SAEs in relation to the study intervention/medication. In the absence of information on causality from the investigator, the sponsor should encourage him to express an opinion. The sponsor should not downgrade the causality assessment given by the investigator. If the sponsor disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor should be provided with the report.

The sponsor, in the light of the reference document (SmPC), will do the assessment on expectedness. However, if information on expectedness has been made available by the investigator, the sponsor should take this into consideration.

10.1.2.2 SAE notification by the investigators

Timeline and contacts

The investigators have to notify:

- All new SAE, immediately and no later than 24 hours of being aware of it (using the *SAE initial notification form*),
- Any SAE worsening, immediately and no later than 24 hours of being aware of it (using the *SAE complementary notification form*),
- All SAE resolutions (upon initial reporting, using the *SAE initial notification form*, if the SAE resolution dates the same day as the SAE initial report; upon SAE resolution, using *SAE complementary notification form*, if SAE resolution dates from after the initial declaration).

- Any relevant change in the data previously reported (eg.: change in diagnosis or in causality assessment, new information on drugs taken, etc.) (using *SAE complementary notification form*).

Initial and complementary reports forms should be sent to: (i) the pharmacovigilance unit of the Inserm-ANRS (email: pharmacovigilance@anrs.fr); (ii) the NECHR.

Notification period: SAEs must be reported if they occur between the time when the subject signed the informed consent form and:

- His/her 48-week visit (V12), or:
- 4 weeks after the last study visit if the event is suspected to be related to the study strategies, or:
- Any time after the completion of the study if the event is suspected related to the medicinal product taken during the study follow-up.

The investigators do not need to actively monitor subjects for adverse events once the study has ended. However, serious adverse events occurring to a subject after his/her study follow-up has ended should be reported to the sponsor if the investigators become aware of them.

Initial report: The immediate report must include at least the following information: the subject ID, the name of the person who reports the SAE, one medicinal product, one SAE, the date, and the signature of the reporter. Whenever incomplete, the immediate report must be followed by a detailed report, which should be sent no later than 8 days after the initial report.

Complete initial reports include the grade, causality and outcome of the event.

SAE follow up: The investigator must follow the SAE until its resolution, or until the patient's death, even if resolution or the patient's death occurs after study termination.

Worsening is defined as the SAE grade becoming higher than the grade initially declared. Any SAE worsening must be reported using the SAE complementary notification form.

Resolution is defined as clinical and/or biological conditions getting back to normal or to the conditions that pre-existed the SAE occurrence. SAE resolution must be reported using the SAE complementary notification form, except when the SAE outcome was already reported as "resolved" on the SAE initial form.

A new episode occurring after complete resolution should be declared as a new SAE.

SAE notification forms filling

The investigator should indicate a specific diagnosis whenever possible, and a syndromic diagnosis otherwise. Symptoms occurring within the framework of a given specific diagnosis or syndrome should not be reported separately (e.g. abdominal pain in a patient with pancreatitis).

The "SAE start" cannot be dated from after the "SAE seriousness".

The outcome indicated on the SAE initial report is the outcome at the time of the report. For all SAEs that are not reported as "resolved" at initial report, any change in the initially reported outcome (resolved, back to previous status, worsening) should be reported using complementary notification form(s). Complementary notification form should also be used for all changes in the investigator judgment regarding causality or expectedness.

The end of seriousness is the date when the adverse event is no longer considered as "serious". For all resolved SAE, the date of resolution corresponds to the date of the event resolution, not necessarily to of the end of seriousness. For all SAE which outcome is "back to previous status" or "improvement", the outcome date is the end of seriousness.

Additional documentation

All relevant documents related to the SAE (e.g. hospitalization report, laboratory results) must be attached to the reports, when existing. Documents must be anonymized before being sent, with patients names being erased and replaced with participant identification number.

Fatal or life-threatening SAE:

In case of fatal or life-threatening SAE, special attention should be paid to reporting timelines. Reports should be made as soon as possible and no later than 24 hours after the investigator has been made aware of the case, and all relevant complementary information should be sent within 8 extra-days.

10.1.2.3 Potential risks of the research and management of selected SAEs

The following risks associated with the use of dolutegravir should be considered: hypersensitivity reactions, immune reactivation syndrome, opportunistic infections, osteonecrosis, hepatotoxicity. More details can be found in the SmPC of dolutegravir on the EMA website.

The following risks associated with the use of darunavir should be considered: severe skin reactions, hepatotoxicity, osteonecrosis, immune reconstitution inflammatory syndrome. More details can be found in the SmPC of darunavir on the EMA website.

If there is any suspicion of drug-induced hepatitis, physicians will measure serum transaminases, alkaline phosphatases and bilirubin levels.

Grade 3 or 4 hepatitis or renal insufficiency or severe mucocutaneous events (such as rash associated with blistering, desquamation, mucosal involvement or fever) will lead to the immediate decision to discontinue all drugs possibly implicated. In case of severe neuropsychiatric disorders, the role of DTG will be systematically brought up, and investigators will also consider discontinuation of these drugs.

Once a drug has been discontinued for any reason, reintroduction will be carefully discussed between the DSC and the country investigators, on a case-by-case basis, taking into account the severity of the event, rapidity of resolution, potential drug causality, and expected benefit of the drug.

10.1.3 Responsibilities of the sponsor

10.1.3.1 SAE Recording and assessment

The sponsor is responsible for:

- Ensuring that all SAE are reported.
- Keeping detailed records of all SAE, which are reported to him by investigators.
- Assessing the causality of the SAE in relation to the study therapy, concomitant medication (e.g. drug-drug interaction) and the research. The sponsor should not downgrade the causality assessment given by the investigator. If the sponsor disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor should be provided with the report.
- Assessing the expectedness of the SAR, by using the applicable RSI. If information on expectedness has been made available by the reporting investigator, the sponsor should take this into consideration.

10.1.3.2 Safety reports

Annual safety report

Once a year the ANRS pharmacovigilance unit will release an annual safety report, the Development Safety Update Report (DSUR). The DSUR will be written in coordination with the monitoring and data management centre, and may be submitted to the coordinating investigators for approval.

The DSUR includes:

- a line-listing of all suspected serious adverse reactions that have occurred over this period (expected and unexpected SAR);
- a cumulative summary tabulation of the SAE, expected SAR, and SUSAR, by System Organ Class (SOC);
- a line-listing of deaths;
- any relevant Independent Data Safety Monitoring Board and study Scientific Committee opinion regarding safety;
- a concise, critical analysis of the subjects' safety.

The RSI in effect at the start of the reporting period serves as RSI during the reporting period. The annual safety report should clearly indicate the version number and date of the IB or SmPC used for this purpose. If there are significant changes to the RSI during the reporting period, they should be listed

in the report. Despite the change to the RSI, the RSI in effect at the start of the reporting period serves as RSI during the reporting period.

Timeline and contacts

The annual DSUR will be sent to: (i) the study coordinating investigators; (ii) the NECHR. The "Development International Birth Date" (DIBD) is the date of the first inclusion of the study. The DSUR is expected to be submitted each year no later than 60 calendar days from the DIBD.

10.1.3.3 SUSAR report

All SUSARs will be reported by the Inserm-ANRS Pharmacovigilance unit to the appropriate country competent authorities, in line with each country's laws and regulations.

In France, this report will be made through the EudraVigilance Clinical Study Module (EVCTM).

For fatal and life-threatening SUSAR, the initial report should be made as soon as possible and in any case no later than 7 calendar days after the sponsor has been made aware of the case. Relevant complementary information should be collected and notified within 8 extra-days.

SUSAR which are not fatal and not life-threatening are to be reported within 15 calendar days. Relevant complementary information should be collected and notified within 8 extra-days.

10.1.3.4 New fact reporting

When a new event is likely to affect the safety of participants, the sponsor and the investigator take appropriate urgent safety measures to protect participants against any immediate hazard.

The sponsor inform without delay the NECHR of safety data that may be relevant in terms of subject safety, or safety issues which might alter the current benefit-risk assessment of the trial.

The safety office shall transmit a written report, within 15 days to the NECHR

If the new fact requires substantial amendments, the sponsor should notify any substantial protocol modification, to the NECHR, within 15 days of the safety measures implementation.

10.1.4 In utero exposure

10.1.4.1 Pregnancy notification

The investigator has to notify all pregnancy, within 24 hours of being aware of it, to the pharmacovigilance unit of the Inserm-ANRS (email: pharmacovigilance@anrs.fr), using the *pregnancy initial report form*.

The "pregnancy report form" must include the following information: estimated date of delivery, obstetrician contact and name of maternity hospital

The investigator has to follow the woman until the end of the pregnancy and notify the outcome to the sponsor using the "pregnancy outcome report form".

Follow-up of pregnant participant continues until the end of the study. Any exceptions, related to the indication of maternal treatment, should be discussed between the investigator, the coordinating investigator, the sponsor and if necessary a specialist in terato-vigilance.

Warning:

- The medical surveillance of the women and their children should be reinforced: a particular attention must be given on serious pathology occurring during pregnancy abnormalities (see section 11.2.2 and 11.2.3).
- Therapeutic interruption of pregnancy, congenital anomalies, birth defects, and miscarriage needing hospitalization are considered and should be notified as SAEs.

10.1.4.2 Pregnancy management

If a participant becomes pregnant, all treatments contraindicated during pregnancy must be discontinued.

In particular, regarding the late information on a potential safety issue related to neural tube defects in infants born to women who were taking DTG at the time of conception, DTG must be switched to an alternative treatment unless there is no suitable alternative, in accordance to EMA recommendations (see also paragraph "Pregnancy occurrence during the study" in chapter "7. Strategies and treatments").

Regarding ART, investigators will carefully weight the benefits/risks balance for the mother and for the baby before taking therapeutic decision. The decision should be compatible with the national guidelines on ART and pregnancy.

- ART regimen will not systematically be modified because of the pregnancy. If a modification is deemed necessary (e.g.: virologic failure, drug intolerance, or ART regimen including a drug contraindicated during pregnancy), the ultimate goal will be to reach viral load undetectability as soon as possible, using drugs that have been proved to be acceptable during pregnancy.
- Cotrimoxazole will not be stopped solely because of the pregnancy.

Clinicians could request rapid advice of the DSC any day of the week when urgent decision is needed (see section 12.2.2).

See also paragraph "Pregnancy occurrence during the study" in chapter "7. Strategies and treatments".

10.1.4.3 Children born to mothers participating in the study

Children born while their mothers are participating in the study will be followed according to the national guidelines.

Newborns who do not have a definitive diagnosis or who have been definitively diagnosed with HIV will be referred to a Paediatrics Department to initiate appropriate care.

11 TRIAL BOARDS AND COMMITTEES

11.1.1 Trial scientific committee

Composition

The trial scientific committee will consist of: (i) the investigators and statistician of the trial; (ii) external HIV and TB experts including PK of the drugs; (iii) an HIV advocacy organization representative; and (iv) sponsor representatives. Members of the scientific committee are listed in annex 1.

Meeting agenda

The trial scientific committee will meet before the beginning of the inclusion phase, as well as once or twice per year until the end of the trial. The trial sponsor or one or several board members may also request a special meeting at any time.

Role

The role of the trial scientific committee is to ensure that the trial is carried out appropriately, not only scientifically but also clinically and ethically. The members of the scientific committee report directly to the sponsor.

- They will ensure that the trial personnel carry out the trial properly, adhere to the trial protocol and maintain patient safety;
- They will guarantee that the trial remains scientifically relevant by ensuring the relevancy of the trial questions and that the methods used are valid and appropriate;
- They will make all decisions regarding necessary and relevant protocol modifications, such as:
 - Actions needed to facilitate patient recruitment;

- Decisions to open or close participating trial sites.
- They will enforce the rules pertaining to access to the trial data as well as reports and publications of the results;
- They will remain in contact with the sponsor, the DSMB and the coordinating investigators, and will make sure investigators and other trial personnel have access to up-to-date information.

At the end of each meeting, a report containing the meeting minutes, signed by the Scientific Committee chair will be sent to the members of the scientific committee, DSMB Members and the director of ANRS.

11.1.2 Data Safety & Monitoring Board (DSMB)

Composition

The DSMB members will be selected in collaboration between the coordinating investigators and the ANRS before the beginning of the inclusion phase.

Meeting agenda

The DSMB will meet once at the beginning of the inclusion phase, and at least every 12 months until the end of the trial. The sponsor, the scientific committee or the DSMB may request to increase the frequency of these meetings.

Role

The DSMB is a consultative board for the scientific committee and to the sponsor. Its members will provide general advice on the progress of the trial, including the rate of inclusions, the quality of follow-up, the overall rate of drug-related adverse events, changes in biological markers, the overall incidence of primary outcomes, and the number of subjects needed.

They will help to make difficult decisions that require an independent assessment while the trial is underway. Advice may be needed regarding:

- Premature discontinuation of the trial (because the rate of adverse events is high, the trial is no longer feasible, or the available data are sufficient);
- Substantial changes to the protocol that becomes necessary during the inclusion or follow-up phases, or to account for new scientific information.

The DSMB will conduct and interpret intermediate analyses, if any.

At the end of each meeting, a written report containing the DSMB's opinion will be sent to the sponsor and the members of the scientific committee.

11.1.3 Decision Support Committee (DSC)

Roles of the DSC

The DSC will provide advices and recommendations to investigators regarding:

- Occurrence of tuberculosis or pregnancy during the study
- Any morbidity event occurring during the study
- Decision on ART management
- Any important clinical issue for which the investigators wish to share experience before taking a decision

Composition of the DSC

The DSC will consist of 1/ Two physicians specialized in HIV infection 2/ One pharmacist specialized in PK 3/ The two principal investigators of the study. Other specialists could be called at DSC's request.

Organization of the DSC

The DSC will meet regularly and must be accessible on rapid request through phone calls, e-mails, private online forum, or any other means in case of morbidity event.

The investigators of each site will be responsible to prepare the documents for the meeting and to send it by e-mail at least two days before the meeting.

The responsibility of DSC is to provide a recommendation on a written document. One member of the DSC will be in charge of the written report. This report will be send to the methodological centre in UHS.

12 COORDINATION, MONITORING, DATA MANAGEMENT

12.1.1 Coordination

The UHS Research Grant Management Office is the referent for the trial methodology and management. The UHS team is responsible, in coordination with the clinical, laboratory and administrative coordinators, for the overall trial management (preparation and organisation of the trial, monitoring, data management and analysis). The team will include the project coordinator, the two trial monitors, one Lab technician and one data manager.

- **Preparation and organisation of the trial:** the UHS team will finalize and review the Case Report Forms (CRF) with all the collaborators, prepare the recruitment procedures, and organize the trial reference documentation. The coordinating centre team and the clinical coordination team will prepare the operation manual describing in details the trial procedures for each collaborators (clinical and laboratory activities, treatment dispensation, data management).
- **Database:** The trial database will be developed and maintained by the coordinating centre in collaboration with NCHADS Data Management Unit. The data manager will also be in charge of the data entry and implementation of data quality insurance plans. H/She has to prepare daily monitoring reports and the data file necessary for the trial analysis.
- **Data analysis and analysis report:** will be coordinated at the UHS coordinating centre.
- **Reporting:** the operational team will be responsible for finalizing:
 - the necessary reports and presentations for the Scientific Advisory Board and the DSMB meetings;
 - the trial yearly progress reports; The operational team will bring its support in preparing any communications on the trial (poster, oral presentation, papers).

12.1.2 Trial documents

Essential trial documents will be retained at the coordinating centre for 15 years. Data will be kept on a secured database installed on a server at the coordinating centre.

12.1.3 Data management

12.1.3.1 Data collection

Data on participants will be collected on duplicated CRF during the trial. A unique CRF will be assigned for each patient. The patient will only be identified by a unique anonymous code. An operation manual

will be provided to help the clinical monitors fill out the CRF. All the information required by the protocol should be provided and any omissions require explanation.

The trial monitor from the coordinating centre will come on a regular basis to check for completeness, accuracy and legibility of data reported on the CRF. He/she will bring back the validated CRF to the coordinating centre for data entry.

12.1.3.2 Data entry and checking

Data will be entered at the coordinating centre (UHS). The trial database will be developed by a database developer which will closely work with the coordination team.

After entry, the database will be checked for consistency. If any inconsistency or question on the data, the coordinating centre monitor will go back to the clinical monitors at sites for clarification during their monitoring visit. The corrections will be entered and followed in the data base.

12.1.4 Monitoring

Monitoring will be conducted according to the Good Clinical Practice (ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996) to guarantee the good quality of the research and safeguard the health and the rights of the patient.

12.1.4.1 On site

The on-site trial activities (patient management; drug management and dispensation; trial monitoring) will be done under the responsibility of the **clinical coordinators**, based at the NCHADS in Phnom Penh. They will be responsible for coordinating all clinical activities for the trial:

- Recruitment, training and management of the clinical monitors;
- Organising and supporting clinical patient follow-up on site;
- Supervision of trial drug management;
- Member of the steering committee;

On each investigational site, a physician known as the “**clinical monitors**” will have the medical responsibility to conduct the trial according to the protocol. They will be responsible, under the supervision of the **clinical coordinator**, in organizing the trial follow-up and management on site:

- Organisation of patient screening, enrollment and follow-up;
- Report clinical and biological data in the Case Report Form (CRF) according to the most updated Methodology onsite MOP that will be provided;
- Report and document all Serious Adverse Events and sent follow-up report when necessary;
- Communication and coordination with the Decision Support Committee (DSC) and coordinating centre;
- Preparation and facilitation of the monitoring visit;

The clinical monitor must give the coordinating centre monitor access to relevant hospital or clinical records, to confirm their consistency with the CRF entries. No information about the identity of the subjects should appear on the CRF. The CRF must be completed within the week following the patient's visit. All CRF must be signed by the physician responsible of the patient follow-up. CRF will be considered as the source document for all the study sites regarding all the informations it contains.

12.1.4.2 Monitoring activities

Monitoring activities will be coordinated by the coordination center. The trial monitors will visit the site regularly during the study:

- Check the adherence to the protocol and to Good Clinical Practice (patient informed

- consent, protocol visit and blood test schedule...);
- Assist in the trial organisation and management on site (communication between collaborators, treatment or material availability, tracing laboratory samples follow-up and result in collaboration with the lab technician);
- Check the completeness and the accuracy of patient data on the CRF;
- Collect and check the Serious Adverse Events reporting, documentation and follow-up, and send the forms to the sponsor;
- Evaluate the progress of enrolment.

13 STATISTICAL ANALYSES

13.1.1 Calculation of number of patients needed

According to data available with the ANRS 139 TRIO and ANRS 143 NEAT studies, we could expect that at least 80% of patients will be in virological success after 6 months and we wish to conclude that treatment will be effective for more than 60% of patients

With a sample size of 45 patients, we could conclude that success rate is at least 60% if the total number of failure does not exceed 12 (alpha 0.05, power 90%, Fleming method with one stage).

Among the 3500 patients on PI-based regimen, and according to the results of the 2PICAM study, we could expect that 5% of patients followed in Cambodian sites could be enrolled in the study (175 patients) and one third of them (60 patients) will need third-line regimen at inclusion. With a 10% refusal ratio, we anticipate to enroll 54 patients for third-line regimen while 100 patients will continue second-line regimen.

13.1.2 Analysis plan

- **Description of the subjects characteristics at inclusion** (including sociodemographic characteristics, medical history, CD4 cell count and plasma viral load). These variables will be described by their median, interquartile range, mean, standard deviation, or frequencies (%), as appropriate.

- **Primary outcome**

The percentage, and its 95% confidence interval, of patients with plasma HIV-1 RNA < 40 copies/mL at 6 months will be given, in a FDA snapshot analysis (patients who had stopped treatment and patients lost to follow-up were considered in virological failure).

An analysis stratified on the level of plasma HIV-RNA at inclusion (thresholds of 4 and 5 Log copies/mL) will also be performed.

Comparisons of the percentage of subjects with plasma HIV-1 RNA <40 copies/mL at 6 months, by the characteristics at inclusion, will be done by a Chi2 test or Fisher exact test, as appropriate.

- **Secondary outcomes**

The immune restoration: The level of CD4 cell count reached at 6 months and 12 months will be described by median, interquartile range, mean and standard deviation. The percentage of subjects with a CD4 level > 350 cells/mm³ and > 500 cells/mm³ will also be given at each of these times.

The results will be given overall, and by group (according to whether subjects switched or not at M6 from DRV/r 600/100 twice daily to DRV/r 800/100 once daily).

The increase in CD4 cell count will also be analyzed by the difference of the value at M6 with that at inclusion, and then by the difference between M12 and M6 (median, interquartile interval, mean and standard deviation).

The analysis will be performed on the available data (without any imputation of missing data).

A sensitivity analysis will be also carried out, with data censored at treatment discontinuation.

Patients with IRIS: The percentage and its 95% confidence interval, of subjects with IRIS will be given.

Treatment discontinuation during the first year: The probability of discontinuation of treatment and its 95% confidence interval, as well as the median time to the occurrence of treatment discontinuation,

will be determined using a Kaplan Meier analysis, to take into account censored data due to lost of follow-up. The analysis will be performed overall, and by group, the time of follow-up since inclusion will be taken into account in the analysis. An analysis will also be performed among those who switched at 6 months, with time of follow-up since M6 that will be considered in the analysis.

Occurrence of grade 3-4 adverse events (ANRS grading table): The percentage and its 95% confidence interval of subjects who had presented at least once a grade 3-4 adverse event will be given.

The incidence rate of grade 3-4 adverse event and its 95% confidence interval will also be determined, overall, and by group.

The type of side effects will be detailed, overall, and by group

Adherence of third-line regimen combination: The percentage and its 95% confidence interval of subjects with adherence > 90% during one year will be given.

Frequency of switch from DRV/r 600/100 twice daily to DRV/r 800/100 once daily at 6 months: The percentage and its 95% confidence interval of subjects who switched at 6 months will be given.

Plasma darunavir concentration: The plasma darunavir concentration will be described (median, interquartile interval, mean, standard deviation) at week 4 in all subjects, at week 30 in the subjects having switched at M6 DRV/r from 600/100 twice daily to 800/100 once daily, and in case of occurrence of coinfection tuberculosis during follow-up, at 2 weeks after Rifampicin initiation.

Effectiveness of switching DRV/r from 600/100 twice daily to 800/100 once daily : The percentage and its 95% confidence interval, of subjects with plasma HIV-RNA < 40 copies/mL at 12 months, among those switched from 600/100 twice daily to 800/100 once daily, will be given.

14 SCIENTIFIC COMMUNICATION

All written or oral communications of the study's results must receive the approval of the coordinating investigators and of the scientific committee of the study.

The coordinating centre will realize data analysis. This analysis will lead to a report, submitted to the scientific committee for approval. This report will help to prepare scientific publications whose final draft must be approved by the scientific committee (SC).

SC shall have sole competence to include – if necessary – additional researchers to the papers related to the research project. All rules related to submission/publication, defined by ANRS, will be followed.

All publications will include the name of sponsor as follows ("Inserm-ANRS. The French National Institute for Health and Medical Research- ANRS [France REcherche Nord&Sud Sida-hiv Hépatites] is the sponsor of the project") followed by the ANRS study number (ANRS 12374 + Acronym), as well as the investigators, the composition of SC

In case of ancillary studies, results will be published after approval by SC. In addition, these results will be published only after the publication of the main results obtained during the research project. The main project will be quoted (ANRS 12374). Results obtained through ancillary studies will be also transmitted to Inserm-ANRS for information.

15 ETHICS AND LEGAL CONSIDERATIONS

• 17.1. Ethics

This research project will be conducted with regard to fundamental ethical principles that are described in the updated version of the Declaration of Helsinki (64th World Medical Association [WMA] General Assembly, Fortaleza, Brazil, October 2013), and in the ANRS ethics charter for research in developing countries (July 2017) (<http://www.anrs.fr/Ressources-et->

[publications/Publications/Publications-ANRS/Charte-d-ethique-de-la-recherche-dans-les-pays-en-developpement](#)).

- **17.2. Policies and legal aspects**

This research project will be also conducted in accordance with national regulations and laws in Cambodia, and according to E6 Good Clinical Practice (GCP).

The protocol, information sheet, and informed consent (**appendix 1 and 2**) will receive the approval from the National Ethics Committee for Health Research (NECHR).

The research will be performed according to the present protocol. All researchers and investigators participating to this project will respect the protocol, especially in obtaining informed consent (see below).

- **17.3. Information and consent**

The consent of each potential participant is a prerequisite before starting any sampling or before obtaining any specific information related to the research project. The informed consent must be signed by each participant after giving full research description:

- What is being studied?
- What is the procedure/protocol?
- Who is sponsoring the study?
- What are the risks and burdens?
- What are the benefits?
- Whom to contact with questions/concerns

Each potential participant will be given time to think about the information before making a decision. Each subject must fully understand that they have the full freedom to accept or refuse to participate in the study. Once one subject is included in the research project, he has to understand that he can also withdraw from it whenever he wants without any problems/consequences and he will continue to benefit from the regular medical care and check-up.

When one person gives his/her consent for participating to the study, he (she) will write his (her) last name and first name, date and sign the informed consent. In case of children, the informed consent will be filled, dated and signed by one parent or the legal guardian.

Each signed informed consent will be kept in a safe manner and in a safe place (MMC) for a total duration of 15 years after the end of the research project.

- **17.4. Data confidentiality**

The data recorded during this study will be the subject of computer processing at UHS and at the ANRS pharmacovigilance unit. Inserm-ANRS declares to the French National Commission of Informatic and Liberty (CNIL) that data management is compliant with the requirements of the modified Law n° 78-17 of January 6 1978 – Law 2018-493 - of June 20 2018 relative to information technology, files and civil liberties.

In order to respect patient confidentiality, all data recorded in the framework of this study will be coded, using a unique study subject identification code. The study subject identification code will be the only patient identifier on any document related to the study, as well as in the electronic study database.

Individual subject medical information obtained as result of this study will be strictly confidential and coded. Study team members are subject to the obligation of professional secrecy. Only persons mandated by the sponsor and involved in the study management and health authorities are able to access to medical files of patients in order to check the accuracy of the collected data.

Individual data will be made available upon request to the study investigators, physicians in charge of patients' care, representatives of the sponsor, and representatives of the ethical and regulatory health authorities in case of external audit or inspection. Disclosure to other third parties is strictly prohibited.

- **17.5. Type of collected data during the research project**

All data obtained through this research project will be recorded in a database at MMC (UHS). All the computer data will be treated in accordance with the Data Protection Act of 6th January 1978 (law n°78-17 Data Protection [DP] Officer) that was modified on 6th August 2004 (law 2004-801 DP Officer),

with the implementing decree numbered 2005-1309 on 20th October 2005. We will follow this procedure in order to be in agreement with the treatment of personal data obtained through medical research programs.

- **17.6. Final report**

MMC will write the final report, in partnership with principal investigators.

This final report will be established within one year following the end of the research and will be transmitted to ANRS.

- **17.7. Archiving system**

Forms and data related to the research project are key documents. They can be useful to demonstrate that researchers and investigators respect GCP and current laws/legislation.

Consequently, all these documents will be archived by MMC and ANRS during 15 years after the end of the research program.

Informed consents will be kept in a safe manner at MMC in sealed envelopes on which ID numbers of the participants will be indicated, as well as name and signature of the coordinating investigators.

No documents must be destroyed without prior authorization from ANRS.

16 ACCESS TO DATA AND SPECIMENS

All research data and all collected samples will be under the responsibility of the MMC and coordinating investigators.

In case of request by other research teams to use results obtained during the research and/or to use stored samples, these teams will write a proposal and send it to the principal investigators, the scientific committee and the NECHR for approval.

After the dissolution of the SC, data and samples will be under the responsibility of ANRS and the coordinating investigators. In case of request by other research teams to use results obtained during the research and/or to use stored samples, these teams will write a proposal and send it to ANRS, which will take a decision in agreement with coordinating investigators and NECHR.

17 DUTIES OF INVESTIGATORS

According to GCP aimed to achieve high quality of the research, each investigator must:

- To respect participants' rights and to ensure subjects' welfare,
- To ensure his availability as well as availability of his team,
- To ensure that enrollment will be feasible according to the research protocol,
- To organize technical infrastructures for the implementation of sampling, filling in of questionnaires and archiving of documents/records during the research study and 15 years after the end of the research,
- To collect and archive in a safe way signed informed consents,
- To ensure that researchers are following the protocol and to allow completed questionnaires to be regularly sent to MMC,
- To accept a possible audit of the research project carried out by ANRS itself or by other agencies if necessary.

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19 ANNEXES

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LISTE DES MEMBRES

CONSEIL SCIENTIFIQUE DE LA RECHERCHE ANRS 12374

V 3.0 du 4 mai 2022

Titre de la recherche : ANRS 12374 3DICAM: Dolutegravir, Darunavir/ritonavir and Optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia

Responsables scientifiques

Sud : Vichea OUK

Nord : Cécile GOUJARD

Nom du Président du Conseil Scientifique: Cécile GOUJARD

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Dr Vichea OUK	HIV infection	NCHADS
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Dr Olivier SEGERAL	HIV infection	UHS
Dr Chandara MOM	Virologist	NCHADS
Dr Janin NOUHIN	Virologist	IPC
Pr Monidarin CHOU	Pharmacologist	UHS
Dr Anne Marie TABURET	Pharmacologist	CHU Bicêtre
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Dr Herb HARWELL	HIV infection	CHAI
Pr F Xavier BLANC	TB infection	CHU Nantes
Représentants ANRS		
Laura FERNANDEZ	Clinical Research	ANRS
Joséphine BALSSA	Pharmacovigilance	ANRS

Date : 04/05/2022

Signature du Directeur de l'ANRS I MIE



Annex 2: Treatments that could not be used because of potential drug-drug interactions

With DRV/r

- amiodarone, quinidine, bepridil, lidocaine systémique
- carbamazépine, phénobarbital, phénytoïne
- rifampicine/rifampine, itraconazole ou kétoconazole > 200 mg/j, voriconazole
- astémizole, terfénadine
- dihydroergotamine, ergometrine, ergonovine, ergotamine, methylergonovine
- simvastatine, lovastatine
- millepertuis et produits contenant l'Hypericum perforatum
- pimozide, sertindole
- midazolam, triazolam
- cisapride

With DTG

- dofetilide
- Phénytoïn, phénobarbitol and carbamazépine
- millepertuis
- concomitant administration with Al/Mg based antacids should be avoided or should be administered 2h before or 6h after antacids
- metformine: may increase metformin levels so that blood sugar levels need to be monitored. Dose should be limited to 1000 mg daily when initiating either dolutegravir or metformin and adjust dose carefully.

Dolutegravir, Darunavir/ritonavir and Optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia – ANRS 12374

NB: this information sheet will be translated in khmer, reviewed with representatives of PLWHIV and back translated in English.

Information sheet

Version 4.0 –04 June, 2020

Sponsor: **ANRS**, France REcherche Nord & sud Sida-hiv Hépatites

Coordinating investigators: **Pr Cécile GOUJARD (CHU Kremlin Bicêtre) – Dr LY Penhsun (NCHADS)**

This information sheet is made to help you to take the decision to participate or not in this study.

You are free to answer “yes” or “no” to the question: do you wish to participate to this study?

You have the right to take time, to discuss about this study and to ask all the questions that you wish

If you don't want to participate to this study, you will continue to receive the best possible care

You can change of mind at any moment and ask to stop the participation to the study. We just ask you to inform investigators as soon as possible.

GLOSSARY and DEFINITION:

- **Adherence:** the fact to take the treatment every day at the same time without missing dose

- **ART:** antiretroviral therapy = drugs active against HIV; according to the target involved in virus destruction, several families are available as:

- **NRTI (Nucleoside reverse-transcriptase inhibitor):** example: retrovir, tenofovir, abacavir
- **NNRTI (Non Nucleoside reverse-transcriptase inhibitor):** example: efavirenz, viramune
- **PI (Protease inhibitor):** example: lopinavir, atazanavir, darunavir
- **Integrase inhibitor:** example: raltegravir, dolutegravir

- **ARV:** antiretroviral

- **Genotype resistance test:** test to know if your virus is resistant to one or several drugs

- **Line regimen:**

- **Fist line regimen:** the first treatment received by the patient.

- **Second line regimen:** the second treatment received if the first one is not effective
- **Third line regimen:** the third treatment received if the second one is not effective

- **Pharmacogenetics:** this is a test to know whether unexpected adverse event could be due to a high amount of drug in your body

- **Viral load:** The viral load measures the quantity of virus circulating in the blood.

- **WHO: World Health Organisation**

What is a health research study?

A health research study is a way to find out new information about a disease and /or the treatment of the disease.

Why your doctor proposed you to participate in this study?

You are suffering of HIV infection and you have received several different drugs as recommended by the national guidelines. Your current treatment is a second line regimen composed of 3 antiretroviral drugs (ART) and one of those drugs is a protease inhibitor but the viral load is not controlled despite adherence counseling.

Without treatment the viral load is high.

The objective of the ART is to suppress the viral load (it becomes “undetectable”).

A viral load could be detectable for many reasons:

- the beginning of the treatment is too recent
- the treatment is not correctly taken
- the treatment is not correctly absorbed by the body
- the treatment is not enough strong and the virus has developed resistance to one or several drugs

The most regular reason is a difficulty with treatment’s dose. For that reason, you have received 3 months of adherence counseling to be sure that your treatment is correctly taken. Despite this adherence counseling, the viral load remains detectable meaning that your current treatment is not enough efficient. That is the reason why, we propose you to participate to this study, in order to possibly start a new ARV treatment call “Third line regimen”.

What is the aim of the study?

The aim of this study is to assess the effectiveness of a third-line regimen including 2 new drugs, darunavir (DRV/r) and dolutegravir (DTG) combined to optimized NRTI recycling, in Cambodian HIV-infected adults, who failed a protease inhibitors (PI)-based second-line regimen after 3 months of boosted adherence counseling (BAC), and evidence of virus resistance to atazanavir (ATV) and/or resistance to all NRTI.

To reach this objective, we plan to enroll 50 participants in one year and to follow them during one year. The total duration of the study is, so, two years.

A genotypic resistance test will be done thanks to a blood exam to detect the presence of HIV resistances to one or several drugs.

If yes, you will be proposed to participate to the study and begin this new treatment.

If no, your doctor will decide the best therapeutic option (to continue your current treatment or to change one drug in case of intolerance) with adherence counseling support and you could not be enrolled in the study. You will continue to receive the support of care and treatment as recommended by the HIV national program.

Who can participate to the study?

The patients who have:

- \geq 15 years old the day of inclusion
- With HIV-1 infection
- Failing a NNRTI-based first-line regimen
- Failing a PI-based second-line regimen after 3 months of adherence boosting with HIV RNA viral load $>$ 1000 copies/mL and evidence of virus resistance to the current treatment
- For women of childbearing age (15-44 years old): acceptance to use effective contraceptive methods
- Agree to participate in the study after oral and written explanation and sign the Informed consent form, at the latest the day of the inclusion

Who cannot participate to the study?**The patients who have:**

- History of antiretroviral treatment including darunavir and integrase inhibitor
- Active pregnancy $<$ 12 weeks of amenorrhea and desire of pregnancy during the duration of the study
- Opportunistic infection in acute phase at inclusion including tuberculosis
- Advanced cirrhosis (Child-Pugh score B or C)
- Creatinine clearance $<$ 50 ml/mn
- Any concomitant medical condition that, according to the clinical site investigator would contraindicate participation in the study
- Concurrent participation in any other clinical study without written agreement of the two study teams

Participation to the study:

You have full freedom in accepting or refusing to participate in this study.

If you refuse to participate in this study, you can still benefit from the current care provided by this center/hospital based on the HIV National Program, and your medical care will not be affected by your refusal.

If you accept to participate in this study, you will sign the consent form that will also be signed by the health care provider who offered you to participate in this study.

For women of childbearing age

Women of childbearing age defines as women aged from 15 to 44 years old.

According to WHO recommendation, pregnancy during the study must be avoided due to neural tube defects of newborn from mothers treated by Dolutegravir. That is why you need to use consistently effective birth control (contraception) while on this treatment.

To be enrolled to the study, women of childbearing age must use effective contraceptive methods to avoid pregnancy during the study period and the urine pregnancy test will be done every month during the total duration of the study. However the women can come at any moment outside the scheduled consultation for the pregnancy test.

For this study, the study will provide free contraceptive method for all women of childbearing age during. You are free to choose between condoms, oral pill, implant or intrauterine device. But you must know that the effectiveness of estroprogestative pills and implants could be decreased because of interaction with your HIV treatment. For that reason, we strongly

encourage you to use intrauterine device or to use condoms even if you take an estroprogestative contraception.

The study will also provide free Folic acid (a B vitamin) as additional preventive treatment to reduce (but not totally eliminate) the risk of neural tube defect for unwanted pregnancy when a woman takes 400 micrograms daily before and during early pregnancy. Nevertheless, it does not replace your contraceptive method.

Visits

- Pre-inclusion visit

During the first visit, we will collect information about personal and familial medical history, perform clinical examination and we will collect 25 mL of blood (5 tubes) to perform the biological tests including genotype resistance test to detect if the virus is resistant or not to your current ARV treatment. For women in childbearing age (15-44 years old), we will also collect urine sample to perform a pregnancy test.

You will not participate to the study if this first evaluation shows that:

- 1) You have an acute infection, or a severe liver disease, or a renal insufficiency or an active pregnancy. In case, the medical problem is solved, it could be possible to you to participate in a second step.
- 2) The virus is still sensitive to the current treatment,

In that case, you will continue to receive the support of care and treatment as recommended by the HIV national program.

You will participate to the study:

- If the first evaluation doesn't detect any medical condition described above
- And confirms that the virus is resistant to atazanavir AND/OR resistant to both retrovir, abacavir and tenofovir: you will receive the new treatment, call third-line regimen, and which include at least two new drugs, darunavir and dolutegravir

In that case, a new consultation will be planned to start the new ARV treatment

- Inclusion visit

During the inclusion visit, the medical investigator will check the results of the pre-inclusion visit tests and verify that you meet all inclusion criteria and exhibit none of the non-inclusion criteria.

During this visit, we will perform clinical examination and we will collect 20 mL of blood (4 tubes) to perform the biological tests including glycemia and lipid profiles. For women in childbearing age, we will also collect urine sample to perform a pregnancy test.

You will receive an adherence counselling and the treatment for one month.

- Follow-up visits for the first 6 months

The total duration of the study is one year and you will have a monthly consultation during the first 6 months + one consultation after 2 weeks of treatment. These consultations are necessary to detect any side effect of the treatment and to help you in case of difficulty to take the treatment. A blood collection will be done after 4, 8 and 12 weeks of treatment: 15 mL (3 tubes) for visits week 4 and 12 and 10 mL (2 tubes) for visits week 8. For women in childbearing age, we will also collect urine sample to perform a pregnancy test every month.

If you miss any of these visits, you will be contacted at home or by phone by the study personal, unless you explicitly refuse such visits or phone calls.

Between study visits, you may come to the hospital whenever you have health problem. If needed, the doctor will undergo additional tests and specific treatment for your health care.

If you have one of these 4 symptoms - persistent cough (several weeks), fever, loss of weight and night sweats – you must contact your doctor to report him and further exams could be done.

- Visit week 24

After 6 months of treatment, a specific evaluation will be done including a viral load. During this visit, we will collect 20 mL of blood (4 tubes) to perform the biological tests including the viral load. The objective is that the quantity of virus in your blood will be undetectable:

In that case, the investigator will propose you to switch from DRV/r 600/100 twice daily to DRV/r 800/100 once daily and to continue other drugs as usual. A new ART-related adherence counseling will be performed to explain the new dose. Plasma HIV-RNA will be performed one month later to confirm the maintenance of undetectable viral load.

If the HIV RNA viral load is still detectable at week 24 or if the virus was possibly resistant to DRV/r at inclusion, the investigator will not change the ART regimen and you will be addressed for systematic monthly adherence counseling during 3 months.

- Visits weeks 36 and 48

You will be systematically asked about your symptoms and drugs intakes, receive a clinical exam, an ART-related adherence counseling and a complete evaluation of adverse events.

Plasma HIV RNA will be performed:

- If you switched to DRV once daily, a reversal switch to DRV twice daily will be done if HIV RNA is > 40 copies/mL on two consecutive analyses

- If you not switched to DRV once daily because of uncontrolled HIV RNA at week 24, a GRT will be performed if HIV RNA is > 1000 copies/mL. If HIV RNA ranges from 40 to 1000 copies/mL, adherence counseling will be done.

Treatment

- Darunavir

Darunavir is a new generation protease inhibitor effective on virus known to be resistant to other protease inhibitors and recommended by WHO. Darunavir is always associated to ritonavir in order to increase the concentration of the drug in the blood. Darunavir could be administered twice daily or once daily. For patients with at least 2 resistance mutations for protease inhibitor, it is recommended to begin the treatment twice daily. For that reason, you will begin the treatment with 600 mg twice daily. But if your viral load is controlled after 6 months, we will propose you to switch to 800 mg once daily. In all cases, tablets must be taken with food.

Darunavir could have side effects. The most frequent are diarrhea, nausea, vomiting, headaches and rash. More serious adverse effects that have been reported include an uncommon but severe hypersensitivity (allergic) reaction with rash, heart attack, lower platelets count, severe disorders of the bones and muscles, and possible effects on the liver and the kidney. This list is non exhaustive and you must report all undesirable effects to your doctor, whether it is included or not in this list.

Darunavir could also present interaction with other drugs. For that reason, a list of forbidden drugs will be delivered and you must ask the authorization to your doctor before taking any new treatment.

- **Dolutegravir**

Dolutegravir is part of a new class of drugs, called integrase inhibitor, for which effectiveness has been reported in case of resistance to others drugs. Dolutegravir is administered once daily and could be taken with or without food. Dolutegravir is recommended by WHO.

Dolutegravir could have side effects. The most frequent are diarrhea, nausea, and headaches. Some psychiatric side effects could occur as depression, anxiety and difficulty to sleep. More serious adverse effects that have been reported include an uncommon but severe hypersensitivity (allergic) reaction with rash and possible effects on the liver. This list is non exhaustive and you must report all undesirable effects to your doctor, whether it is included or not in this list.

Dolutegravir could also present interaction with other drugs. For that reason, a list of forbidden drugs will be delivered and you must ask the authorization to your doctor before taking any new treatment.

Recently, the World Health Organization has sent an alert regarding the use of dolutegravir for women of childbearing age. An ongoing study in Botswana found 4 cases of neural tube defects out of 426 women who became pregnant while taking dolutegravir. Neural tube defects are serious birth defects of the brain and spine of the baby and can cause lifelong disabilities (that range from mild to severe) and deaths. They happen very early in pregnancy (first month). That is why active pregnancy < 12 weeks of amenorrhea and desire of pregnancy are considered as non-inclusion criteria for the study.

It is extremely important to avoid any pregnancy during the study and to report immediately any delay of menstruation in order to detect a potential pregnancy very early. Urine pregnancy test will be done every month during the total duration of the study but you can come at any moment outside the scheduled consultation.

Constitution of a biobank

Your biological samples (blood) will be frozen and kept anonymously in the freezers in the laboratory Rodolphe Merieux in University of Health Sciences of Phnom Penh, Cambodia and will be used in accordance with the protocol. They might be used for other purposes that the ones directly related to this study including genetic research studies, provided that explicit authorization from the National Authorities and from the sponsor will be obtained.

Pharmacokinetic analyses

We could propose you to participate to a pharmacokinetic sub-study. You are free to refuse to participate to this sub-study and you will continue to receive the follow-up of the main study. If you accept, analysis of concentration of dolutegravir and darunavir/ritonavir present in your blood will be conducted in a laboratory of the University of Health Sciences. A specific information sheet and consent form will be given to you.

If you have an unexpected adverse event with ART regimen or introduction of a possible interactive treatment, a pharmacogenetic test could be performed to better understand the reason of this adverse event or to document a possible interaction. You are free to refuse pharmacogenetic test. If you are agree, you must answer “yes” in the consent form. In that case, a supplementary blood sample will be collected (one tube of 5 mL).

Costing Analysis

Cost analysis based on the design of the 3DICAM study is an opportunity to inform national authorities about the relative costs of the third-line implementation and the share of this cost in the funds allocated to HIV-related services globally. A cost analysis could be useful to compare the 3DICAM strategy with and without DRV dose reduction after 6 months of treatment. Impact of extra costs as safety or morbidity management, out of pocket expense for the patients within the ANRS 12374-3DICAM strategy with and without DRV dose reduction after 6 months of treatment will be estimated within the 2 scenarios. All these information's are relevant for government policies. At the international level, cost analysis based on the design of the 3DICAM study could be compared to other third-line programs and can contribute to more accurate international estimates of the resources needed for HIV programs. We suggest you take part in this study that will last about 30 minutes for the interview, one day at your convenience. If you are selected for the study, you will be interviewed by a member of research team. All of the answers you give will be confidential and will not be shared with anyone other than members of our study team. You don't have to be in the study, but we hope you will agree to answer the questions since your views are important. If there is any question you don't want to answer, just let us know or you can stop the interview at any time.

What are the advantages and disadvantages of participating?

Advantages

- You could receive new drugs, effective for resistant virus
- You could expect to have an undetectable viral load
- You could improve your immunity and so, decrease the risk of infections
- You will benefit the very closely monitored medical care with additional clinical and biological examination
- You will be reimbursed for the transportation fees used to come to the hospital for your protocol visits

Possible risks and disadvantages

- You will have to come more frequently to the hospital for the purpose of the study.
- Inconvenient could be associated with the drawing of blood. That includes light-headedness, bleeding and/or bruising at the puncture site and minor infection at the vein puncture site.
- The drugs can produce side effects (as explained above)
- It is possible that the treatment does not work as expected.
- For women of childbearing age, you will need to use a contraceptive method

For your safety, several Committees will meet regularly to examine all information related to the study.

Withdrawal

You can withdraw from this study whenever you wish without having to give any explication for your decision or having any consequences on your medical follow up. Moreover, your health care provider can stop your study participation if she/he thinks it is necessary. National Health Authorities or the Scientific Committee who monitor this study may also decide to end it.

If you withdraw from the study, you can express the will for your data to be removed from the database and your laboratory samples be destroyed. If you do not express such will, the study team could use data and samples collected prior to the date of your consent withdrawal.

Alternatives to Participating

If you do not wish to take part in the research, you will be provided with the established standard treatment available at the ART sites.

What happens when the study ends?

After this study ends, your medical care will continue at the same hospital and under the same condition as before through the HIV National Program. You could continue the same treatment delivered by the HIV National Program.

Confidentiality

All your personal information collected during the study period (data related to clinical assessment, biological testing) will be kept under an identification study code, which will not allow your identification. These data will be registered and coded in a computerized database. The health care providers and scientists have access to your medical record on the condition that they respect its confidentiality. When results of this study will be published, your identity will be kept confidential.

Expenses and compensations

You will not pay any costs related to this study: the costs of the biological and radiological diagnosis, of hospitalization and transportation expenses for protocol visits will be covered by the study during the entire duration of your follow-up.

Your participation is entirely voluntary and you will not receive any financial compensation.

Data processing and rights associated

The study sponsor, Inserm-ANRS, is a French research agency that is responsible for carrying out a public interest mission. This justifies the processing of personal health data and data collected for scientific research purposes, in accordance with European, French and local regulations.

Your personal health data will be collected to the extent that it is necessary for the research. The main source from which the data is collected is your medical file.

All your information obtained in this study will be kept and handled in a confidential manner, in accordance with applicable laws and/or regulations. Your data will be identified by a code. Your name, date of birth and address will not be entered into the database so you cannot be identified. Sponsor or health authorities' representatives may conduct inspections and consult your personal data. These people will keep the information strictly confidential.

Data transfer

Your data will be safely transferred to a database located at UHS for storage and analysis. These data will be managed in accordance with European and French regulations on personal data protection.

Your data may be transferred to national or French health authorities and other private or public, national or international research teams. The data transferred will be agreed upon a sharing agreement between ANRS and the recipient guaranteeing the confidentiality of your data.

Your rights

In accordance with european, french¹ and national laws you have the right of access, right of rectification of your data collected, right to oppose (right of opposition) or to limit their use (right of limitation of data processing), directly or through a doctor of your choice.

You can withdraw from the study whenever you wish without giving any reason and without any consequences on your medical follow-up. Please note that as the erasure of data may make it impossible or seriously jeopardize the achievement of treatment goals, data collected prior to the withdrawal of your consent may not be erased and may continue to be processed under conditions that will ensure their confidentiality².

The knowledge we will receive from this study will be shared with you if you wish. We will publish or present the results of the ANRS 12374 3-DICAM study so that other people interested can learn from our research. Confidential information will not be shared and your identity will never be mentioned. Part of data collected during the study may be shared with other partners, in accordance with the agreements signed under the security rules stated below.

Finally, your data will be kept as long as necessary for analysis and publication purposes; and then your data will be archived according to existing regulations.

To exercise your rights, or for any information about the use of your data, you can contact the data protection officer (dpo@inserm.fr, Déléguées à la protection des données, 101, rue de Tolbiac - 75654 Paris Cedex 13).

In addition, you have the right to lodge a complaint with the National Commission for Information Technology and Civil Liberties - CNIL (French data protection Authority).

What happens if there is a problem?

In case of a problem, you should report it to your health care provider.

As a research sponsor, the ANRS purchase liability insurance for damage. This insurance guarantees compensation in case you experience any harm as the result of your involvement in this study including side effects of treatment.

Contact for further information

During the study period, you can ask any questions or request additional information from your health care provider or the person in charge of the study at your hospital or Health Centre. You have access to all the results of this research during and at the end of the research study.

Dr/ Mr/ Mrs

Phone number:

¹ Conformément aux dispositions de la loi n° 78-17 du 6 janvier 1978 modifiée relative à l'informatique, aux fichiers et aux libertés et au Règlement Général sur la Protection des Données (RGPD - Règlement (UE) 2016/679)

² In accordance with Article 17 of the General Data Protection Regulations (GDPR) to ensure compliance with Article L.1122-1-1 of the Public Health Code

Glossary :

(Personal) data: any information about your person collected in the course of the research.

Right of access: right to know your data and to obtain a copy.

Right of opposition: right to object at any time to the processing and transmission of your data and to have them no longer processed in the future. The exercise of this right leads to the termination of participation in research.

Right of rectification: right to ask for your data to be corrected in the event of an error.

Right to limitation: right to temporarily block the use of some of your data: no operations can be carried out on them.

Dolutegravir, Darunavir/ritonavir and Optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia – ANRS 12374

NB : this information sheet will be translated in khmer, reviewed with representatives of PLWHIV and back translated in English.

Consent form

Version 2.0 – August 18th, 2021

Version 1.0 approved by the National Ethics Committee for Health Research of Cambodia on July 02th,2018

Sponsor: **ANRS**, France REcherche Nord & sud Sida-hiv Hépatites

Coordinating investigators: **Pr Cécile GOUJARD (CHU Kremlin Bicêtre) – Dr OUK Vichea (NCHADS)**

I, (full name)....., the undersigned, declare that I have read or have been read the information sheet for pharmacokinetic analyses attached with this form and I have clearly understood the objectives, advantages and disadvantages of this sub-study.

I had the opportunity to ask all questions and discuss this research sub-study with the health care provider who proposed me to participate, whose name and signature are shown below, as well as with his/her staff.

They answered all my questions in an understandable language. The risk and benefits have been explained to me. I believe that I have not been unduly influenced by any team member to participate in the research sub-study by any statement or implied statements. A time for thought is possible if I wish to take time before making my decision.

I understand that I may be given, if I wish, a copy of this consent form after signing it. I understand that my participation in this clinical sub-study is voluntary, that I may choose to refuse to participate, that, if I accept, I may withdraw my consent in any time with no penalty or disadvantage to my routine care and that if I withdraw my consent, I have the right to ask that my personal data and samples would be removed from the study.

I understand that my samples and data might be used for further researches provided authorization from the National Ethics Committee and including genetic analyses.

Therefore, I fully agree to participate in this study. Yes No

I agree to be contacted by phone or at home in relation to this study. Yes No

I agree that my stored samples would be further used, for other studies approved by the National Ethics Committee Yes No

I agree that my stored samples would be further used for genetic research studies Yes No

I understand that information regarding my personal identity will be kept confidential and coded. I authorize the inspection of my medical records by the team in charge of the study. Yes No

I agree to be interviewed and voice recorded by the team in charge of the study for costing analysis of the third line treatment. Yes No

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature _____ **Date** |__|_| / |__|_| / |__|_|_|_|

I, Dr. / Mr/ Mrs, the undersigned, have fully explained the relevant details of this research to the participant named above and believe that the participant have understood and has knowingly given his(her) consent.

I solemnly promise I will respect all the terms and conditions mentioned in this consent form, kept full confidentiality, and respect the individual's rights and freedom as well as the requirements of the scientific work.

Investigator Signature _____ **Date**|__|_| / |__|_| / |__|_|_|_|

Dolutegravir, Darunavir/ritonavir and Optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia – ANRS 12374

NB : this information sheet will be translated in khmer, reviewed with representatives of PLWHIV and back translated in English.

**Information sheet for Pharmacokinetic analyses
Version 1.0 – October 19th, 2017
approved by the National Ethics Committee for Health Research of Cambodia on**

.....

Sponsor: **ANRS**, France REcherche Nord & sud Sida-hiv Hépatites
Coordinating investigators: **Pr Cécile GOUJARD (CHU Kremlin Bicêtre) – Dr LY Penhsun (NCHADS)**

This information sheet is made to help you to take the decision to participate or not in this sub-study for pharmacokinetic analyses.

You are free to answer “yes” or “no” to the question: do you wish to participate to this sub-study?

You have the right to take time, to discuss about this sub-study and to ask all the questions that you wish

If you don't want to participate to this sub-study, you will continue to receive the follow-up of the main study

You can change of mind at any moment and ask to stop the participation to the sub-study. We just ask you to inform investigators as soon as possible.

Why your doctor proposed you to participate in this sub-study?

You have accepted to participate in a study entitled “dolutegravir, darunavir/ritonavir and Optimized NRTI recycling as a third-line antiretroviral regimen in Cambodia” and you will be treated for your HIV infection with antiretroviral drugs including dolutegravir and darunavir/ ritonavir. The HIV drug activity and tolerability depends on the drug concentration in your blood. We would like to understand precisely how your body absorbs and eliminates dolutegravir and darunavir/ritonavir. Analysis of concentration of dolutegravir and darunavir/ritonavir present in your blood will be conducted in a laboratory of the University of Health Sciences. We suggest you take part in a study that will last ten hours, one day at your convenience one month after initiation of treatment.

Role in the sub-study

The day before your visit to the hospital, you will take your dolutegravir or darunavir/ritonavir and other medicines prescribed by your doctor. On the morning of your arrival in the hospital, you will not take any medicine but you will bring them to the hospital (with the dolutegravir or darunavir/ritonavir that you did not take). A nurse will tell you when to take these medications.

To facilitate blood sampling and introduce a small needle in your vein only once, a catheter will be placed and then a first blood sample (4mL x 2 tubes) will be drawn. You will then take your medication with the nurse and samples of 4 mL x 2 tubes will be drawn at 1h, 2h, 6h, and 10h after taking the drugs.

Risk

Inconvenient could be associated with the drawing of blood. That includes light-headedness, bleeding and/or bruising at the puncture site and minor infection at the vein puncture site. Overall, certified medical doctors or nurses will perform these collection methods. During these 10 hours, you will be under medical observation and we ask you to stay in your hospital room. A surveillance or medical check-up will be done during your stay. A breakfast and lunch will be served.

Compensation

The cost of transportation to the hospital, room and meals will be borne by the study. A sum of 20 USD will be given to compensate for the day you will spend in the hospital.

Frozen samples

Some biological samples will be frozen and kept anonymously in the freezers in the laboratory Rodolphe Merieux in University of Health Sciences of Phnom Penh, Cambodia. They might be used for other purposes that the ones directly related to this study including genetic research studies, provided that explicit authorization from the National Authorities and from the sponsor will be obtained.

Contact for further information

During the study period, you can ask any questions or request additional information from your health care provider or the person in charge of the study at your hospital or Health Centre. You have access to all the results of this research during and at the end of the research study.

Dr/ Mr/ Mrs

Phone number:

**Dolutegravir, Darunavir/ritonavir and Optimized NRTI recycling as a
third-line antiretroviral regimen in Cambodia – ANRS 12374**

NB : this information sheet will be translated in khmer, reviewed with representatives of PLWHIV and back translated in English.

**Consent form for pharmacokinetic analyses
Version 1.0 – October 19th, 2017
approved by the National Ethics Committee for Health Research of Cambodia on**

.....

Sponsor: ANRS, France REcherche Nord & sud Sida-hiv Hépatites
Coordinating investigators: Pr Cécile GOUJARD (CHU Kremlin Bicêtre) – Dr LY Penhsun (NCHADS)

I, (full name)....., the undersigned, declare that I have read or have been read the information sheet attached with this form and I have clearly understood the objectives, advantages and disadvantages of the study.

I had the opportunity to ask all questions and discuss this research study with the health care provider who proposed me to participate, whose name and signature are shown below, as well as with his/her staff.

They answered all my questions in an understandable language. The risk and benefits have been explained to me. I believe that I have not been unduly influenced by any team member to participate in the research study by any statement or implied statements. A time for thought is possible if I wish to take time before making my decision.

I understand that I may be given, if I wish, a copy of this consent form after signing it. I understand that my participation in this clinical study is voluntary, that I may choose to refuse to participate, that, if I accept, I may withdraw my consent in any time with no penalty or disadvantage to my routine care and that if I withdraw my consent, I have the right to ask that my personal data and samples would be removed from the study.

I understand that my samples and data might be used for further researches provided authorization from the National Ethics Committee and including genetic analyses.

Therefore, I fully agree to participate in this study. Yes No

I agree to be contacted by phone or at home in relation to this study. Yes No

I agree that my stored samples would be further used, for other studies approved by the National Ethics Committee Yes No

I agree that my stored samples would be further used for genetic research studies Yes No

I agree that one of my stored blood sample be used for a pharmacogenetic test which could be performed whether I happen to have an unexpected adverse effect with the new ARV regimen? Yes No

I understand that information regarding my personal identity will be kept confidential and coded. I authorize the inspection of my medical records by the team in charge of the study. Yes No

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature _____

Date |__|_| / |__|_| / |__|_|_|_|_|

I, Dr. / Mr/ Mrs, the undersigned, have fully explained the relevant details of this research to the participant named above and believe that the participant have understood and has knowingly given his(her) consent.

I solemnly promise I will respect all the terms and conditions mentioned in this consent form, kept full confidentiality, and respect the individual's rights and freedom as well as the requirements of the scientific work.

Investigator Signature _____

Date|__|_| / |__|_| / |__|_|_|_|_|

Annex 7: Costing analysis Proposal

Cost analysis on Third-line implementation in Cambodia

1. Research question

- a. What would a third-line program design as the ANRS 12374-3DICAM study cost in Cambodia using DRV and DTG as a backbone?
- b. And in which proportion of the funds allocated to HIV-related services globally?
- c. What is the part related to morbidity and toxicity monitoring and management?
- d. What are the costs of different scenarios: GRT with DRV dose reduction and GRT without DRV dose reduction?
- e. How much future funding is needed to expand the program to more people in need?

2. Background

Third-line regimen implementation is an area of considerable uncertainty worldwide. Many countries are reluctant to implement third-line regimen at the national level because of limited availability and high prices of the drugs. Low market volume and necessity to new lab tests as genotyping test and new policies are also significant national barriers.

However, WHO made the assumption that the number of people on third-line treatment will increase to approximately half a million by 2025.

Additional funds may be needed to face third-line regimen scaling up. Making high-quality treatment as affordable as possible and optimizing the allocation of existing HIV funding across specific interventions could maximize the impact of each dollar spent, reducing the size of the resource gap and encouraging increased investments from donors and national governments. For that, a better understanding of the current costs of treatment and the identification of potential areas where efficiency gains are possible is needed.

In Cambodia, DRV/DTG combination was retained as the preferred third-line combination, which is in phase with the recent WHO guidelines revised in July 2018. DTG is available as a generic drug in single tablet (44 USD per patient, per year) or in fixed-dose combination with TDF/3TC (75 USD per patient, per year). No generic drugs for DRV are available so far. DRV is available as single 400 mg and 600 mg dose (700 to 900 USD per patient, per year) and must be associated with RTV 100 mg. However, generic fixed-dose combination (FDC) of Darunavir/Ritonavir tablets (400/50) is expected in 2019 with a significant decrease of the price (estimation of 300 USD per patient, per year). As nearly three-quarters of the cost per patient were reported attributable to drugs for second-line regimen, a prospective cost analysis taking into account the evolution of the price of the drugs could be of great interest.

Despite the cost of these 2 drugs, others factors could influence the costs of a third-line program:

- The use of genotyping test before switching. WHO recommends to use it, if possible, to optimize the treatment, specifically for NRTI recycling. It is also a way to avoid unnecessary switching among patients with low adherence and no PI-mutations (27% in the ANRS THILAO study). That's why we decide to use it as an inclusion criteria in the 3DICAM study. But many countries face the challenge of difficulties in access to GRT with high price of the exam.
- DRV dose. According to ANRS table, DRV/r 800/100 once-daily could be used for patients with < 2 mutations among: V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V,

I84V, L89V. For patients with 2 mutations or more or in absence of GRT available, DRV/r 600/100 twice daily must be used. But Asian population seems to have higher antiretroviral concentrations than those who are not Asian (ex. EFV, ATV) and the possibility to switch from twice-daily to once-daily dose after reaching virological suppression could be an interesting option to improve adherence and reduce the cost as planned in the 3DICAM study.

- Monitoring toxicity. Few data are reported on the safety of third-line regimen.
- IRIS: Few data are reported on IRIS occurrence after initiation of third-line regimen.

Cost analysis based on the design of the 3DICAM study is an opportunity to inform national authorities about the relative costs of the third-line implementation and the share of this cost in the funds allocated to HIV-related services globally. A cost analysis could be useful to compare the 3DICAM strategy with and without DRV dose reduction after 6 months of treatment. Impact of extra costs as safety or morbidity management, out of pocket expense for the patients within the ANRS 12374-3DICAM strategy with and without DRV dose reduction after 6 months of treatment will be estimated within the 2 scenarios. All these information's are relevant for government policies. At the international level, cost analysis based on the design of the 3DICAM study could be compared to other third-line programs and can contribute to more accurate international estimates of the resources needed for HIV programs.

3. General Objectives

- To conduct a cost analysis of the global strategy for third-line program in Cambodia.
- To generate a base of evidence to inform policy around the implementation of national HIV/AIDS third line program.

4. Specific Objectives

- To conduct a cost analysis of third-line program according to different strategies
- To assess separately the cost of the training process and its implementation

5. Materials and methods

The present study will be nested into the ANRS12374-3DICAM project. A cost analysis will be conducted over different scenarios of third-line ART implementation to provide evidence about most advisable strategy. We will estimate, for each scenario, the cost of providing third-line ART to patients in ART sites setting in Cambodia.

Second, we will conduct a qualitative interview with 15 patients among the patients enrolled in the ANRS12374-3DICAM to understand actual and additional spending for seeking care of third-line treatment with the different scenarios. Then information emerged from the qualitative interview will be used to develop a structured questionnaire which will be used to interview face to face with all patients enrolled in the ANRS12374-3DICAM to assess on their additional expense and financial burden related to different scenarios of third-line ART implementation (with and without DRV dose reduction after 6 months of treatment). The information from interview will enable the program national to understand how the third-line treatment impact on the patients, specifically on their expense to seek care at ATR site.

A qualitative interview will be also conducted with health system staffs at national, provincial and district level to identify the information on the resources relevant to third-line treatment implementation. Then all resources and cost items identified from the interview will be listed in structured questionnaire which will be used to collect the costs of third-line treatment implementation.

5.1 Study sites

The study will be conducted in the 3 main health service levels - national, provincial and district hospitals - with one or two ART sites by level. The ART sites will be chosen to epitomize the program in Cambodia and to reflect the diverse range of HIV treatment and care facilities. At each ART site, the care service center will be divided into 5 categories including 1/ Outpatient clinic 2/ Inpatient ward 3/ Laboratory 4/ Pharmacy 5/ Social service.

5.2 Data collection

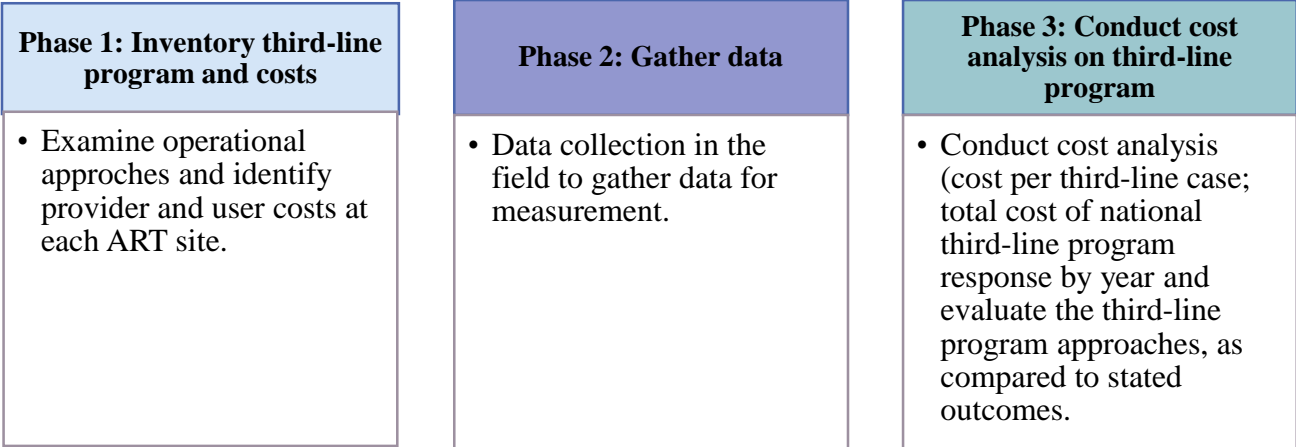
At each ART site, the records of patient on third-line regimen will be selected to review and all resources used by provider between pre-third-line regimen initiation and at 12 months follow up on third-line regimen will be collected to measure the resource usage for 12 months.

A mix method both qualitative and quantitative interview will be conducted to collect indirect costs relevant to third-line treatment and care among patients enrolled in the ANRS12374-3DICAM. All patients enrolled in the ANRS12374-3DICAM and two health care workers by each ART site at each level (national, provincial and district) will be purposively selected for face to face interview using a structured questionnaire. The programmatic support service costs provided to each ART site by HIV National Program will be also collected, both retrospectively and prospectively, in addition to the cost of ART site levels and patients indirect cost. This will include the service chains such as commodities (drug procurement, laboratory materials, others medical supplies, others non-medical supplies), monitoring and evaluation including technical working group and drug quality control, human resources, training and communication. Costing will be estimated according to the observed prices for each provider, taking into account the most recent prices.

The study will consider the specific mix of resources used by each ART site to be a feature of the treatment delivery model represented by each ART site. The study therefore will make no effort to standardize the resources included at each ART site in order to be closer to the reality. Resources will be captured for all or some of the ART sites, depending on the site resource mix, including ARVs, non-ARV medications, laboratory tests, outpatient visits, inpatient visits, infrastructure (buildings), equipment, data capture and program management. However, if for example, there is a site that without laboratory tests, this will imply a more important cost for the patient as he/she has to go to another place to do the blood tests and this will imply collecting information on these costs from a given source which will also imply for patients to spend in transport, etc. Quantities of variable resources (defined as all resources utilized for an individual patient) will be summed from medical records. Fixed resources (defined as annual resource utilization required for the existence of the treatment program as a whole) will be identified for each facility and will be adjusted to exclude resources not directly associated with the AIDS treatment program and then will be divided by the total number of active patients in the study period.

Unit costs for each resource utilized will be estimated from financial information provided by the ART sites and from interviews with ART site managers and others relevant stakeholders.

5.3 Study framework



5.4 Analysis design

Cost analysis will be conducted over different scenarios defined according to the following strategies of implementation of the third-line ART in Cambodia:

- Strategy 1:** third-line implementation with the ANRS 12374-3DICAM strategy: with GRT at inclusion and with DRV dose reduction at 6 months.
- Strategy 2:** third-line implementation with GRT at inclusion but without DRV dose reduction.

5.5 Cost analysis

The costs will include ARV prices, lab test costs, staff salaries and time requirements, opportunistic infection costs and other drug costs, psychological support cost, cost related to the patients such as transportation, food, the programmatic support service costs by HIV National Program including drug procurement, laboratory materials, others medical supplies, others non-medical supplies, monitoring and evaluation including technical working group and drug quality control, human resources, training and communication.

The costs related to providers such as ARV prices, lab test costs, staff salaries and time requirements, opportunistic infection costs and other drug costs, psychological support cost and the programmatic support service costs will be estimated by reviewing relevant available documents and through the interviews and discussions with health system staffs at national, provincial and district level. The Global Price Reporting Mechanism GPRM of WHO will also be used as another source for prices of ARVs to estimate the ARV costs.

The costs of resources used by patients and family to participate in third-line treatment such as travel, food and accommodations will be estimated by face to face interview with the patients.

The characteristics of the participants including the clinical characteristics, the number of lab test, number of consultations and number of adverse events will be available in the ANRS 12374-3DICAM study database.

Costs for per third-line ART phases will be analyzed cross-sectionally, and the costs incurred in the period of 3DICAM study will be estimated.

Unit costs which will be used in the analysis will be the most recent costs to the ART sites, either reported in 3DICAM study will be adjusted to the inflation rate.

Resource Needs Model (RNM) will be used to calculate the resources required for the implementation of HIV/AIDS third line program at the national level.

The cost of providing third-line ART to a patient for a year will be calculated by estimating the resources required to provide ART to a single patient as follow:

Per patients cost= (Drugs cost × doses per patient per year) + (lab cost × tests per patient per year) + [(staff salary × Hours per consultation/working hours per year) × consultation per patient per year] + other costs

All the above information will be used to calculate a cost of 3rd line implementation per patient according to each ART site level (district, provincial, national).

For each ART site level (district, provincial, national) participating in the ANRS 12374-3DICAM study, the per-patient cost will be used to calculate the ART site costs.

These costs will be aggregated to have finally the total 3rd line program implementation at the national level. This will account for the individual specificities and the differences among ART site levels.

5.6 Sensitivity Analysis

The sensitivity analyses will be conducted for each strategy in order to identify the elements that impact the most the cost of the 3rd line implementation. The study will also calculate a mean cost per patients (for 1 year of treatment) and will use univariate and multivariate linear regression to understand the importance of patients characteristics and ART site characteristics on the cost of the 3rd line program.

6. Ethic consideration

The study will be nested to ANRS12374-3DICAM study which was approved by the National Ethic Committee for Human Health Research in Cambodia (NECHR) on July 7, 2018.

Ethical considerations, including data confidentiality, anonymity and necessary clearance will be respected. The data collected for this study will be kept confidential and will be used only for the study purpose. The individual data and the results will be kept only by the research team. All the results from this study will be presented using pseudonyms or numbers.

7. Expected outputs

1. Information on costs of a third-line program design as the ANRS 12374-3DICAM study in Cambodia using DRV and DTG as a backbone which will inform national authorities about the relative costs of the third-line implementation and the share of this cost in the funds allocated to HIV-related services globally.
2. Information which is relevant for government policies on the impact of extra costs such as out of pocket expense within the ANRS 12374-3DICAM strategy with and without DRV dose reduction after 6 months of treatment
3. At the international level, information on costs of a third-line program based on the design of the ANRS 12374-3DICAM study could be used to compare with other third-line programs and could be contributed to more accurate international estimates of the resources needed for HIV programs.

8. Timeframe for the study

- a. April 2022-June 2022: data collection
- b. July 2022-December 2022: cost analysis
- c. December 2022-March 2023: writing and submission of the third manuscript

**Information letter for participants being involved in
ANRS 12374 3-DICAM Study**

April 19th 2022

**Subject: Informing about the replacement of the Cambodian coordinating investigator
for project ANRS 12374 3-DICAM Study**

Dear Sir, Madam

You agreed to participate in **ANRS 12374 3-DICAM study**, whose sponsor (legal entity responsible for the study) is Inserm-ANRS, and thank you for your participation.

When you consented to participate in the ANRS 12374 3-DICAM study, on the day of your enrolment, the consent form that you signed was with the name of **Dr. Ly Penh Sun**, the Cambodian Coordinating Investigator of the project. With this letter, Inserm-ANRS wants to inform you that the new coordinating investigator is **Dr. OUK Vichea** since **Dr. Ly Penh Sun**, passed away.

Dr OUK Vichea is the new Director of National Center for HIV/AIDS, Dermatology and STD (NCHADS), if you want to contact him regarding your participation in the trial, please find below his contact details:

Tel: +855 12 512 425

Address: #245H, Sreet 6A, Phum Kean Khlang, Sangkat Prekleap, Phnom Penh Cambodia

Please note that all the information mentioned in the consent form that signed conforming your participant remains the same.

Sincerely yours,



Yazdan YAZDANPANA
Director of ANRS MIE

RESEARCH

Open Access



Hepatitis C seroprevalence among people living with HIV/AIDS and pregnant women in four provinces in Cambodia: an integrated bio-behavioral survey

Phearavin Pheng^{1,2*}, Laurence Meyer², Olivier Ségéral¹, Phalla Chea¹, Siyan Yi^{3,4,5}, Sovannary Tuot^{3,7,8}, John M. Kaldor⁶ and Vonthanak Saphonn¹

Abstract

Background: Understanding the extent of viral hepatitis burden in specific subgroups, such as pregnant women and people living with HIV/AIDS (PLWHA), and their geographic distribution is essential for evidence-informed policy and mobilizing resources for targeted treatment and prevention efforts. However, in Cambodia, the epidemiology of hepatitis C remains uncertain. We estimated the hepatitis C virus (HCV) burden and transmission risk factors among PLWHA and pregnant women attending antenatal care (ANC) in Cambodia.

Methods: Between March and April 2016, we conducted a cross-sectional survey in four diverse geographical areas: the capital city of Phnom Penh and three provinces. We collected information on demographic characteristics and risk behaviors and performed HCV antibody (Anti-HCV) testing among pregnant women attending public ANC clinics and among those receiving HIV care at the hospitals. We computed the prevalence of HCV among the two population subsets and performed logistic regression analyses to identify risk factors associated with HCV antibody positivity.

Results: Of 935 participants enrolled, 510 (54.6%) were pregnant women and 425 (45.4%) were PLWHA. Anti-HCV prevalence was significantly higher in PLWHA than in pregnant women (29/425, 6.8% vs 5/510, 0.9%, $P < 0.001$). Of the geographic regions, Preah Sihanouk province (Southwest) had the highest anti-HCV prevalence among PLWHA (12.0%, $P = 0.031$). There was no significant geographic difference in anti-HCV prevalence among pregnant women. In multivariable analyses (data subset to PLWHA), HCV infection was significantly associated with having a family member positive for HCV (OR = 7.6 [95% CI: 1.01–57.84], $P = 0.048$) and a history of intravenous medication injection in the last 5 years (OR = 7.1 [95% CI: 2.79–18.10], $P < 0.001$).

Conclusions: HCV infection is relatively common among Cambodian PLWHA, likely related to intravenous medication injection and intra-familial viral transmission. Systematic HCV testing and care among PLWHA (and possibly their family members) might be necessary. Setting up a surveillance system for HCV might also be beneficial for some geographical regions and populations.

Keywords: HCV infection, Prevalence, People living with HIV/AIDS, Pregnant women, Cambodia

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Background

Hepatitis C virus (HCV) infection is a major public health concern, with an estimated 130–150 million chronic hepatitis C virus infection worldwide [1] and 350,000 deaths each year due to hepatitis C-related causes, mostly cirrhosis and hepatocellular carcinoma (HCC) [2]. The prevalence of HCV varies substantially around the world. The estimated global prevalence of HCV is 2–3% [3] and ~ 8% of pregnant women have HCV infection [1], with the highest prevalence in low- and middle-income countries (LMICs) in Africa and Southeast Asia [4]. The World Health Organization (WHO) Global Health Sector Strategy for Viral Hepatitis calls for the elimination of viral hepatitis by 2030, aiming at a 90% reduction in incidence and a 65% reduction in mortality. To reach this target, 90% of chronic hepatitis cases need to be diagnosed, and 80% of eligible cases treated [1].

HCV treatment with direct-acting antivirals (DAA), is generally curative and success rates in LMICs are similar to those in high-income countries (HICs). Improved access to HCV testing and better epidemiological surveillance data are important steps in increasing HCV case detection and identifying those eligible for treatment. In particular, serological surveys can improve the understanding of the distribution of HCV infection in the general population and identify specific high-risk groups.

Globally, about 2.9 million people living with HIV/AIDS (PLWHA) are co-infected with hepatitis C virus [1]. The major cause of morbidity and mortality among people co-infected with HIV and viral hepatitis is potentially related to liver diseases [5]. It is recommended that this population should be prioritized for screening and providing with an appropriate and effective treatment for both HIV and hepatitis. In Cambodia, addressing HCV infection in PLWHA could possibly be done through expanding access to treatment for hepatitis C for people with HIV who are co-infected with HCV in the existing National HIV program. However, the epidemiology of hepatitis C remains uncertain, and prevalence data are limited with a wide variation in reported estimations among this population ranging from 7.6% [6] to 78.5% [7] and the data restricted in selective areas. The most at-risk population seems to be patients who have household member with liver disease, aged over 40 years old, possibly because of historical exposures to unsafe medical injections or transfusions [6–8].

In Cambodia, the HCV prevalence was high in people who inject drugs (30.6%) [9] and low prevalence were found among young adult aged less than 45 years old (0.6%) [10]. However, no data on the HCV prevalence among pregnant women are available in Cambodia. Due to the lack of appropriate laboratories and high cost of HCV testing and treatment, screening for HCV has not

been routinely provided in health care settings including ANC clinics to prevent vertical transmission.

Despite there were a few studies on HCV prevalence among PLWHA in the past, the data have been limited to some selected areas (the Northwestern Cambodia and to a hospital-based cohort in the Capital city). Assessing viral hepatitis burden in other regions across the Cambodia to have broader understanding of the extent of viral hepatitis at the national level is essential to inform policy development and resource mobilization to address this infection.

In order to fill a knowledge gap in Cambodia, we undertook a study to estimate HCV burden and transmission risk factors in four provinces and two specific populations, PLWHA and pregnant women attending antenatal care (ANC) in Cambodia. This study attempted to assess the HCV prevalence rates in different regions across the country to have representative HCV prevalence rates for the overall population in Cambodia.

Methods

Study design

This study was an integrated bio-behavioral (cross-sectional) survey undertaken to assess the prevalence of HCV among PLWHA receiving HIV care at the ART clinics and pregnant women attending public ANC clinics in Cambodia.

Study settings

This study was carried out between March and April 2016 in antiretroviral therapy (ART) sites for PLWHA and in ANC clinics for pregnant women, located in four diverse geographical areas, including the capital city of Phnom Penh and three provinces in the West (Battambang), the Northwest (Siem Reap) and the Southwest regions (Preah Sihanouk) of Cambodia.

Eligibility criteria

Individuals were eligible for the survey if they were at least 18 years old and either living with HIV/AIDS and had visited the ART clinics or pregnant women who visited the public ANC clinics during the study period.

Sample size, sampling and recruitment

We used Open Epi software version 3 to calculate the sample size. Assuming the prevalence of HCV is 5% among pregnant women and 10% among PLWHA, to achieve a 2% margin of error for the pregnant women and 3% for PLWHA, power fixed at 80%, at 5% alpha-level, a design effect of 1, and a non-response rate of 10%, 502 participants were required for pregnant women and 423 for PLWHA.

A two-stage cluster sampling method was employed to select the eligible participants. Firstly, the probability-proportional-to-size-sampling method was used to select 51 clusters (34/153 clusters for pregnant women and 17/21 clusters for PLWHA) from the selected geographical regions. A cluster was a health center for pregnant women and an ART clinic for PLWHA. Secondly, a consecutive sampling strategy was used to invite 15 pregnant women and 25 PLWHA from each cluster. Each participant signed the consent form if they agreed to participate in the study. The participants underwent face-to-face interviews using a structured questionnaire, following by a blood sample collection for HCV testing. Prior to data collection, all interviewers attended a training course to ensure they understood and followed standard procedures. The participants were only identified by a unique anonymous code (no identifiable information were collected). The code was used to link each participant to their sociodemographic information, their HCV results and their appointment sleep when they were back to the clinics to get their HCV results. The questionnaire was checked for completeness, accuracy and legibility, and validated questionnaires were sent to the University of Health Sciences (UHS) for data entry.

Behavioral questionnaire

The questionnaire was used to collect information on socio-demographic characteristics of participants such as age, sex, marital status, occupation and education. Multiple-choice questions were used to assess participants' knowledge, attitudes and practices related to HCV infection. The knowledge questions explored four broad dimensions: basic information about HCV infection, mode of transmission, vaccination and treatment availability. Information on potential risk factors of HCV infection included history of intramuscular and intravenous injections, blood transfusion, and cases of HCV in the household. For each question, participants were offered three response options: "No", "Yes" and "Don't know".

Laboratory testing

A 5 ml blood sample was drawn from participants for HCV testing. Samples were kept at room temperature (18–25 °C) for a maximum of 6 h and then sent to the UHS laboratory in Phnom Penh. After centrifugation, plasma specimens were stored at – 80 °C for analysis. HCV antibody testing was done with a third-generation (G3) HCV ELISA assay (MONOLISA ANTI HCV PLUS version 3, Bio-Rad), according to the manufacturer's instructions. Specimens showing a signal-to-cutoff (SCO) ≥ 1 were considered as positive for the anti-HCV antibody. Those with a SCO value < 1 were considered

negative. All samples with a SCO ≥ 1 were further tested to confirm chronic HCV infection with a commercial HCV RNA viral load (VL) assay (OMUNIS, Clapiers, France). All runs were done on Bio-Rad CFX96 real-time PCR machines (Bio-Rad). HCV genotyping was performed in NS5B gene, using an in-house semi-nested RT-PCR and ANRS protocol.

Statistical analyses

The prevalence of HCV infection among pregnant women and among PLWHA were presented as percentages and 95% confidence intervals (CIs), computed using exact methods (for binomial distribution). Categorical variables were presented as frequencies and percentages, while continuous variables were described with means and standard deviation (SD). Comparisons of categorical variables between HCV+ and HCV– participants across different socio-demographic and behavioral risk factors were performed using chi-squared or Fisher's exact tests accordingly. Multivariable logistic regression analysis was performed to identify risk factors associated with HCV antibody positivity. All non-collinear variables with a $P \leq 0.2$ in bivariate analyses or considered to be potential confounders based on previous studies were included in the models. Data were managed and analyzed using STATA 16 (StataCorp, USA), and p -values less than 0.05 were considered statistically significant.

Results

Characteristics of study participants

The characteristics of the 935 study participants (510 pregnant women and 425 PLWHA) are summarized in Table 1. The mean age of the participants was 42.9 years (SD, 9.3) for PLWHA and 27.6 years (SD, 5.3) for pregnant women. Approximately half of participants in both groups reported not having formal schooling or having Less than or primary school (46.6% in PLWHA and 45.3% in pregnant women), about 98.0% of pregnant women and only 64.0% of PLWHA were married. More than half of participants in both groups were farmers/self-employed and around 40% reported ever migrated to another country or to other places.

Knowledge/attitudes on hepatitis C prevention and care

Participants indicated high levels of willingness to get tested for HCV and to go for further investigations or treatment if HCV test positive. Correct answers to questions about whether blood should be screened for hepatitis C before transfusion, and barbers should use new blades or safe equipment for ear and nose piecing were reported by more than 90% of participants in both groups (Table 2).

Table 1 Characteristics of study participants by population group (n = 935), Cambodia, 2016

Characteristics	PLWHA (N = 425)		Pregnant women (N = 510)	
	n	%	n	%
Mean age in years (\pm SD)	42.95 (\pm 9.37)		27.68 (\pm 5.33)	
Age group, years				
\leq 30	28	6.59	363	71.18
31–40	137	32.24	142	27.84
> 40	260	61.18	5	0.98
Sex				
Male	189	44.47	–	–
Female	236	55.53	510	100
Education				
No formal schooling/less than or primary school	198	46.59	231	45.29
Secondary school	100	23.53	148	29.02
High school/University or higher	127	29.88	131	25.69
Marital status				
Never married	37	8.71	10	1.96
Currently married	272	64.00	499	97.84
Separated/divorced/widow	116	27.29	1	0.20
Main occupation				
Employee (Government/Non-government)	111	26.12	110	21.57
Retired/unemployed/home duties	76	17.88	129	25.29
Farmer/self-employed/others	238	56.00	271	53.14
Residential mobility				
Always live here	240	56.47	328	64.31
Ever migrated (to another country/to other places)	184	43.29	181	35.49
Geographic area				
Battambang (West)	75	17.65	165	32.35
Siem Reap (Northwest)	75	17.65	135	26.47
Preah Sihanouk (Southwest)	25	5.88	30	5.88
Phnom Penh (Central)	250	58.82	180	35.29

Table 2 Knowledge/attitudes on hepatitis prevention and care

Variables	PLWHA (N = 425)		Pregnant women (N = 510)	
	n	%	n	%
Willing to get tested for hepatitis C	420	98.82	509	99.80
Willing to go for further investigations or treatment if HCV test positive	423	99.53	509	99.80
Asking for or use a new/sterilized syringe or needle	344	80.94	396	77.65
Getting the blood screened for hepatitis C before transfusion	389	91.53	394	77.25
Asking barber to use new blades or for safe equipment for ear and nose piercing	394	92.71	399	78.24

Table 3 Healthcare and behavioral factors potentially related to hepatitis C infection

Variables	PLWHA (N = 425)		Pregnant Women (N = 510)	
	n	%	n	%
Healthcare and behavioral factors ^a				
Ever been admitted to a hospital or clinic	115	27.06	167	32.75
Ever received intra-muscular medication injection	196	46.12	419	82.16
Ever received intravenous medication injection	330	77.65	368	72.16
Ever received blood transfusion	12	2.82	4	0.78
Ever undergone any plastic surgery procedure	12	2.82	7	1.37
Ear/nose piercing or tattoo	10	2.35	21	4.12
Ever visited dental clinic for dental care	203	47.76	207	40.59
Ever used any illicit drug injection	2	0.47	0	0.00
Having a family member positive for HCV	31	7.29	20	3.92
HCV testing				
Ever tested for viral hepatitis infection	181	42.59	169	33.14
Ever tested for hepatitis C	85	20.00	27	5.29

^a Healthcare and behavioral factors reported over the past 5 years

Healthcare and behavioral factors potentially related to hepatitis C infection

Within the past 5 years, high proportions of participants had received intravenous medication (77.6% in PLWHA, 72.1% in pregnant women) or intramuscular injection (46.1% in PLWHA, 82.1% in pregnant women), or visited the dentist (47.7% in PLWHA, 40.5% in pregnant women (Table 3).

Prevalence of HCV infection

HCV antibody prevalence was as high as (29/425) 6.8% [95% CI: 4.6–9.6] in PLWHA and was low (5/510) 0.9% [95% CI: 0.3–2.7] in pregnant women. Of the 34 anti-HCV-positive individuals, 24 (70.6%) had detectable HCV RNA, with substantially higher proportions of chronic infection in PLWHA compared to pregnant women (23/29) 79.3% [95% CI: 60.2–92.0] vs. (1/5) 20.0% [95% CI: 0.5–71.6], *P* = 0.007. A phylogenetic analysis of 24 samples with a detectable HCV RNA showed that (11/24) 45.8% were genotype 1b; (1/24) 4.1% were genotype 2a and (8/24) 33.3% were genotype 6 (6a:4.1%, 6e:12.5%, 6q:8.3%, 6r:4.1%) and 16.6% failed to amplify. In PLWHA, anti-HCV prevalence generally increased with age, from (1/28) 3.5% [95% CI: 0.0–18.3] in the youngest group (18–30 years) to (21/260) 8.0% [95% CI: 5.0–12.0] in the oldest group (> 40 years old), but the difference was not statistically significant (*P* = 0.51). In pregnant women, the anti-HCV prevalence was (4/363) 1.1% [95% CI: 0.3–2.7] in the 18–30 years age group and (1/142) 0.7% [95% CI: 0.0–3.8] in the 31–40 years age group (Additional file 1: Table S1).

We observed that in PLWHA group, Preah Sihanouk province (Southwest) had the highest anti-HCV prevalence among the four studied geographic areas: Preah Sihanouk province (3/21) 12.0% [95% CI: 2.5–31.2], Battambang (West) (2/75) 2.6% [95% CI: 0.3–0.9.3], Siem Reap (Northwest) (1/75) 1.3% [95% CI: 0.0–7.2], Phnom Penh (Central) (23/250) 9.2% [95% CI: 5.9–13.4] (Fig. 1). There was no significant difference in anti-HCV prevalence across the four regions in pregnant women. **Fig. 1** Geographical distribution of HCV prevalence among PLWHA (n = 425) AND pregnant women (n = 510) in Cambodia, 2016

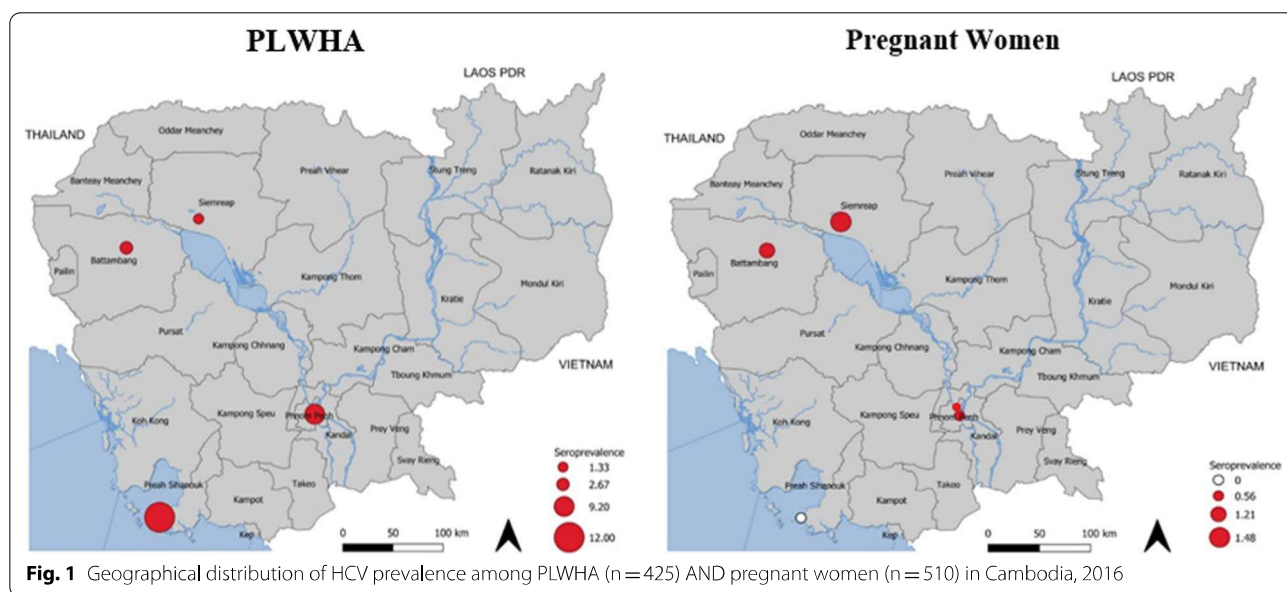


Table 4 Prevalence of Hepatitis C virus antibody per demographic characteristics of participants (n = 935), Cambodia, 2016

Characteristics	PLWHA (N = 425)		P	Pregnant women (N = 510)		P ^a
	Anti HCV positive			Anti HCV positive		
	n/N	%		n/N	%	
Education						
No formal schooling/less than or primary school	15/198	7.58	0.85	4/231	1.73	0.39
Secondary school	6/100	6.00		1/148	0.68	
High school/University or higher	8/127	6.30		0	0.00	
Marital status						
Never married	2/37	5.41	0.84	0	0.00	1.00
Currently married	20/272	7.35		5/499	1.00	
Separated/divorced/Widow	7/116	6.03		0	0.00	
Main occupation						
Employee (Government/Non-government)	8/111	7.21	0.59	0/110	0.00	0.71
Retired/unemployed/home duties	7/76	9.21		1/129	0.78	
Farmer/self-employed/others	14/238	5.88		4/271	1.48	
Ever received intra-muscular injection in the past 5 years						
No	12/229	5.24	0.16	0	0.00	0.59
Yes	17/196	8.67		5/419	1.19	
Ever received blood transfusion in the past 5 years						
No	28/413	6.78	0.83	5/506	0.99	1.00
Yes	1/12	8.33		0	0.00	
Ever visited dental clinic in the past 5 years						
No	12/222	5.41	0.23	4/303	1.32	0.65
Yes	17/203	8.37		1/207	0.48	
Ever received intravenous medication injection in the past 5 years						
No	1/95	1.05	0.01	0	0.00	0.33
Yes	28/330	8.48		5/368	1.36	
Having a family member positive for HCV						
No	20/394	5.08	<0.001	5/490	1.02	1.00
Yes	9/31	29.03		0	0.00	
Geographic area						
Battambang (West)	2/75	2.67	0.031	2/165	1.21	0.810
Siem Reap (Northwest)	1/75	1.33		2/135	1.48	
Phreah Sihanouk (Southwest)	3/25	12.00		0	0.00	
Phnom Penh (Central)	23/250	9.20		1/180	0.56	

^a Fisher's exact test

In PLWHA population, anti-HCV prevalence was significantly higher among those who had a history of intravenous medication injection in the past 5 years, at (28/330) 8.4% [95% CI: 5.7–12.0] compared to (1/95) 1.0% [95% CI: 0.0–5.7]) in those who had not. The same was observed in pregnant women, where all five HCV-infected women reported intravenous medication injection in the past 5 years (5/368) 1.3% [95% CI: 0.4–3.1] in those with this history vs. (0/142) 0.0% [95% CI: 0.0–0.2.5] in those without. In PLWHA, the anti-HCV prevalence was significantly higher in those who had a family member positive for HCV than in those who did

not (9/31) 29.0% [95% CI: 14.2–48.0] vs. (20/394) 5.0% [95% CI: 3.1–7.7] (Table 4).

Risk factors for HCV

Table 5 shows the results of crude and adjusted logistic regression on factors associated with HCV serological status among PLWHA. In bivariate analysis, participants who reported ever received intravenous medication injection in the last 5 years (OR = 8.7, 95% CI: 1.1–64.9, $P = 0.04$) and having a family member positive for HCV (OR = 7.6, 95% CI: 3.1–18.7, $P < 0.001$) were associated with an increased risk of HCV infection. In

Table 5 Univariate and multivariate analysis of HCV risk factors for people living with HIV, Cambodia, 2016

Characteristics	Anti HCV positive		Unadjusted		Adjusted ^a	
	n = 29	%	OR (CI 95%)	P	OR (CI 95%)	P
Age group, years						
18–30	1	3.57	Ref.		Ref.	
31–40	7	5.11	1.45 [0.17–12.30]		1.42 [0.15–12.77]	
> 40	21	8.00	2.37 [0.30–18.33]	0.20	2.52 [0.30–20.88]	0.28
Sex						
Male	13	6.88	Ref.		Ref.	
Female	16	6.78	0.98 [0.46–2.10]	0.97	0.89 [0.38–2.03]	0.78
Ever received intravenous medication in the past 5 years						
No	1	1.05	Ref.		Ref.	
Yes	28	8.48	8.71 [1.17–64.91]	0.04	7.67 [1.01–57.84]	0.048
Having a family member positive for HCV						
No	20	5.08	Ref.		Ref.	
Yes	9	29.03	7.65 [3.12–18.75]	< 0.001	7.10 [2.79–18.10]	< 0.001

^a Variables included in the multivariate model were: age, gender, history of intravenous medication injection, having a family member positive for HCV

a multivariable model (adjusted for age and sex), HCV infection remained significantly associated with having a family member positive for HCV (OR = 7.6, 95% CI: 1.0–57.8, $P=0.048$) and a history of intravenous medication injection in the last 5 years OR = 7.1 (95% CI: 2.7–18.1, $P<0.001$). A multivariable analysis could not be performed among pregnant women due to the small number of HCV-infected women.

Discussion

To our knowledge, this study represents the first HCV seroprevalence survey conducted in four different provinces of Cambodia evaluating the burden of HCV infection and its risk factors in these two different groups of population (PLWHA and pregnant women).

For PLWHA, HCV seroprevalence was similar to that found in a hospital-based HIV cohort in Phnom Penh at the same calendar period (7.6%) [6]. The anti-HCV prevalence was slightly higher than that reported in Cambodia in 2014 [11] and in neighboring countries in a regional HIV cohort [12], excluding countries with a high proportion of people who reported that they injected drugs. Chronic HCV infection prevalence among HCV antibody positive was 79% among PLWHA our study, in line with the rates generally found in newly diagnosed HCV patients [13]. These results might be useful to guide the local strategy development in prioritizing the routine screening plan focusing on most at risk population for HCV case detection and identifying those eligible for treatment.

Our study showed that HCV seroprevalence among pregnant women was 0.9% [95% CI: 0.3–2.2]. This study could not provide the evidence of different distribution across the regions due to small number of positive cases. In a recent seroprevalence study conducted in Battambang province among the general population, anti-HCV prevalence was 0.6% [95% CI: 0.3–0.9] in adults 18–44 years old [14]. These results confirm the low anti-HCV prevalence (below 1%) in people under 40. The currently available literature shows that HCV seroprevalence in the general population reported in previous studies conducted in Cambodia varied over time with a decreasing trend in the most recent years, from 6.5% in 1993 [14] to 5.8% in 2012 [15] and 0.6% in 2018 [10]. The decrease in prevalence of HCV over time may be explained by a variety of reasons. Since 2000, there has been major improvements in clinical practice in the country, with emphasis on sterilization of medical equipment, use of disposable needles and systematic screening of donated blood, all of which contribute to the reduction of blood borne agents such as HCV [8]. It should also be noted that the availability of HCV testing and treatment, both in private sectors and public hospitals with

the implementation of HCV program of Médecins Sans Frontières, could also have contributed to the decreasing prevalence of HCV by reducing the pool of infectious persons [10, 16]. In addition to that, the low anti-HCV prevalence among young age-group could be explained by the age cohort effect for which people in the old-age group were exposed to HCV longer than those in the young-age group. The decreased prevalence in younger population was also observed in Thailand [17, 18].

In multivariate analyses, ever received intravenous medication injection in the past 5 year and having a family member positive for HCV were strong, independent risk factors for HCV infection among Cambodian PLWHA, findings that are consistent with previous studies [6, 8, 10]. A history of intravenous therapeutic injection was a major risk factor since 33 out of the 34 anti HCV positive participants reported this practice in the past 5 years, as compared to 665/901 among the negatives. Therapeutic injections are a frequent practice in Cambodia as evidenced by our results showing that three-quarters of the participants, with both PLWHA and pregnant women reporting having received intravenous medication injection over the past 5 years. The overall treatment injection usage rate in Cambodia was 5.9 per person-year (women had a higher rate than men (7.5 vs. 4.3 per person-year), reported as one of the highest overall treatment injection usage rates worldwide [19]. Moreover, having an HCV-positive family member was an independent risk factor for HCV infection. HCV familial clustering and intra-familial viral transmission have been demonstrated previously in a study by Indolfi et al. [20] and in a Chinese study [21]. In Cambodia, sharing hygienic products, such as shaving razors, toothbrushes and nail clippers or ear/nose piercing or tattoo are common due to ignorance about sanitation and lack of resources. Our results argue in favor of the need of HCV screening for the whole family if one of member is positive and more particularly for children who are currently mostly unaware of access to HCV care programs. The risk factors of HCV infection could not be analyzed in pregnant women due to sample size issue. Of note, all five HCV-infected pregnant women also reported a history of intravenous medication injection in the previous 5 years.

HCV genotype 1b, 2a and 6 variants were identified. HCV genotype 1b (47.8%) and 6 (30.4%) were predominant in the current study. This finding is consistent with several previous studies in Cambodia, which reported HCV genotype 1b and 6 as the common genotypes observed in Cambodia [6, 15, 16, 22, 23]. The genotype 6 variant was also found as the most common in Vietnam and Laos [22], in blood donors in some regions of Thailand [16] and in intravenous drugs users in China [17, 24, 25].

Limitations of the study

This study has several limitations. Our study attempted to assess the anti-HCV prevalence rates in different regions across the country to have representative anti-HCV prevalence rates for the overall population in Cambodia. However, our small sample size in Preah Sihanouk (South-western region) may limit the generalizability of the results for this region. Our findings may have been subject to recall bias or social desirability bias resulting in the underreporting of some risky behaviors and practices over the last 5 years such as the experience of injecting illicit drugs. Last, due to the small number of HCV positive in pregnant women, the risk factors of HCV infection in this group could not be assessed. However, in this study, we can rule out differential misclassification for assessing the risk factors of HCV infection linked to the history of risky behaviors and practices, since interviews were done before the result of HCV serology was known.

Conclusions

In conclusion, we report a high HCV seroprevalence in PLWHA in Cambodia, likely related to past intravenous medication injection and/or intra-familial viral transmission. Our results led the national Cambodia program to offer systematic HCV testing in PLWHA and underline the necessity of care of HCV positive populations, measures which had already been initiated by National Program for adults. Children of HCV-infected parents living in the same household are likely the next target for testing and envisaging care access.

The low prevalence of HCV infection in the population under 40 years old seems to be confirmed but the anti-HCV prevalence varied across geographical area and specific populations, suggesting the need to set up a surveillance system to assess the anti-HCV prevalence across different population groups.

Abbreviations

PLWHA: People living with HIV/AIDS; HIV: Human immuno-deficiency virus; HCV: Hepatitis C virus; ANC: Antenatal care; Anti-HCV: HCV antibody; HCC: Hepatocellular carcinoma; LMICs: Low-and middle-income countries; WHO: World Health Organization; DAA: Direct-acting agents; HICs: High-income countries; ART: Antiretroviral therapy; UHS: University of Health Sciences; SCO: A signal-to-cut-off; VL: Viral load.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12879-022-07163-2>.

Additional file 1: Table S1: seroprevalence and viremic prevalence per sex and per age category (n = 935), Cambodia, 2016.

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Authors' contributions

VS, PP, JK, SY, ST conceptualized, designed the study, developed the research protocol and substantively reviewed the manuscript. PP, VS, LM, OS analyzed, interpreted the data and were a major contributor in writing the manuscript. PP, ST, PC have made substantial contributions to the project implementation and the acquisition of data. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the National Ethics Committee for Health Research in Cambodia on 22/09/2015 (338 NECHR) and in compliance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before the interview and the blood sample collection.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Study Site: Calmette Hospital

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2	Vann Pisey	Investigator
3	Pao Samaun	Nurse
4	Oem Him	Nurse
5	Kim Bopiseth	Counselor

Study Site: Takeo Provincial Referral Hospital

1	Kuoch Sokhan	Investigator
2	Neak Vibol	Investigator
3	Am Sreynich	Nurse
4	Meas Huch	Nurse
5	Tieng Sophary	Counselor

Study Site: Kampong Cham Provincial Referral Hospital

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ANRS 12374 3DICAM Study Teams

N° Name Role

Study Site: Prey Veng Provincial Referral Hospital

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| 2 | Sam Kea | Nurse |
| 3 | Has Channa | Counselor |
| 4 | Nav Chandyna | Pharmacist |

Study Site: Preah Sihanouk Provincial Referral Hospital

- | | | |
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| 1 | Som Dara | Investigator |
| 2 | Nhok Meth | Investigator |
| 3 | Ouk Pheary | Nurse |
| 4 | Say Sokha | Counselor |
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| 6 | Tath Thida | Pharmacist |

Study Site: Sampov Meas Provincial Referral Hospital(Pursat)

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1 **Effectiveness and pharmacokinetics of a third-line cART regimen combining dolutegravir,**
2 **ritonavir-boosted darunavir and optimized NRTI in HIV-infected treatment-experienced**
3 **adults in Cambodia: the ANRS 12374 3DICAM study**

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43 (PK), Cambodia.

44 **Word count:**

45 Abstract: 305/350

46 Main text: 3496/3500

47 **Abstract**

48 **Background**

49 WHO recommends that third-line regimens include drugs with minimal risk of cross-
50 resistance to previously used regimens. The objective of the ANRS 1237 3DICAM study was to
51 evaluate the efficacy, safety and pharmacokinetics of a genotypic-driven third-line regimen
52 combining dolutegravir (DTG), ritonavir-boosted darunavir (DRV/r) and optimized NRTI in
53 Cambodian HIV-infected treatment-experienced adults who failed a protease inhibitor-based
54 second-line regimen.

55 **Methods**

56 This single-arm multicentre 48-week trial included 49 patients with plasma HIV-RNA
57 levels >1000 copies/mL and resistance to atazanavir and/or to all NRTIs between October 2018
58 and April 2021 in 13 ART clinics. The primary outcome was the proportion of patients with
59 plasma HIV-RNA levels <40 copies/mL at W24. The pharmacokinetics of DRV (600/100 mg)
60 twice daily and DTG (50 mg) once daily at steady-state were evaluated for the first 20 enrolled
61 participants.

62 **Results**

63 Of the 49 participants, 61% were male; the median age was 40 years (IQR: 24-46). The
64 median baseline plasma HIV-RNA levels and CD4 counts were 4.4 Log₁₀ copies/mL (3.6-5.1)
65 and 195 cells/μL (36-353), respectively. The median time spent on first- and second-line
66 treatment was 4 years (3-7) and 5 years (3-7), respectively. At baseline, 75.5% of patients were
67 resistant to atazanavir, and 43% had resistance to AZT/TDF/ABC. At W24, 59% of participants
68 (95%CI: 44-73) had plasma HIV-RNA levels <40 copies/mL and 81.6% (95% CI: 68-91) had
69 levels <1000 copies/mL. Response rates were of similar magnitude at W48, 55.1% (40.2-69.3)
70 and 79.5% (65.6-89.7), respectively. No patient stopped the trial treatment. The median trough
71 concentrations of DRV and DTG were close to those previously reported in other populations.
72 Risk factors for viral failure at W48 included baseline CD4 counts<200/μL and infection through
73 mother-to-child transmission.

74 **Conclusions**

75 In these heavily pretreated multidrug-resistant HIV-infected patients, the combination of
76 DTG and DRV/r was effective and well tolerated. Youths infected through mother-to-child
77 transmission need innovative support services, including counselling, to improve their adherence.

78 **Clinical Trial Number:** NCT03602690

79 **Main text**

80 **Introduction**

81 In 2021, the World Health Organization (WHO) recommended the development of
82 policies by national programs for third-line ART regimens that should include new drugs with
83 minimal risk of cross-resistance to previously used regimens, such as integrase inhibitors or
84 second-generation nonnucleotide reverse transcriptase inhibitors (NNRTIs) and protease
85 inhibitors (PIs) [1]. Randomized controlled trials and observational cohorts support the use of
86 ritonavir-boosted darunavir, etravirine, dolutegravir and raltegravir-containing regimens in
87 treatment-experienced adults in high-income countries [2-4]. However, few studies have been
88 conducted in low- and middle-income countries, particularly with dolutegravir as the integrase
89 inhibitor.

90 In 2013, a nationwide cross-sectional study in Cambodia reported a 90% rate of
91 virological suppression with a PI-based second-line regimen (either LPV/r or ATV + 2NRTIs)
92 and underlined the importance of adherence boosting before deciding any change in the ART
93 regimen [6]. Following this result, the National Center for HIV/AIDS, Dermatology and STD
94 (NCHADS) implemented enhanced adherence counselling (EAC) in 2016 by forming a technical
95 working group to help clinicians to manage patients failing a PI-based second-line regimen with
96 a plasma HIV RNA above 1000 copies/mL and to improve the quality of adherence support.
97 Guidance for implementing adherence counselling was finalized in 2017, and HIV genotyping
98 resistance tests appeared necessary to guide decisions for third-line regimens. In this context, the
99 ANRS12374 study was implemented to evaluate the efficacy and safety of a third-line ART
100 regimen combining dolutegravir (DTG), ritonavir-boosted darunavir (DRV/r) and optimized
101 NRTI in Cambodian HIV-infected treatment-experienced volunteers with genotypic resistance to
102 ATV and/or to all NRTIs.

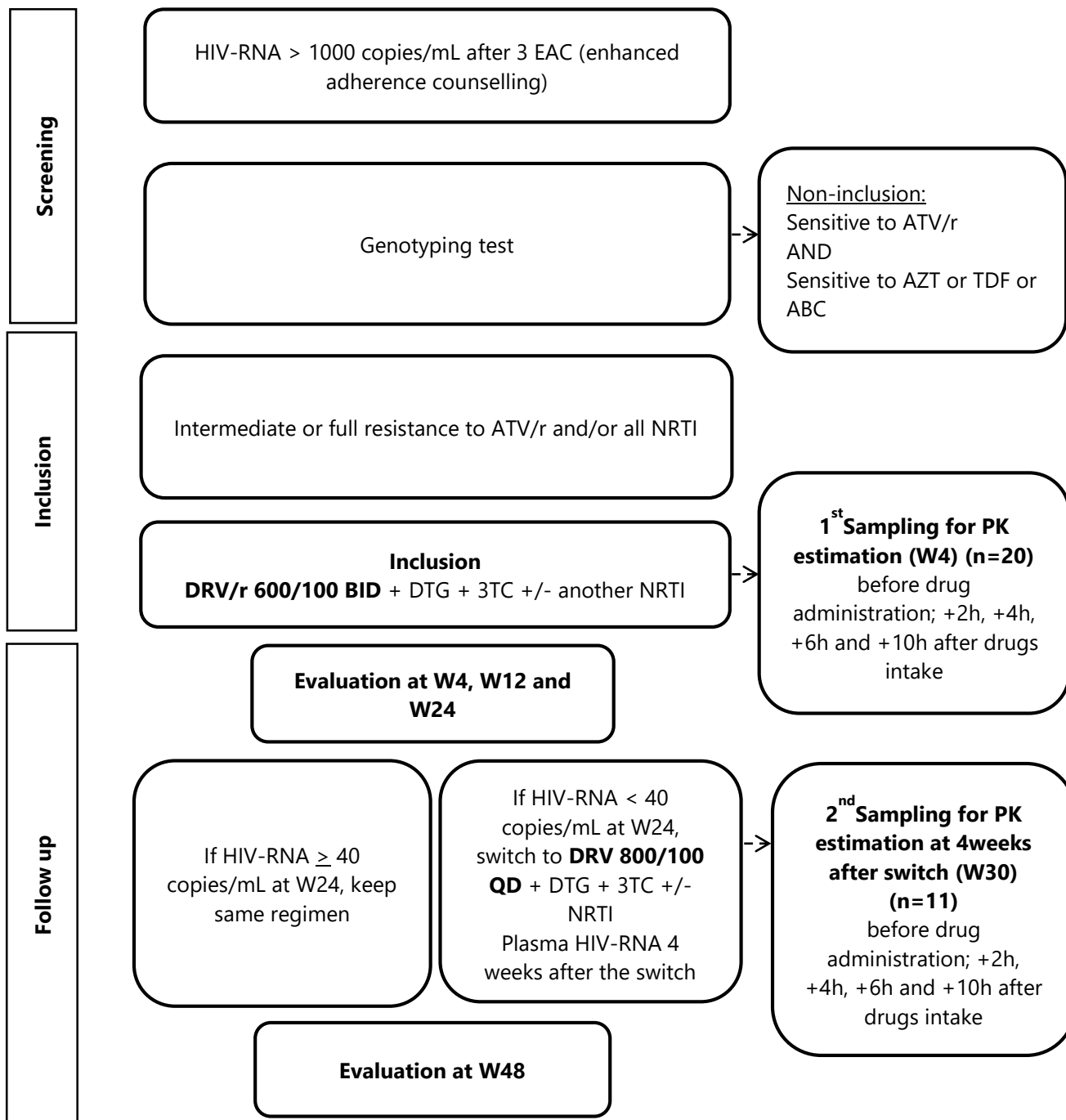
103 **Methods**

104 **Study design and participants**

105 The ANRS12374 study is a one-arm, multicentre, phase II study that evaluates the efficacy
106 and safety of a third-line ART regimen combining dolutegravir (DTG), ritonavir-boosted
107 darunavir (DRV/r) and optimized NRTI in Cambodian adult HIV-infected treatment-experienced
108 volunteers. The first 24 weeks constituted in an evaluation of the efficacy and safety of this
109 combination using DRV 600 mg twice daily boosted ritonavir 100 mg. Then, the assessment of

110 the efficacy and safety at 48 weeks of a dose adjustment phase of switching to 800 mg DRV once
 111 daily was planned for participants with HIV RNA <40 copies/mL at week 24 (Figure 1).

Figure 1: Study design



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115 The primary objective was to assess virological success at week 24 of this strategy.
116 Secondary objectives included the assessment of virological success at week 48 and the factors
117 related to virological success, safety, adherence, and pharmacokinetic parameters.

118 Study participants were screened from 13 ART clinics across Cambodia between October
119 2018 and April 2021. Participants aged 15 years or over who failed a protease inhibitor (PI)-based
120 second-line regimen (defined by plasma HIV RNA > 1000 copies/mL) despite 3 months of
121 boosted adherence counselling were screened and a genotyping resistance test (GRT) was
122 performed. Participants with evidence of intermediate or full resistance to atazanavir (ATV) or
123 sensitivity to ATV but resistance to all NRTIs were eligible the study.

124 The study was approved by the Cambodian National Ethics Committee for Health
125 Research. Written informed consent was obtained from each study participant before the initiation
126 of the study procedures. A data and safety monitoring board and a scientific advisory board
127 annually reviewed the study progress and safety data.

128 **Procedures**

129 The third-line regimen was initiated, including DRV/r 600/100 twice daily + DTG 50 mg
130 once daily + 3TC 300 mg once daily +/- one full or intermediate sensitive NRTI among TDF,
131 ABC, and AZT, according to the HBsAg status, the result of the GRT and the medical history of
132 the patient. Briefly, a second NRTI (either TDF or ABC or AZT) was added to 3TC if the patient
133 had no full resistance to this NRTI and no contraindication. Participants were followed every two
134 weeks for the first month, then every four weeks until week 24, at week 36 and week 48. Blood
135 tests were performed through the first 12 weeks and then at week 24, week 36 and week 48. An
136 HIV-RNA assessment was done at screening, at weeks 4, 12, 24, 36 and 48 for all participants;
137 for those who switched to darunavir 800 mg/ritonavir 100 mg once daily, an additional virological
138 assessment was performed 4 weeks after the switch. Plasma HIV RNA levels were measured at
139 the NCHADS laboratory using an automated Abbott real-time HIV-1 assay. GRT and
140 biochemical analyses were performed at the laboratory of the Pasteur Institute in Cambodia.
141 Adherence was measured at each visit by a visual adherence scale adapted for Cambodian
142 patients. The value of > 90% at the visual scale was chosen to define “optimal adherence”.

143 Blood samples for pharmacokinetics of DRV and DTG for the first 20 enrolled
144 participants at steady-state (4 weeks after starting treatments) and 4 weeks after the switch for
145 those who switched after week 24 were drawn pre dose and up to 10 h post-dosing. We measured

146 the plasma levels by high-performance liquid chromatography (HPLC), using adaptations of
147 previously published methods [7, 8]. The lower limit of quantification of DRV and DTG was 500
148 ng/mL; the standard curves were linear up to 10000 ng/mL for both drugs. The observed
149 maximum concentration (C_{max}), time to C_{max} (T_{max}) and predosing concentration (C₀) were
150 reported. We collected data on adverse events at all study visits and graded them by severity on
151 a scale from 1 to 4 using the ANRS scale.

152 **Sample size calculation and statistical analysis**

153 By using the Fleming method with one stage, we estimated that with a sample size of 45
154 patients, we could conclude that the success rate is at least 60% if the total number of failures
155 does not exceed 12 (alpha at 0.05, power at 0.90, expected success rate of 80%). We summarized
156 baseline characteristics, CD4 cell count and plasma HIV RNA by their median and interquartile
157 range (IQR), or mean and standard deviation, or frequencies with % and 95% confidence intervals
158 (CI), as appropriate.

159 The primary outcome of this study was the percentage of participants with plasma HIV
160 RNA < 40 copies/mL by FDA Snapshot analysis at week 24, presented with its 95% confidence
161 interval. The participants who died, were lost to follow-up or had a missing plasma HIV RNA at
162 week 24 were considered to have treatment failure for the primary outcome. The comparison of
163 participants with plasma HIV RNA < 40 copies/mL and ≥ 40 copies/mL across different baseline
164 categorical characteristics was performed using chi-squared or Fisher's exact tests accordingly.

165 The percentage of participants with plasma HIV-1 RNA < 40 copies/mL by FDA Snapshot
166 analysis at 48 weeks, the median change in CD4 cell count, the incidence of grade 3-4 adverse
167 events, discontinuation of study treatment, adherence to treatment strategy, the percentage of
168 participants who switched from DRV/r 600/100 twice daily to DRV/r 800/100 once daily at week
169 24, and plasma drug concentrations (darunavir and dolutegravir) were assessed as secondary
170 outcomes.

171 Data management and data analysis were performed using STATA 15 (StataCorp, USA);
172 p-values of less than 0.05 were considered statistically significant. The 95% confidence intervals
173 for percentages were computed using a binomial distribution.

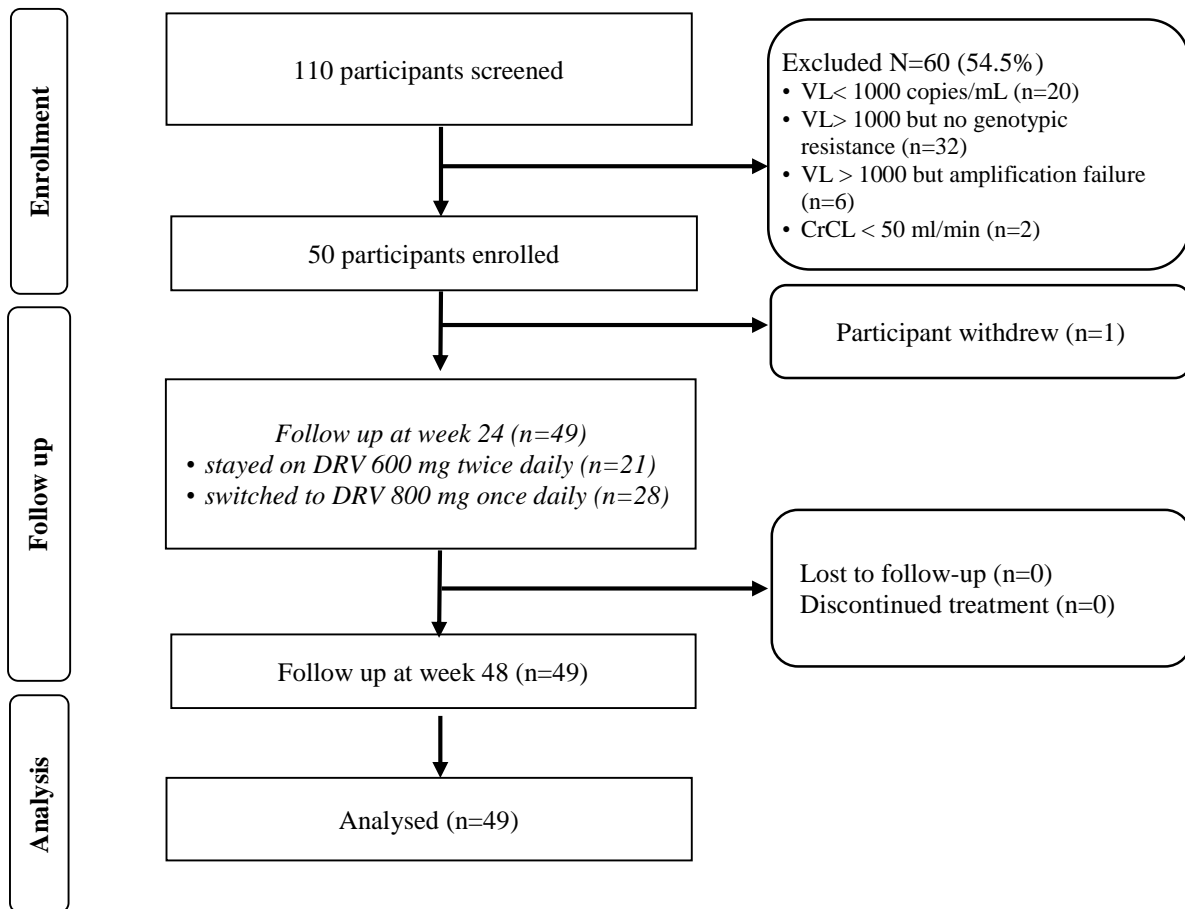
174

175 **Results**

176 **Baseline characteristics**

177 Between October 2018 and April 2021, 110 patients were pre-enrolled, and 50 participants were
178 enrolled in the study in 13 clinics. The reasons for nonenrolment are reported in Figure 2. One
179 participant withdrew from the study at inclusion before any antiretroviral uptake, leading to a
180 total of 49 participants included in the analysis (Figure 2).

Figure 2. participant flow



181
182 The baseline characteristics of the study participants are summarized in Table 1. Of the 49
183 participants, 61% were male. The median age was 40 years (IQR: 24-46). The median CD4 level
184 at baseline was 195 cells/ μ L (36-353). The median plasma HIV RNA level was 4.4 log₁₀
185 copies/mL (3.6-5.2). The median number of baseline resistance-associated mutations (RAMs) in
186 NRTIs, NNRTIs and PIs were 4 (2-5.5), 3 (2-4) and 8 (6-9), respectively. Thirty-seven
187 participants (75.5%) had full or intermediate resistance to ATV/r. Only one patient had full
188 resistance to DRV/r. The majority of the participants (81.6%) had full or intermediate resistance

189 to 3TC, 53.0% to AZT, 47.0% to TDF and 67.4% to ABC. In total, 43% of participants had full
 190 or intermediate resistance to AZT/TDF/ABC. All participants started third-line treatment with
 191 twice-daily DRV, once-daily DTG, in combination with only 3TC for 36.8%, 3TC plus TDF for
 192 57.1% or 3TC plus AZT for 6.1%.

Table 1. Characteristics of participants enrolled in the ANRS 3DICAM Study (n=49)

Characteristics	N=49	
General and demographic data		
Gender, Male, n (%)	30	(61.2)
Age (years), median (IQR)	40	(24-46)
Body Mass index, ^{kg/m²} , Median (IQR)	21	(19-24)
HBsAg Positive, n (%)	2	(4.0)
Anti-HCV Positive, n (%)	2	(4.0)
Baseline Adherence assessment, n (%)		
Good	36	(73.4)
Moderate	10	(20.4)
Poor	3	(6.1)
Route of infection, n (%)		
Mother to child transmission	13	(26.5)
Heterosexual	33	(67.4)
MSM	1	(2.0)
Other/unknown	2	(4.0)
Previous duration of cART (years), median (IQR)		
First-line ARV regimen [†]	4	(3-7)
Second-line ARV regimen [†]	5	(3-7)
Overall	12	(8-14)
History of opportunistic infection (clinical stage), n (%)		
B	4	(8.2)
C	20	(40.9)
No history	25	(51.0)
Prescribed third-line regimen, n (%)		
DRV/r/DTG/3TC/TDF	28	57.1
DRV/r/DTG/3TC/AZT	3	6.1
DRV/r/DTG/3TC	18	36.8
Laboratory data, median (IQR)		
CD4 cell-count (cells per μ L)	195	(36-353)
Plasma HIV RNA (Log_{10} copies/mL)	4.4	(3.6-5.1)

IQR interquartile range; [†] Number of patient on first- and second-line, n=48; DRV/r: Darunavir/ritonavir; DTG: Dolutegravir; AZT: Zidovudine; 3TC: Lamivudine; TDF: Tenofovir disoproxil fumarate

194 **Patient outcome**

195 No patient died, and none were lost to follow-up or stopped treatment. At week 24, 59.1% [44.2-
196 73.0] had an HIV-1 RNA <40 copies/mL, and 81.6% [67.9-91.2] had an HIV-1 RNA <1000
197 copies/mL (Table 2). At week 48, 55.1% [40.2-69.3] patients had HIV-1 RNA < 40 copies/mL
198 and 79.5% [65.6-89.7] had HIV-1 RNA < 1000 copies/mL (Table 2). Among the 20 patients who
199 had plasma HIV-1 RNA \geq 40 copies/mL at week 24, 11 had a plasma HIV RNA below 1000
200 copies/ml, and 10 out of these 11 patients remained below 1000 at week 48. Two out of the 9
201 patients who had HIV RNA above 1000 copies/mL at week 24 decreased below 1000 copies/mL
202 at week 48. At W48, the CD4 count had increased to >350 cells/ μ L in 38.8% [25.1-53.7] of
203 patients and above 500 cells/ μ L in 22.4% [11.7-36.6] (Table 2). The median CD4 change was
204 higher in those who reached a plasma HIV RNA <40 at week 48 than in the others (+140 vs.
205 +55/ μ L, $p < 10^{-4}$).

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Table 2: Outcomes at Week 24 and Week 48 in the ANRS 3DICAM study (n=49)

Characteristic	Week 24 (N=49)			Week 48 (N=49)		
	n/N	%	95% CI	n/N	%	95% CI
Plasma HIV RNA (copies/mL)						
< 40	29	59.1	[44.2-73.0]	27	55.1	[40.2-69.3]
< 200	37	75.5	[61.1-86.6]	36	73.4	[58.9-85.0]
< 1000	40	81.6	[67.9-91.2]	39	79.5	[65.6-89.7]
CD4 >350 cells per μ L	23	46.9	[32.5-61.7]	19	38.8	[25.1-53.7]
CD4 >500 cells per μ L	9	18.3	[08.7-32.0]	11	22.4	[11.7-36.6]
Adherence > 90% during study period	46	93.8	[83.1-98.7]	44	89.8	[77.7-96.6]
Switch from DRV/r 600/100 mg twice daily to DRV/r 800/100 mg once daily	28	57.1	[42.2-71.1]	-	-	-
HIV RNA <40 copies/mL in patient staying with DRV/r600/100 mg twice daily	-	-	-	7/21	33.3	[14.5-56.9]
HIV RNA <40 copies/mL in patient switched to DRV/r800/100 mg once daily	-	-	-	20/28	71.4	[51.3-86.7]
Patients with at least one grade 3-4 adverse events or SAE	9	18.4	[08.7-32.0]	13	26.5	[14.9-41.0]

220 Over the study period until week 48, 26.5% [14.9-41.0] of the patients presented at least
221 one grade 3-4 adverse event or a serious adverse event (SAE) (Table 3). One participant
222 experienced cryptococcal meningitis at W4 (CD4 85 cells/ μ L and plasma HIV RNA 3.0 log₁₀
223 copies/mL), and one participant had a zona grade 1 at W12 (baseline CD4: 199 cells/ μ L and
224 plasma HIV RNA 4.9 Log₁₀ copies/mL). Three participants stopped TDF due to a decrease in
225 creatinine clearance, and two participants were referred for diabetes treatment. No patient stopped
226 the trial treatment (DRV/r + DTG) due to an adverse event.

Table 3: Factors associated with virological success below 40 copies/mL at Week 48 (n=49)

Characteristic	Week 48							
	<40 copies/mL		Univariate			Multivariate ^s		
	n/N	%	OR [†]	95% CI	P	AOR	95% CI	P
Gender								
Female	13/20	65.0	Ref			Ref		
Male	14/29	48.2	0.50	[0.15-1.62]	0.25	0.42	[0.11-1.68]	0.22
Age (years)								
<25	5/14	35.7	Ref			Ref		
≥25	22/35	62.8	3.04	[0.83-11.07]	0.09	0.67	[0.06-7.82]	0.75
CD4 cell-count (cells per µL)								
<200	11/26	42.3	Ref			Ref		
≥200	16/23	69.5	3.11	[0.95-10.15]	0.06	3.64	[0.93-14.2]	0.06
HIV RNA Viral load (Log ₁₀ copies/mL)								
≥ 5 Log ₁₀ copies/mL	7/16	43.7	Ref					
< 5 Log ₁₀ copies/mL	20/33	60.6	1.97	[0.59-6.63]	0.27			
Infected through mother-to-child route								
no	24/36	66.7	Ref			Ref		
yes	3/13	23.1	0.15	[0.04-0.65]	0.01	0.07	0.005-1.09	0.06
First-line ARV regimen Duration (years)								
≥4 years	10/18	55.6	Ref					
<4years	17/31	54.8	1.02	[0.32-3.31]	0.961			
Second-line ARV regimen Duration (years)								
≥5 years	16/28	57.1	Ref					
<5 years	11/21	52.3	0.82	[0.26-2.57]	0.74			
Third-line regimen								
DRV/r / DTG / 3TC	12/18	66.6	Ref					
DRV/r / DTG / 3TC / TDF or AZT	15/31	48.3	0.46	[0.14-1.56]	0.219			
Adherence assessment atW24								
<90%	0/5	0	Ref					
≥90%	27/44	61.3	NC [‡]					
Full resistance to ABC/AZT/TDF								
Yes	10/16	51.52	Ref					
No	17/33	62.5	0.63	[0.18-2.16]	0.47			

[†]OR: Odds-Ratio; [‡]NC: Not Computable; [§]adjusted Odd Ratios (AOR) were obtained from multiple logistic regression models including all variables in the column

228 **Factors associated with virological failure at Week 24**

229 In univariate analysis, no factor was associated with virological failure, including gender, age,
230 mode of transmission, CD4 cell count and HIV RNA at baseline, duration of previous ART and
231 resistance mutations at baseline. The proportion of patients with HIV-1 RNA <40 copies/mL
232 tended to be greater in patients with baseline CD4 count ≥ 200 cells per μL than in those who had
233 CD4 counts below, 69.5% [47.0-86.7] vs 50.0% [29.9-70.0], but the difference was not
234 statistically significant ($P=0.16$). In a multivariate analysis comprising gender, age and CD4
235 count, both an age ≥ 25 years and baseline CD4 counts ≥ 200 cells/ μL tended to be associated
236 with a higher probability of virological success, although not significantly (Table 4). When
237 considering a threshold of 1000 copies/mL, the association with baseline CD4 count (≥ 200 vs.
238 < 200 cells per μL) was statistically significant (95.6% vs. 69.2%, $p=0.026$). There was a
239 predominance of subjects < 25 years of age in patients > 1000 copies/mL (5/9, 56% vs. 9/40 23%,
240 $p=0.047$).

Table 4: Factors associated with virological success below 40 copies/ml at Week 24 (n=49)

241

Characteristic	Week 24							
	<40 copies/mL		Univariate analysis			Multivariate analysis		
	n/N	%	[†] OR	95% CI	P	[‡] AOR	95% CI	P
Gender,								
Female	12/20	60.0	Ref			Ref		
Male	17/29	58.6	0.94	[0.29-3.01]	0.9	0.95	[0.27-3.26]	0.93
Age (years)								
<25	6/14	42.8	Ref			Ref		
≥25	23/35	65.7	2.55	[0.71-9.08]	0.15	2.81	[0.75-10.53]	0.13
CD4 cell-count (cells per μL)								
<200	13/26	50	Ref			Ref		
≥200	16/23	69.5	2.28	[0.70-7.39]	0.17	2.47	[0.72-8.41]	0.15
Plasma HIV RNA (Log ₁₀ copies/mL)								
≥ 5 Log ₁₀ copies/mL	8/16	50.0	Ref					
< 5 Log ₁₀ copies/mL	21/33	63.6	1.75	[0.52-5.86]	0.37			
Infected through mother-to-child route								
no	23/36	63.9	Ref					
yes	6/13	46.1	0.49	[0.13-1.75]	0.27			
First-line ARV regimen Duration (years)								
≥4 years	12/18	66.7	Ref					
<4years	17/31	54.8	1.64	[0.50-5.51]	0.42			
Second-line ARV regimen Duration (years)								
≥5 years	16/28	57.1	Ref					
<5 years	13/21	61.9	1.2	[0.38-3.87]	0.74			
Third-line regimen								
DRV/r/DTG/3TC	9/18	50.0	Ref					
DRV/r/DTG/3TC/TDF or AZT	20/31	64.5	1.81	[0.55-5.92]	0.32			
Adherence assessment at W24								
<90%	1/4	25.0	Ref					
≥90%	28/45	62.2	4.9	[0.47-51.4]	0.18			
Full resistance to ABC/AZT/TDF								
Yes	9/16	56.2	Ref					
No	20/33	60.6	1.2	[0.35-4.01]	0.77			

[†]OR: Odds-Ratio [‡] adjusted ORs were obtained from multiple logistic regression models including all variables in the column

245 **Factors associated with virological failure at Week 48**

246 Similarly, to week 24, we found at week 48 that patients with baseline CD4 count $\geq 200/\mu\text{L}$ had
247 a better virological response than those < 200 , the difference being of borderline significance
248 $p=0.056$ (Table 4). The youngest individuals (<25 years) again tended to have less often an HIV
249 RNA < 40 copies/mL compared to the older ones: 35.7% vs 62.8%, respectively, $p=0.09$. Subjects
250 infected through mother-to-child transmission were significantly less likely to be virological
251 responders at week 48 than the others (23.1% vs. 66.7%, $p=0.007$). In multivariate analysis, the
252 role of age below 25 years disappeared after adjustment for the route of HIV acquisition (Table
253 5). Baseline CD4 cell count $>200/\mu\text{L}$ remained borderline significantly associated with a better
254 virological response. The duration of previous treatment was not associated with virological
255 success at week 48.

Table 5: Factors associated with virological success below 40 copies/mL at Week 48 (n=49)

Characteristic	Week 48							
	<40 copies/mL		Univariate			Multivariate		
	n/N	%	†OR	95% CI	P	‡AOR	95% CI	P
Gender								
Female	13/20	65.0	Ref			Ref		
Male	14/29	48.2	0.50	[0.15-1.62]	0.25	0.42	[0.11-1.68]	0.22
Age (years)								
<25	5/14	35.7	Ref			Ref		
≥25	22/35	62.8	3.04	[0.83-11.07]	0.09	0.67	[0.06-7.82]	0.75
CD4 cell-count (cells per µL)								
<200	11/26	42.3	Ref			Ref		
≥200	16/23	69.5	3.11	[0.95-10.15]	0.06	3.64	[0.93-14.2]	0.06
HIV RNA Viral load (Log ₁₀ copies/mL)								
≥ 5 Log ₁₀ copies/mL	7/16	43.7	Ref					
< 5 Log ₁₀ copies/mL	20/33	60.6	1.97	[0.59-6.63]	0.27			
Infected through mother-to-child route								
no	24/36	66.7	Ref			Ref		
yes	3/13	23.1	0.15	[0.04-0.65]	0.01	0.07	[0.005-1.09]	0.06
First-line ARV regimen Duration (years)								
≥4 years	10/18	55.6	Ref					
<4years	17/31	54.8	1.02	[0.32-3.31]	0.96			
Second-line ARV regimen Duration (years)								
≥5 years	16/28	57.1	Ref					
<5 years	11/21	52.3	0.82	[0.26-2.57]	0.74			
Third-line regimen								
DRV/r/DTG/3TC	12/18	66.6	Ref					
DRV/r/DTG/3TC/TDF or AZT	15/31	48.3	0.46	[0.14-1.56]	0.22			
Adherence assessment at W48								
<90%	0/5	0.0	Ref					
≥90%	27/44	61.3	§NC		0.01*			
Full resistance to ABC/AZT/TDF								
Yes	10/16	51.5	Ref					
No	17/33	62.5	0.63	[0.18-2.16]	0.47			

†OR: Odds-Ratio; §NC: Not Computable; *Fisher's exact test; ‡adjusted ORs were obtained from multiple logistic regression models including all variables in the column

257 **Pharmacokinetic parameters**

258 The characteristics of the 20 patients included in the PK substudy were similar to those of the 49
259 patients. The median baseline HIV RNA and CD4 cell levels were 4.4 Log₁₀ cp/mL (3.9-5.2)
260 and 192 cells/ μ L (58-354), respectively. Participants received DRV/r600/100 mg BID plus
261 3TC/TDF (50%), 3TC (45%), or AZT/3TC (5%). Plasma drug concentrations of DRV and DTG
262 are presented in Table 6. Steady-state plasma exposures of DRV and DTG during a dosing interval
263 showed wide interpatient variability. The median pre-dose concentration(C₀) of darunavir at W4
264 was 2483 μ g/L (1344-4836). The median dolutegravir C₀ at W4 was 865 μ g/L (500-1051).
265 Eleven patients switched after W24 to DRV/r800/100 mg QD since they had reached a plasma
266 HIV RNA <40 copies/mL. There was no difference between the 2 dosing regimens (DRV/r
267 600/100 mg BID vs DRV/r 800/100 mg QD), notably in the median C₀ pre-dosing concentrations
268 2488 μ g/L (1372-4880) and 2660 μ g/L (574-3549), respectively in the 11 patients who switched.

Table 6: Pharmacokinetic parameters of darunavir/ritonavir 600mg twice daily (Period 1, n=20) and darunavir/ritonavir 800mg once daily (Period 2, n=11) and dolutegravir 50mg once daily (n=20)

Pharmacokinetic Parameter†	Period 1	Period 2	Median (Range) DTG 50mg QD (n=20)
	Median (Range) DRV/r 600/100 mg BID‡ (n=20)	Median (Range) DRV/r 800/100 mg QD§ (n=11)	
Cmax, µg/L	4434 (3694-6658)	4697(3366-7523)	2682 (1922-3444)
Tmax, h	2 (2-4)	2 (2-4)	2 (2-4)
C0, µg/L	2483(1344-4836)	2660(574-3549)	865(500-1051)

† Cmax: maximum concentration ; Tmax: time to achieve maximum concentration; C0: concentration at time 0 pre-dosing;

‡ BID: twice daily-dosing, § QD: once daily-dosing

269

270

271 Discussion

272 This study reports the results of a programmatic evaluation of a third-line regimen strategy
273 implemented within the framework of the Cambodian HIV national program. In this heavily
274 pretreated, multidrug-resistant population of patients aged over 15 years, a genotypic-driven
275 third-line regimen led to a virological response below 40 copies/mL in 59% and 55% at 24 weeks
276 and 48 weeks of treatment, respectively. Notably, 80% of the patients were below <1000 copies
277 at week 24 and week 48. No patient stopped the DRV/DTG combination due to an adverse event.

278 Patients recruited in the study had experienced three months of boosted adherence
279 counselling before enrolment. Adherence was identified as a major factor of second-line
280 therapeutic failure and must be enhanced before switching from second- to third-line therapies
281 and of virological response to third-line therapies [5, 6, 9, 10]. We previously reported in the
282 2PICAM study in Cambodia that 47% of patients with second-line therapeutic failure (> 1000
283 copies/mL) were rescued with adherence boosting, and among those who were not rescued, 29%
284 had no RAM on genotyping tests [6]. In Western Africa, Eholie et al. reported that adherence
285 reinforcement resulted in viral resuppression in 67% of patients who therefore continued with
286 second-line ART [5]. In our study, the 110 patients pre-enrolled, already received adherence
287 boosting before pre-enrolment. Among them, 32 patients still had plasma HIV RNA > 1000
288 copies/mL, while the GRT showed sensitivity to the current therapeutic regimen. This result
289 highlights the difficulty of fully supporting adherence in some patients and the possible necessity
290 of evaluating alternative strategies of maintain in care, adherence counselling and if possible
291 therapeutic simplification.

292 In our study, the ART median duration was 12 years, including five years with second-
293 line regimens. Patients exhibited high rates of RAMs to NRTI, NNRTI and PI. All patients were
294 naive to integrase inhibitor (ISTI). All but one patient had a viral strain sensitive to DRV/r, even
295 after exposure to several PIs, mostly LPV/r and ATV/r, in their second-line regimen. Optimized
296 NRTIs were chosen to be combined with DRV/r and DTG, taking into account RAMs; lamivudine
297 was prescribed in all even when the genotypic resistance test did not show sensitivity, as proposed
298 in previous studies [11, 12]. The virological response observed at 24 and 48 weeks, for a threshold
299 of 40 copies/ml, was lower than that observed in intensified regimens assessed in highly
300 experienced patients in high-income countries [13]. However, the rates of virological response at
301 week 24 and week 48 for the thresholds of 200 and 1000 copies/mL were close to 75% and 80%,
302 respectively.

303 In low- and middle-income countries, studies have shown contrasting results according to patient
304 profiles and salvage drugs prescribed. In studies of third-line therapy, modification of ART using
305 DRV/r and/or INSTI (dolutegravir or raltegravir) and/or etravirine led to similar results [5, 14-
306 19]. Approximately 80% of patients with plasma HIV RNA > 1000 copies/mL at third-line
307 initiation achieved HIV RNA < 1000 copies/ml between 24 and 48 weeks, according to the
308 studies. In the multisite ACTG A5288, viral suppression to < 200 copies/mL was observed in 74
309 to 100% of patients according to their viral profile and drugs prescribed [16]. In a prospective
310 study in Western Africa, 41% of patients achieved < 50 copies/mL at week 24 and 59% at week
311 48 [5]. In South Africa, 58% of patients suppressed their plasma HIV RNA to < 50 copies/mL at
312 least 6 months after third-line initiation approval [18]. In our study, even with at least two drugs
313 fully active on their viral strains (DRV/DTG), 20% of patients had no decline or rapid relapse in
314 viral load, indicating a probable adherence issue. A majority of our patients were included or
315 followed in the trial during the SARS-CoV-2 pandemic. Even if ARV delivery was preserved,
316 individual socioeconomic factors may have interfered with their daily life and adherence to ART.
317 Unexpectedly, adherence measurements (pill count and visual analogic scale) failed to detect low
318 levels of adherence in failing patients, as a white coat effect cannot be excluded. These patients
319 evolving from persistent virological failure on second-line ART to ineffective third-line ART
320 may represent patients with more preeminent adherence difficulties.

321 The observed concentrations of DRV and DTG in this Cambodian population were in the
322 expected range as compared to previous studies conducted in other populations of HIV-infected
323 subjects, whether DRV/r was administered BID or QD and combined to NRTI, raltegravir or
324 dolutegravir (20-25). There is a huge between patient variability especially in pre dose
325 concentrations, likely because timing of drug intake or food intake the day before the PK-day
326 could not be controlled. Interestingly, switching to OD dosing did not impair exposure of DRV
327 in our study, although the small number of patients who switched does not allow bioavailability
328 comparison. Importantly, concentrations remained well above the protein-adjusted IC90
329 (64ng/mL) for dolutegravir and above the protein-adjusted EC90 (200ng/mL) for darunavir (22).
330 Among the 28 patients who switched to 800 mg DRV at 24 weeks, eight had a virological rebound
331 after the switch. Even if for six of them, plasma HIV RNA remained below 200 copies/mL, the
332 relevance of such a switch with the aim of lightening treatment and maintaining adherence
333 remains questionable. The continuation of a twice-daily regimen could be the most effective

334 option for this population of heavily pretreated patients, even if once daily PK parameters were
335 reassuring.

336 Apart from low baseline CD4 counts, the only risk factor for virological failure at week 48 was
337 mother-to-child infection. We did not find a role of the number of resistance mutations at baseline
338 or of baseline HIV RNA. As discussed above, DRV and DTG concentrations were close to those
339 of non-Cambodian populations [20-22, 25]. Taken together, these findings argue for persistent
340 poor adherence in some of these patients. This poor adherence in the younger ones appeared
341 attributable to their history of mother-to-child HIV transmission and, for some, to family issues
342 incidentally reported by the caregivers. A European study reported that the risk of triple-class
343 virologic failure was higher in perinatally infected children and adolescents than in a distinct
344 group of heterosexually infected adults [26]. A French study also found them at higher risk of
345 having viral failure than persons infected during adulthood with the same duration of HIV
346 infection, after adjusting for therapeutic history. This difference was even more obvious in
347 teenagers and young adults <25 years and was likely due to problems of adherence specific to
348 these periods of life [27]. Transitioning from paediatric to adult care has been shown to make
349 HIV-infected adolescents and young adults more vulnerable to virological failure [28]. In
350 Cambodia, HIV-infected adolescents go to adult consultations from 15 years of age, and this may
351 have been too early for some of them [29].

352 The ANRS12374 study is a programmatic evaluation of a third-line strategy conducted within the
353 national HIV program. This strategy allowed us to achieve undetectability at the threshold of 1000
354 copies/mL for 80% of patients at 24 and 48 weeks, the threshold used by the national program to
355 define therapeutic failure. These results are comparable to those reported by the South African
356 national public sector HIV program [18, 30] and confirmed the evidence of the effectiveness of
357 the third-line ART regimen provided in a public sector programmatic setting. Some lessons can
358 be learned. The small number of patients eligible for the third-line regimen at each ART site
359 challenges the national program in making decisions to invest in the nationwide training of health
360 care workers in the use of these new drugs. A dedicated Technical Working Group has been
361 created within the national program, and a decision to switch from second-line to third-line
362 regimens can only be made after the agreement of this group, which can also serve as clinical
363 support to the physicians. The procurement and distribution of this drug in small quantities are
364 also challenging, especially for darunavir, which is only used in Cambodia for this indication.
365 The procurement option should be explored to ensure the availability of the drugs for the patients.

366 The logistical and financial impact of such a third-line strategy for the national HIV program
367 deserves to be further assessed by a cost analysis.

368 **Conclusion**

369 A third-line regimen combining DRV/r, DTG and an optimized background of NRTI appears to
370 be effective in obtaining a significant decrease in plasma HIV RNA after one year for the majority
371 of these multi-experienced patients and is associated with immunological response. Adherence
372 strategies at ART initiation, repeated during HIV care, particularly in young patients infected
373 through mother-to-child transmission with suboptimal virological response, remain a key element
374 in life-long HIV therapy, even in the context of recent powerful salvage antiretroviral drugs.

375 **Authors' contributions**

376 PP, OS, SS, MC, AMT, PL, CG conceptualized, designed the study, developed the research
377 protocol.

378 PP, OS, RS, ATM, VO, LM, CG analysed, interpreted the data and were a major contributor
379 in writing the manuscript.

380 PP, CC, CM, CLP, SM, BNG, KL have made substantial contributions to the project
381 implementation and the acquisition of data and substantively reviewed the manuscript.

382 All authors read and approved the final manuscript.

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389 Supporting Information. The authors declare that they have no competing interests.

390 **Data Availability Statement**

391 The data that support the findings of this study are available from the ANRS | Emerging
392 Infectious Diseases. Restrictions apply to the availability of these data, which were used under
393 license for this study. Data are available from the corresponding author with the permission of
394 the ANRS|MIE.

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538 **Additional Files**

539 File S1: Members of the ANRS 12374 3DICAM study Group

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541

Titre : Evaluation de l'implantation d'un programme de 3^{ème} ligne pour des sujets infectés par le VIH au Cambodge

Mots clés : VIH, traitement de troisième ligne, darunavir(DRV), Dolutegravir(DTG), Pharmacocinétique(PK), Cambodge.

Résumé : Les données probantes pour guider les stratégies de troisième ligne dans les pays à ressources limitées, dont le Cambodge, sont rares. L'étude 3DICAM visant à évaluer l'efficacité et la pharmacocinétique d'un régime de troisième ligne associant le DTG, le DRV et un INTI optimisé chez les PVVIH en échec d'un traitement de deuxième ligne. A la semaine 24, 59% des patients avaient une charge virale indétectable. Les concentrations de DRV et de DTG étaient proches de celles précédemment rapportées dans d'autres populations. Les facteurs de risque d'échec virologique étaient un taux de CD4<200 à l'initiation de la 3^{ème} ligne et une infection VIH transmise par voie mère-enfant. En ce qui concerne l'infection par le VHC, des données sont nécessaires pour atteindre les objectifs de l'OMS d'éliminer le VHC d'ici à 2030.

Dans une enquête transversale sur la séroprévalence du VHC de 425 PVVIH et 510 femmes enceintes, la séroprévalence du VHC était de 6,8% contre 0,9%. L'infection par le VHC était significativement associée au fait d'avoir un membre de la famille positif pour le VHC et des antécédents d'injection de médicaments par voie intraveineuse. En conclusion, un régime de 3^{ème} ligne combinant DRV, DTG et un INTI optimisé est efficace pour diminuer significativement la charge virale. Nos résultats soulignent la nécessité d'un dépistage et d'une prise en charge systématique du VHC chez les PVVIH et les membres de leur famille.

Title: Programmatic assessment of a third-line regimen implementation for adult HIV-infected in Cambodia

Keywords: HIV, Third-line regimen, darunavir (DRV), Dolutegravir(DTG), Pharmacokinetic(PK), Cambodia.

Abstract: There is limited evidence to guide HIV third-line strategies in resource-limited settings, including Cambodia. The 3DICAM study evaluated the effectiveness of a third-line regimen with dolutegravir, darunavir and optimized NRTI in 49 patients failing a second-line. At week 24, 59% of patients had viral load suppressed. Plasma concentrations of antiretrovirals were close to those in other populations. Risk factors for virological failure included low CD4 counts<200 and infection through MTCT. Regarding HCV infection, data are needed to meet WHO targets of eliminating HCV by 2030 in Cambodia.

In a cross-sectional survey of HCV seroprevalence among 425 PLHIV and 510 pregnant women, HCV seroprevalence was 6.8% vs. 0.9%. In PLHIV, HCV infection was associated with having a family member positive for HCV and intravenous medication injection. To conclude, a third-line regimen combining DRV, DTG and optimized NRTI is effective to significantly decrease viral load. Our results highlight a need for systematic HCV testing and care among PLHIV and their family members.