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Virological outcomes of a third-line antiretroviral therapy to inform Cambodian public health policy: the ANRS 12374 3DICAM study

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Abstract

Background. Several countries including Cambodia have achieved important milestones in the response to HIV. Sustainability of this progress greatly depends on the capacity of countries to maintain virological suppression and, by consequence, to avoid transmission. In Cambodia, evidence concerning the management of virological failure for first-line regimens suggested the necessity of enhancing adherence and decision-making based on genotype resistance tests to guide the switch into second-line regimens. In addition, a non-negligible number of PLHIV in virological failure for second-line regimens was found in previous research urging the need of developing policy for managing transitions to third-line ART.

Methods. The ANRS 12374 3DICAM study conducted between October 2018 and April 2021, provided Cambodian authorities with evidence concerning the virological outcomes of the regimen recommended as (salvage) third-line ART.

Results. Among 110 patients in virological failure for second-line regimens, 49 were eligible including around 59.1% and 55.1% virologically supressed at the threshold of <40 copies/mL at weeks 24 and 48. Virological suppression rate was around 80% at the threshold of <1000 copies/mL for both weeks 24 and 48. None of the participants discontinued the prescribed third-line ART due to drugs toxicity.

Conclusions. The present study was of valuable importance for the Cambodian National HIV Program in order to adapt guidelines. It confirms the importance of guiding the management of PLHIV by enhancing adherence and decision-making based on GRT; and respond to the need of assessing a third-line regiment in Cambodia suggested in previous research.

Trial registration. ClinicalTrials.gov, NCT03602690 on October 4, 2018.

Introduction

In 2023, the remarkable progress of Cambodia in the response to HIV is reflected by 89% of the 76 000 people living with HIV (PLHIV) diagnosed, of whom 98% were on ART including 98% with achieved viral load.[1] However, the sustainability of this progress greatly depends on the capacity of the country to maintain virological suppression as a cornerstone to avoid transmission.[2] Since the release of the guidelines for HIV treatment by the World Health Organization (WHO) in 2010, Cambodian authorities have attached special attention to the development of evidence-based policies for PLHIV in virological failure for second-line ART. In this regard, the ANRS 12276 2PICAM study demonstrated in 2013 a rate of 90% of virological suppression with a protease inhibitor (PI) based second-line regimen (either LPV/r or ATV + 2NRTIs) and underlined the importance of adherence boosting before deciding any change in the ART regimen as well as the importance of genotyping resistance testing (GRT) to guide decisions.[3,4] However, the abovementioned study has also highlighted that 5.5% (among 1 317 patients enrolled) had confirmed virological failure, including 12 patients (near 1%) eligible for third-line regimen.[3] Although the durability of first- and second-line ART has been demonstrated in among adult PLHIV in the Asia-Pacific region including Cambodia, the reported rates of failure to second-line ART suggest an increasing need for third-line regimens[5–7]. A forecast study has estimated that 2% of people receiving ART in low- and middle-income countries would be concerned by third-line regimens in 2025.[8] In Cambodia, this would represent around 1 353 PLHIV, raising important public health challenges in terms of management of patients failing second-line regimens.

In 2017, the National Centre for HIV/AIDS, Dermatology and STD (NCHADS) implemented a technical working group to formalize the guidance for the improvement of the quality of adherence and the GRT process. In the event of second-

line virological failure, it was recommended to enhance counselling for adherence and to conduct GRT among patients with persistent virological failure. For patients confirmed with resistant mutations the proposed ART combination is dolutegravir (DTG), ritonavir-boosted darunavir (DRV/r) and one or two nucleoside reverse transcriptase inhibitor (NRTI).[9] This is in line with the WHO recommendations of 2021 reiterating their call for developing policies for third-line ART, especially with regimens including new drugs with minimal risk of cross-resistance to previously used regimens.[10] In these policies, genotype profile is cornerstone as it allows optimizing regimens and timely third-line ART initiation, with important benefits in terms of viral suppression and patients' quality of life.[2] However, overall, there is a lack of evidence to guide third-line ART strategies in resource-limited settings. Furthermore, the few existing studies about virological outcomes for patients in third-line ART have been conducted principally in sub-Saharan African countries. Only two studies demonstrating effectiveness and tolerability of third-line regimens were identified in Thailand.[11,12] In a context of rapid increase of resistance mutations to second-line drugs recently reported in the region[13], producing evidence for an effective management of switches to third-line regimens is urgent.

In this context the ANRS 12374 3DICAM study provided Cambodian authorities with evidence concerning the virological outcomes of the regimen recommended as (salvage) third-line ART. It provided a better understanding of the effectiveness of this regimen as well as the factors associated with virological suppression. These elements are crucial to guide treatment strategies and for the improvement of HIV patient-centred public policy.

Methods

Study design and population

The ANRS 12374 3DICAM study is a one-arm, multicentre study aiming to evaluate the feasibility, efficacy and safety of a third-line ART regimen combining DTG, DRV/r and optimized with 1 or 2 NRTI among Cambodian adult PLHIV treatment-experienced volunteers (Figure 1). It was conducted in 18 ART clinics across Cambodia between October 2018 and April 2021. Patients were eligible for pre-inclusion into the study if they were ≥ 15 years old and presented virological failure (defined by plasma HIV-1 RNA levels >1000 copies/mL) to PI-based second-line ART regimen despite three months of enhanced adherence counselling. GRT was performed for these patients and those with evidence of intermediate or full resistance to atazanavir (ATV) or sensitivity to ATV but resistance to all NRTIs were eligible for inclusion into the study. Exclusion criteria included patients previously treated with DRV and integrase inhibitor, pregnancy, opportunistic infections in acute phase including tuberculosis, and any other concomitant medical condition contraindicating participation. The protocol with the complete inclusion and exclusion criteria is available in Supplementary file 1.

Third-line ART strategy

At inclusion into the study, the third-line regimen consisted of DRV/r (600mg/100mg) twice daily + DTG (50mg) once daily + 3TC (300mg, lamivudine) once daily. Furthermore, depending on the hepatitis B status (HBsAg), the medical history of the patient and the GRT results, the regimen was optimized with a full or intermediate sensitive NRTI among tenofovir disoproxil fumarate (TDF), abacavir (ABC), and zidovudine (AZT). TDF was preferred in case of positive HBsAg and the absence of full resistance to this drug.

A first assessment was conducted at week 24 (W24). For patients with plasma HIV-1 RNA levels < 40 copies/mL and without DRV/r resistance at inclusion, the strategy included a switch from DRV/r (600mg/100mg) twice daily to DRV/r (800mg/100mg) once daily with no changes concerning the other drugs. The switch started at W26 and plasma HIV-1 RNA levels were measured four weeks later (W30). A second assessment was conducted for all patients at week 48 (W48).

Procedures

Participants were followed-up every two weeks during the first month, then every four weeks until W24, and then at W36 and W48 (Figure 1). Blood tests were performed every 4 weeks during the first quarter (W4, W8, W12) and then at W24, W36 and W48. Plasma HIV-1 RNA levels were measured at inclusion, then at weeks 4, 12, 24, 36 and 48 for all participants; for those who switched to darunavir 800 mg/ritonavir 100 mg once daily, an additional virological assessment was performed 4 weeks after the switch (W30). The NCHADS laboratory measured plasma HIV-1 RNA levels using an automated Abbott real-time HIV-1 assay and performed CD4 cell counts. GRT and biochemical analyses were performed by the Pasteur Institute laboratory in Cambodia. Creatinine clearance (CrCL) was calculated using the Cockcroft-Gault equation. Adherence was measured at each visit by a visual adherence scale. The value of > 90% on the visual scale was chosen to define “optimal adherence”. We collected data on adverse events at all study visits and graded them by severity on a scale from 1 to 4 defined according to the French Agency for Research on AIDS, Viral Hepatitis and Emerging Infectious diseases grading table (https://www.scribd.com/document/152634847/ANRS-GradeEI-V1-En-2008).

Sample size

According with previous studies concerning multi-drug resistances[14] and ART initiation with NRTI-sparing regimens[15], it can be expected that at least 80% of participants in the study will be in virological success after 6 months. Under the statistical assumptions of 5% of significance level (alpha) and a power of 90%, the Fleming’s procedure indicated that a sample size of 45 participants would be required to conclude a success rate of at least 60% if the total number of failures does not exceed 12. At the time of the study 3 500 patients were on PI-regimen in Cambodia and according to the results of the ANRS 12276 2PICAM study, it was expected that 5% of patients (n=175) could be in second-line virological failure (i.e. eligible for pre-inclusion into the present study) and one third of them (n=60) would need third-line ART (i.e. eligible for inclusion). With the assumption of a refusal rate of 10%, the present study required the recruitment of 54 participants.

Outcomes

The primary outcome of this study was the percentage of participants with plasma HIV-1 RNA levels <40 copies/mL (i.e. virological suppression) by FDA Snapshot analysis at W24. Participants with missing plasma HIV-1 RNA levels at W24 were considered to have treatment failure for the primary outcome. Pre-defined secondary outcomes included: i) the percentage of participants with plasma HIV-1 RNA levels < 40 copies/mL by FDA Snapshot analysis at W48, ii) the median change in CD4 cell count; iii) the percentage of participants who switched from DRV/r (600mg/100mg) twice daily to DRV/r (800mg/100mg) once daily with no changes concerning the other drugs at W26 (i.e. two weeks after the clinical assessment at W24); iv) adherence to treatment strategy and discontinuation ; and v) the incidence of grade 3-4 adverse events. Other secondary outcomes included the percentage of participants with plasma HIV-1 RNA levels <200 and 1000 copies/mL by FDA Snapshot analysis at W24 and W48.

Statistical analysis

The sample was described using percentages (%) or medians [IQR: interquartile ranges] for categorical and continuous variables respectively. The primary and secondary outcomes were described at W24 and W48 using percentages with 95% confidence intervals (CI) computed using a binomial distribution. Adverse events and the number of occurrences were listed. Finally, univariate analysis and multivariable logistic regression was used separately at W24 and W48 to estimate the factors associated with virological suppression (=1 if HIV-1 RNA<40 c/mL vs =0 if HIV-1 RNA≥40). Factors with p-value<0.2 in the univariate analysis were tested in the multivariable estimation.

All analyses were performed using STATA 15 software (StataCorp, USA).

Ethical considerations

The ANRS 12374 3DICAM study (ClinicalTrials.gov, NCT03602690) was conducted in accordance with the Declaration of Helsinki and was approved by the Cambodian National Ethics Committee for Health Research (No.273NECHR). Written informed consent was obtained from each study participant before beginning the study procedures. A data and safety monitoring board and a scientific advisory board annually reviewed study progress and safety data.

Results

Between October 2018 and April 2021, 110 patients were pre-enrolled in 18 ART clinics. Among them, 50 participants (45.5%) from 13 clinics were eligible and included into the present study. Reasons for the exclusion of 60 patients are detailed in Figure 2. One participant withdrew from the study at inclusion before any antiretroviral intake, leading to a total of 49 participants included in the analysis (Figure 2). None of participants was lost to follow-up and none of participants discontinued treatment.

Table 1 shows that among the 49 participants, 61.2% were male and median [IQR] age was 40 years [24 – 46]. The most common routes of infection were heterosexual sex intercourses (67.4%) and mother to child transmission (26.5%). Overall, previous duration of ART was in median 12 years [8 – 14]. At baseline, the median CD4 level was 195 cells/µL [36 - 353] and 4.4 Log_{10} copies/mL [3.6 - 5.2] for plasma HIV-1 RNA level. The number of resistance-associated mutations in NRTIs, NNRTIs and PIs were 4 [2 - 6], 3 [2 – 4] and 8 [6 – 9], respectively. Finally, most of participants were prescribed with DRV/r+DTG+3TC+TDF (57.1%) followed by DRV/r+DTG+3TC (26.8%) as third-line regimen.

Table 1. Baseline characteristics of participants enrolled in the study (N=49)

Characteristics	n (%) Median [IQR]
Male	30 (61.2)
Age (years)	40 [24 – 46]
Body weight (kg)	55 [48 – 63]
Body Mass index (kg/m²)	21 [19 – 24]
HBsAg Positive	2 (4.0)
Anti-HCV Positive	2 (40)
Route of infection	
Mother to child transmission	13 (26.5)
Heterosexual	33 (67.4)
Men who have sex with men	1 (2.0)
Other/unknown	2 (4.1)
Baseline Adherence assessment	
Good	36 (73.5)
Moderate	10 (20.4)
Poor	3 (6.1)
Previous duration of ART (years)	
First-line ARV regimen[†]	4 [3 – 7]
Second-line ARV regimen[†]	5 [3 – 7]
Overall	12 [8 – 14]
History of opportunistic infection (clinical stage)	
C	20 (40.8)
B	4 (8.2)
A	25 (51.0)
Laboratory data	
CD4 cell-count (cells per µL)	195 [36 – 353]
Plasma HIV RNA level (Log_{10}copies/mL)	4.4 [3.6 – 5.1]
Number of resistance-associated mutations	
NRTI	4 [2 – 6]
NNRTI	3 [2 – 4]

PI	8 [6 – 9]
Prescribed third-line regimen	
DRV/r+DTG+3TC+TDF	28 (57.1)
DRV/r+DTG+3TC+AZT	3 (6.1)
DRV/r+DTG+3TC	18 (36.8)

IQR interquartile range; [†]Number of patients on first- and second-line therapy, n=48

Genotypic resistances

Thirty-seven participants (75.5%) had full or intermediate resistance to ATV/r. Only one patient had full resistance to DRV/r and none had partial resistance to DRV/r. The majority of the participants (81.6%) had full or intermediate resistance to 3TC, 53.0% to AZT, 47.0% to TDF and 67.4% to ABC. In total, 42.9% of participants had full or intermediate resistance to AZT/TDF/ABC. All participants started third-line treatment with twice-daily DRV/r, once-daily DTG, combined with only 3TC for 36.8%, 3TC plus TDF for 57.1% or 3TC plus AZT for 6.1%. The major resistance mutations accumulated are reported in the Supplementary file 2.

Virological outcomes

Of the 49 participants, the primary outcome was observed for 59.1% 95%CI[44.2 – 73.0] participants whose HIV-RNA level was <40 copies/mL at W24 (Table 2). Among the 20 participants with plasma HIV-1 RNA level ≥40 copies/mL at W24, 11 (55% 95%CI[40.2 – 69.3]) had levels <1000 copies/mL including 10 maintaining these levels at W48 (not shown in the table). Of the remaining 9 participants with HIV-1 RNA level ≥1000 copies/mL at W24, two participants were virologically controlled at W48 with <1000 copies/mL. This led to an overall rate of virological control of 79.5% 95%CI [65.6 – 89.7] at W48 (Table 2). Furthermore, at W48, the CD4 count was >350 cells/µL in 38.8% 95%CI[25.1 – 53.7] of participants and >500 cells/µL in 22.4% [11.7 – 36.6] (Table 2). The median CD4 change was higher in those who reached a plasma HIV-1 RNA level<40 copies/mL at W48 than in the others (+140 vs. +55/µL, p<0.001) (not shown in the table).

The switch from DRV/r (600mg/100mg) twice daily to DRV/r (800mg/100mg) once daily (with no changes concerning the other drugs) concerned 28 participants (57.1% 95%CI[42.2-71.1]) of the 49 included in the study (Table 2). Among them, 20 (71.4% 95%CI[51.3-86.7]) were virologically supressed with plasma HIV-1 RNA level <40 copies/mL at W48. Virological rebound was observed for the remaining 8 participants after the switch including 6 with plasma HIV-1 RNA level <200 copies/mL (Table 2).

Adherence, discontinuation and adverse events

A score of adherence higher than 90% was observed for most of participants: 93.8% 95%CI[83.1 – 98.7] and 89.8% 95%CI[77.7 – 96.6] at W24 and W28 respectively (Table 2). None of the participants discontinued the third-line treatment prescribed in the study due to drugs toxicity. Patients presenting at least one grade 3-4 adverse events or serious adverse events (SAE) passed represented 18.4% 95%CI[08.7-32.0] at W24 and 26.5% 95%CI[14.9 – 41.0] at W48. Furthermore, 10.2% 95%CI[3.3 – 22.2] of participants presents at least one drug-related adverse event. However, the total number of patients presenting adverse events (non-serious and serious) during the whole follow-up was

higher (Table 3). One participant presented cryptococcal meningitis at W4 (CD4: 85 cells/µL and plasma HIV-1 RNA level: 3.0 Log_{10} copies/mL), and one participant had grade 1 shingles at W12 (baseline CD4: 199 cells/µL and plasma HIV-1 RNA level: 4.9 Log_{10} copies/mL). Three participants stopped TDF due to a decrease in CrCL, and two participants were referred for diabetes treatment. However, it is important to notice that none of the patients stopped the trial treatment (DRV/r + DTG) because of adverse events.

Factors associated with virological suppression

Univariate analysis showed that participants aged ≥ 25 years (OR: 2.55, 95%CI[0.71 – 9.08], p=0.15), those with a CD4 cell-count ≥200 at baseline (OR: 2.28, 95%CI[0.70 – 7.39], p=0.17) and those with adherence assessment ≥ 90% at W24 (OR: 4.9, 95%CI[0.47 – 51.4], p=0.18) were associated with virological suppression at W24 (Table 4). However, multivariable estimation resulted only in tendencies for age and CD4 cell-count, indicating that participants aged ≥ 25 years (AOR: 2.81, 95%CI[0.75 – 10.53], p=0.13), those with a CD4 cell-count ≥200 at baseline (OR: 2.47, 95%CI[0.72 – 8.41], p=0.15) might be more likely to be in virological suppression at W24.

Concerning virological suppression at W48, univariate estimations in Table 5 show that it was more likely for patients aged ≥ 25 years (OR: 3.04, 95%CI[0.83 – 11.07], p=0.09), those with a CD4 cell-count ≥200 at baseline (OR: 3.11, 95%CI[0.95 – 10.15], p=0.06), and those concerned by mother-to-child transmission (OR: 0.15, 95%CI[0.04 – 0.65], p=0.01). Associations of CD4 cell-count ≥200 at baseline (AOR: 3.88, 95%CI[1.02 – 14.78], p=0.047) and mother-to-child transmission (AOR: 0.12, 95%CI[0.03 – 0.60], p=0.01) with virological suppression at W48 were confirmed in the multivariable estimation.

Table 2. Virological outcomes at week 24 and week 28 (N=49)

Characteristics	Week 24 (N=49)			Week 48 (N=49)		
	n	%	95% CI	n	%	95% CI
Plasma HIV-1 RNA level (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 µL	23	46.9	[32.5-61.7]	19	38.8	[25.1-53.7]
CD4 >500 cells per µL	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/r 600/100 mg twice daily (n=21)	-	-	-	7	33.3	[14.5-56.9]
HIV RNA <40 copies/mL in patient switched to DRV/r 800/100 mg once daily (n=28)	-	-	-	20	71.4	[51.3-86.7]

Table 3. Adverse Events reported during study follow-up (N=49)

Type of AE	Number of participants with non-serious or serious AE	Patients with AEs Grade 3-4 or SAEs
Infections and infestations		
Cryptococcal meningitis	1	1/1
Investigations		
Alanine aminotransferase increase	22	3/22
Aspartate aminotransferase increase	29	2/29
Creatinine clearance decrease	38	5/38
Fasting blood glucose increase	15	2/15
LDL cholesterol increase	2	1/2
Platelet count decrease	5	1/5

Table 4. Factors associated with virological suppression below 40 copies/mL at Week 24 (n=49)

Covariates	<40 copies/mL		Univariate analysis			Multivariable analysis		
	n/N	%	OR†	95% CI	p-value	AOR‡	95% CI	p-value
Gender								
Female	12/20	60.0	Ref			Ref		
Male	17/29	58.6	0.94	[0.29-3.01]	0.9	0.95		
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
Baseline CD4 cell-count (cells per µL)								
<200	13/26	50.0	Ref			Ref		
≥200	16/23	69.5	2.28	[0.70-7.39]	0.17	2.47	[0.72-8.41]	0.15
Baseline 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 the variables in the column.

Table 5. Factors associated with virological suppression below 40 copies/mL at Week 48 (n=49)

Covariates	<40 copies/mL		Univariate analysis			Multivariable analysis		
	n/N	%	OR†	95% CI	p-value	AOR‡	95% CI	p-value
Gender								
Female	13/20	65.0	Ref					
Male	14/29	48.2	0.50	[0.15-1.62]	0.25			
Age (years)								
<25	5/14	35.7	Ref					
≥25	22/35	62.8	3.04	[0.83-11.07]	0.09			
Baseline 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	3.88	[1.02-14.78]	0.047
Baseline 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	0.12	[0.026-0.60]	0.01
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	-		-
	>=90%	27/44	61.3	-		-
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; ‡adjusted ORs were obtained from multiple logistic regression models including all variables in the column

Discussion and conclusions

This study demonstrated the effectiveness and safety of a third-line ART strategy integrated in the routine activities of the Cambodian HIV national program, ensuring its feasibility and sustainability. The DRV/r + DTG + 3TC +/- 1 NRTI prescribed to patients in virological failure for second-line regimens led to virological suppression at the threshold of 40 copies/mL in 59.1% and 55.1% at 24 weeks and 48 weeks of treatment respectively. Furthermore, at the threshold of 1000 copies/mL the virological control rate was around 80% at weeks 24 and 48. In low- and middle-income countries, these rates have been estimated to be on average 75.5% at week 24 and 78.6% at week 48 with important variations according to patient profiles and the prescribed salvage drugs.[2] However, the majority of studies conducted in different settings included etravirine (ETV) in their third-line regimen,[16–21] which was not the case in our study as this molecule was not procured for the HIV National Program in Cambodia. Indeed, our study intended to propose a third-line strategy including molecules available and procured in the country in order to ensure the feasibility and sustainability of its implementation. Therefore, 3TC was prescribed for all patients, even when the GRT did not show sensitivity as proposed in previous studies,[22,23] although it was optimized with other NRTIs (i.e. ABC, AZT or TDF) taking into account resistance-associated mutations (RAM) and combined with DRV/r and DTG.

The meticulous screening by the technical working group of the 110 PLHIV in virological failure for second-line ART at pre-enrolment, allowed targeting accurately the patients in need for third-line ART. In this screening process, the enhancement of adherence played a crucial role for the rationalization of switches from second- to third-line therapies. Indeed, 20 patients were maintained in second-line ART (among the 60 excluded from the study) as they were virally controlled (<1000 copies/mL) after three months of enhanced adherence. Although, the importance of adherence in rescuing patients in second-line virological failure has already been demonstrated in Cambodia and in other low- and middle-income countries,[24,25] the resuppressed viral load among some of the pre-enrolled participants, suggests that tailoring adherence support to specific profiles is necessary. Indeed, the inadequacy of the adherence support could explain the 20% of participants included into the study with no decline or rapid relapse in viral load in spite of the use of drugs fully active on their viral strains such as DRV and DTG. The adherence issues suggested by our results contrast with the high levels of adherence measured by counsellors with scores ≥90% for the majority of participants, although these could be reflecting a social desirability bias. In fact, our multivariable estimations seem to be in line with adherence issues as participants with CD4 count lower than 200 cells/µL at baseline were associated with lower odds of virological suppression. Indeed, immune reconstitution has been found to be a factor reflecting low adherence especially among patients with high viral load at baseline.[26,27] On the other hand, our multivariate estimations also

highlight the lower odds of virological suppression associated with participants concerned by mother-to-child HIV transmission. This suggests the need of services specific to this population, especially at the time of transitioning from paediatric to adult care as the vulnerability of perinatally infected children and adolescents to virological failure has been found to be higher. [28–30] This could be explained by adherence problems specific to these periods of life, but also by greater delays in ART initiation and other structural factors leading to relatively poorer health HIV-related outcomes.[31]

Combining enhanced adherence with GRT greatly contributed to the durability of the second-line as demonstrated by the 32 patients still in virological failure at the pre-enrolment stage (≥1000 copies/mL) for which second-line was maintained in the absence of no genotypic resistance. Furthermore, integrating systematic GRT contributed to the rationalization of third-line ART initiation by guiding the technical working group decision-making concerning the choice of the strategy. In spite of the high rates of RAMs to NRTI, NNRTI and PI among patients, the effectiveness and safety of the third-line ART combinations prescribed in this study was demonstrated by the absence of discontinuations related to adverse events. Our results confirmed that the guidance by the technical working group, the adherence enhancement and the GRT implementation are the main pillars for the effectiveness of the proposed third-line ART strategy. They are crucial for the management of ART in low- and middle-income countries for the optimization of resources, but also for the improvement of durability of second-line regimens and the rationalization of the initiation of PLHIV into third-line regimens.[8,18]

Overall, the simplification of treatment by switching from DRV (600mg) twice per day to DRV (800mg) once per among virologically suppressed participants at week 24 did not appear relevant in our study. In spite of the sustained HIV-RNA levels below 200 copies/mL for 6 out of the 28 patients concerned by the switch, virological rebound was observed for 8 of them after the switch. Our results contrast with existing literature demonstrating the efficacy of the switch of DRV from twice to once per day in maintaining virological suppression among treatment-experienced patients.[32–35] However, these studies have been conducted under the framework of clinical trials, and therefore not accounting for structural barriers that may exist in the 'real-life' settings, especially in low- and middle-income. Indeed, in Cambodia DRV is procured for the HIV National Program only in the 600mg and 400mg versions. Thus, the switch to DRV (800mg) once per day was not really a simplification as patients had to uptake 2 pills of DRV (400mg) per day. This result demonstrates that the effectiveness of treatment simplification has to adopt a comprehensive approach tailored to low- and middle-income contexts including structural barriers. In the Cambodian context, maintaining the ART strategy with DRV (600mg) per day has not impact over the effectiveness and safety of third-line strategies as DRV once- or twice- per day have been demonstrated to be comparable with similar rates of virological failure and emergence of resistances.[33,34]

This study has been conducted no without some limitations. First, the majority of participants were included or followed-up during SARS-CoV-2 pandemic. Even if ART delivery was ensured, individual and structural factors may have interfered with patients' daily life and adherence to the prescribed third-line ART. However, the 80% rates of virological control at the threshold used by the HIV National Program (<1000 copies/mL) at weeks 24 and 48 are comparable to those reported by other national programs of the public sector in low- and middle- income countries before the SARS-CoV-2 pandemic.[2,29,36] This suggests that the pandemic did not exacerbated adherence issues as the virological failure rates are similar to those in the abovementioned studies. Second, the study was based on a small sample with an inclusion rate of 45.5% out of 110 pre-enrolled patients raising the question about the statistical power to conduct in-depth analyses according to sub-groups in terms of demographic, geographical or clinical characteristics. However, the sample was calculated with power enough to estimate the percentage of participants with plasma HIV RNA levels <40 copies/mL. Although the small sample could lead to underestimating some results, especially concerning

secondary outcomes, this study could be seen as a 'pilot' real-life experience of the implementation of a third-line strategy proved to be effective and feasible.

In spite of these limitations, the ANRS 12374 3DICAM study provided Cambodian authorities with evidence supporting the development of policies for PLHIV in virological failure for third-line ART. The creation of the technical working group in charge of guiding the switch of these patients to third-line regiments, the enhanced adherence activities and the GRT were integrated in the routine activities of the HIV national program ensuring their feasibility, scale-up and sustainability. In a context of financial and human resources shortage, the small number of patients eligible for third-line regimen at the level of ART clinics challenges national programs as healthcare workers have to be trained in the use of third-line therapies. However, as a public health concern, the management of PLHIV in virological failure for second-line ART is crucial for the sustainability of the achievements of the Cambodian HIV national program. Adherence support is crucial upon ART initiation and throughout HIV follow-up, even in the context of recent powerful salvage antiretroviral drugs. In addition, alternative strategies accounting not only for individual-related factors, but also for structural issues such as the lack human resources trained to provide support tailored to specific PLHIV's behaviours affecting adherence including mental health issues. The present study was of valuable importance for the Cambodian National HIV Program in order to adapt guidelines. It confirms the importance of guiding the management of PLHIV by enhancing adherence and decision-making based on GRT; and respond to the need of assessing a third-line regiment in Cambodia suggested in previous research. Future work should focus on the logistical and financial cost of the third-line strategy proposed in this study.

Declarations

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Disclosure statement

The authors declare that they have no competing interests, financial or otherwise, in completion of this work.

Author contributions

PP, OS, SS, MC, AMT, PL, CG conceptualized, designed the study, developed the research protocol. PP, OS, RS, ATM, VO, LST, LM, CG analyzed, interpreted the data. PP, LST, LM and OS were major contributors in writing the manuscript. CC, CM, CLP, BNG, KL made substantial contributions to project implementation and the acquisition of data and substantively reviewed the manuscript. All authors read and approved the final manuscript.

Data availability statement

The study protocol is provided in Supplementary file 1. Data collection documents will be made available upon reasonable request. Data reported in this article can be requested by submitting a study proposal to the scientific

board of the ANRS 12374 3DICAM study (phearavinpheng@uhs.edu.kh). Proposals will be evaluated for compatibility with the 3DICAM study and overlap with ongoing work, and according to the Cambodian and French Ethics Committees.

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Figures

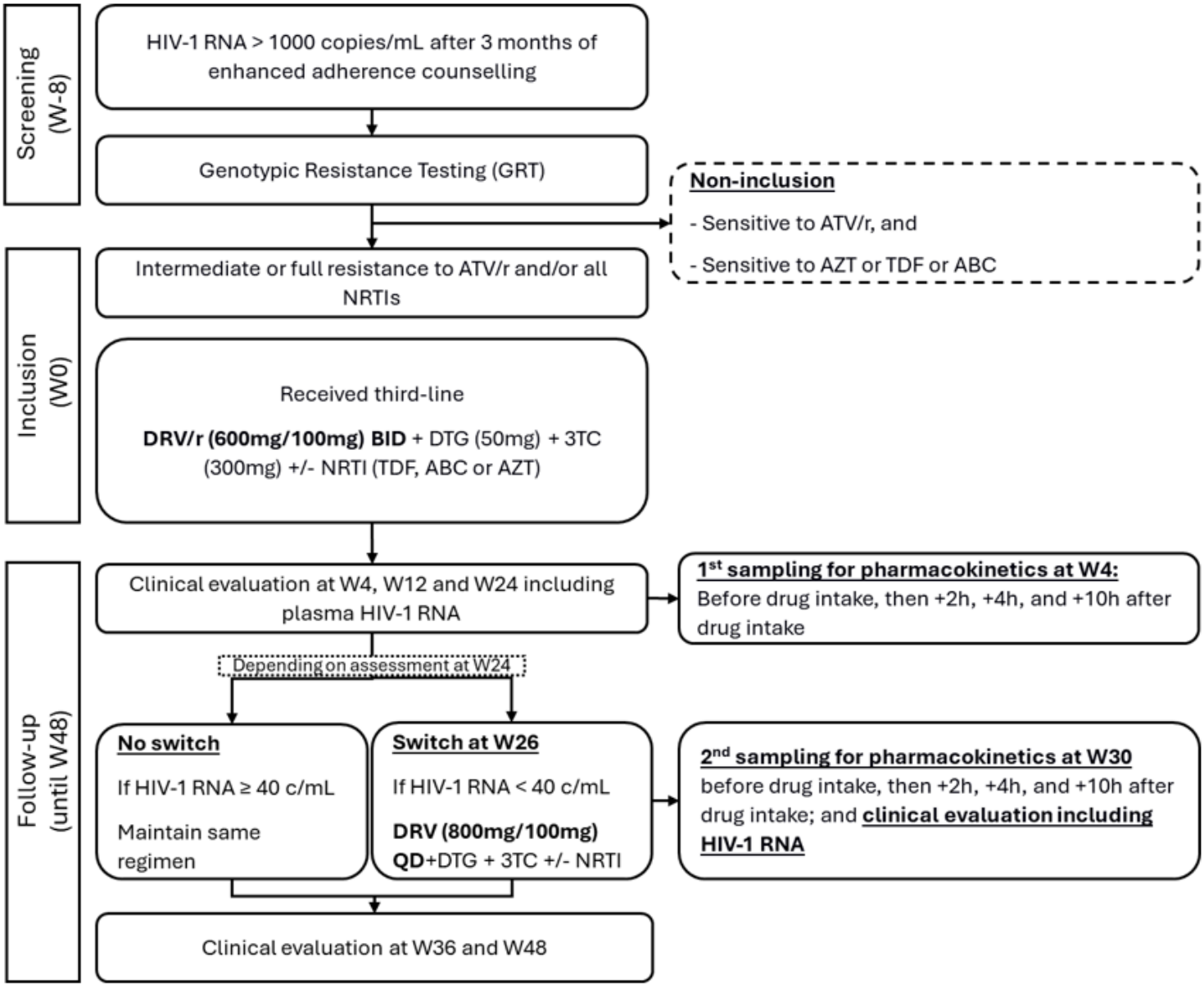


Figure 1

ANRS 12 3DICAM study design*

*The detailed schedule for the activities included in screening, inclusion and the clinical assessments are presented in the Supplementary file 1 (page 25/48).

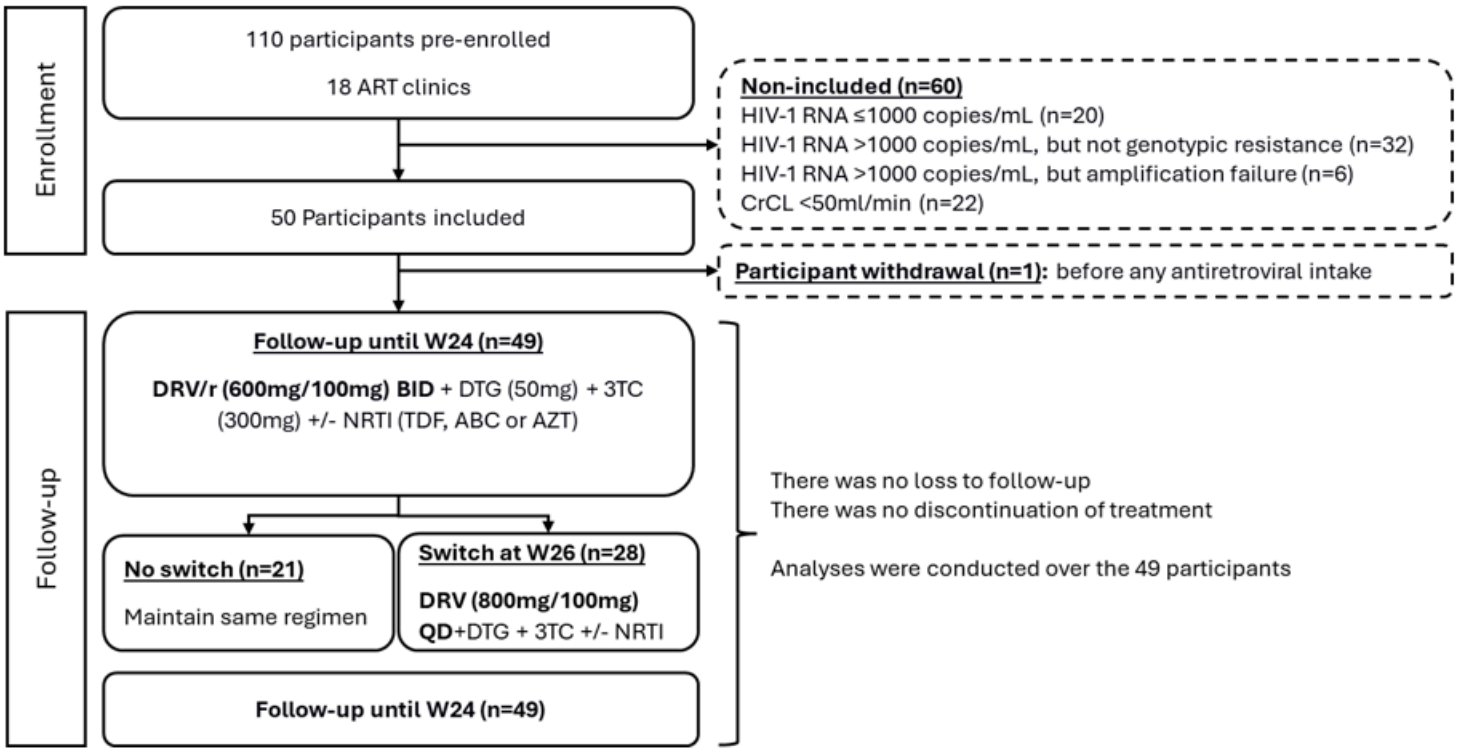


Figure 2

Participants flowchart

Supplementary Files

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- ANRS12374Protocolversion1Ethicsubmission.pdf
- S2Majorresistancemutationsaccumulated.docx
- S3studygroup.pdf