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Prevalence of pretreatment HIV resistance to integrase inhibitors in West African and Southeast Asian countries

Avelin F. Aghokeng [1]*, Nicole Ngo-Giang-Huong[1,2], Thu H. K. Huynh[3], Anoumou Y. Dagnra[4], Thomas D'Aquin Toni[5], Almoustapha I. Maiga[6], Kania Dramane[7], Sabrina Eymard-Duvernay[8], Marie-Laure Chaix [9], Vincent Calvez[10] and Diane Descamps[11]; on behalf of the ANRS12425 Study Group†

[1]MIVEGEC, Université de Montpellier, CNRS, IRD, Montpellier, France; [2]LMI Presto, IRD-Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand; [3]HIV/AIDS Laboratory, Pasteur Institute, Ho Chi Minh City, Vietnam; [4]Faculté des Sciences de la Santé, Centre de Biologie Moléculaire et d'Immunologie, Université de Lomé, Lomé, Togo; [5]Programme PACCI, Virology Laboratory CIRBA, Abidjan, Côte d'Ivoire; [6]UCRC/SEREFO, FMOS, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali; [7]Department of Biomedical Sciences, Virology Laboratory, Centre MURAZ, Bobo-Dioulasso, Burkina Faso; [8]IRD, UMR MoISA, Montpellier Interdisciplinary Center on Sustainable Agri-food Systems, Montpellier, France; [9]INSERM U941, Université Paris Diderot, AP-HP, Hôpital Saint-Louis, Laboratoire de Virologie, Paris, France; [10]Department of Virology, AP-HP, Hôpital Pitié Salpêtrière, Laboratoire de Virologie, Paris, F-75013, France; [11]IAME, UMR 1137, Université Paris Diderot, Sorbonne Paris Cité et INSERM, AP-HP, Hôpital Bichat, Paris, France

*Corresponding author. E-mail: avelin.aghokeng@ird.fr
†Members are listed in the Acknowledgements section.

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Objectives: Integrase strand transfer inhibitors (INSTIs) have been recently recommended as the preferred first-line option for antiretroviral treatment initiators in low- and middle-income countries (LMICs) in response to the growing circulation of resistant HIV to non-nucleoside reverse transcriptase inhibitors (NNRTIs). In this study, we estimated the frequency of pretreatment drug resistance (PDR) to INSTIs in West Africa and Southeast Asia.

Materials and methods: Using samples collected from 2015 to 2016, and previously used to assessed PI, NRTI and NNRTI resistance, we generated HIV integrase sequences and identified relevant INSTI PDR mutations using the Stanford and ANRS algorithms.

Results: We generated 353 integrase sequences. INSTI PDR frequency was low, 1.1% (4/353) overall, ranging from 0% to 6.3% according to country. However, frequency of PDR to any drug class was very high, 17.9% (95% CI: 13.9%–22.3%), and mostly associated with a high level of NNRTI PDR, 9.7%, and a moderate level of NRTI PDR, 5.3%.

Conclusions: Our results support the recent introduction of INSTIs in LMICs to improve treatment outcome in these settings, but also stress the need for effective actions to prevent uncontrolled emergence of drug resistance to this drug class.

Introduction

HIV mutants with the ability to escape antiretroviral drugs are common. These drug resistant strains can significantly affect the management of this infection and are recognized as a major health problem for HIV treatment and future elimination. HIV drug resistance can be selected under drug pressure, and thus defined as acquired HIV drug resistance (ADR), or can be present before antiretroviral treatment or therapy (ART) initiation and known as pretreatment HIV drug resistance (PDR). Both ADR and PDR are associated with poor response to treatment and premature virological failure, as well as increased transmission rate and mortality.[1–3] The public health consequence of drug resistance is also of high concern, as it can significantly affect the efficacy of some drugs or full drug classes, and therefore reducing treatment options. This scenario has been recently illustrated with non-nucleoside reverse transcriptase inhibitors (NNRTIs),

Table 1. Study population and characteristics

	BF	CI	ML	TG	TH	VN	Overall
Total recruited	26	74	32	69	75	77	
Female[a]	14 (53.8%)	30 (40.5%)	19 (59.4%)	43 (62.3%)	25 (33.3%)	16 (20.8%)	147 (41.6%)
Age (years)[b]	37.5 (32.0–42.0)	38.3 (33.0–42.0)	41.4 (32.0–48.0)	41.2 (34.0–47.0)	32.1 (23.0–38.0)	31.4 (26.0–35.0)	36.1 (28.0–42.0)
CD4 (cells/mm^3)[c]	187 (68–333)	206 (140–268)	102 (24–197)	149 (61–255)	466 (296–550)	388 (239–492)	245 (102–443)
First-line (planned)							
TDF+3TC+EFV	2 (7.7%)	42 (56.8%)	32 (100.0%)	54 (78.3%)	61 (81.3%)	71 (92.2%)	262 (74.2%)
TDF+FTC+EFV	16 (61.5%)	2 (2.7%)	0 (0.0%)	0 (0.0%)	1 (1.3%)	2 (2.6%)	21 (5.9%)
TDF+3TC+NVP	2 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.6%)
AZT+3TC+EFV	4 (15.4%)	1 (1.4%)	0 (0.0%)	0 (0.0%)	6 (8.0%)	0 (0.0%)	11 (3.1%)
AZT+3TC+NVP	0 (0.0%)	3 (4.1%)	0 (0.0%)	11 (15.9%)	0 (0.0%)	0 (0.0%)	14 (4.0%)
Other	0 (0.0%)	4 (5.4%)	0 (0.0%)	4 (5.8%)	7 (9.3%)	0 (0.0%)	15 (4.2%)
Missing	2 (7.7%)	22 (29.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (5.2%)	28 (7.9%)
Previous exposure to ARV							
No	23 (88.5%)	52 (70.3%)	31 (96.9%)	67 (97.1%)	72 (96.0%)	67 (87.0%)	312 (88.4%)
Yes	1 (3.8%)	0 (0.0%)	1 (3.1%)	2 (2.9%)	3 (4.0%)	8 (10.4%)	15 (4.2%)
Missing	2 (7.7%)	22 (29.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.6%)	26 (7.4%)
Type of exposure to ARV							
ART	1 (3.8%)	0	1 (3.1%)	0	0	6 (7.8%)	8 (2.3%)
PMTCT	0	0	0	2 (2.9%)	3 (4.0%)	1 (1.3%)	6 (1.7%)
Other	0	0	0	0	0	1 (1.3%)	1 (0.3%)

Data are median (IQR) or *n* (%).
TDF, tenofovir; 3TC, lamivudine; FTC, emtricitabine; AZT, zidovudine; EFV, efavirenz; NVP, nevirapine; PMTCT, prevention of mother-to-child transmission.
[a]Data available for 329 participants.
[b]Data available for 329 participants.
[c]Data available for 302 participants.

the currently most resistance-affected drug class.[4,5] As a consequence, decision to replace these drugs by a more robust option in terms of virological efficacy and a better genetic barrier to resistance has been taken, leading to the recent introduction of second-generation integrase strand transfer inhibitors (INSTIs)-based first-line in low- and middle-income countries (LMICs).[6] Millions of people worldwide are now receiving a dolutegravir-based first-line regimen, stressing the need for investigations to estimate baseline and background resistance to this strategy, especially among ART initiators, and to better appreciate the potential impact of PDR on the efficacy of the new strategy.

In this study, we conducted a complementary investigation of INSTI PDR in ART initiators attending major ART clinics in four countries in West Africa (Burkina Faso, Cote d'Ivoire, Mali and Togo) and in two countries in Southeast Asia (Thailand and Vietnam).

Material and methods

This study was designed as a complementary investigation of a previously conducted study from December 2015 to November 2016, in several African and Asian countries, including Burkina Faso (BF), Cote d'Ivoire (CI), Mali (ML) and Togo (TG) from West Africa, and Thailand (TH) and Vietnam (VN) from Southeast Asia.[7] In the previous study, we recruited 1153 adult ART initiators and analysed 1020 HIV-1 genomic sequences in the protease (PR) and reverse transcriptase (RT) regions. For the purpose of this study, we randomly selected 353 stored plasma samples from the previously collected 1153 samples, distributed per country as followed; BF ($n=26$), CI

Table 2. Frequency of HIV sequences presenting drug resistance mutations

	BF	CI	ML	TG	TH	VN	Overall
PI DRM	0% (0/17)	0% (0/53)	3.3% (1/30)	2.9% (2/69)	5.4% (4/74)	5.3% (4/76)	3.4% (11/319)
NRTI DRM	0% (0/17)	5.7% (3/53)	0% (0/31)	11.6% (8/69)	5.4% (4/74)	2.6% (2/77)	5.3% (17/321)
NNRTI DRM	0% (0/17)	9.4% (5/53)	3.2% (1/31)	18.8% (13/69)	8.1% (6/74)	7.8% (6/77)	9.7% (31/321)
INSTI DRM	0% (0/26)	0% (0/74)	6.3% (2/32)	1.4% (1/69)	1.3% (1/75)	0% (0/77)	1.1% (4/353)
NRTI, NNRTI, or INSTI DRM	0% (0/17)	11.3% (6/53)	9.7% (3/31)	26.1% (18/69)	14.9% (11/74)	9.1% (7/77)	14.0% (45/321)
NRTI + NNRTI DRM	0% (0/17)	3.8% (2/53)	0% (0/31)	4.3% (3/69)	0% (0/74)	1.3% (1/77)	1.9% (6/321)
NRTI + NNRTI + INSTI DRM	0% (0/17)	0% (0/53)	0% (0/31)	0% (0/69)	0% (0/74)	0% (0/77)	0% (0/321)
HIVDR % [95% CI]	0% [–/–] (0/17)	13.2% [6.3–26.0] (7/53)	13.3% [4.7–30.7] (4/30)	29.0% [19.4–40.9] (20/69)	20.3% [12.5–31.1] (15/74)	14.3% [8.0–24.2] (11/76)	17.9% [13.9–22.3] (57/319)

PI, protease inhibitor; DRM, drug resistance mutation; HIVDR, HIV drug resistance.

(*n* = 74), ML (*n* = 32), TG (*n* = 69), TH (*n* = 75) and VN (*n* = 77). All these samples were known as HIV-1 non-B positive samples and the viral genetic diversity was reported previously, and was predominantly represented by CRF02_AG and CRF01_AE strains in Africa and Asia, respectively.[7]

For all the 353 selected samples, we performed HIV drug resistance genotyping in the viral integrase (IN) region using the National Agency for AIDS Research (ANRS) in-house protocol. Details of this protocol including primer sequences and the polymerase chain reaction conditions are available online (https://hivfrenchresistance.org/wp-content/uploads/2022/10/amplification-sequencing-procedures-04102022.pdf). Relevant drug resistance mutations were identified using the Stanford algorithm, v.9.4 (available at https://hivdb.stanford.edu/hivdb/by-sequences/). Additional investigations included mutations interpretation with the ANRS algorithm for HIV-1, v.33, available at (https://hivfrenchresistance.org/wp-content/uploads/2022/10/algo-2022-V33.pdf). In the interpretation process, we considered not only mutations in the IN region, but also mutations in the already sequenced PR and RT regions to generate a comprehensive and updated overview of the PDR for drug classes targeting these regions; PR inhibitors (PIs), RT inhibitors (NRTIs and NNRTIs) and INSTI.

Ethics

The study protocol was approved by the Ethics Review Board or National Ethics Committee in each of the countries where it was implemented. Anonymous identifiers were used throughout the study to safeguard participant confidentiality.

Results and discussion

The characteristics of the participants included in this study are described in Table 1. Overall, female participants predominated in participants recruited in Africa, while most of those recruited in Asia were male, correlating with the major HIV transmission routes in the two regions: mostly heterosexually in Africa and intravenous drug users and MSM routes in Asia.[8] Most of the participants recruited in the African countries were initiating ART at lower CD4 levels, the median ranged between 102 and 206 cells/mm^3, compared to participants from Asian countries, where the median CD4 count ranged between 388 and 466 cells/mm^3. At the time these samples were collected, most of the study participants (up to 74%) were initiating or re-initiating a first-line ART including TDF/3TC/EFV, and almost 90% of the participants did not report previous exposure to ARVs (Table 1). Interpretable IN sequences were generated for all the 353 samples and among these samples, we previously generated PR (*n* = 319) and RT (*n* = 321) sequences.[7] Therefore, PR, RT and IN regions were fully available for 319 sequences.

Using both the Stanford and ANRS interpretation algorithms, the overall frequency of PDR was 17.9% (95% CI: 13.9%–22.3%), representing 57 out of 319 sequences assessed (Table 2), and was close to the frequency we reported on this sample set previously, but by assessing only PR and RT regions, that was 15.9% overall.[7] Although high, this overall PDR frequency is similar to data generated recently in many HIV treatment programmes from LMIC. Indeed, data from different programmes in Africa, the Americas, Southeast Asia and the

Western Pacific has confirmed significant increase of PDR over the last 10 years, especially to NNRTI drugs.[5] Many other recent studies have also confirmed this high level of resistant viruses among ART initiators in different settings in LMIC.[4,9] We also confirmed this important contribution of NNRTI resistant mutations in this study that reached 18.8% in sites such as Togo, with an overall frequency of 9.7% (Table 2): a frequency close to the warning threshold defined by WHO in 2017.[10] Considering the alarming evolution of this NNRTI PDR over recent years, WHO has recommended transitioning to INSTIs and the phase out of NNRTI regimens.[6] In this study, we aimed to assess the frequency of INSTI PDR, in addition to PI, NRTI and NNTI PDR, and we found a very low level of resistance to this drug class, 1.1% overall. This finding correlates with most reports conducted in LMIC to date.[9,11–14] Participants identified with INSTI mutations carried Q95K+ E157Q, G163R, G163K and R263K mutations. Q95K, E157K and G163R/K are mostly associated with first-generation integrase inhibitors, while R263K was the sole mutation identified with potential interactions with second-generation integrase inhibitors. Alone, this mutation reduces dolutegravir, bictegravir and carbotegravir susceptibility by about 2-fold.[15] Overall, these results indicate that baseline resistance to second-generation integrase inhibitors is still low in LMICs and should currently not represent a limitation for the ongoing scale-up of dolutegravir-based ART, or the introduction of other second-generation INSTIs such as carbotegravir and bictegravir.

Although we should recognize some limitations of this study —such as the sampling period, 2015–2016, and the fact that the number of sequences analysed was relatively low for some study sites—the results we report here are still of high importance, as the implementation of the dolutegravir-based ART strategy is still in the scaling-up phase in many LMICs. Also, it is currently estimated that up to 25 million people are receiving dolutegravir-based ART worldwide, stressing the need for robust future surveillance strategies to safeguard this drug class in contexts where treatment management and virological monitoring are still to be improved. Indeed, a recent study conducted in South Africa has predicted that, with the current management of ART in LMICs and the recent introduction of dolutegravir-based ART, the increase in NNRTI PDR will be halted, but dolutegravir PDR will increase over time and could reach 10% in 2040.[16]

The recent experience with NNRTI drugs and the current impressive levels of PDR and acquired drug resistance, regardless the low genetic barrier of this ARV class, should not be ignored and call for additional measures to limit virological failures and emergence of resistant viruses to better protect new ART strategies.

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Transparency declarations

The authors declare no conflicts of interest.

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