Insertion as a Resistance Mechanism Against Integrase Inhibitors in Several Retroviruses

TO THE EDITOR—We read with great interest a recent report by Le Hingrat and colleagues [1] of a new mechanism of human immunodeficiency virus (HIV)-2 resistance against integrase inhibitors through the development of 5 amino acid insertions within integrase coding sequences. Integrase inhibitors are currently the leading class for antiretroviral treatment initiation [2]. Insertions in the integrase of HIV-2 conferred various levels of resistance against integrase strand transfer inhibitors [1]. Insertions had been previously described in the context of HIV-1 drug resistance against protease and reverse transcriptase, but not integrase inhibitors [3]. To the best of our knowledge, there has not been any report of integrase insertion in HIV-1, which suggests that this type of genetic alteration is specific to HIV-2. Altogether, the findings by Le Hingrat and colleagues are very significant.

Intriguingly, this paper was not the first to report insertions in the integrase coding sequence of retroviruses (Table 1). The first report was made in a Simian Immunodeficiency Virus (rhesus

macaque) strain 251 (SIVmac251)-infected rhesus macaque unsuccessfully treated with a long-acting nano-suspension of cabotegravir, an integrase strand transfer inhibitor currently in advanced phases of development [4]. The insertion consisted of 5 amino acids after position 232 in the integrase of SIVmac251, and could not be introduced in either SIVmac239 or HIV-1 without severely impairing fitness [4]. More recently, we also reported the frequent emergence of various 5 or 6 amino acid insertions in the SIVmac251 integrase in rhesus macaques treated with dolutegravir monotherapy [5]. Integrase insertions in this setting were heterogeneous in sequence, but all contained 2 positively charged amino acids, and all were found in the immediate vicinity of arginine (R) 231, a residue that was shown by cryogenic electron microscopy (cryo-EM) to be the only one outside of integrase catalytic domain to interact with host target DNA strongly [6]. This specific residue is also involved in interactions with viral DNA. Based on structural data [6], it is thus expected that insertions that extend the length of the loop carrying residue R231, while at the same time increasing the local, positive charge, may bestow flexibility in DNA binding that could confer resistance against integrase

strand transfer inhibitors. In agreement with this hypothesis, all insertions reported to date also contained the small amino acid glycine (G), which could contribute to loop flexibility. Importantly, in our study, insertions could be found in circulating strains as well as proviruses, indicating that both replicative capacity and integration were conserved [5]. The replicative competence of retroviruses carrying integrase insertions is now confirmed by Le Hingrat et al's report of evolution from 2 to 5 amino acid insertions in 1 patient [1].

Finally, phylogenetic studies have concluded that HIV-2 is very closely related to the SIV strains found in *Sooty mangabeys* [7]. This genetic proximity may help to explain the evolutionary convergence in resistance via insertion between HIV-2 and SIVmac. For structural reasons [6], HIV-1 is less likely to become resistant via this pathway. However, Le Hingrat et al's results indicate that patients living with HIV-2 may need special clinical attention when using integrase inhibitors. Future studies will have to investigate the potential for the transmission of such resistant strains, a question that could be critical given the relatively high prevalence of insertions in Le Hingrat et al's report, compared to reverse transcriptase (RT) and protease (PR) insertions [3].

Note

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Table 1. Summary of Integrase Insertions Associated With Resistance Against Integrase Strand Transfer Inhibitors

Amino Acid Insertion	Position (-1)	Organism	Reference
GK	231	HIV-2	Le Hingrat et al [1]
SREGK/K			
YREGR			
GYKGK			
GYRGR			
YREGR	232	SIVmac251	Andrews et al [4]
EGRGIK	228	SIVmac251	Van Rompay et al [5]
EGRYK	228		
SREGK	234		
EGRYK	235		
GKGR	235		

Positively charged amino acids (at neutral pH) are indicated in bold.

Abbreviations: HIV, human immunodeficiency virus; SIVmac251, simian immunodeficiency virus (rhesus macaque) strain 251.

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Lack of Convincing Evidence of Artemisinin Resistance in India

TO THE EDITOR—We read the article by Das et al [1] with interest. However, we are concerned by several misinterpretations potentially leading to inaccurate conclusions.

The presence of malaria parasites at day 3 without fever should not be classified as early treatment failure. The use of day 3 positivity alone to define early treatment failure means that Kaplan-Meier probability estimates for cure and failure rates were probably overestimated. Furthermore, early treatment failures do not provide the evidence needed to confirm presence of artemisinin resistance.

Confirmed partial artemisinin resistance is defined as $\geq 5\%$ of patients

carrying *PfKelch13* resistance-validated mutations, all of whom have been found, after treatment with artemisinin-based combination therapy or artesunate monotherapy, to have either persistent parasitemia by microscopy on day 3, or a parasite clearance half-life of > 5 hours [2] (note that this threshold was recently suggested changed by the WorldWide Antimalarial Resistance network to > 5.5 hours [3]). Currently, 9 *PfKelch13* mutations are validated. The G625R mutation identified by Das et al is yet neither a validated nor a candidate marker for artemisinin partial resistance [4]. In vitro resistance assessed by ring-stage survival assay (RSA)_{0–3h} is defined by persistence of a parasite survival rate $> 1\%$ [2] (and not 10% as used in a previous version of the article and in another publication of this study [5]).

The data contrast with other available data on drug efficacy and resistance in India. Artesunate-sulfadoxine-pyrimethamine efficacy (outside the northeastern states of India) is on average 99.3% (ranges 93.8–100%) between 2010 and 2017 (number of studies = 52) and 100% in West Bengal in 2014 (number of patients = 55) [6]. *PfKelch13* mutations are rare in India without any clonal expansion. Among 832 Indian isolates collected 2014–2016, 1.4% were found to have *PfKelch13* mutations. Globally G625R has only been described in one isolate in Gabon [6]. In the Greater Mekong subregion where artemisinin partial resistance is almost fixed, early treatment failures are exceptionally reported and artemisinin partial resistance does not lead to treatment failure in absence of partner drug resistance.

Because parasites are culture adapted, external quality control should be conducted for in vitro RSA_{0–3h} and *PfKelch13* sequencing done by a reference laboratory. Data must be reanalyzed using corrected definitions and thresholds, and the slope calculated with different software by an independent entity. Whole genome sequencing is required to analyze the genetic background of these parasites to confirm potential spread of R539T

mutants from Cambodia. It is surprising that R539T is proposed to have spread, because R539T was mostly replaced by C580Y in Cambodia in 2014 (maximum prevalence of R539T was 2.1% in 11 Cambodian studies in 2014 compared to 37.5% in 13 studies in 2012 [6]).

We agree that G625R could be a potential new marker but before claiming emergence of artemisinin resistance in India more information is needed about this mutation in particular transfection studies with additional in vitro RSA_{0–3h}. Surveillance alone will not stop the spread of resistance, but surveillance is crucial to inform appropriate action. It is critical that information is shared as soon as data indicate a potential resistance problem and that data and samples are made available for external quality control.

Notes

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