Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother 2017; **72**: 1546–1550
doi:10.1093/jac/dkx014
Advance Access publication 31 January 2017

Comment on: Integrase strand-transfer inhibitor polymorphic and accessory resistance substitutions in patients with acute/recent HIV infection

Claudia Fabrizio*†, Luciana Lepore†, Antonella Lagioia, Grazia Punzi, Annalisa Saracino, Gioacchino Angarano and Laura Monno

Clinic of Infectious Diseases, University of Bari, Piazza Giulio Cesare 11, Bari, Italy

*Corresponding author. Tel: +39-080-5592473; Fax +39-080-5478333; E-mail: claudia.fabrizio.82@gmail.com
†C. Fabrizio and L. Lepore contributed equally to the work.

Sir,

We read with interest the paper of Ambrosioni *et al.*[1] in naive patients with recent HIV infection, the authors reported a 13.9% prevalence of integrase strand-transfer inhibitor (INSTI) polymorphisms or substitutions (E157Q and Q95K), conferring low-level resistance to raltegravir and elvitegravir, by using high-throughput sequencing. Pre-treatment E157Q has also been reported as the only INSTI mutation responsible for non-virological response in a patient receiving a dolutegravir-based regimen,[2] thereby reinforcing the need for genotypic analysis encompassing the integrase (IN) gene in all naive patients.

According to current guidelines,[3] plasma samples coincident with the first HIV-positive testing of patients referring to our clinic are regularly subjected to population sequencing for evaluation of primary resistance to NRTIs, NNRTIs and PIs. Since 2009, sequences including IN, gp41 and gp120 have also been obtained. At present, IN sequences from 370 consecutive patients with new HIV diagnosis are available. Among these patients, we retrospectively selected those with acute/recent HIV-1 infection (detectable HIV-1-RNA in plasma in the setting of a negative/indeterminate HIV-antibody test; $\leq$0.2% nucleotide ambiguity of reverse transcriptase and protease regions,[4] respectively) diagnosed between January 2015 and June 2016, to mimic the same conditions as those of Ambrosioni *et al.*,[1] although by means of slightly different selection criteria. The WHO[5] 2009 SDRM and the IAS lists (2015) were used to identify transmitted drug resistance-associated mutations for NRTIs/NNRTIs/PIs and relevant INSTI and fusion inhibitor mutations, respectively. IN substitutions/polymorphisms with $\geq$10 points according to the HIV Drug Resistance Database of Stanford University (https://hivdb.stanford.edu/) were also recorded. HIV-1 tropism was inferred with the geno2pheno algorithm (false-positive rate 10%). Epidemiological and clinical laboratory data were retrieved from our database. According to local regulations, approval of the Ethics Committee was not required due to the retrospective nature of the study.

Thirty naive patients (86.7% males; 96.7% Italians; 56.7% MSM; median age 32 years) were included, eight (26.7%) with an acute HIV infection. At diagnosis, the median HIV-RNA was 40 500 copies/mL; the median absolute CD4+ count and percentage were 501 cells/mm^3 and 26%, respectively. A subtype B was attributed to 83.3% of clinical strains; most patients (73.3%) were infected with R5-tropic HIV-1.

Overall, four patients (13.3%) had the E157Q (three patients) and T97A (one patient) IN mutations; the latter patient also had the unusual N155Y mutation (at this position N155H reduces raltegravir susceptibility >10-fold and elvitegravir susceptibility >30-fold; while N155S/T have somewhat less effect on raltegravir/elvitegravir susceptibility).[6] Three additional patients had L74I that, combined with primary INSTI-resistance mutations, appears to contribute to reducing susceptibility to INSTIs, including dolutegravir.[7]

Substitutions/polymorphisms in regions different from the integrase were observed in an additional seven patients; in particular, two epidemiologically unrelated subjects had a similar pattern of multiple NRTI mutations and a third person had the revertant T215S. Interestingly, two further patients with acute HIV infection had, respectively, the Q40H and V38E mutations for enfuvirtide. Finally, E138A and V108I for NNRTI resistance were detected in two additional subjects (Table 1). No clinical or virological variable (risk factor for HIV-1 transmission, acute infection, subtype, tropism, median CD4 count and viral load) appeared to correlate with the occurrence of INSTI mutations.

Our data confirm the observations of Ambrosioni *et al.*[1] describing a higher than previously reported[8] frequency of INSTI substitutions/polymorphisms in a recent and relatively short period; in fact, we obtained a similar percentage despite using Sanger sequencing, with a detection threshold corresponding to a variant population frequency of ~20%.

Moreover, one seroconverter with a CRF02_AG HIV strain had the N155Y mutation, which, although not associated *per se* with reduced INSTI susceptibility, could evolve into the major N155H through a single nucleotide transition rather than a transversion. When analysing 215 IN sequences downloaded from the Los Alamos database (query: CRF02_AG subtype, sample data before 2007), an Asn155 was always found (https://www.hiv.lanl.gov/; data not shown). Overall, these data must warn about the possibility of an incoming wave of virological failure with first-line INSTIs.

The detection in four patients of transmitted drug resistance-associated mutations for NRTIs and signature mutations for enfuvirtide confirms the high stability of certain mutations[9] (even in the absence of drug pressure and fitness advantages). In fact, it seems highly improbable that enfuvirtide mutations were transmitted

Table 1. Mutational patterns in patients with acute/recent HIV infection (2015–16)

Patient	HIV-1 subtype	Co-receptor tropism (false-positive rate 10%)	Acute HIV-1 infection	NRTIs	NNRTIs	PIs	Fusion inhibitors	INSTIs
1	CRF02_AG	R5	yes	–	–	–	–	T97A, N155Y
2	B	R5	no	–	–	–	–	E157Q
3	B	R5	yes	–	–	–	–	E157Q
4	B	R5	no	–	–	–	–	E157Q
5	B	R5	no	D67NS, T69D, L210W, T215S	–	–	–	–
6	B	R5	yes	–	–	–	Q40H	–
7	B	R5	no	D67N, T69D, L210W, T215S	–	–	–	–
8	CRF60_BC	R5	yes	–	–	–	V38E	–
9	B	R5	no	–	E138A	–	–	–
10	B	R5	no	T215S	–	–	–	–
11	G	R5	no	–	V108I	–	–	–

from individuals still receiving this drug, which is no longer in use. Similarly, the NRTI mutation patterns detected in our patients evoke almost outdated drugs; conversely, we never found M184V, which has been positively correlated with HIV-RNA levels[10] of patients treated with drugs in current regimens.

Overall, these observations indicate that nowadays non-responding patients cannot be considered the unique origin of transmitted drug resistance; instead, we are convinced that we are facing an unexpected scenario, in which young, newly infected patients, mainly MSM, represent an unaware source of HIV-1 strains, continuously evolving during transmission, with drug susceptibility profiles that may become unpredictable.

Funding

This study was partly supported by PRIN (2012L783TW_003).

Transparency declarations

None to declare.

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J Antimicrob Chemother 2017
doi:10.1093/jac/dkx071
Advance Access publication 7 March 2017

Integrase strand-transfer inhibitor polymorphic and accessory resistance substitutions in patients with acute/recent HIV infection—authors' response

Juan Ambrosioni[1]*, María M. Mosquera[2] and José M. Miró[1]

[1]Infectious Diseases, Hospital Clinic-IDIBAPS, Barcelona, Spain; [2]ISGlobal Institute for Global Health, Microbiology Department, Hospital Clinic-IDIBAPS, Barcelona, Spain

*Corresponding author. E-mail: jambrosioni@intramed.net

Sir,

We thank Fabrizio *et al*.[1] for their interest in our paper,[2] recently published in the *Journal of Antimicrobial Chemotherapy*, and for their valuable comments.