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Susceptibility to HIV-1 integrase inhibitors in HIV-1 sub-subtype A6 isolates

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Introduction/Summary

- Regimens based on second generation integrase inhibitors (INSTIs) such as dolutegravir (DTG) and bictegravir (BIC) are currently recommended as the preferred first-line HIV-1 therapy.
- Considering the recent introduction of second generation INSTIs in the Russian Federation, this study aimed to evaluate the natural susceptibility of INSTIs in viral strains belonging to sub-subtype A6, which is the most prevalent genetic variant circulating in Russia and surrounding countries.

Materials and methods

- The identification of plasma samples harboring viral strains belonging to sub-subtype A6 was carried out through phylogenetic analysis of HIV-1 sequences generated during routine HIV-1 drug resistance testing at the HIV monitoring laboratory in Siena, Italy. Viral sequences assigned to subtype A according to sequence homology were aligned with representative sequences of each subtype including A6 retrieved from the HIV Database of the Los Alamos National Laboratory.
- Phylogenetic analysis was performed with Mega 7 software.
- Plasma samples harboring viral strains assigned to sub-subtype A6 were used for the generation of NL4-3 based recombinant viruses carrying patient derived integrase coding region.
- *In vitro* susceptibility to the INSTIs raltegravir (RAL), DTG, BIC and cabotegravir (CAB) was determined through a TZM-bl cell line based phenotypic assay as described previously[1] and fold-change (FC) values were calculated with respect to the IC_{50} value obtained with the wild-type NL4-3 strain.

Results – 1

- Twenty out of eighty-one (24.7%) viral sequences originally labelled as subtype A were assigned to sub-subtype A6 (**figure 1**). Residual plasma available in eight cases was successfully used for the construction of recombinant viruses in addition to two and five A6 samples received from the Institute of Virology of Cologne and Gamaleya Center of Moscow, respectively.

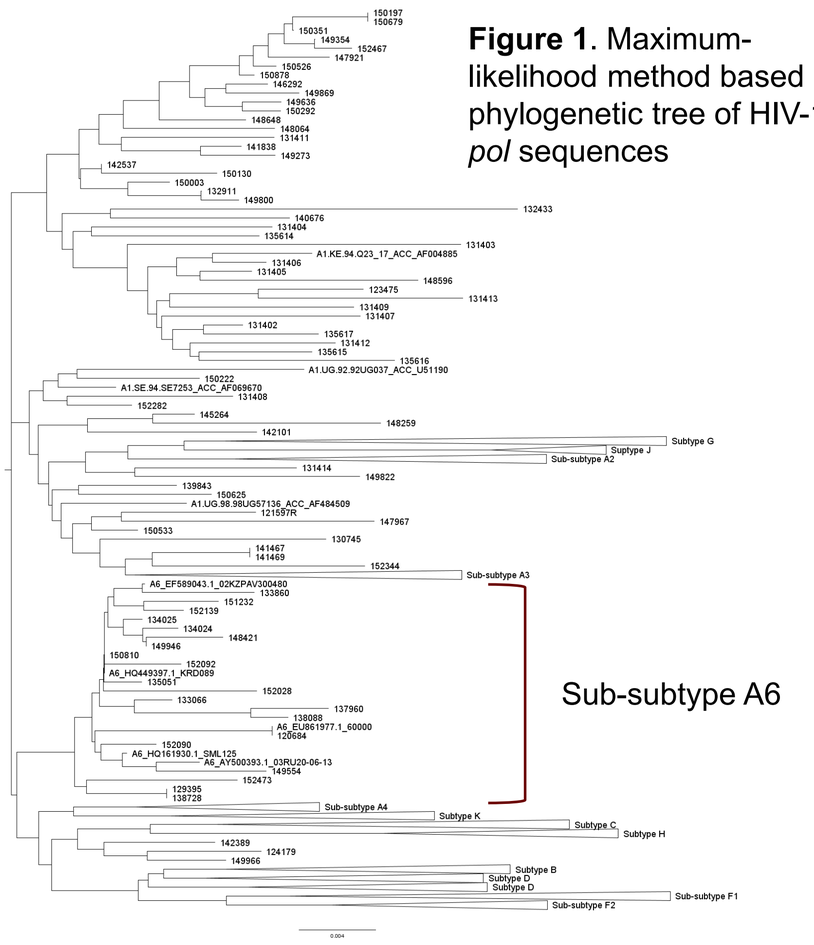

Figure 1. Maximum-likelihood method based phylogenetic tree of HIV-1 *pol* sequences

Sub-subtype A6

Results – 2

- None of the fifteen A6 sequences harbored major INSTIs RAMs while 14/15 (93%) sequences harbored the L74I variant, which is the consensus aminoacid in subtype A and found to be weakly selected in patient under INSTI therapy with no impact on INSTIs susceptibility when alone (**figure 2**).

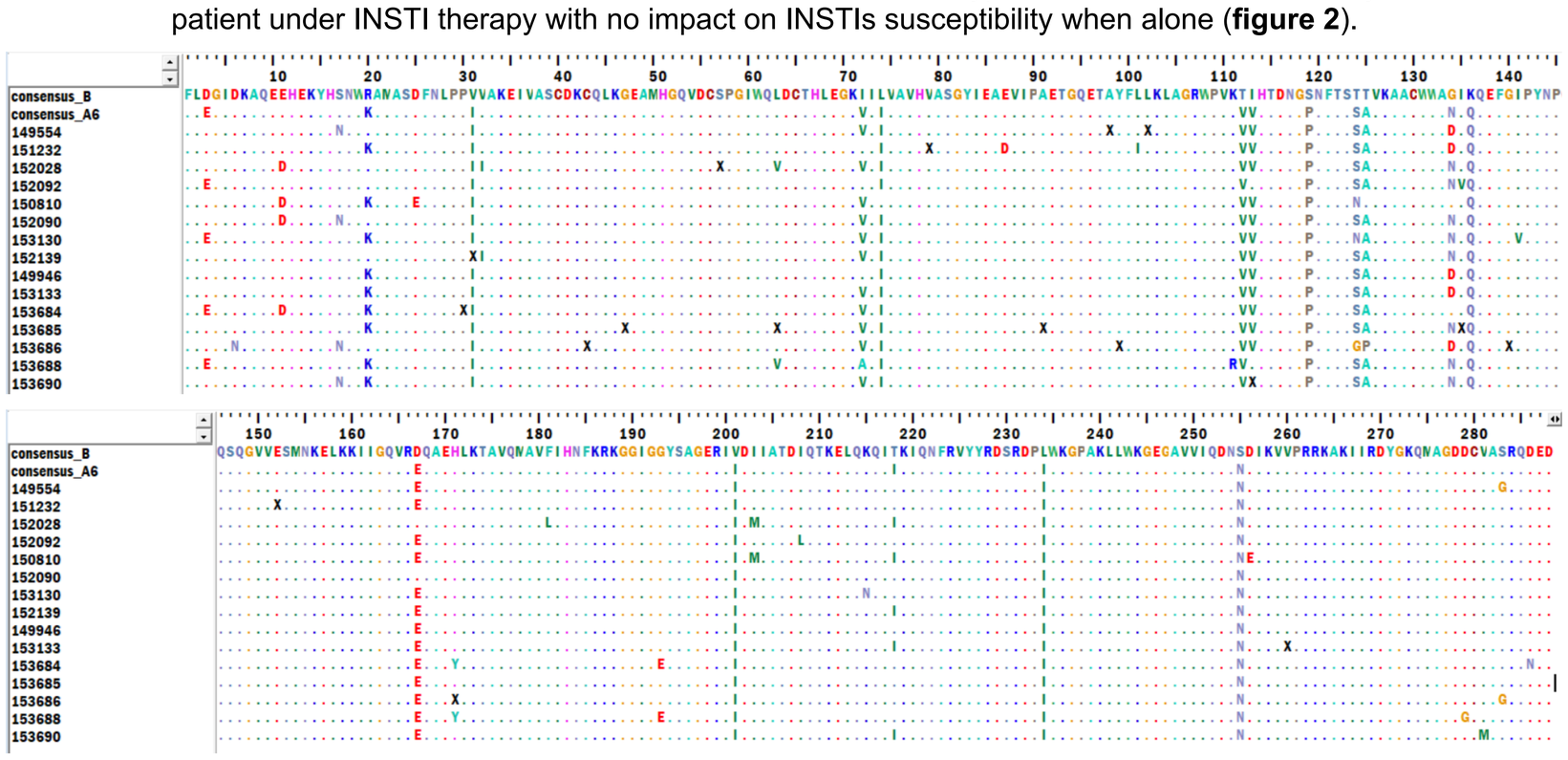

Figure 2. Alignment of aminoacidic sequences of integrase coding region from 15 A6 sub-subtype samples. "X" indicates mixed viral populations.

Results – 3

Median FC values for RAL, DTG, BIC and CAB were 1.2 (IQR 1.0-1.7), 1.1 (IQR 0.9-1.4), 0.8 (IQR 0.7-1.1), and 1.0 (IQR 0.6-1.2), respectively. According to the available biological or clinical FC cut-offs established by the reference Phenosense Assay, all FC values calculated for RAL and DTG were below the FC threshold associated with reduced susceptibility.

Table 1 **Mean half maximal inhibitory concentration (IC50) values calculated with A6 recombinant viruses against INSTI raltegravir (RAL), dolutegravir (DTG), bictegravir (BIC) and cabotegravir (CAB). Fold-change (FC) values are calculated as compared to the IC_{50} value calculated with the HIV-1 wild-type strain NL4-3.**

	RAL		DTG		BIC		CAB	
ID	**IC_{50} ± SD nM**	**FC**	**IC_{50} ± SD nM**	**FC**	**IC_{50} ± SD nM**	**FC**	**IC_{50} ± SD nM**	**FC**
149554	6.6 ± 1.0	1.1	1.6 ± 0.4	1.4	1.6 ± 1.0	1.0	0.4 ± 0.3	0.4
151232	7.6 ± 3.2	1.2	1.4 ± 0.6	1.2	1.6 ± 1.4	0.9	1.4 ± 0.7	1.4
152028	4.4 ± 1.7	0.7	1.8 ± 0.8	1.5	0.8 ± 0.2	0.5	1.4 ± 0.7	1.4
152092	12.6 ± 5.4	**2.1**	0.9 ± 0.3	0.8	2.1 ± 0.6	1.2	0.6 ± 0.2	0.6
150810	12.1 ± 3.8	**2.0**	1.8 ± 0.4	1.5	1.2 ± 0.7	0.7	1.2 ± 1.1	1.2
152090	11.1 ± 1.8	**1.8**	1.7 ± 0.2	1.4	1.1 ± 0.5	0.6	1.2 ± 0.6	1.2
153130	9.9 ± 4.8	**1.6**	1.0 ± 0.3	0.9	4.0 ± 1.7	2.4	1.1 ± 0.5	1.1
152139	7.8 ± 3.0	1.3	1.0 ± 0.1	0.8	2.6 ± 1.0	1.5	1.0 ± 0.5	1.0
149946	9.6 ± 5.1	**1.6**	0.7 ± 0.3	0.6	0.7 ± 0.4	0.4	1.1 ± 0.4	1.1
153133	10.3 ± 5.8	**1.7**	1.3 ± 0.4	1.1	1.3 ± 0.1	0.7	1.4 ± 0.2	1.4
153684	6.5 ±1.3	1.1	1.3 ± 0.6	1.1	1.8 ±0.3	1.1	0.9 ± 0.3	0.9
153685	5.6±2.8	0.9	2.5 ± 0.4	2.1	1.9 ± 0.2	1.1	1.0 ± 0.2	1.0
153686	6.3±2.8	1.0	1.3 ± 0.5	1.1	1.2 ± 0.4	0.7	0.8 ± 0.2	0.8
153688	7.2±3.9	1.2	1.1 ± 0.4	0.9	1.1 ± 0.3	0.7	0.4 ± 0.2	0.4
153690	3.3±1.7	0.5	1.2 ± 0.4	1.0	1.3 ± 0.6	0.8	0.6 ± 0.3	0.6

In bold FC above the biological or clinical cut-offs (RAL 1.5 – biological; DTG lower 4, upper 13 – clinical; BIC lower 2.5, upper 10 – clinical, estimated; CAB not available)

Conclusion

- Re-analysis of viral sequences originally assigned to subtype A revealed the presence of A6 sub-subtype strains in nearly 25% of cases.
- The A6 sequences considered in this study did not include major INSTI RAMs, while all but one sequences include the L74I variant, which is the consensus aminoacid in A6 subtype.
- All the recombinant viruses showed FC values indicating full susceptibility to RAL, DTG, BIC and CAB, except for 6 viruses with FC values associated with a possible minimal reduction in RAL susceptibility.
- Based on this panel of recombinant viruses, phenotypic susceptibility to INSTI in sub-subtype A6 does not appear to differ from that of the reference subtype B NL4-3 virus. Further analysis are required to test the genetic barrier to resistance to INSTI and the role of A6 genetic background outside the integrase coding region to conclude that sub-subtype A6 does not increase the risk of failure of INSTI based therapy.

Reference

1. Saladini F et al. *J Clin Lab Anal*. 2018.

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