The Genetic Barrier to Resistance to LEN Is Not Affected by Non-B Subtypes or Treatment Exposure

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BACKGROUND

Lenacapavir (LEN) is a novel, first-in-class, multistep HIV-1 capsid inhibitor approved for the treatment of heavily-treatment experienced (HTE) people with HIV (PWH). *In vitro* and *in vivo* resistance mutations were identified at position 56, 66, 67, 70, 74, 105 and 107 of the p24 coding region. To further define LEN resistance profile, we evaluated the LEN susceptibility and the genetic barrier to resistance in B and non-B subtypes derived from HTE and treatment naïve (TN) PWH.

METHODS

Thirty-one NL4-3 based recombinant viruses harbouring clinically derived GAG-PR region were generated from plasma samples collected from TN (n=20) and HTE (n=11) PWH enrolled in the Italian PRESTIGIO registry (https://registroprestigio.org/), which includes PWH experiencing resistance to the four main antiretroviral classes (**Fig. 1**).

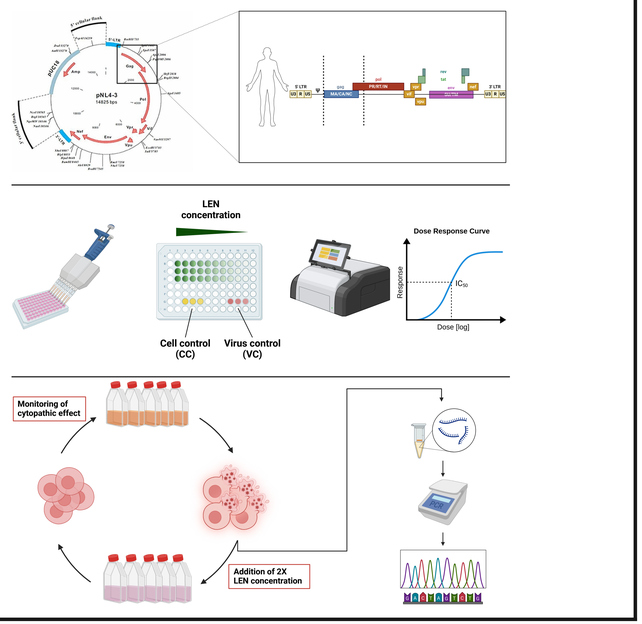
LEN half-maximal inhibitory concentration (IC_{50}) was measured for each recombinant virus through a TZM-bl cell line-based luciferase assay. Fold-change (FC) susceptibility values were calculated with respect to the IC_{50} value of the wild type NL4-3 strain (**Fig. 2**).

In vitro resistance selection (IVRS) experiments were performed by infecting MT-2 cells with NL4-3 or recombinant viruses in presence of increasing concentrations of LEN. Cultures were stopped when viral breakthrough was observed at approximately 100X LEN IC_{50} (2.56 nM) or after 105 days from the start of IVRS. Sanger sequencing of the p24 coding region was performed at each viral breakthrough to detect emerging mutations (**Fig. 3**)

Generation of recombinant viruses *(Figure 1)*

Determination of phenotypic susceptibility to LEN *(Figure 2)*

***In vitro* resistance selection workflow** *(Figure 3)*



The *in vitro* **genetic barrier to resistance to LEN** was **generally low** but not affected by long-time exposure to antiretroviral therapy. Viruses harbouring non-B GAG-PR showed delayed breakthrough compared to entirely subtype B viruses.

RESULTS

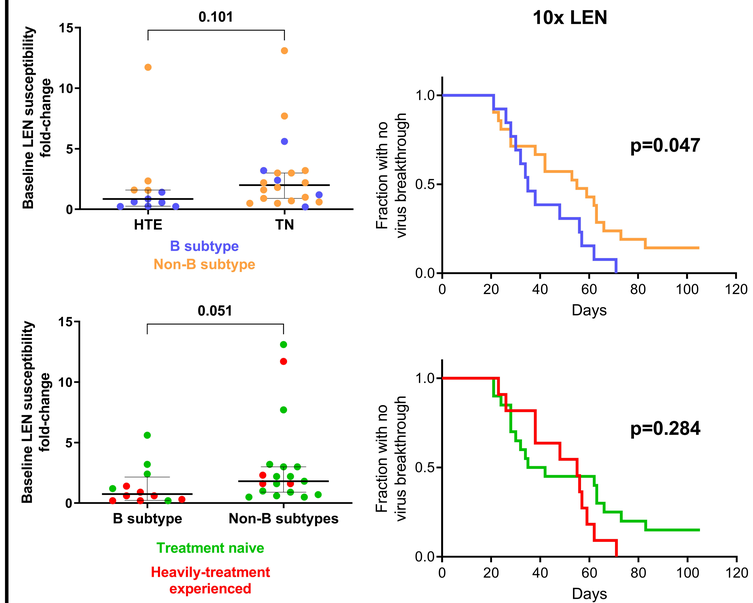


Figure 4

Figure 5

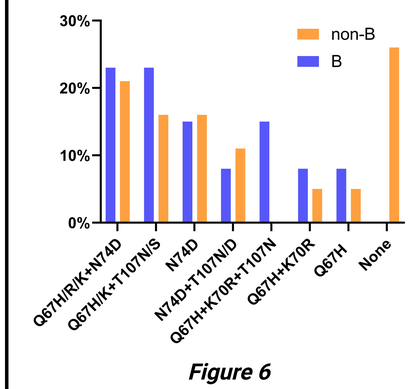


Figure 6

None of the viruses harboured known LEN resistance mutations.

Baseline susceptibility to LEN was comparable between HTE vs. TN (median FC 0.9 [IQR 0.3-1.6] vs. 2.0 [0.8-3.2], p=0.101, Mann-Whitney test) and between B (n=12) vs. non-B (n=19; 3 each F1 and CRF02_AG, 2 each C, D, CRF01_AE and CRF06_cpx, 1 each A1, A6, G, CRF40_BF, URF D/B) subtypes (median FC 0.75 [0.2-2.2] vs. 1.8 [0.9-3.0], p=0.0514) (**Fig. 4**).

By Kaplan-Meier survival analysis, the time to viral breakthrough was comparable among HTE vs. TN PWH but not B vs. non-B subtypes at approximately 10X (p=0.284 and p=0.047, respectively, log-rank test) and 100X LEN IC_{50} (p=0.131 and p=0.026, respectively), with lower time to viral breakthrough observed with B viruses (**Fig. 5**).

Known LEN resistance mutations emerged in 27/32 cultures including Q67H/R/K+N74D (n=7), Q67H/K+T107N/S (n=6), N74D (n=5), N74D +T107N/D (n=3), Q67H +K70R+T107N (n=2), Q67H+K70R (n=2), and Q67H (n=2), while in 5 cases no emergent mutations were identified.

Non-polymorphic substitutions F169L (with Q67H+T107TN), V86M (with Q67H +K70R) and E213D (with Q67H+T107N) were detected in three distinct cases each (**Fig. 6**).

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PRESTIGIO

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