Accumulation of HIV-1 Env Mutations Leads to High-Level Resistance to Dolutegravir

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Background

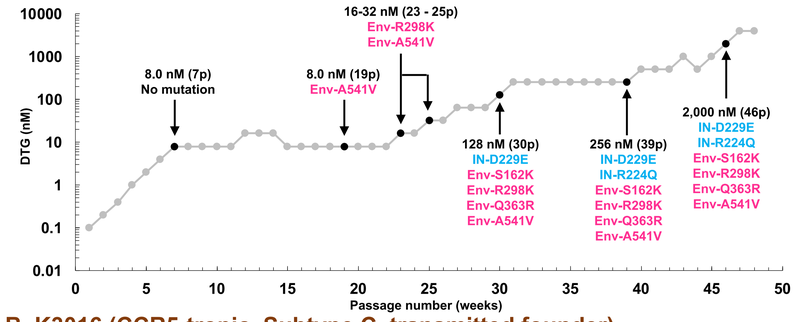
We recently reported that mutations in the HIV-1 envelope glycoprotein (Env) can broadly reduce viral susceptibility to ARVs by enhancing virus to spread in vitro via cell-cell transfer. We also identified multiple Env mutations in participants failing an integrase (IN) strand transfer inhibitor (INSTI)-containing regimen (ACTG study A5273) in the absence of resistance mutations in IN. The aim of the current study was to examine whether HIV-1 can develop high-level resistance to the INSTI dolutegravir (DTG).

Methods

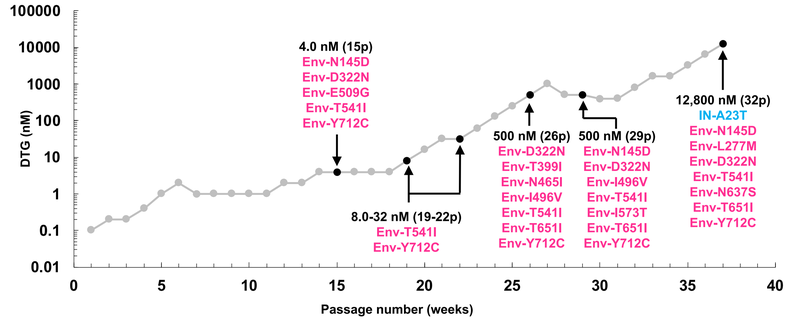
In vitro selection experiments using the SupT1 T-cell line and several HIV-1 strains [subtype B NL4-3 and NL(AD8); and subtype C transmitted founder K3016] were performed over nearly one year with increasing concentrations of DTG (0.1 – 2,000 nM). Sequence analysis of IN/Env-coding regions was performed longitudinally. The env amplicons from virus replicating at 256 nM DTG were cloned into NL4-3 and the replication kinetics and cell-free infectivity were examined.

Result 1: Long-term passaging of HIV-1 in the presence of DTG

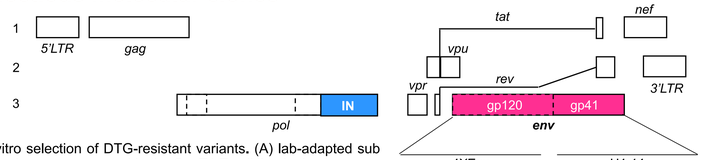
A. NL4-3 (CXCR4-tropic, Subtype B, lab-adapted strain)



B. K3016 (CCR5-tropic, Subtype C, transmitted founder)



C. Infectious molecular clones



4XEnv	
NL4-3 (256 nM, 39p)	
V85A	gp120 C1
S162K	gp120 V2
R298K	gp120 V3
Q363R	gp120 C3
A541V	gp41 HR1
V693I	gp41 TM
G825E	gp41 CT

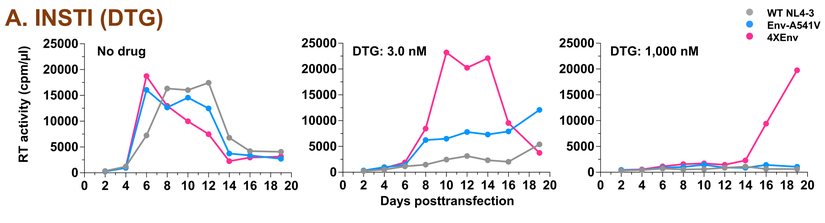
H4-14	
K3016 (500 nM, 29p)	
N145D	gp120 V1
D322N	gp120 V3
T541I	gp41 HR1
S619N	gp41 DSL
T651I	gp41 HR2
Y712C	gp41 CT

Fig.1. In vitro selection of DTG-resistant variants. (A) lab-adapted sub B NL4-3 strain was propagated in the SupT1 T-cell line in the presence of increasing concentrations of DTG. Each viral passage was done in a cell-free manner. At the time point indicated by the arrow, genomic DNA was extracted from the DTG-treated culture and the IN- and Env-coding regions were sequenced by Sanger sequencing. (B) transmitted-founder sub C K3016 was propagated in SupT1R5 cells in the presence of DTG. (C) Env-coding regions at the indicated time point were cloned into the parental strain. 4XEnv and H4-14 are infectious molecular clones.

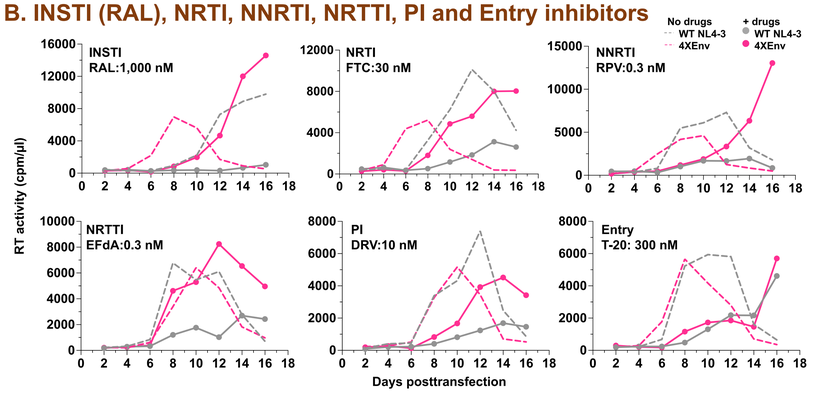
- HIV-1 accumulated multiple Env mutations under the pressure of DTG.
- Clones with heavily mutated Env exhibit **high-level resistance to DTG.**
- The Env mutations reduce cell-free infectivity, but enhance the efficiency of **cell-cell transfer.**
- 4XEnv alters sensitivity to NAbs

Result 2: Spreading Infection of clones with heavily mutated NL4-3 Env

A. INSTI (DTG)

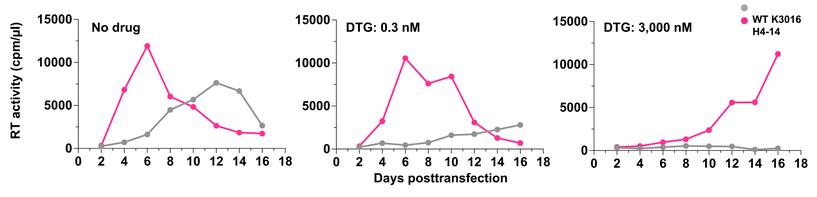


B. INSTI (RAL), NRTI, NNRTI, NRTTI, PI and Entry inhibitors



Result 3: Spreading Infection of a clone with heavily mutated K3016 Env

A. INSTI (DTG)



B. INSTI (RAL) and NNRTI (RPV)

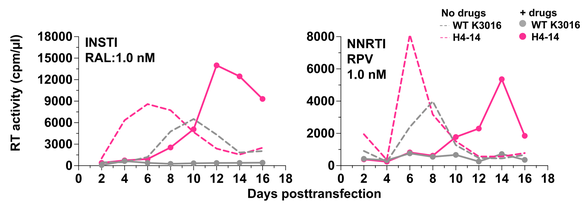
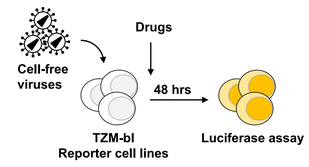


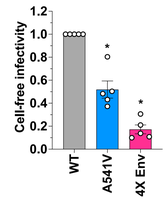
Fig. 2 and 3. SupT1 cells (SupT1R5 cells for K3016 variants) were transfected with the indicated proviral clones in the presence of the indicated concentrations of INSTI, non-nucleoside/nucleoside reverse transcriptase inhibitor (NNRTI/NRTI), protease inhibitor (PI) and entry inhibitor (Ent-Is).

Result 4: Cell-free viral infectivity of 4XEnv

A. Infectivity assay



B. infectivity



C. DTG sensitivity

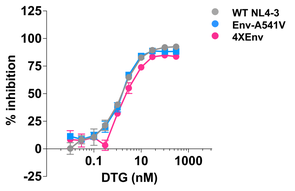
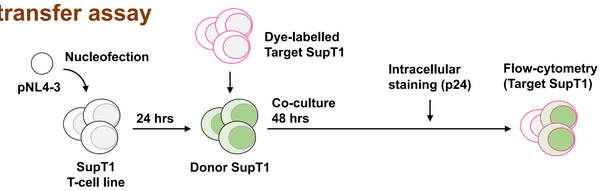


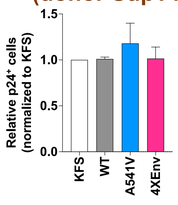
Fig. 4. (A) Overview of infectivity assay using TZM-bl reporter cell line. Virus stocks were normalized for RT activity and used to infect TZM-bl cells to measure cell-free infectivity. (B) Cell-free infectivity in the absence of DTG. (C) DTG sensitivity of cell-free virus. TZM-bl cells were exposed to 100 $TCID_{50}$ of the indicated virus. Data are shown as mean ± SEM. Statistical significance was assessed by one-way ANOVA and Tukey's multiple-comparison test.

Result 5: Cell-cell transmission of 4XEnv

A. Cell-cell transfer assay



B. p24 expression (donor SupT1)



C. p24 expression (target SupT1)

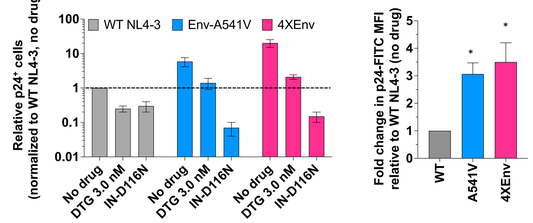


Fig. 5. (A) Overview of cell-cell transfer assay. Donor SupT1 cells are nucleofected with proviral pNL4-3 variants 24 hrs prior to the assay. Target SupT1 are labeled with cell proliferation dye. The donor and the target SupT1 are co-cultured at 1:1 ratio in the absence or presence of DTG. 48 hrs after co-culture, intracellular p24 antigen is stained with FITC-conjugated Ab. (B) Fold change in p24 expression in the donor SupT1 24 hrs after nucleofection. (C) Fold change in p24-positive population (left) and median fluorescence intensity (MFI) (right) in the absence of DTG, respectively. Gating is based on pNL4-3 KFS (Δenv). A catalytically inactive IN mutant (IN-D116N) does not show cell-cell transfer. Data are shown as mean ± SEM. Statistical significance was assessed by one-way ANOVA and Tukey's multiple-comparison test.

Result 6: Neutralization sensitivity of 4XEnv to sCD4 and NAbs

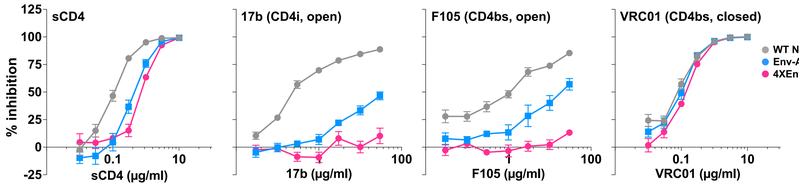


Fig. 6. Sensitivity of NL4-3 Env mutants to neutralizing antibodies (NAbs) recognizing different Env conformation and soluble CD4 (sCD4). TZM-bl cells were exposed to 100 $TCID_{50}$ of indicated viruses in the presence of various concentrations of NAbs. Luciferase activity was measured at 48 hrs postinfection. Data from three independent experiments are shown as means ± SEMs.

Conclusion

High-level DTG resistance in culture can arise via the accumulation of multiple Env mutations. These findings advance the understanding of how HIV-1 can evolve resistance to antiretrovirals including the potent INSTI DTG in the absence of mutations in genes targeted by the drug.

Acknowledgement

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