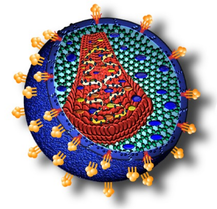
HIGH-LEVEL RESISTANCE TO INTEGRASE INHIBITORS CONFERRED BY MUTATIONS OUTSIDE INTEGRASE

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Background

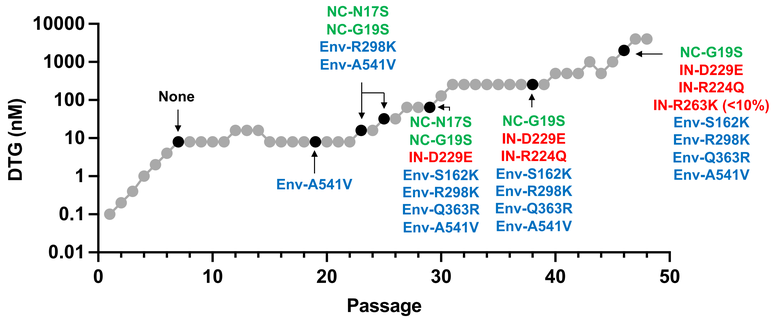
We recently reported that mutations in the HIV-1 envelope glycoprotein (Env) can broadly reduce viral susceptibility to ARVs by enhancing virus to spread in vitro via cell-cell transfer. We also identified multiple Env mutations in participants failing an integrase (IN) strand transfer inhibitor (INSTI)-containing 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

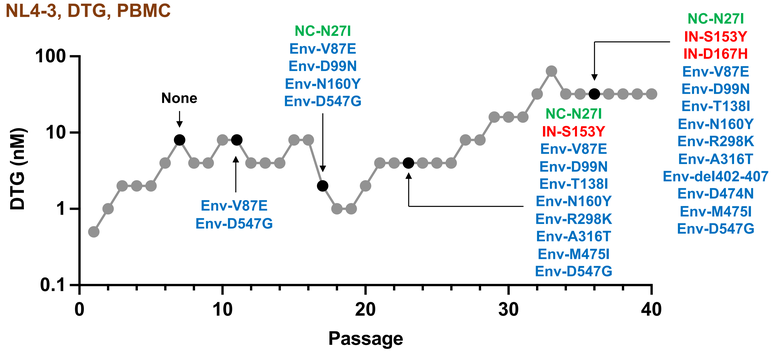
In vitro selection experiments using the SupT1 T-cell line and several HIV-1 strains [subtype B NL4-3 and NL(AD8); and subtype C transmitted founder K3016] were performed over nearly one year with increasing concentrations of DTG (0.1 – 2,000 nM). Sequence analysis of IN/Env-coding regions was performed 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, DTG, SupT1 T-cell line



B. NL4-3, DTG, PBMC



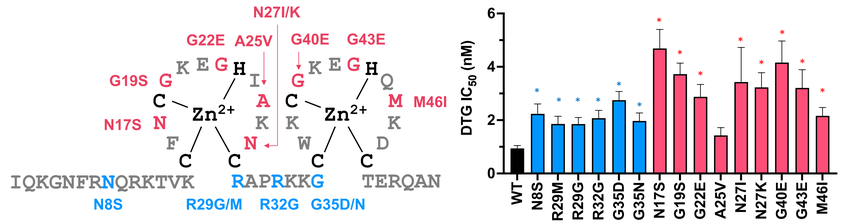
In vitro selection of DTG-resistant variants in (A) SupT1 and (B) PBMC. Lab-adapted subtype B NL4-3 strain was propagated in the SupT1 T-cell line or PBMC in the presence of increasing concentrations of DTG. At the time point indicated by the arrow, genomic DNA was extracted from infected cells, and the Gag, Pol- and Env-coding regions were sequenced.

Reference

Van Duyne et al., PNAS, 2019
Hikichi et al., mBio, 2021
Hikichi et al., Sci. Adv. 2024

- HIV-1 sequentially acquire mutations in Env, NC, and IN in the presence of DTG.
- NC mutations reduce sensitivity to INSTIs.
- Env mutations confer resistance to INSTIs by increasing infection capacity through cell-free infection or cell-cell transmission.
- **Multiple regions in the HIV-1 genome (Env, NC, and IN) collectively contribute to INSTI resistance**

Result 2: NC mutations reduce sensitivity to INSTIs



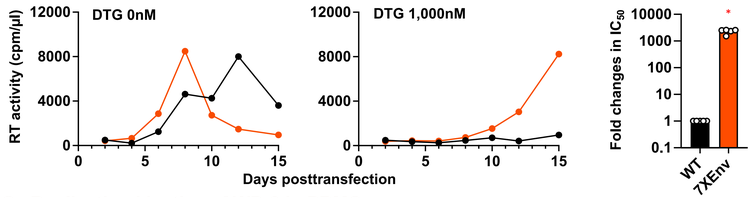
(Left) The NC mutations selected in the presence of DTG were highlighted in red (within the zinc-finger domain) or blue (outside the zinc-finger domain). NC mutations frequently emerged in multiple viral strains in the presence of DTG. (Right) DTG sensitivity of the selected NC mutants in TZM-bl reporter cell line.

Result 3: Env mutations confer resistance to INSTIs

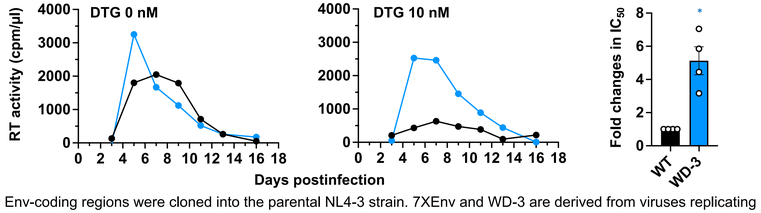
A. NL4-3 Env mutants (infectious molecular clones)

		gp120												gp41			
		C1			V1/V2			V3		C3	V4		C4	HR1		MD	CT
		V85A	V87E	D99N	T138I	N160Y	S162K	R298K	A316T	Q363R	Δ 400-405	M475I		A541V	D547G	V693I	G825E
7XEnv	SupT1 256 nM																
WD-3	PBMC 16 nM																

B. Replication kinetics of 7XEnv in SupT1 cells



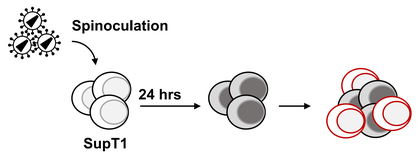
C. Replication kinetics of WD-3 in PBMC



(A) Env-coding regions were cloned into the parental NL4-3 strain. 7XEnv and WD-3 are derived from viruses replicating in SupT1 and PBMC, respectively. (B) Replication kinetics of 7XEnv in the absence or presence of DTG. The SupT1 T-cell line was transfected with the indicated infectious molecular clones. (C) Replication kinetics of WD-3 in PBMC in the absence or presence of DTG. PBMCs were infected with the indicated viruses. IC_{50} values were calculated on the basis of the area under the curve (AUC) of the replication kinetics.

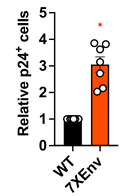
Result 4: 7XEnv exhibits enhanced cell-cell transfer capacity

A. Cell-cell transfer assay

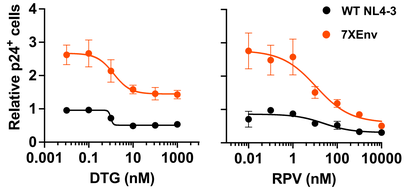


(A) Overview of single-cycle cell-cell transfer assay
(B) Cell-cell transfer of 7XEnv in the absence of drugs
(C) Drug sensitivity in the context of cell-cell transmission
(D) Cell-free infectivity in TZM-bl reporter cell

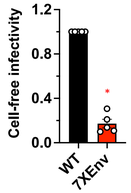
B. p24 expression



C. Drug sensitivity

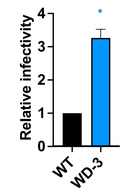


D. Cell-free infectivity

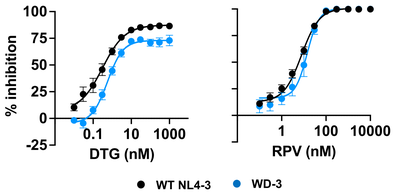


Result 5: WD-3 exhibits enhanced cell-free viral infectivity

A. Cell-free infection



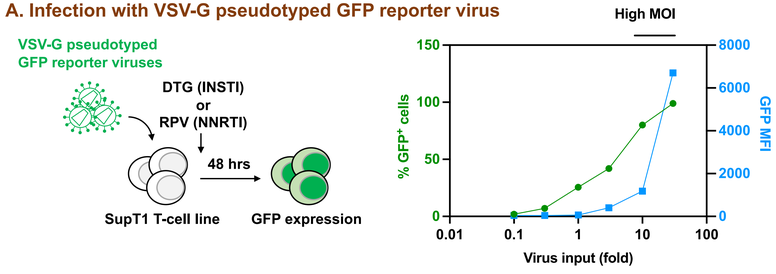
B. Drug sensitivity



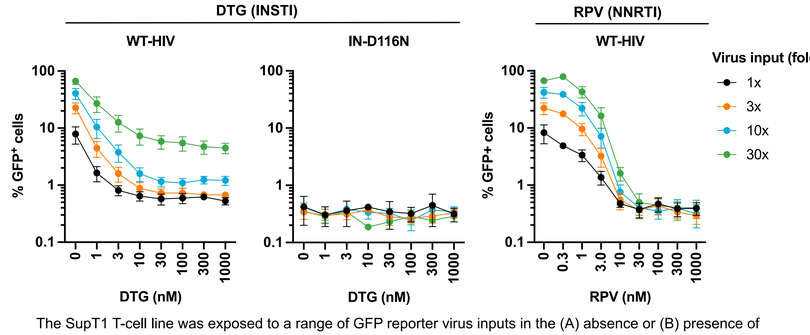
(A) Cell-free infectivity in TZM-bl reporter cell line
(B) Drug sensitivity in the context of cell-free infection

Result 5: INSTIs are readily overwhelmed by high MOI

A. Infection with VSV-G pseudotyped GFP reporter virus



B. Drug sensitivity in the context of high multiplicity of infection (MOI)



The SupT1 T-cell line was exposed to a range of GFP reporter virus inputs in the (A) absence or (B) presence of antiretrovirals. MFI; median fluorescence intensity, IN-D116N is an IN-deficient mutant.