Poster #548

Characterization of the Dolutegravir Monotherapy-Acquired S230R Resistance Mutation

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Background

- Dolutegravir (DTG) is a potent and well tolerated integrase strand-transfer inhibitor (INSTI) with a high barrier to resistance, which makes it a suitable candidate for antiretroviral maintenance monotherapy and simplified regimens (1).
- The relevance of the S230R substitution was reported in a recent study, where DTG monotherapy failed to durably suppress HIV viremia in humanized mice.
- Here, we report the characterization of a S230R substitution that emerged in two patients experiencing virological failure after switching to DTG monotherapy from triple therapy in regard to integrase (IN) enzyme activity, viral infectivity and drug resistance.

Purpose of the study

- Our study aims to explain the underlying mechanism related to the emergence of a S230R substitution in patients who experienced virological failure while using DTG monotherapy.

Materials and methods

- IN-resistance associated mutations were evaluated by sequencing both prior to and at the time of virologic failure.
- The pET15b-IN overexpression and pNL4.3 proviral vectors containing S230R were generated by site-directed mutagenesis. Biochemical strand-transfer and tissue culture assays were performed to characterize enzyme activity, viral infectivity and to measure resistance to DTG and other INSTIs.

Results

- The first case of S230R was found in the DOMONO study (NCT02401828) in a patient who experienced virologic failure at week 30 (HIV-1 RNA was 1570 copies/mL). The patient had a CD4 nadir of 330cells/mm^3 and had been virologically suppressed on EFV/TDF/FTC for 25 months before switching to DTG 50 mg once daily.
- A second case involved a patient who had been virologically suppressed on DTG/ABC/3TC for 8 months before switching to DTG monotherapy. Viral load remained <20 copies/mL but increased to 700 copies/mL with the presence of S230R in IN at week 29.

1. Strand transfer activity and susceptibilities to INSTIs of purified recombinant IN_{WT} and IN_{S230R} enzymes

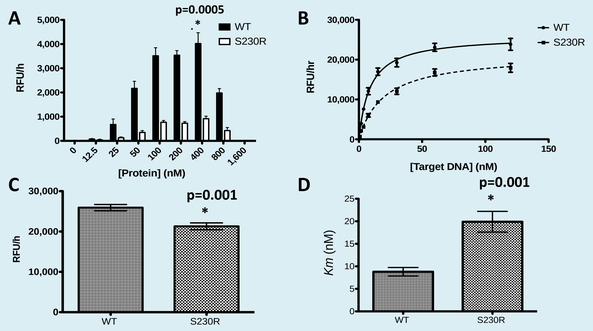


Figure 1. Strand transfer activity of purified recombinant wild-type (IN) and S230R (IN_{S230R}) integrases.
For A-D, means and standard errors are presented. *p<0.05 defined significant difference from WT using Student's t-test, OpenEpi Toolkit.
(A) Strand transfer activity expressed in relative fluorescent units per hour (RFU/h) in the presence of 20 nM target DNA and increasing concentrations of purified INs (Δ protein).
(B) Strand transfer activity in the presence of 400 nM purified IN_{WT} or IN_{S230R}, and increasing concentrations of target DNA (Δtarget DNA).
(C) Calculated maximum strand transfer activities (*Vmax*) for IN_{WT} and IN_{S230R} with Δ protein from (A).
(D) Calculated *1/2maxDNA* values for purified IN_{WT} and IN_{S230R} enzymes from (B).

Table 1. Comparison of trand transfer activity of purified recombinant wild-type (IN_{WT}) and S230R (IN_{S230R}) integrases.

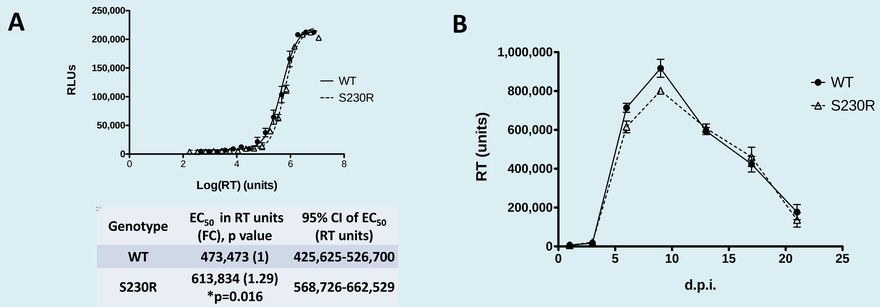
Enzymes	Vmax (RFU/h), p value	95% CI of Vmax	1/2MaxDNA in nM (FC), p value	95% CI of 1/2MaxDNA (in nM)	Vmax/ 1/2MaxDNA ratio (FC)
WT	25,917	24,341 – 27,494	8.9 (1)	6.97 - 10.80	2,918 (1)
S230R	21,287 *p=0.001	19,572 – 23,001	19.9 (2.26) *p=0.001	15.26 - 24.55	1,070 (0.37)

Table 2. Susceptibilities of purified recombinant integrase proteins for DTG, CAB, RAL and EVG

Enzymes	DTG		CAB		RAL		EVG	
	Ki (nM) (mean ± SEM) p value	Relative FC	Ki (nM) (mean ± SEM) p value	Relative FC	Ki (nM) (mean ± SEM) p value	Relative FC	Ki (nM) (mean ± SEM) p value	Relative FC
WT	1.27 ± 0.13	1	1.76 ± 0.41	1	6.40 ± 0.5	1	2.30 ± 0.13	1
S230R	3.33 ± 0.22 *(p=0.004)	2.62	3.38 ± 0.55 ns(p=0.08)	1.92	13.8 ± 5.5 ns(p=0.3)	2.16	7.34 ± 0.47 *(p=0.009)	3.19

(*) indicates statistically significant difference from WT (Student's t-test, p<0.05),); (ns), no statistically significant difference from WT; Ki, the inhibitor constant which is the concentration required to yield 50% maximal inhibitory activity

2. Viral infectivity and replicative capacity of pNL4.3-S230R virus



Genotype	EC_{50} in RT units (FC), p value	95% CI of EC_{50} (RT units)
WT	473,473 (1)	425,625-526,700
S230R	613,834 (1.29) *p=0.016	568,726-662,529

Figure 2. Relative infectivity and replication kinetic of WT and S230R viruses.
(A). Relative infectivity in TZM-bl cells of WT and S230R viruses measured by luciferase fluorescence 48 h post infection.
(B). Viral replication kinetics over a 21-day period in PM1 cells. PM1 cells were infected with the same amount of RT for all viral stocks. Levels of RT in cell-free culture fluids were monitored by RT assay.

3. Susceptibility of S230R virus to DTG, CAB, RAL and EVG in cell-based assay

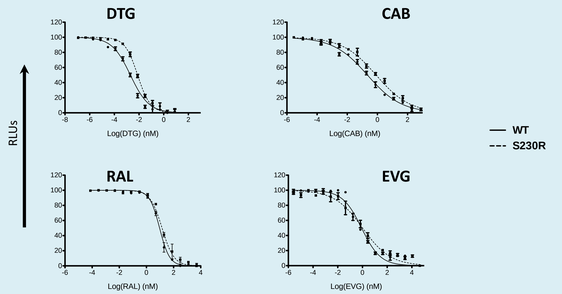


Figure 3. Curves of the dose-response of selected drugs DTG, CAB and RAL of WT and S230R viruses.

Table 3. IC50s in TZM-bl cells DTG, CAB and RAL of S230R virus

Virus	DTG		CAB		RAL		EVG	
	Relative IC_{50} in nM (FC), p value	95% CI of IC_{50}	Relative IC_{50} in nM (FC), p value	95% CI of IC_{50}	Relative IC_{50} in nM (FC), p value	95% CI of IC_{50}	Relative IC_{50} in nM (FC), p value	95% CI of IC_{50}
WT	2 (1)	1.63-2.45	0.22 (1)	0.18-0.27	9.33 (1)	8.71-9.99	0.84 (1)	0.60-1.18
S230R	7.69 (3.85) *p=0.02	6.74-8.8	0.82 (3.72) *p=0.019	0.68-1	14.18 (1.52) *p=0.013	13.24-16.68	1.02 (1.21) nsp=0.57	0.66-1.59

(*) indicates statistically significant difference from WT (Student's t-test, p<0.05); (ns), no statistically significant difference from WT; IC_{50}, 50% inhibitory concentrations of drugs

Conclusions

- S230R substitution conferred 63% reduction in enzyme efficiency.
- The S230R virus was 1.29-fold less infectious than wildtype , but could replicate in PM1 cells without significant delay.
- Resistance levels against DTG, CAB, RAL and EVG in tissue culture were 3.85-, 3.72-, 1.52-, and 1.21-fold, respectively.
- ➢ Virological failure under DTG monotherapy can occur through the development of S230R mutation without the need for high levels DTG resistance.

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Acknowledgements

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