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Raltegravir-associated Mutations in HIV-2 Confer Cross-resistance to Dolutegravir In Vitro

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Background

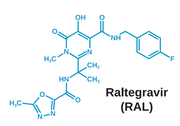
HIV-2 infection is a significant public health problem in West Africa and has been reported in other countries with ties to the region.

Historically, clinical outcomes of antiretroviral therapy in HIV-2 and HIV-1/HIV-2 dually positive patients have been poor, with high rates of immunovirologic failure and emergent multidrug resistance.

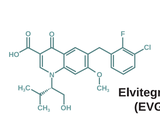
Newer classes of antiretrovirals (ARV) with anti–HIV 2 activity could represent substantial improvements to the current therapeutic picture.

Recent data suggest that integrase strand transfer inhibitors (INSTIs) may be useful for treating HIV-2 infection.

FDA-approved INSTI

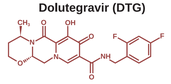


Raltegravir (RAL)



Elvitegravir (EVG)

Dolutegravir (DTG)



Wild-type HIV-1 and HIV-2 isolates show similar susceptibilities to RAL, EVG and DTG in culture. Limited data suggest that RAL is active against HIV-2 (in combination with other ARV). Outcomes of treatment with EVG- or DTG-containing regimens have not been reported for HIV-2.

HIV-1 and HIV-2 acquire resistance to RAL via similar genetic pathways.

Amino acid changes observed in integrase sequences from raltegravir-treated HIV-2 patients

Author, year	Number of patients[a]	Primary INSTI-associated changes observed[b]	Additional changes reported
Garrett et. al. 2008	1	N155H	none
Roquebert et. al. 2008	1	Q148K, Q148R	none
Salgado et. al. 2009	1	N155H	A33A/G, H51H/R, V72I, I84V, A153G, N160K, S163S/G
Xu et. al. 2009	1	N155H, E92Q+N155H, T97A+Y143C[c]	E92G, Q91R, S147G, A153G, H157R, M183I
Charpentier et. al. 2011	7	T97A+Y143C[d], Q148K[e], Q148R, G140S+Q148R, E92E/Q+Y143H/R+N155He, T97A+N155H	none
Treviño et. al. 2011	3	N155H, E92Q, T97A[f]	A153G, S163G/D
Treviño et. al. 2013	8[g]	N155H E92Q+T97A+N155H N155H T97A+Y143G N155H G140A+Q148R	I84V+A153G+Q214H I84V+Q214H I84V+A153G+Q214H Q214H A153G
Raugi 2014 (unpublished)	1	E92A+N155H	T60I+I72V+I84V+A153G+ +N160T+R164K+ E167D+I172V+M183L

[a]Patients that did not receive raltegravir-containing treatment are not included in this tally.
[b]Genotypic analyses were performed on consensus (bulk) sequences unless otherwise indicated.
[c]These changes appeared in cloned PCR products amplified from a single patient.
[d]This combination of replacements was observed in two study subjects.
[e]Replacements listed for Charpentier et. al. are the changes observed at time of virologic failure. Upon further raltegravir treatment, the predominant genotypes in the two patients harboring Q148K/R changed to G140S+Q148R, and the consensus sequence of the patient with E92E/Q+Y143H/R+N155H changed to E92E/A/P/Q+N155H.
[f]N155H variants emerged in two study subjects. The exact combinations of changes were not specified.
[g]Two patients in this study had no genotypic evidence of selection for raltegravir resistance.

Raltegravir-resistant mutants of HIV-2 are broadly cross-resistant to elvitegravir in culture. The extent of cross-resistance to dolutegravir is unclear.

Aim

To determine the extent of cross-resistance between raltegravir and dolutegravir in HIV-2

Approach

- Site-directed mutagenesis of HIV-2 integrase.
- Single-cycle drug susceptibility assays using HeLa-CD4 indicator cells (MAGIC-5A).
- Multicycle assays measuring DTG sensitivity in an immortalized T cell line (CEMss).

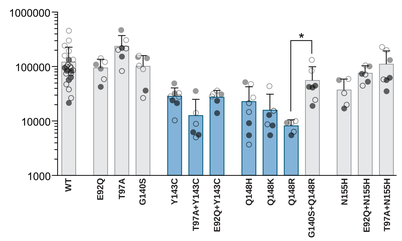
Results

Wild-type HIV-1 and HIV-2 strains show similar sensitivities to DTG in the single cycle assay

HIV Type	Group/ Strain	Subtype	EC_{50} (nM)[a]	Fold Δ[b]	n[c]
HIV-1	NL4-3	M/B	0.9 ± 0.5		3
	LAI	M/B	1.3 ± 0.7	1	2
	92UG029	M/A	1.1 ± 0.5	1	2
	MVP5180-91	O	0.9 ± 0.8	1	3
HIV-2	ROD9[e]	A	2.5 ± 0.7	3	
	7924A	A	1.5 ± 0.8	2	4
	MVP15132	A	1.7 ± 0.4	2	2
	60415K	A	3.0 ± 0.9	3	2
	CBL20	A	1.4 ± 0.7	1	3
	EHO	B	2.6 ± 1.1	3	4
	CDC301319	B	3.0 ± 0.0	3	2

[a]50% effective concentration (mean ± standard deviation) determined via single-cycle focus reduction assay.
[b]Fold change in EC50 value relative to HIV-1 NL4-3 (for wild-type HIV-1 and HIV-2 strains) or wild-type HIV-2 ROD9 (for site-directed mutants).
[c]Number of assay runs performed for each strain.
[d]Virus generated by transfection of wild-type pROD9 (a full-length molecular clone of HIV-2 ROD) in 293T/17 cells.
[e]Mutations encoding the indicated amino acid changes in integrase were introduced into pROD9 via site-directed mutagenesis. Entries in boldface type indicate a statistically significant increase in $LOG_{10}(EC_{50})$ relative to the wild-type HIV-2 ROD9 clone ($p < 0.05$, analysis of variance with Tukey's post-test).

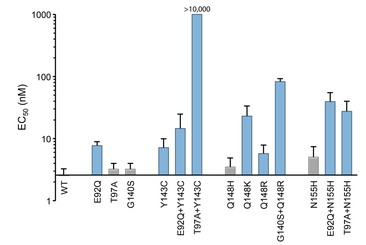
INSTI resistance changes compromise HIV-2 replication capacity



Each datum point is the infectious titer [MAGIC-5A focus-forming units (FFU)/ml] produced by an independent transfection of full-length HIV-2 plasmid DNA into 293T-17 cells. Bars indicate the mean titers for each wild-type (WT) or mutant strain. Blue bars indicate variants that are significantly different from WT ($P \leq 0.05$) and * indicates a significant difference between Q148R and G140S+Q148R HIV-2 ($P \leq 0.01$) (ANOVA of log_{10}-transformed titers with Tukey's post-test). Filled and open circles represent the titers produced by two independent plasmid DNA preparations for each genotype; titers from a third preparation of wild-type DNA are shown as filled, outlined circles. Error bars indicate standard deviations.

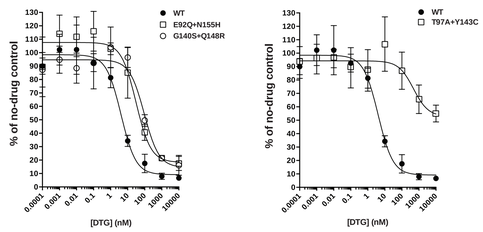
Results

Several RAL-resistant mutants of HIV-2 are cross-resistant to DTG in the single-cycle assay



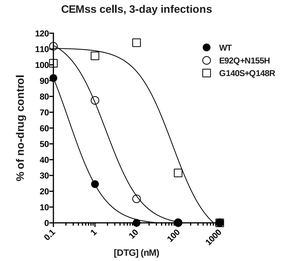
Bars are the means ± standard deviations from 3–5 dose-response assays for each site-directed mutant and 15 assays for wild-type HIV-2 ROD9. Blue bars indicate statistically-significant resistance to DTG ($p \leq 0.05$, ANOVA of $Log_{10}(EC_{50})$ with Tukey's post-test.

The combination of T97A+Y143C results in high-level DTG resistance



Representative dose-response data for WT ROD9 and site-directed ROD9 mutants encoding two amino acid changes in the integrase protein. Results are from a single assay run in which all five strains were tested in parallel. Titers are expressed as the percentage of those seen in the absence of drug (i.e., % of solvent-only controls) and are the means of three independent cultures at each drug concentration. Error bars indicate standard deviations.

Mutants E92Q+N155H and G140S+Q148R are resistant to DTG in short-term spreading infections



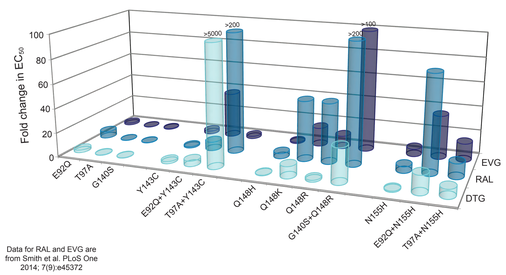
EC_{50} (nM) for Dolutegravir	
Wild-type	0.24 nM
E92Q+N155H	1.9 nM
G140S+Q148R	73 nM

T97A+Y143C = no detectable growth in CEMss cells

Assay measures the level of replication-competent HIV-2 (cell-free plus cell-associated) generated in the presence of varying concentrations of DTG. Each datum point represents the titer of a single DTG-treated culture, expressed as the percentage of the titer in the corresponding solvent-only control. Titers in the solvent-only control cultures were 523000, 22850 and 1100 MAGIC-5A FFU/ml for WT, E92Q+N155H and G140S+Q148R, respectively.

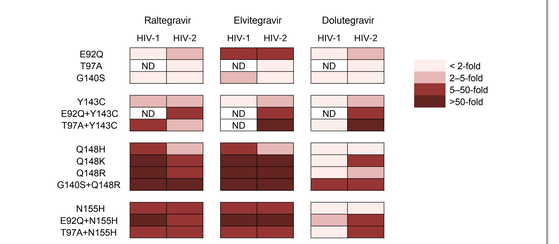
Summary

Resistance Profiles for RAL, EVG and DTG in HIV-2



Data for RAL and EVG are from Smith et al. PLoS One 2014; 7(9):e45372

Equivalent changes in HIV-1 and HIV-2 integrase have differing effects on DTG susceptibility



HIV-1 data are from Kobyashi AAC 55(2):813 except T97A+Y143C (RAL, DTG) from Canducci JID 204:1811

Conclusions

- In HIV-2, all three of the canonical pathways to raltegravir resistance (anchored by changes at Y143, Q148 and N155) confer cross-resistance to dolutegravir.
- In contrast, in HIV-1, dolutegravir is fully active against variants with multiple changes in the Y143 and N155 pathways. HIV-1 mutants with Q148H/K/R + 2° changes are resistant to the drug.
- Dolutegravir may not be an effective option for HIV-2–infected patients that have developed resistance to raltegravir. Additional studies of HIV-2 isolates from RAL-treated patients are needed.

Acknowlegments

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