Bictegravir confers long-term viral suppression against drug-resistant viruses in cell culture

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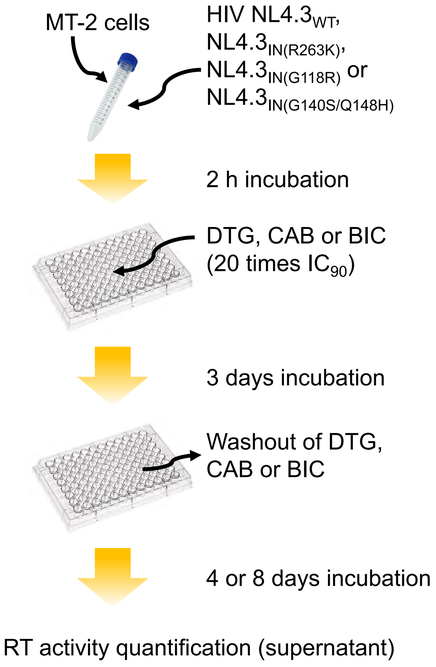
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Background

- Integrase (IN) strand transfer inhibitors (INSTIs) are the latest class of HIV antiretrovirals to have been approved by the FDA.
- Resistance mutations to first generation INSTIs raltegravir (RAL) and elvitegravir (EVG) confer cross-resistance to INSTIs and have resulted in virologic failure in some patients.[1-3]
- Second-generation INSTIs dolutegravir (DTG) and bictegravir (BIC) display superior kinetics and higher genetic barriers than RAL and EVG, and have helped patients who have failed previous antiretroviral therapy.[4,5]
- DTG exhibits longer residual activity than RAL and EVG after drug washout from MT-2 cells infected with wild-type (WT) HIV-1 and the G140S/Q148H RAL-resistant mutant.[6]
- Here, we investigated the potential long-term effects of BIC and of the investigational second-generation INSTI, cabotegravir (CAB), before and after drug washout, using the WT-NL4.3 strain and clones harboring the R263K, G118R, or G140S/Q148H mutations.

Materials and Methods

- IN mutations R263K, G118R and G140S/Q148H were introduced in the laboratory HIV-1 vector pNL4.3 by site-directed mutagenesis.
- Viral clones were produced by transduction of HEK293T cells with pNL4.3 containing WT, R263K, G118R or G140S/Q148H integrase, and used to infect MT-2 cells subsequently treated with DTG, BIC or CAB at 20 times the IC_{90} for 3 days to ensure maximal suppression of viral replication.
- Following drug washout at day 3, viral replication at days 3, 7 and 11 was assessed by measuring reverse-transcriptase activity in culture fluids.



Results

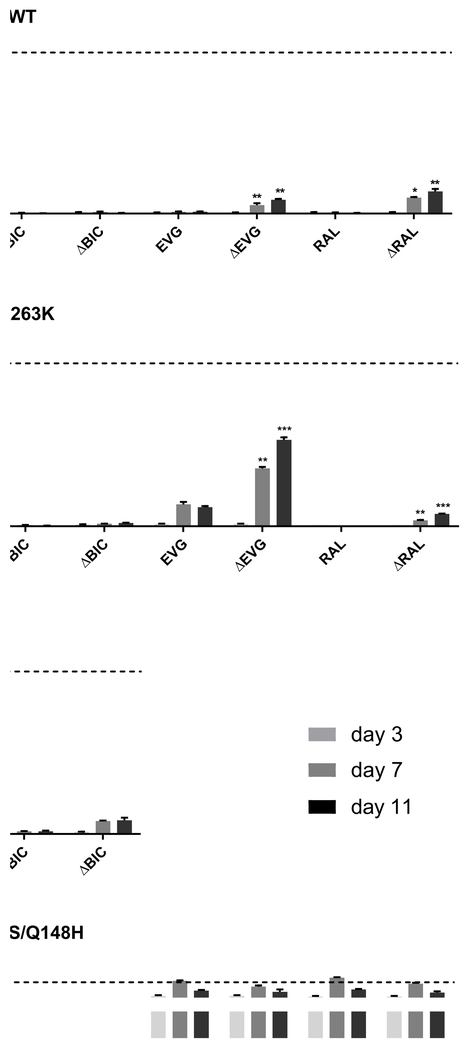


Figure 1. Viral replication of WT, R263K, G118R and G140S/Q148H variants after treatment with DTG, CAB and BIC following drug washout (Δ) at day 3. Replication was assessed by measuring RT activity in culture supernatant without and after drug washout. Bar graphs show the mean ± SEM. Statistical significance of drug washout with drug control conditions were assessed by adjusted P value with Tukey's test.
* < 0.05, *** < 0.001 and **** < 0.0001.

- There were no significant viral replication under DTG, CAB or BIC selective pressure for WT and R263K viruses. The R263K virus showed low-level resistance to EVG.
- There was significant viral rebound of the G118R variant 8 days after DTG washout, minor rebound after CAB washout and almost no rebound after BIC washout.
- There was significant viral rebound of the highly resistant G140S/Q148H variant 8 days after DTG washout, minor rebound after BIC washout. The G140S/Q148H were resistant to CAB, EVG and RAL.

Conclusion and Discussion

DTG, CAB and BIC sustain strand transfer inhibition after their washout against WT and R263K viruses. BIC suppression persists after drug washout from cells infected with INSTI-resistant G118R and G140S/Q148H viruses whereas viral rebound occurred with DTG and CAB.

Second generation INSTIs show a high genetic barrier to resistance. One of the potential dangers of using these drugs in monotherapy or dual therapy is the selection of resistance mutations that may confer cross-resistance to all INSTIs. We demonstrated this using in vitro selections with CAB but not DTG or BIC (Table 1 and 2).[8]

Our results are consistent with the reported biochemical residence half-time of these drugs on the IN-DNA complex.[5] The enhanced resistance profile of BIC might be due to van der Waals interactions of the larger oxazepine ring with the α2B4 loop in Integrase that may act as an additional anchor for Q148H/G140S.[9]

Table 1. Selection of resistance to INSTIs in cell culture

Virus	Clade	Acquired mutations at week 43-46 (Final Passages)			
		DTG	BIC	CAB	EVG
3 isolates	B	none	none	none	T66I (2); E92Q (1)
C050.64	B	none	none	H51HY	T66I
CAE 11.12	AE	None	None	None	H51HY, T66I
4742	C	None	None	R263K	E92EG, R263KR
C185.09	B	R263K	R263K	R263K	T66I, E157Q, R263K
C185.15	B	R263K	R263K	R263K, S153A	T66I, E138EK, S147G, Q148R
196.02	B	H51H/Y	S153Y	L74M, G140S, S147G, Q148K	R263K, S153A
4742	C	None	None	R263K	E92EG, R263KR
96USSN20	AG	R263K	S153FS, E157EK	L74I, E138K, Q148R, R263K	T66I, Q146R, S147G, Q195K
CAE 13.01	AE	R263K	S153Y	S153Y, G163R	T66I, R263K
CC002.02	C	R263K	R263K	S147G	E92V, R263K
pNL4.3	B	R263K, M50I	R263K	S153Y	T66I

Table 2. Phenotypic resistance conferred by mutations selected under escalating pressure with cabotegravir (CAB) and elvitegravir (EVG)

Isolate	Drug	[Drug]	Resistance mutations	[nM]				
				BIC	DTG	CAB	EVG	RAL
5326	wt	0	wt	0.41	0.77	0.20	0.25	1.12
	EVG	1 μM	R263K (S153A)	0.53 1.3x	0.52 0.7x	0.19 1.0x	53 212x	nd
	CAB	0.01 μM	Q148K	0.90 2.2x	1.57 2.0x	0.61 3.1x	2.96 11.8x	3.57 3.2x
	CAB	0.5 μM	G140S, S147G, Q148K	0.72 1.8x	3.6 4.7x	8.07 40x	180 720x	66.5 60x
	CAB	1 μM	L74M, G140S, S147G, Q148K	49 120x	125 162x	140 700X	3429 >1000X	1000 900x
96USSN20	wt	0	wt	0.77	0.58	0.29	0.20	1.6
	CAB	0.025μM	Q148R, R263K,R E138EK	5.83 7.6x	8.08 13.9x	2.4 8.3x	22.35 112x	12.01 7.5x
	CAB	0.25μM	Q148R, R263K, E138K, L74LM	9.01 11.7x	10 17.2x	2.4 8.3x	>300 >1500x	>300 >200x
	CAB	0.5μM	L74I, E138K, Q148R, R263K	13.4 17.4x	13.98 24x	33.73 >100x	1612 8060x	568 355x
	EVG	2.5μM	T66I, T97A, S147G	0.84 2X	0.43 0.5X	1.18 24x	>100 >102	nd

References and Acknowledgements

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