Poster 546

Integrase Inhibitor Resistance Selections Initiated with Drug Resistant HIV-1

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Introduction

- Integrase strand transfer inhibitor (INSTI) resistance substitutions develop during treatment failure with raltegravir (RAL) or elvitegravir (EVG) and often confer cross-resistance to both drugs. Primary INSTI resistance (-R) substitutions commonly observed include E92Q, Y143R/H/C, Q148R/H/K, or N155H[1]
- Development of drug resistance to dolutegravir (DTG) also occurs in patients
  - Treatment-naïve or INSTI-naïve patients: is rare and consists of R263K[1]
  - INSTI-experienced patients: often selects additional complex INSTI-R patterns[1]
- Bictegravir (BIC) is a novel, potent INSTI with a high barrier to resistance
  - No viral breakthrough under physiologically relevant BIC concentrations in cell culture[2]
  - Previous *ex vivo* dose escalation selections of BIC resulted in development of R263K ± M50I with low fold changes in phenotypic susceptibility, comparable to selections with DTG[2]
- BIC has an improved resistance profile compared to other INSTIs and retains antiviral efficacy against many HIV-1 isolates with primary and secondary INSTI-R substitutions[2]
- A single tablet regimen containing BIC coformulated with the nucleos(t)ide reverse transcriptase inhibitors (NRTIs) emtricitabine (FTC) and tenofovir alafenamide (TAF) has recently been approved in the US for HIV-1 treatment in antiretroviral (ARV)-naïve and virologically suppressed adults (B/F/TAF)
  - Among 1271 B/F/TAF-treated patients, no resistance developed to the components of B/F/TAF through 24 weeks of a phase 2 clinical trial and through 48 weeks of 4 phase 3 clinical trials[3-7]

Objectives

- Assess BIC resistance pathways in HIV-1 with preexisting INSTI-R
  - Compare resistance substitutions selected in wild-type (WT) and INSTI-R viruses under increasing concentrations of BIC, DTG, EVG, and RAL in parallel
  - Characterize the phenotypic susceptibility of HIV-1 with selected substitutions to INSTIs and other ARVs as applicable

Methods

Virus Production

- The INSTI-R substitutions E92Q, Q148R, N155H, and R263K in integrase (IN) and the common NRTI-R substitution M184V in reverse transcriptase (RT-M184V) were introduced into a full-length HIV-1 xxLAI-based DNA plasmid9[8,9] by site-directed mutagenesis using standard protocols. Viruses were produced by transient transfection of xxLAI plasmids into 293T cells (TransIT-293, Mirus Bio LLC) and harvested after 2 days.

Resistance Selections

- *Ex vivo* dose-escalation resistance selections were initiated with WT strain xxLAI or mutant HIV-1 at 1X the half-maximal effective concentration (EC_{50}) of the INSTIs BIC, DTG, EVG, and RAL and the NRTI FTC, as a control, in MT-2 cells. At the onset of extensive cytopathic effect, the viral passage (P) was increased: viral supernatants were harvested for analysis and used to infect fresh MT-2 cells at 2-fold higher drug concentrations.

Genotypic Analyses

- Deep sequencing of protease (PR), RT, and IN was performed on amplified viral DNA from select passages (SeqIT GmbH). Reads in the form of FASTQ files were processed and aligned via a multi-step method in a Gilead-developed analysis pipeline.[10] RT and/or IN substitutions present in ≥2 aliquots of viral supernatant at a frequency of ≥2% for INSTI-R associated substitutions or ≥15% for other/polymorphic substitutions are reported. Population sequencing of RT and/or IN was performed on amplified viral DNA from all final passages of selections (Monogram Biosciences). Additional population sequencing of IN was performed on viral DNA from select passages amplified by standard protocols (Elim Biopharmaceuticals). All sequences were aligned to parental xxLAI sequences.

Construction of Representative Mutants

- Emergent IN substitutions were introduced into parental xxLAI plasmids by site-directed mutagenesis as described above. Mutated coding regions of xxLAI *pol* genes were cloned into HIV-1 NL4-3-based test vectors for phenotypic analysis (Monogram Biosciences).

Phenotypic Analyses

- To determine initial selection concentrations, EC_{50} values were derived from the mean antiviral dose responses of a multi-cycle drug susceptibility assay in MT-2 cells described previously.[11] Viral pools from final passages and representative clonal mutants were analyzed for phenotypic susceptibility to ARV inhibitors, including BIC, by the PhenoSense® IN assay or PhenoSense GT® *Plus* IN assay (Monogram Biosciences). EC_{50} values were compared to that of the intra-assay NL4-3 WT virus (final passages) or the xxLAI WT virus (site-directed mutants) to calculate fold change.

Table 1. Drug Concentrations at Selection Initiation

Starting Viral Genotype	Selection Initiation Concentrations (1X EC_{50}) BIC	DTG	EVG	RAL	FTC	Control
WT	0.7 nM	0.7 nM	1.5 nM	6 nM	0.7 µM	No drug
E92Q	1.8 nM	1.8 nM	86 nM	48 nM	0.7 µM	No drug
Q148R	0.9 nM	0.9 nM	230 nM	250 nM	0.7 µM	No drug
N155H	1.4 nM	1.4 nM	90 nM	130 nM	0.7 µM	No drug
R263K	2.8 nM	2.8 nM	8 nM	8 nM	0.7 µM	No drug
RT-M184V	0.7 nM	0.7 nM	1.5 nM	6 nM	NA	No drug

Results

Figure 1. INSTI Selections Initiated with Wild-type HIV-1

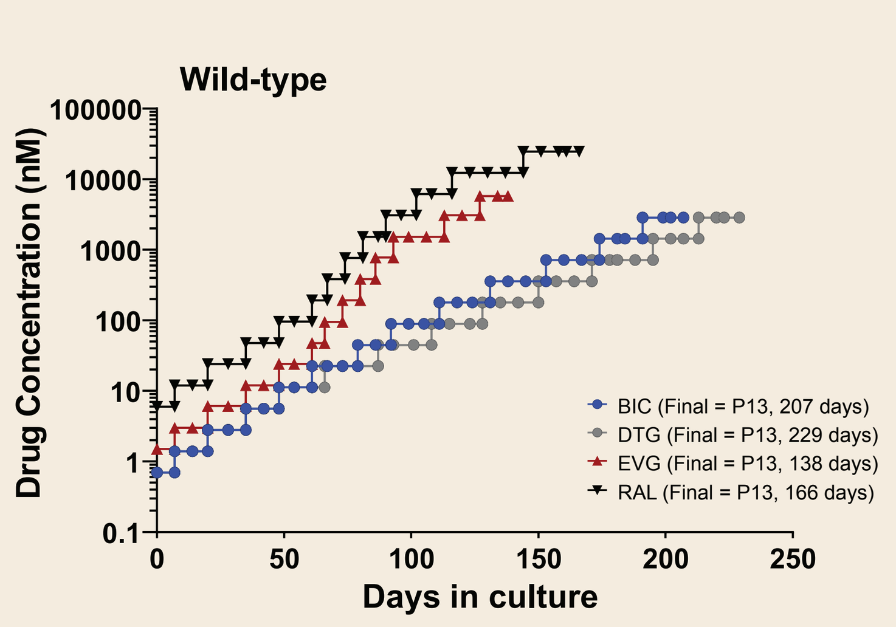


Table 2. Genotypic and Phenotypic Profiles of Final Viral Pools from INSTI Selections Initiated with Wild-type HIV-1

WT Selection (Passage)	IN Substitutions (Population Sequence)	EC_{50} Fold Change Compared to HIV-1 NL4-3 WT: BIC	DTG	EVG	RAL	RC
Start (P0)	None	1.0	1.1	1.5	1.3	102%
BIC (P13)	S24S/G, S153F	1.4	1.7	2.8	1.4	15%
DTG (P13)	S153Y, L234F	2.0	1.9	2.9	1.4	7.2%
EVG (P13)	T66T/I, E138E/K, Q148Q/R/K	2.9	3.3	>145	61	12%
RAL (P13)	G118G/D, Q148K	0.8	0.8	71	47	3.3%

IN = integrase; P= passage; RC= replication capacity compared to HIV-1 NL4-3

Table 3. Genotypic and Phenotypic Profiles of Wild-type HIV-1 under Selective Pressure of INSTIs

Selecting Drug	Key IN Substitutions at Select Passages by Deep Sequencing (Frequency)[a]: P4 (48 days)	P6 (66-87 days)[b]	P8 – P9 (80-131 days)[c]	P10 – P12 (113-191 days)[d]	P13 (138-229 days)[e]	IN Genotype of Representative SDM	EC_{50} Fold Change Compared to HIV-1 xxLAI WT: BIC	DTG	EVG	RAL	RC
BIC	None	**T66I** (8.0%) **S153F** (2.4%)	**T66I** (3.1%) **S153F** (38%) **E157K** (3.4%)	**S153F** (97%) **E157K** (3.2%) S24G (32%)	**S153F** (>99%) S24G (11%)	T66I	0.4	0.4	8.6	0.8	76%
						S153F	1.4	1.1	2.2	1.4	52%
						T66I/S153F	0.6	0.6	30	0.6	12%
						E157K	1.0	0.8	0.8	1.3	16%
DTG	None	**S153Y** (34%)	**S153Y** (87%) L234F (72%)	**S153Y** (90%) L234F (87%)	**S153Y** (>99%) L234F (>99%)	S153Y	2.0	1.7	2.4	1.1	45%
						L234F	1.2	1.2	1.3	0.6	120%
						S153Y/L234F	2.2	1.9	3.0	0.7	33%
EVG	T66I (4.7%)	**T66I**[f]	**T66I** (79%) **Q146L** (19%) Q148R (2.9%) E92V (12%)	**T66I** (63%) **Q146L** (19%) **Q148R** (24%) Q148K (22%) **E138K** (25%) **E92V** (5.0%)	**T66I** (50%) **Q146L** (12%) **Q148R** (23%) **Q148K** (61%) **E138K** (55%) **E92V** (5.3%)	T66I/Q148K	2.1	1.0	>114	>105	3.1%
						E138K/Q148K	4.4	3.2	>114	>105	29%
						T66I/E138K/Q148K	54	34	>114	>105	3.7%
						T66I/E138K/Q148R	3.7	3.3	>114	103	12%
RAL	**T66K** (3.2%)	T66T/**K**[f] Q148Q/**K**[f]	**T66K** (30%) **Q148K** (71%)	**Q148K** (>99%)	**Q148K** (98%) **E138K** (2.5%) **G118D** (21%)	Q148K	0.9	0.7	69	22	29%
						E138K	0.8	0.7	0.9	0.7	60%
						G118D/Q148K	ND	ND	ND	ND	ND
Control	P7 (47 days)	P13 (86 days)	P20 (138 days)	P28 (192 days)	P34 (233 days)						
No drug	None	None	None	None	None	WT	1.0	1.0	1.0	1.0	102%

a. Substitutions at amino acid positions associated with INSTI resistance are in bold.
b. Passage 6 for BIC (79 days), DTG (87 days), EVG (66 days), RAL (67 days)
c. Passage 8 for DTG (128 days), EVG (80 days), and RAL (81 days); passage 9 for BIC (131 days)
d. Passage 10 for DTG (171 days), passage 11 for EVG (113 days) and RAL (116 days), passage 12 for BIC (191 days)
e. Passage 13 for BIC (207 days), DTG (229 days), EVG (138 days), and RAL (166 days)
f. Population sequencing data.

P = passage; ND = not determined; RC = replication capacity compared to HIV-1 NL4-3; SDM = site-directed mutant

Figure 2. INSTI Selections Initiated with HIV-1 RT-M184V Mutant

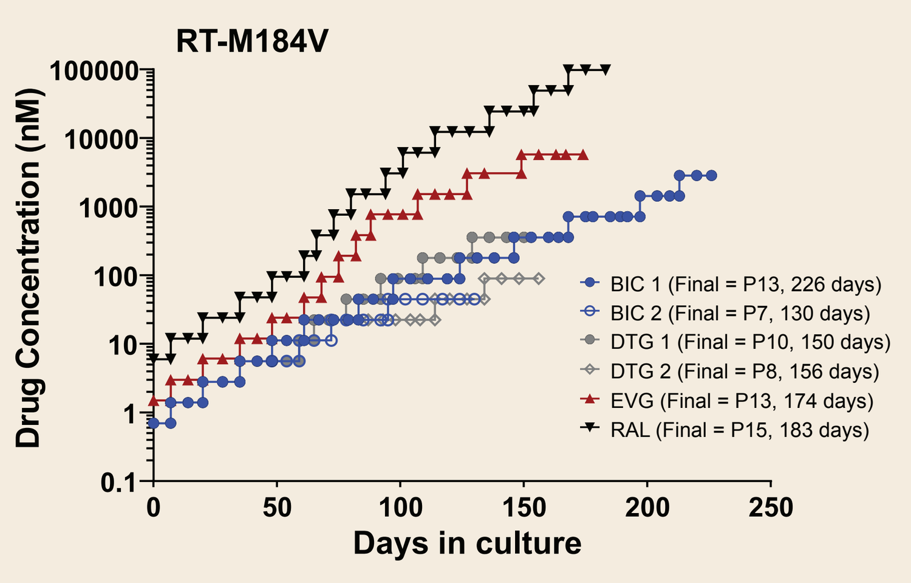


Table 4. Genotypic and Phenotypic Profiles of Final Viral Pools from INSTI Selections Initiated with HIV-1 RT-M184V Mutant

E92Q Selection (Passage)	IN Substitutions (Population Sequence)	EC_{50} Fold Change Compared to HIV-1 NL4-3 WT: BIC	DTG	EVG	RAL	RC
Start (P0)	None	1.0	1.1	1.5	1.3	102%
BIC 1 (P13)	V72I, S153Y	1.6	1.6	3.3	1.4	5.2%
BIC 2 (P7)	R263K	1.4	1.8	4.0	1.2	6.6%
DTG 1 (P10)	S153Y	1.6	1.8	2.7	1.4	20%
DTG 2 (P8)	N144D	1.5	2.1	3.5	1.5	28%
EVG (P13)	T66I	0.3	0.3	9.8	1.0	25%
RAL (P15)	V79V/I, G140A, Q148R	2.1	2.7	>145	159	13%

IN = integrase; P= passage; RC= replication capacity compared to HIV-1 NL4-3

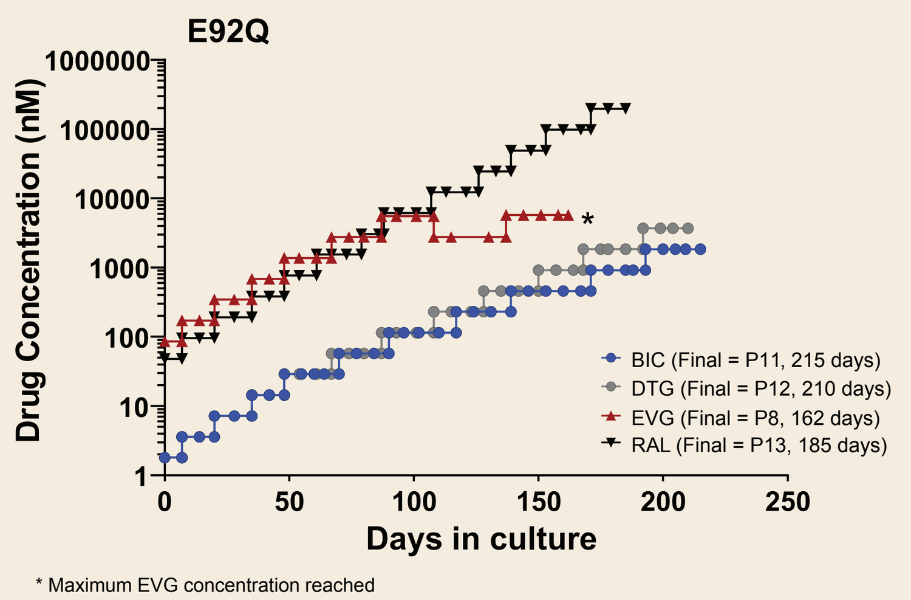
Table 5. Genotypic and Phenotypic Profiles of HIV-1 RT-M184V Mutant under Selective Pressure of INSTIs

Selecting Drug	Gene	Key Substitutions at Select Passages by Deep Sequencing (Frequency)[a]: P4 (48-59 days)[b]	P6 – P8 (80-114 days)[c]	P7 – P11 (114-156 days)[d]	P12 – P14 (168-213 days)[e]	P13 – P15 (183-226 days)[f]	IN Genotype of Representative SDM	EC_{50} Fold Change Compared to HIV-1 xxLAI WT: BIC	DTG	EVG	RAL	RC
BIC 1	RT	M184V (>99%)	M184V (>99%)	M184V (>99%)	M184V (>99%)	M184V (>99%)	V72I	0.5	0.6	1.0	1.1	98%
	IN	None	**V72I** (11%) **S153Y** (88%)	**V72I** (>99%) **S153Y** (>99%)	**V72I** (>99%) **S153Y** (>99%)	**V72I** (>99%) **S153Y** (>99%) **G140E** (9.8%)	V72I/S153Y	2.0	1.2	2.1	0.7	46%
BIC 2	RT	M184V (>99%)	M184V (>99%)	M184V (>99%)	NA	NA	S153Y	2.0	1.7	2.4	1.1	45%
	IN	**S153Y** (14%)	**S153Y** (17%)	**S153Y** (>99%)	NA	NA						
DTG 1	RT	M184V (>99%)	M184V (>99%)	M184V (99%)	NA	NA	N144D	ND	ND	ND	ND	ND
	IN	None	**N144D** (5.9%)	N144D (>99%)	NA	NA						
DTG 2	RT	M184V (>99%)	M184V (>99%)	M184V (99%)	NA	NA	N144D	ND	ND	ND	ND	ND
	IN	None	N144D (5.9%)	N144D (>99%)	NA	NA						
EVG	RT	M184V (99%)	M184V (>99%)	M184V (>99%) E302K (30%)	M184V (>99%)	NA	T66I	0.4	0.4	8.6	0.8	76%
	IN	None	**H51Y** (14%) **T66I** (42%) **V72G** (14%) **E92V** (4.9%)	**H51Y** (17%) **T66I** (82%) **V72G** (26%) **E92V** (4.3%)	**T66I** (>99%)	NA						
RAL	RT	M184V (>99%)	M184V (>99%)	M184V (54%)	M184V (4.6%) E297K (78%)	M184V (4.8%) E297K (90%)	G140A	0.6	0.6	2.8	1.2	53%
	IN	None	**Q148R** (>99%)	**Q148R** (>99%) V79I (2.7%) **G140A** (>99%)	**Q148R** (>99%) V79I (18%) **G140A** (>99%)	**Q148R** (>99%) V79I (19%) **G140A** (>99%) **E138K** (2.4%)	G140A/Q148R	2.3	2.8	>114	77	55%
							E138K/G140A/Q148R	18	13	>114	105	ND
Control		P7 (47 days)	P13 (86 days)	P20 (138 days)	P28 (192 days)	P34 (233 days)						
No drug	RT	M184V (>99%) V75L (35%)	M184V (>99%)	M184V (>99%)	M184V (>99%) V75L (2.3%)	M184V (>99%)	WT	1.0	1.0	1.0	1.0	102%
	IN	None	None	**G163E** (2.6%)	**G163E** (3.0%)	**G163E** (4.2%)						

a. Substitutions at amino acid positions associated with INSTI resistance are in bold.
b. Passage 4 for BIC 1 (48 days), BIC 2 (48 days), DTG 1 (59 days), DTG 2 (48 days), EVG (48 days), and RAL (48 days).
c. Passage 6 for BIC 1 (83 days), DTG 2 (114 days), passage 7 for DTG 1 (92 days), passage 8 for EVG (82 days) and RAL (80 days).
d. Passage 7 for BIC 2 (130 days), passage 8 for BIC 1 (124 days) and DTG 2 (156 days), passage 10 for DTG 1 (150 days), passage 11 for EVG (127 days) and RAL (114 days).
e. Passage 12 for BIC 1 (213 days), passage 13 for EVG (174 days), passage 14 for RAL (168 days).
f. Passage 13 for BIC 1 (226 days), passage 15 for RAL (183 days).

NA = not applicable; ND = not determined; P = passage; RC = replication capacity compared to HIV-1 NL4-3; RT = reverse transcriptase; SDM = site-directed mutant

Figure 3. INSTI Selections Initiated with HIV-1 E92Q Mutant



* Maximum EVG concentration reached

Table 6. Genotypic and Phenotypic Profiles of Final Viral Pools from INSTI Selections Initiated with HIV-1 E92Q Mutant

RT-M184V Selection (Passage)	IN Substitutions (Population Sequence)	EC_{50} Fold Change Compared to HIV-1 NL4-3 WT: BIC	DTG	EVG	RAL	RC
Start (P0)	E92Q	1.2	1.5	33	5.5	38%
BIC (P11)	E92Q, G140G/E, M154M/I	0.9	1.7	25	9.4	2.0%
DTG (P12)	E92Q	1.1	1.4	26	4.9	13%
EVG (P8)	E92Q	1.1	1.5	22	5.3	13%
RAL (P13)	L74L/M, E92Q, G140G/A	1.2	1.8	63	27	5.7%

IN = integrase; P= passage; RC= replication capacity compared to HIV-1 NL4-3

Table 7. Genotypic and Phenotypic Profiles of HIV-1 E92Q Mutant under Selective Pressure of INSTIs

Selecting Drug	Key IN Substitutions at Select Passages by Deep Sequencing (Frequency)[a]: P4 (48 days)	P6 (87-117 days)[b]	P8 – P9 (108-171 days)[c]	P10 – P12 (162-215 days)[d]	IN Genotype of Representative SDM	EC_{50} Fold Change Compared to HIV-1 xxLAI WT: BIC	DTG	EVG	RAL	RC
BIC	**E92Q** (>99%)	**E92Q** (>99%) **G140E** (2.3%)	**E92Q** (>99%) **G140E** (14%) M154I (11%)	**E92Q** (>99%) **G140E** (58%) M154I (37%) **V151I** (7.2%)	G140E	1.1	0.9	2.4	1.0	5.6%
					E92Q/G140E	>104	>232	>114	>105	0.6%*
DTG	**E92Q** (>99%)	**E92Q** (>99%)	**E92Q** (>99%)	**E92Q** (>99%) **S147G** (4.7%) **E157K** (2.2%)	S147G	1.1	1.1	3.3	0.7	44%
EVG	**E92Q** (>99%)	**E92Q** (>99%)	**E92Q** (>99%)	E92Q (>99%)	E92Q	1.1	1.3	24	4.3	38%
RAL	**E92Q** (>99%)	**E92Q** (>99%) **L74M** (13%) **G140A** (41%)	**E92Q** (>99%) **L74M** (11%) **G140A** (72%)	**E92Q** (>99%) **L74M** (14%) **G140A** (62%)	L74M	0.4	0.5	1.0	1.0	93%
					E92Q/G140A	1.4	2.3	54	25	22%
Control	P7 (47 days)	P13 (86 days)	P20 (138 days)	P34 (233 days)						
No drug	**E92Q** (>99%)	**E92Q** (>99%)	**E92Q** (>99%) **V151I** (2.2%)	**E92Q** (>99%)	E92Q	1.1	1.3	24	4.3	38%

*** Very low replication capacity variants may not be viable *in vivo*; did not replicate in multi-cycle phenotyping assay**

a. Substitutions at amino acid positions associated with INSTI resistance are in bold.
b. Passage 6 for DTG (87 days) and EVG (87 days), passage 7 for BIC (117 days) and RAL (88 days).
c. Passage 7 for EVG (108 days), passage 9 for BIC (171 days) and RAL (126 days), passage 10 for DTG (168 days).
d. Passage 8 for EVG (162 days), passage 11 for BIC (215 days), passage 12 for DTG (210 days), passage 13 for RAL (185 days).

P = passage; RC = replication capacity compared to HIV-1 NL4-3; SDM = site-directed mutant

Figure 4. INSTI Selections Initiated with HIV-1 Q148R Mutant

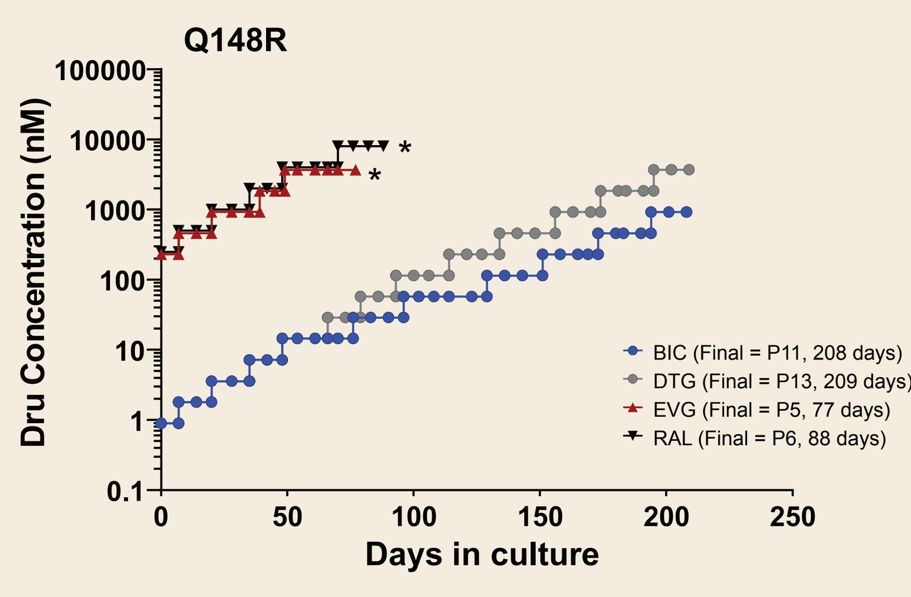


Table 8. Genotypic and Phenotypic Profiles of Final Viral Pools from INSTI Selections Initiated with HIV-1 Q148R Mutant

N155H Selection (Passage)	IN Substitutions (Population Sequence)	EC_{50} Fold Change Compared to HIV-1 NL4-3 WT: BIC	DTG	EVG	RAL	RC
Start (P0)	Q148R	0.7	0.7	113	34	39%
BIC (P11)	Q148R	0.6	0.6	32	24	4.9%
DTG (P13)	G52G/E, E138K, Q148R, H171H/Y	1.4	1.4	>145	58	3.5%
EVG (P5)	Q148R	0.7	0.7	44	25	7.9%
RAL (P6)	Q148R	0.7	0.7	67	29	16%

IN = integrase; P= passage; RC= replication capacity compared to HIV-1 NL4-3

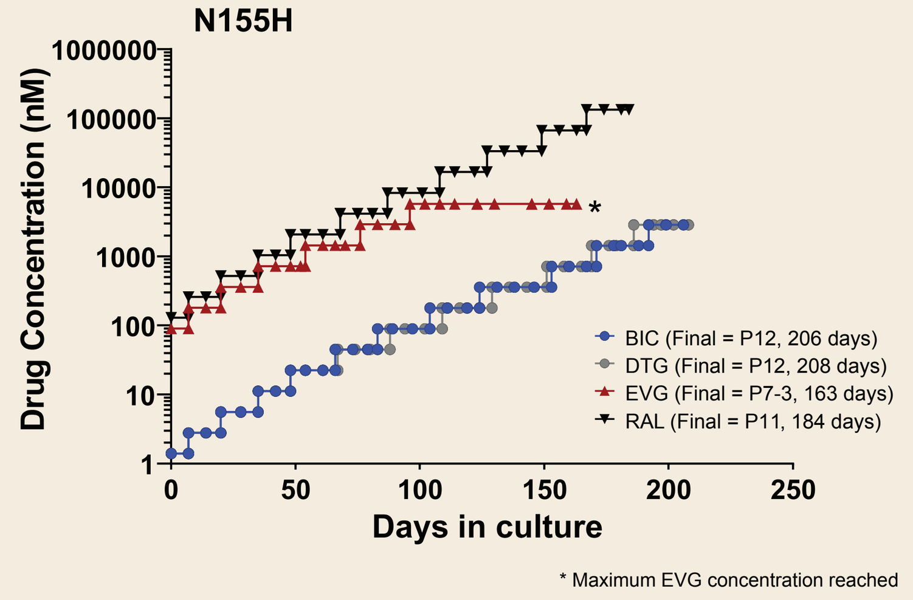
Table 9. Genotypic and Phenotypic Profiles of HIV-1 Q148R Mutant under Selective Pressure of INSTIs

Selecting Drug	Key IN Substitutions at Select Passages by Deep Sequencing (Frequency)[a]: P4 (48-49 days)[b]	P5-P6 (77-88 days)[c]	P7– P9 (129-134 days)[d]	P9 – P11 (173-174 days)[e]	P11-P13 (208-209 days)[f]	IN Genotype of Representative SDM	EC_{50} Fold Change Compared to HIV-1 xxLAI WT: BIC	DTG	EVG	RAL	RC
BIC	**Q148R** (>99%)	ND	**Q148R** (>99%)	**Q148R** (98%) **G140E** (4.2%)	**Q148R** (>99%) **G140E** (3.0%) **E157K** (3.2%	G140E/Q148R	ND	ND	ND	ND	ND
						Q148R/E157K	ND	ND	ND	ND	ND
						G140E/Q148R/E157K	ND	ND	ND	ND	ND
DTG	**Q148R** (87%) **E138K** (20%) **N155H** (9.2%)	**Q148R** (92%) **E138K** (87%) **N155H** (9.7%)	**Q148R** (97%) **E138K** (97%) **N155H** (3.5%)	**Q148R** (99%) **E138K** (>99%) G52E (3.6%) H171Y (2.4%)	**Q148R** (>99%) **E138K** (>99%) G52E (40%) H171Y (22%)	E138K/Q148R	1.6	1.7	>114	51	ND
						Q148R/N155H	2.1	2.2	>114	99	ND
EVG	**Q148R** (>99%)	**Q148R** (99%) **G140R** (17%) **E157K** (2.6%)	NA	NA	NA	G140R/Q148R	ND	ND	ND	ND	ND
RAL	**Q148R** (>99%)	**Q148R** (98%)	NA	NA	NA	Q148R	0.6	0.6	81	27	39%
Control	P7 (47 days)	P13 (86 days)	P20 (138 days)	P28 (192 days)	P34 (233 days)						
No drug	**Q148R** (99%)	**Q148R** (82%)	**Q148R** (15%)	**Q148R** (5.2%)	None (R148Q >99%)	Q148R	0.6	0.6	81	27	39%

a. Substitutions at amino acid positions associated with INSTI resistance are in bold.
b. Passage 4 for BIC (48 days), DTG (48 days), EVG (49 days), and RAL (48 days).
c. Passage 5 for EVG (77 days), passage 6 for DTG (79 days) and RAL (88 days).
d. Passage 7 for BIC (129 days), passage 9 for DTG (134 days).
e. Passage 9 for BIC (173 days), passage 11 for DTG (174 days).
f. Passage 11 for BIC (208 days), passage 13 for DTG (209 days).

NA = not applicable; ND = not determined; P = passage; RC = replication capacity compared to HIV-1 NL4-3; SDM = site-directed mutant

Figure 5. INSTI Selections Initiated with HIV-1 N155H Mutant



* Maximum EVG concentration reached

Table 10. Genotypic and Phenotypic Profiles of Final Viral Pools from INSTI Selections Initiated with HIV-1 N155H Mutant

Q148R Selection (Passage)	IN Substitutions (Population Sequence)	EC_{50} Fold Change Compared to HIV-1 NL4-3 WT: BIC	DTG	EVG	RAL	RC
Start (P0)	Q148R	0.7	0.7	113	34	39%
BIC (P11)	Q148R	0.6	0.6	32	24	4.9%
DTG (P13)	G52G/E, E138K, Q148R, H171H/Y	1.4	1.4	>145	58	3.5%
EVG (P5)	Q148R	0.7	0.7	44	25	7.9%
RAL (P6)	Q148R	0.7	0.7	67	29	16%

IN = integrase; P= passage; RC= replication capacity compared to HIV-1 NL4-3

Table 11. Genotypic and Phenotypic Profiles of HIV-1 N155H Mutant under Selective Pressure of INSTIs

Selecting Drug	Key IN Substitutions at Select Passages by Deep Sequencing (Frequency)[a]: P4 (48-54days)[b]	P6 (83-88 days)[c]	P7– P8 (123-129 days)[d]	P7 – P11 (163-186 days)[e]	P12 (206-208 days)[f]	IN Genotype of Representative SDM	EC_{50} Fold Change Compared to HIV- 1xxLAI WT: BIC	DTG	EVG	RAL	RC
BIC	**N155H** (99%)	**N155H** (>99%)	**N155H** (>99%)	**N155H** (>99%) L45Q (35%)	**N155H** (>99%) L45Q (85%)	L45Q/N155H	1.5	1.5	23	7.2	59%
DTG	**N155H** (>99%)	**N155H** (>99%)	**N155H** (>99%)	**N155H** (>99%) **S147N** (26%)	**N155H** (>99%) **S147N** (50%)	S147N	ND	ND	ND	ND	ND
						S147N/N155H	ND	ND	ND	ND	ND
EVG	**N155H** (>99%) **V72A** (15%) **E170K** (5.5%)	ND	**N155H** (>99%) **V72A** (72%) **E170K** (4.9%) **Q95K** (32%)	**N155H** (>99%) **V72A** (89%) **Q95K** (6.6%) **G140E** (27%)	NA	V72A	0.7	0.7	3.2	0.6	90%
						V72A/N155H	1.0	1.1	36	8.2	55%
						V72A/G140E/N155H	ND	ND	ND	ND	ND
RAL	**N155H** (>99%) **V151I** (3.3%)	**N155H** (>99%) **V151I** (34%)	**N155H** (>99%) S24G (87%) D253E (81%)	**N155H** (99.7%) S24G (99%) D253E (>99%)	NA	S24G/N155H/D253E	1.4	1.5	17	4.6	19%
Control	P7 (47 days)	P13 (86 days)	P20 (138 days)	P28 (192 days)	P34 (233 days)						
No drug	**Q148R** (99%)	**Q148R** (82%)	**Q148R** (15%)	**Q148R** (5.2%)	None (R148Q >99%)	N155H	1.2	1.2	30	12	60%

a. Substitutions at amino acid positions associated with INSTI resistance are in bold.
b. Passage 4 for BIC (48 days), DTG (48 days), EVG (54 days), and RAL (48 days).
c. Passage 6 for BIC (83 days), DTG (88 days), and RAL (87 days).
d. Passage 7-1 for EVG (123 days), passage 8 for BIC (124 days), DTG (129 days), and RAL (127 days).
e. Passage 7-3 for EVG (163 days), passage 10 for BIC (171 days), passage 11 for DTG (186 days) and RAL (184 days).
f. Passage 12 for BIC (206 days) and DTG (208 days).

NA = not applicable; ND = not determined; P = passage; RC = replication capacity compared to HIV-1 NL4-3; SDM = site-directed mutant

Figure 6. INSTI Selections Initiated with HIV-1 R263K Mutant

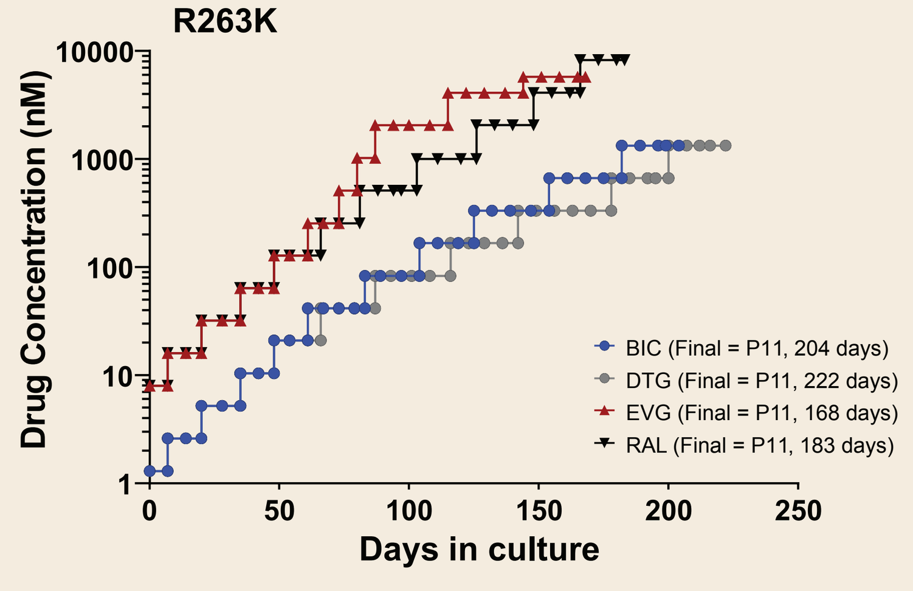


Table 12. Genotypic and Phenotypic Profiles of Final Viral Pools from INSTI Selections Initiated with HIV-1 R263K Mutant

R263K Selection (Passage)	IN Substitutions (Population Sequence)	EC_{50} Fold Change Compared to HIV-1 NL4-3 WT: BIC	DTG	EVG	RAL	RC
Start (P0)	R263K	1.7	1.7	3.8	1.3	50%
BIC (P11)	R263K	1.7	1.6	4.4	1.2	AF
DTG (P11)	R263K	1.6	1.8	3.9	1.3	7.5%
EVG (P11)	T66I, R224R/G, R263K	1.4	1.3	88	1.4	1.5%
RAL (P11)	R263K	1.6	1.5	3.3	1.2	9.9%

AF = assay failure; IN = integrase; P= passage; RC= replication capacity compared to HIV-1 NL4-3

Table 13. Genotypic and Phenotypic Profiles of HIV-1 R263K Mutant under Selective Pressure of INSTIs

Selecting Drug	Key IN Substitutions at Select Passages by Deep Sequencing (Frequency)[a]: P4 (48-54days)[b]	P6 (83-88 days)[c]	P7– P8 (123-129 days)[d]	P7 – P11 (163-186 days)[e]	P12 (206-208 days)[f]	IN Genotype of Representative SDM	EC_{50} Fold Change Compared to HIV-1xxLAI WT: BIC	DTG	EVG	RAL	RC
BIC	**R263K** (>99%)	**R263K** (>99%)	**R263K** (99%)	**R263K** (>99%)	**R263K** (>99%)	R263K	1.6	1.5	2.6	1.0	50%
DTG	**R263K** (>99%)	**R263K** (>99%)	**R263K** (>99%)	**R263K** (>99%) **E157K** (2.7%)	**R263K** (>99%) **E157K** (2.4%)	E157K	1.0	0.8	0.8	1.3	16%
						E157K/R263K	ND	ND	ND	ND	ND
EVG	**R263K** (>99%)	**R263K** (>99%) **T66I** (99%)	**R263K** (99%) **T66I** (98%)	**R263K** (>99%) **T66I** (>99%)	NA	T66I/R263K	1.5	1.3	84	0.7	15%
RAL	**R263K** (>99%)	**R263K** (>99%)	**R263K** (99%)	**R263K** (>99%) **V151A** (6.3%)	NA	V151A/R263K	ND	ND	ND	ND	ND
Control	P7 (47 days)	P13 (86 days)	P20 (138 days)	P28 (192 days)	P34 (233 days)						
No drug	**R263K** (>99%)	**R263K** (>99%)	**R263K** (95%)	**R263K** (86%)	**R263K** (69%) (K263R 31%)	R263K	1.6	1.5	2.6	1.0	50%

a. Substitutions at amino acid positions associated with INSTI resistance are in bold.
b. Passage 6 for BIC (83 days), DTG (87 days), and RAL (81 days), passage 7 for EVG (80 days).
c. Passage 8 for BIC (125 days), DTG (142 days), and RAL (126 days), passage 9 for EVG (115 days).
d. Passage 9 for DTG (178 days), passage 10 for BIC (182 days), passage 11 for EVG (168 days) and RAL (183 days).
e. Passage 11 for BIC (204 days) and DTG (222 days).

NA = not applicable; ND = not determined; P = passage; RC = replication capacity compared to HIV-1 NL4-3; SDM = site-directed mutant

Figure 7. FTC Selections Initiated with Wild-type and Mutant HIV-1

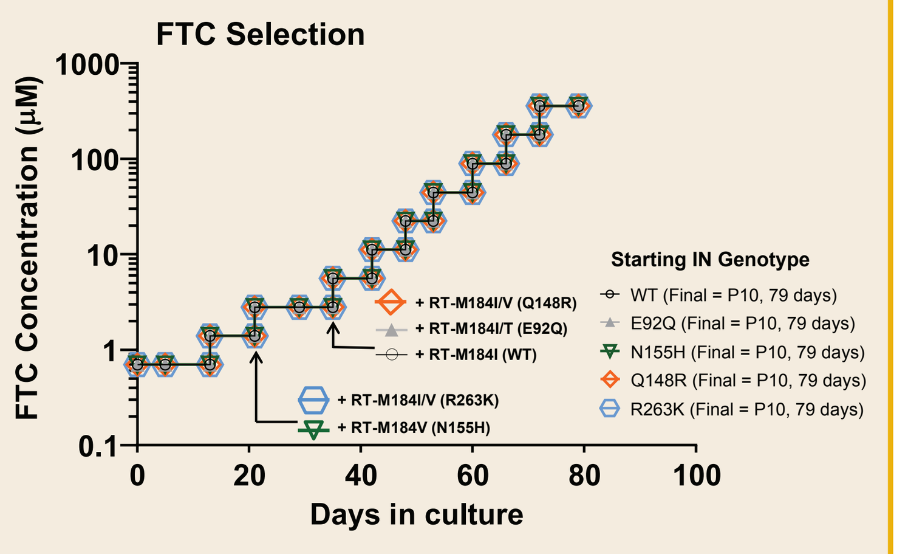


Table 14. Genotypic and Phenotypic Profiles of Final Viral Pools from FTC Selections

FTC Selection (Final Passage)	Genotype (Population Sequence): RT	IN	EC_{50} Fold Change Compared to HIV-1 NL4-3 WT: NRTI FTC	NRTI TFV	INSTI BIC	INSTI DTG	INSTI EVG	INSTI RAL
WT (P10)[a]	M184V	None	>123	1.6	1.5	1.6	2.0	1.4
E92Q (P10)	M184M/I/V	E92Q	>86	0.5	0.9	1.1	15	4.5
Q148R (P10)	M184M/I/V	Q148Q/R	>86	0.6	1.4	1.0	1.8	1.4
N155H (P10)[b]	M184V	N155H	>96	0.6	1.4	1.5	41	17
R263K (P10)	M184V	R263K	>186	0.5	2.4	1.7	3.0	1.2

a. Fold change values derived from multi-cycle phenotyping assay and compared to HIV-1xxLAI WT (Gilead).
b. Data pending for final viral pool. Data shown here is extrapolated from deep sequencing (Table 15) and historical SDM data

FTC = emtricitabine; P= passage; RT = reverse transcriptase; SDM = site-directed mutant; TFV = tenofovir

Table 15. Genotypic Profiles by Deep Sequencing of HIV-1 under Selective Pressure of FTC

Starting Virus	Gene	Key Substitutions at Selected Passages of FTC Selection (Frequency)[a]: P2 (21 days)	P3 (35 days)	P4 (42 days)	P6 (53 days)	P10 (79 days)
WT	RT	None	**M184I** (37%)	**M184I** (70%) **M184V** (12%) **M184T** (11%)	**M184I** (63%) **M184V** (28%) **M184T** (7.6%)	**M184I** (4.5%) **M184V** (94%) **M184T** (2.4%)
	IN	None	None	None	None	None
E92Q	RT	None	**M184I** (49%) **M184T** (5.2%)	**M184I** (72%) **M184T** (13%)	**M184I** (93%) **M184T** (4.5%)	**M184I** (25%) **M184T** (3.1%) **M184V** (72%)
	IN	**E92Q** (>99%)	**E92Q** (>99%)	**E92Q** (>99%)	**E92Q** (>99%)	**E92Q** (>99%)
Q148R	RT	None	**M184I** (88%) **M184V** (4.9%)	**M184I** (96%)	**M184I** (95%) **M184V** (5.1%)	**M184I** (22%) **M184V** (78%)
	IN	**Q148R** (>99%)	**Q148R** (96%)	**Q148R** (87%)	**Q148R** (74%)	**Q148R** (25%)
N155H	RT	**M184V** (4.7%)	**M184V** (89%)	**M184V** (>99%)	**M184V** (>99%)	**M184V** (>99%)
	IN	**N155H** (>99%)	**N155H** (>99%) D207E (74%)	**N155H** (>99%) D207E (62%)	**N155H** (>99%) D207E (59%)	**N155H** (>99%) D207E (38%)
R263K	RT	**M184I** (17%) **M184V** (6.1%)	**M184I** (59%) **M184V** (16%)	**M184V** (>99%)	**M184I** (8.6%) **M184V** (91%) **T215I** (2.6%)	**M184V** (99%) **T215I** (4.8%)
	IN	**R263K** (99%)	**R263K** (>99%)	**R263K** (>99%)	**R263K** (>99%)	**R263K** (99%)

a. Substitutions at amino acid positions associated with INSTI or NRTI resistance are in bold.
IN = integrase; P = passage; RT = reverse transcriptase; WT = wild-type

Conclusions

- The *ex vivo* BIC resistance profile from wild-type HIV-1 has been further defined
  - Two patterns of resistance substitutions in IN after extended culture with BIC
    - R263K ± M50I (<3-fold reduced susceptibility to BIC)[2]
    - S153F or S153Y (≤2-fold reduced susceptibility to BIC)
  - Selected variants remained sensitive or had low-level reduced susceptibility to BIC
- BIC continued to demonstrate a high barrier to resistance with slow, if any, development of additional substitutions in IN after *ex vivo* selections with INSTI-R HIV-1
  - All selected viral pools remained sensitive to BIC and DTG
  - One variant with E92Q + G140E was identified with high level resistance to all INSTIs (single cycle phenotyping assay), had very low replication capacity and did not replicate in multi-cycle phenotyping assay; this isolate may not be viable *in vivo*
- FTC selected M184V/I in RT in HIV-1 with or without INSTI-R substitutions, including the R263K mutant
- Slow development of resistance by BIC in HIV-1 with or without INSTI-R confirms BIC's high barrier to resistance and supports future studies of B/F/TAF in patients with preexisting resistance substitutions

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Acknowledgements

The authors would to thank Martin Daeumer and Alexander Thielen from SeqIT and Tim Persyn from Monogram Biosciences for their technical and project management support.

Presented at the Conference on Retroviruses and Opportunistic Infections, Boston, MA, March 4-7, 2018