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Novel Nucleotide Inhibitor GS-9148 Selects for a K70E Mutation in HIV-1 Reverse Transcriptase and Low-Level Resistance *In Vitro*

G Laflamme, D Grant, K White, K Stray, C Boojamra, L Zhang, R Mackman, A Ray, M Miller, and T Cihlar

Gilead Sciences, Inc., Foster City, CA

GILEAD
Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Tel: (650)574-3000
Fax: (650)578-9264

Introduction

- GS-9148 (Fig. 1) is a novel nucleotide HIV RT inhibitor with a favorable *in vitro* resistance profile against HIV-1 strains with clinically relevant mutations including M184V, K65R, L74V, and ≥ 4 TAMs (Fig. 2)[1].
- GS-9131 (Fig. 1), an orally bioavailable phosphonoamidate prodrug of GS-9148 capable of effectively delivering GS-9148 diphosphate into PBMCs *in vivo*[2] is an attractive candidate for clinical development for the treatment of HIV-infected patients with NRTI resistance.
- Here we report on the evolution and characterization of HIV-1 variants selected *in vitro* in the presence of nucleotide inhibitors GS-9148, tenofovir (TFV), and their combination.

Figure 1. Structure of GS-9148 and its Prodrug GS-9131

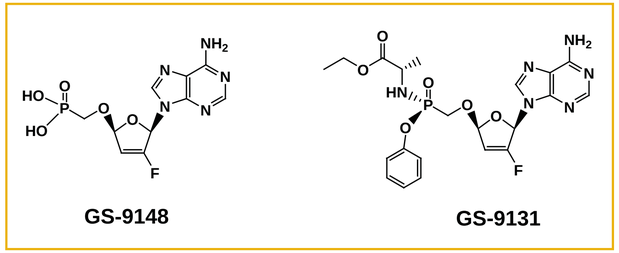
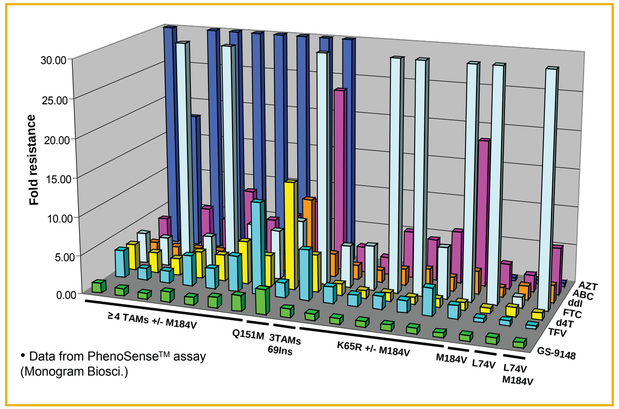


Figure 2. Resistance Profile of GS-9148 and Marketed NRTIs



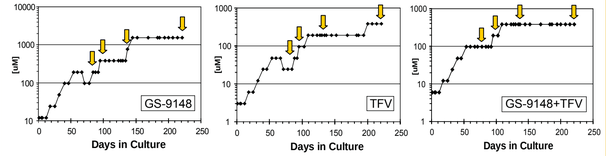
• Data from PhenoSense™ assay (Monogram Biosci.)

Methods

- Parallel selections with GS-9148, TFV, and their combination were conducted in MT-2 cells infected with HIV-1(IIIB). Virus cultures were passaged at least once a week for 32 weeks in the presence of gradually increasing concentrations of selecting inhibitors.
- Both the population and clonal sequencing of PCR-amplified fragments of RT gene (aa1-320) from the selected HIV-1 isolates was performed by Elim Biopharmaceuticals (Hayward, CA).
- Recombinant HIV-1 strains containing mutations selected by GS-9148 were constructed by a site-directed mutagenesis of an infectious chimeric LAI (backbone)/IIIB(RT) cDNA clone of HIV-1.
- Susceptibilities of selected isolates and recombinant strains to GS-9148 and marketed NRTIs were determined in MT-2 cells using an XTT-based cytopathic assay[3].
- The frequency of GS-9148 selected RT mutations among patients HIV-1 isolates was evaluated via an on-line search of the Stanford HIV Drug Resistance Database[4].

Results

Figure 3. Parallel *In Vitro* Resistance Selection with GS-9148, TFV, and their Combination



- Arrows mark specific time points at which the genotypic analysis was performed. Combination panel depicts only GS-9148 concentration with the ratio of GS-9148 to TFV being constant (4:1 based on relative EC_{50} values for the respective agents) throughout the selection.

Table 1. RT Mutations Selected by GS-9148, TFV, and their Combination

Time in culture	Selecting inhibitor	Conc. reached [µM]	Mutations in RT gene[a]
85 days	GS-9148	192	Not detected
	TFV	48	G45R, K65R, L214F
	Combination	24 + 96	K70K/N
110 days	GS-9148	384	K70K/N, T165T/I
	TFV	96	G45R, K65R, L214F
	Combination	48 + 192	K70K/N
140 days	GS-9148	768	K70E/N, D123N, T165I
	TFV	192	G45R, K65R, L214F
	Combination	96 + 384	K70K/N/E
220 days	GS-9148	1,536	K70E, D123N, T165I
	TFV	384	G45R, K65R, S68N, L214F
	Combination	96 + 384	K70E

a. Amino acid changes in RT gene (amino acid 1-320) detected by population sequencing relative to the sequence of virus passaged in parallel in the absence of inhibitors.

Results (cont'd)

Table 2. Clonal Sequencing Analysis of RT from the Selected HIV-1 Strains

Day	Selection	Frequency of mutations in RT gene[a]								
		G45R	K65R	S68N	K70E	K70N	D123N	T165I	R172K	L214F
110	None	-	-	-	-	-	-	-	6/10	4/10
	GS-9148	-	-	-	-	3/9	1/9	6/9	-	2/9
140	None	-	-	-	-	-	-	-	4/10	4/10
	GS-9148	-	-	-	6/12	6/12	6/12	10/12	-	1/12
	Combination	-	-	-	3/12	6/12	-	-	-	1/12
220	None	-	-	-	-	-	-	-	9/10	2/10
	Tenofovir	10/10	10/10	10/10	-	-	-	-	-	10/10
	GS-9148	-	-	-	10/10	-	8/10	9/10	-	-
	Combination	-	-	-	10/10	-	-	-	-	-

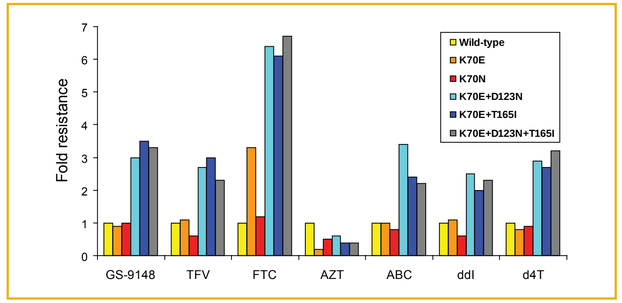
a. Frequency of mutations calculated as the ratio of the number of mutant clones relative to the number of analyzed clones from each selected viral isolate.

Table 3. Phenotypic Analysis of the Selected HIV-1 Isolates

Inhibitor	Wild-type EC_{50} [µM]	Fold resistance[a]		
		GS-9148 selection	TFV selection	Combination selection
GS-9148	3.5 ± 1.1	2.9	3.0	2.0
GS-9131	0.09 ± 0.02	2.6	2.9	2.4
TFV	1.2 ± 0.2	2.6	9.5	3.0
FTC	0.5 ± 0.3	4.0	13.0	2.8
AZT	0.15 ± 0.04	0.3	1.7	0.2
ABC	0.25 ± 0.05	2.4	5.6	2.7
ddI	2.5 ± 0.1	1.6	3.1	1.6
d4T	5.1 ± 1.8	1.6	2.8	1.3

a. Fold resistance relative to the control wild-type HIV-1 (IIIB). Values are means from n ≥ 3 and changes in susceptibility of ≥ 2-fold are highlighted in red.

Figure 4. Phenotypic Analysis of HIV-1 Site-Directed Recombinant Mutant Strains



Values are means from n = 3. No resistance to any of the tested NRTIs was observed with recombinant strains containing D123N, T165I, or their combination in the absence of K70E (data not shown).

Table 4. Frequency of Mutations Selected by GS-9148 Among HIV-1 Clinical Isolates

Mutation		Frequency [%] [a]		
		Treatment-naïve	NRTI-experienced	
GS-9148 selected	K70E	0	0.3	Primary resistance
	K70N	0.04	0.2	
	D123N	5.6	9.1	Polymorphism
	T165I	2.3	5.3	
	K70E+D123N and/or T165I	0	0	
Major NRTI mutations	TAMs[b]	0 - 0.2	22.0 - 44.0	
	K65R	< 0.1	2.8	
	L74V	< 0.1	7.1	
	M184V/I	0.4	46.8	

a. N > 5,000 for both naïve and NRTI-experienced.
b. TAMs: M41L, D67N, K70R, L210W, T215Y/F, K219Q.

Conclusions

- **GS-9148 selected for K70E+D123N+T165I in RT and 3-fold resistance to both GS-9148 and GS-9131. The selected virus showed low level cross-resistance to TFV, FTC and ABC and was hypersensitive to AZT. Only combinations of K70E with D123N and/or T165I, but not individual selected mutations, confer detectable reduced susceptibility to GS-9148**
- **Virus selected in parallel by TFV contained the expected K65R mutation and exhibited a higher degree of NRTI cross-resistance than the virus selected by GS-9148**
- **The combination of GS-9148+TFV selected for a single K70E mutation, indicating the ability of GS-9148 to shift the TFV resistance pathway away from K65R. This is consistent with the lack of GS-9148 resistance due to K65R[1]**
- **Analysis of the Stanford HIV Resistance Database showed a low frequency of K70E in NRTI-experienced patients (0.3%); combinations of K70E with either D123N or T165I have not been found in the database samples**
- **In summary, prolonged *in vitro* exposure to GS-9148 leads to the selection of a rare combination of RT mutations and low-level resistance**

References

1. Cihlar T. et al. Abstr. 45, 13th CROI, 2007.
2. Ray A. et al. Abstr. 498, 13th CROI, 2007.
3. Weislow O.S. et al. J Natl Cancer Inst 81: 577-86, 1989.
4. Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu/), 2006.