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Resistance Mechanisms of the K70E HIV-1 Reverse Transcriptase Mutant to GS-9148 and Other NRTIs

K White, J Ly, A Eastoak-Siletz, G Laflamme, N Kutty, A Ray, T Cihlar, and M Miller

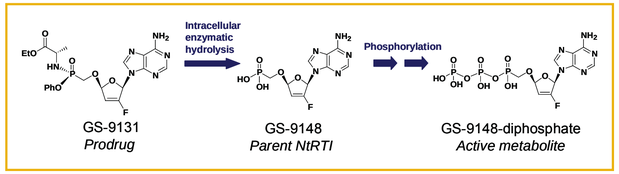
Gilead Sciences, Inc., Foster City, CA, USA

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 structural analog of dAMP that acts as a nucleotide HIV reverse transcriptase (RT) inhibitor (NRTI)[1]
- Both GS-9148 and its phosphonoamidate prodrug, GS-9131, exhibit potent activity *in vitro* against a broad range of NRTI-resistant HIV clinical isolates, including those with TAMs, M184V, or K65R[2]
- In cell culture, GS-9148 selects for HIV-1 mutations in RT at codon 70, where K70N develops first and is then replaced by a triple mutant containing K70E+D123N+T165I[3]
  - K70E is present at a low frequency in NRTI-experienced patients (0.3%)[3]
  - K70R is a thymidine analog-associated mutation (TAM) involved in excision of NRTIs[4]
- There are two major mechanisms of RT resistance to NRTIs: (1) decreased binding/incorporation, and (2) increased excision after incorporation

Figure 1. Structures of GS-9131, GS-9148, and the Active Metabolite GS-9148-Diphosphate



Objective

- To characterize the molecular mechanisms by which the K70N, K70E, and K70E+D123N+T165I mutations in HIV-1 reverse transcriptase cause reduced virus susceptibility to GS-9148 and other NRTIs

Methods

- Drug susceptibilities in cell culture were determined for wild-type (WT) and site-directed mutant HIV-1 strains in MT-2 cells using an XTT-based assay[5]
- The steady state kinetic constants K_i and K_m were determined for WT and mutant RT enzymes for the natural substrates dATP and dTTP and for the active metabolites of NRTI: GS-9148-DP, tenofovir-DP, ddATP (ddI), and AZT-TP using a heteropolymeric DNA template[5]
- The efficiency of excision of incorporated NRTI by the ATP-mediated excision mechanism was determined using WT and mutant RT enzymes and increasing concentrations of the next complementary nucleotide. Reactions were initiated by the addition of 3.5 mM ATP and primers with NRTI excised were extended in a rescue of polymerization assay with dNTPs and the Klenow polymerase[6]

Results

Figure 2. Progression of RT Genotypic Changes Induced by GS-9148 *In Vitro*[3]

WT ⟶ K70N ⟶ K70E ⟶ K70E+D123N+T165I

Table 1. Susceptibilities of WT and Recombinant Mutant HIV-1 to NRTIs

Inhibitor	Wild-type[a]	K70N[b]	K70E	K70E+D123N+T165I[b]	K65R[b]
GS-9148	7.8 ± 1.7	1.0	0.9	**3.3**	1.3
GS-9131	0.11 ± 0.01	1.5	1.1	**3.3**	ND
tenofovir	3.4 ± 0.7	0.6	1.1	2.3	**4.2**
ddI	3.4 ± 1.4	0.6	1.1	2.3	2.3
FTC	0.6 ± 0.2	1.2	**3.3**	**6.7**	**6.8**
abacavir	0.5 ± 0.2	0.8	1.0	2.2	**4.0**
d4T	6.1 ± 1.8	0.9	0.8	**3.2**	2.4
AZT	0.18 ± 0.06	0.5	0.2	0.4	1.0
adefovir	10 ± 0.6[c]	ND[d]	**9.0**	ND	ND
amprenavir	0.27 ± 0.08	0.7	0.8	1.4	ND

a. EC_{50} [µM] values are mean values ± s.d. from n ≥ 2.
b. Fold change in the susceptibility relative to the control wild-type HIV-1. Fold changes greater than 3-fold are shown in bold.
c. Cherrington et al., (1996) Antimicrobial Agents & Chemotherapy 40(9):2212-2216.
d. ND = not done.

Figure 3. Mechanisms of NRTI Resistance

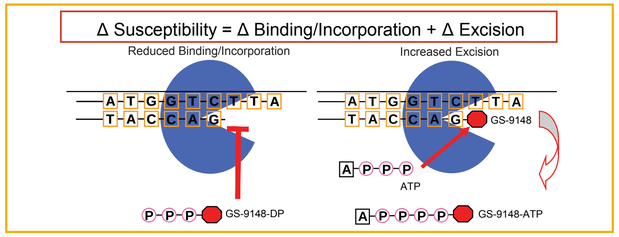
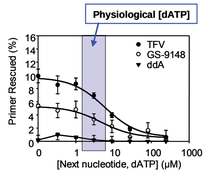


Table 2. Binding/Incorporation Using Steady State Kinetic Constants K_i and K_i/K_m for WT and Mutant HIV-1 RT Enzymes

Inhibitor	Wild-type		K70N	K70E	K70E+ D123N+ T165I	K70R	K65R
	K_i [µM][a]	K_i/K_m[b]	Fold K_i/K_m	Fold K_i/K_m	Fold K_i/K_m	Fold K_i/K_m	Fold K_i/K_m
GS-9148-DP	0.75 ± 0.12	3.1	2.4	2.6	4.0	0.8	7.4
TFV-DP	0.25 ± 0.09	1.0	2.1	4.2	3.5	1.1	13
ddATP	0.046 ± 0.004	0.19	2.6	4.2	3.7	1.6	15
ADV-DP	0.14 ± 0.05	0.6	2.3	4.3	3.2	1.0	18
AZT-TP	0.024 ± 0.005	0.10	1.7	1.8	2.3	1.5	8.0

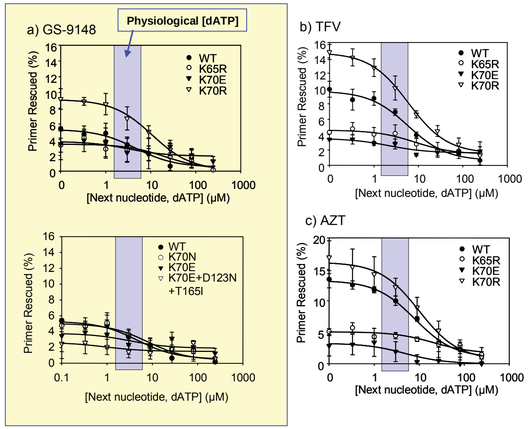
a. K_i values are mean values ± s.d. from n ≥ 3.
b. Mean K_m values of wild-type, K70N, K70E, K70E+D123N+T165I, K70R, and K65R RT for dATP were 0.24, 0.28, 0.38, 0.48, 0.18, and 0.32 µM, respectively and for dTTP were 0.25, 0.35, 0.54, 0.66, 0.20, 0.28 µM, respectively.

Figure 4. ATP-mediated Excision and Rescue of NRTI Chain-Terminated Primers by WT RT



Relative to tenofovir, GS-9148 shows decreased excision potential by WT RT

Figure 5. ATP-mediated Excision and Rescue of NRTI Chain-Terminated Primers by WT and Mutant RT



Excision Relative to WT
- K70R (TAM): increased excision
- K70N = WT excision
- K70E and K65R: reduced excision

Table 3. Summary of GS-9148 Resistance Mechanisms

	K70N	K70E	K70E+D123N+ T165I	
Virus susceptibility (fold vs WT)	1.0	0.9	3.3	
RT inhibition by K_i/K_m (fold vs WT)	**2.4**	**2.6**	**4.0**	**Primary Resistance Mechanism**
RT primer excision	<5%	<5%	<5%	

Conclusions

- **Viruses containing K70E+D123N+T165I, but not K70N or K70E alone, showed low-level decreases in susceptibility to GS-9148 (3.3-fold) and other NRTIs (2- to 3-fold), a 6.7-fold decrease to FTC, but were hypersensitive to AZT (0.4-fold) in cell culture**
- **The progression of K70N to K70E to K70E+D123N+T165I mutant RTs corresponded to decreased binding/incorporation (increased K_i/K_m) for GS-9148-diphosphate (2.4-, 2.6-, and 4.0-fold, respectively) relative to wild-type**
- **GS-9148 exhibited low levels of excision by both wild-type and K70E-containing RT**
- **This mechanistic study confirmed the role of the K70E RT mutation in the resistance phenotype of GS-9148**
  - **For GS-9148 and other NRTIs, the resistance due to K70E, like K65R, was mediated primarily by decreased binding/ incorporation of the inhibitors**
  - **The K70E RT mutant, like K65R and in contrast to the TAM K70R, showed reduced excision of NRTIs with measurable excision (tenofovir and AZT) relative to wild-type, that appears to counteract the incorporation defects for these NRTIs**
- **These data suggest that the K70E mutant utilizes a resistance mechanism similar to that of K65R, but shows lower levels of resistance against NRTIs in cell culture and in biochemical assays**

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