GS-9131 is a Novel NRTI with Activity Against NRTI-Resistant HIV-1

Poster 436

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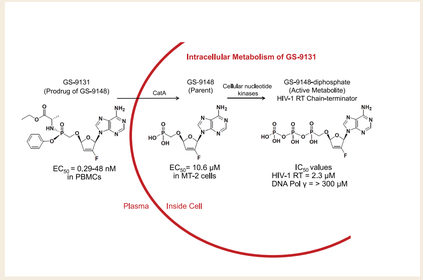
Introduction

- Although there are many potent antiretrovirals available for use around the world, there remain individuals with multi-class drug resistance and limited treatment options[1]
- GS-9131 (Figure 1) is a prodrug of the novel NtRTI GS-9148 that is phosphorylated and inhibits HIV-1 reverse transcription by chain termination[2]
- GS-9131 has low potential for mitochondrial toxicity and renal accumulation[3]
- GS-9131 has broad in vitro activity against HIV-1 and HIV-2[2]
- Notably, GS-9131 maintains potent in vitro activity against HIV-1 with most NRTI resistance patterns for which there are no other NRTI options

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



Methods

- **HIV-1 Antiviral Phenotypic Profiling:** A large panel of HIV-1 with high-level NRTI resistance were profiled for susceptibility to GS-9131 and all other approved NRTIs using the PhenoSense assay (Monogram Biosciences, South San Francisco, CA).
- **HIV-1 Antiviral assay in PBMCs:** The antiviral activity of GS-9131 and control NRTIs were determined for 18 HIV-1 strains spanning subtypes A, B, C, D, E, F, G, and group O and N in PBMCs using an RT assay (Southern Research Institute, Frederick, MD).
- **Drug Resistance Selections:** Dose-escalation drug resistance sections were conducted with GS-9148 and sequenced by population sequencing. Site-directed mutant HIV-1 containing patterns of selected NRTI resistance substitutions were phenotyped (Monogram Biosciences).
- **Drug Combination Studies:** The combination effect of each tested pair of inhibitors was determined by the analysis of datasets from the anti-HIV-1 cytopathic assay using MacSynergy II program (University of Michigan, Ann Arbor, MI).

Results

Table 1. HIV-1 Antiretroviral Activity and Selectivity in MT-2 Cells

Compound	EC_{50} (µM)	CC_{50} (µM)	Selectivity Index
GS-9131	0.16 ± 0.02	>50	>300
GS-9148	10.6 ± 2.4	>1000	>95
TAF[a]	0.005 ± 0.002	42	>8000
TFV	3.5 ± 1.5	>1000	>285
FTC	0.4 ± 0.2	>1000	>2500
ZDV	0.12 ± 0.04	ND	ND
3TC	1.0 ± 0.5	>1000	>1000
ABC	0.3 ± 0.2	55 ± 7	183
ddI	3.1 ± 1.7	ND	ND
d4T	4.8 ± 2.3	286 ± 122	59

Cytotoxicity in HepG2 and human renal proximal tubule epithelial cells (RPTECs; 2 donors) were also >50 µM
a Callebaut et al., Antimicrobial Agents and Chemotherapy (2015) 59 (10): 5909-5916

- GS-9131 exhibits potent activity against HIV-1 with low cytotoxicity and high selectivity

Table 2. Susceptibility of Different HIV-1 Subtypes and HIV-2 to GS-9131 in PBMCs

HIV-1 Subtype	Isolate ID	Susceptibility of HIV-1 and HIV-2 to ARVs (EC_{50} in nM)			
		GS-9131	**AZT**	**DRV**	**BIC**
A	92RW016	3.12	24.6	1.05	0.74
A	92UG037	2.45	<0.15	1.24	1.71
B	89BZ_167	48.1	11.0	2.78	0.88
B	91 US001	8.11	<0.15	2.22	0.87
B	91US004	8.19	2.90	3.01	0.82
B	Ba-L	7.38	26.3	2.02	0.35
C	93IN905	7.33	1.36	1.55	0.15
C	98US_ MSC5016	18.0	15.9	1.80	1.4
D	98UG_57128	15.3	13.9	1.79	0.31
D	99UG_ A07412M1	10.7	12.9	0.98	1.06
E	96TH_M02138	44.8	11.0	2.06	0.8
E	96TH_MNI1046	5.08	6.43	1.09	0.28
F	93BR020	42.9	18.9	2.17	1.13
G	01CM1475MV	8.88	3.41	<0.15	<0.05
N	HIV-1 YBF30	0.29	2.18	1.34	<0.08
O	BCF01	20.4	5.60	4.89	<0.08
O	BCF02	27.1	9.28	0.71	0.09
O	BCF03	15.1	7.14	2.45	<0.08
HIV-2	CDC310319	20.6	51.8	18.8	1.11

- GS-9131 exhibited potent activity against HIV-1 subtype A, B, C, D, E, F, G, and group O and N (EC_{50} 0.29-48 nM) and HIV-2 (EC_{50} = 21 nM)

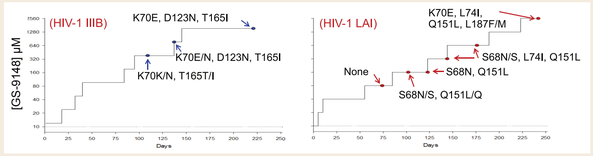
Table 3. Susceptibility of HIV-1 with NRTI Resistance to GS-9131

NRTI Mutation	Susceptibility of HIV-1 to ARVs (Fold-change vs WT)							
	GS-9131	**GS-9148**	**TFV**	**FTC**	**ABC**	**ddI**	**ZDV**	**d4T**
K65R	0.56	0.66	1.98	8.22	2.46	1.87	0.23	1.22
M184V	0.31	0.43	0.47	>110	2.82	1.29	0.19	0.61
L74V	0.61	0.66	0.57	1.30	1.93	1.34	0.22	0.86
L74I	0.67	0.75	0.82	0.92	1.13	0.90	0.40	0.74
K65R+M184V	0.40	0.42	1.20	>110	7.72	3.16	0.17	0.85
K70E+M184V	0.28	0.31	0.58	>110	6.19	1.52	0.10	0.60
L74V+M184V	0.40	0.38	0.35	>110	6.41	2.35	0.12	0.73
4-TAM (4Y)	0.68	0.69	1.69	3.60	2.15	1.05	9.85	1.51
4-TAM (4F)	0.73	0.83	1.36	3.88	1.57	0.96	9.60	1.38
4-TAM (4Y)+M184V	0.41	0.45	0.85	>110	4.66	1.35	1.91	1.35
6 TAMs	1.50	1.65	4.2	5.70	4.70	1.86	379	3.20
6TAMs+M184V	0.75	0.85	2.07	>110	9.60	1.96	32	2.54
T69-insertion+4TAMs	1.11	1.39	5.70	7.68	5.59	3.1	>664	3.57
Q151M	0.97	0.89	0.78	1.40	3.59	3.99	1.97	3.20
Q151M Complex	3.79	3.79	1.53	4.67	6.96	8.22	46	10
Q151M Complex+M184V	0.88	0.96	1.24	>110	>35	13	88	7.06

Color coding uses Monogram PhenoSenseGT cut-offs, with GS-9131 and its prodrug GS-9148 arbitrarily set at 2.5-fold.
Green: Fold change < lower cut-off or <2.5 if not defined
Yellow: Fold change ≥ lower cut-off < upper cutoff
Red: Fold change ≥ upper cut-off or 10-fold if not defined

- GS-9131 exhibited potent activity against HIV-1 with most patterns of NRTI resistance

Table 4. HIV-1 Resistance Selection Experiments with GS-9131



NRTI Mutation	Susceptibility of HIV-1 Mutants (Fold-change vs WT)							
	GS-9131	**GS-9148**	**TFV**	**FTC**	**ZDV**	**ABC**	**ddI**	**d4T**
K70E	1.1	1.1	1.1	2.2	0.2	0.9	1.4	nd
K70N	1.5	1.0	0.6	1.2	0.5	0.8	0.6	0.9
K70E+T165I+D123N	3.3	3.3	2.3	6.7	0.4	2.2	2.3	3.2
Q151L	21	15	0.2	1.2	0.2	1.4	1.4	1.4
Q151L+K70E+L74I+L187F	>62	>39	0.3	8.4	0.3	2.9	1.6	2.9
Q151L+K70E+L74I+L187M	>100	>52	0.5	nd	nd	0.2	nd	nd

Q151L has poor replication in vitro and is rare in patients[4]

Green: Fold change < lower cut-off or <2.5 if not defined
Yellow: Fold change ≥ lower cut-off < upper cutoff
Red: Fold change ≥ upper cut-off or 10-fold if not defined

Table 4. Drug Combination HIV-1 Antiviral Studies of GS-9131 (or GS-9148) with ARVs

Drug Combination	Other Class	Synergy/Antagonism Volumes (µM2.%)		
		Mean Synergy Volume	**Mean Antagonism Volume**	**Combination Effect**
GS-9131 + TAF	NRTI	7 ± 9	-6 ± 5	Additive
GS-9131 + TFV	NRTI	27 ± 27	-6 ± 8	Additive
GS-9131 + d4T	NRTI	16 ± 15	-18 ± 11	Additive
GS-9131 + ddI	NRTI	6 ± 5	-12 ± 8	Additive
GS-9148 + AZT	NRTI	194 ± 95	-0.6 ± 0.8	Highly Synergistic
GS-9148 + FTC	NRTI	79 ± 17	-2.1 ± 2.5	Slightly Synergistic
GS-9148 + ABC	NRTI	112 ± 27	-9 ± 13	Highly Synergistic
GS-9131 + EFV	NNRTI	190 ± 17	-3 ± 3	Highly Synergistic
GS-9131 + NVP	NNRTI	174 ± 23	-8 ± 10	Highly Synergistic
GS-9148 + LPV	PI	53 ± 18	-3.7 ± 2.1	Slightly Synergistic
GS-9131 + DRV	PI	152 ± 19	-3 ± 4	Highly Synergistic
GS-9131 + DTG	INSTI	119 ± 32	-3 ± 6	Highly Synergistic
GS-9131 + BIC	INSTI	124 ± 18	-4 ± 6	Highly Synergistic
Controls of Additive, Synergistic, and Antagonistic Antiviral Activity				
GS-9131 + GS-9131	-	7± 8	-27± 7	Additive
TAF + EVG	-	197 ± 21	-13 ± 13	Highly Synergistic
RBV + d4T	-	0 ± 0	-561 ± 129	Highly Antagonistic

The combination effect was defined as volumes of ≥100 (highly synergistic); ≥ 50 to <100 (slightly synergistic), ≥ 50 to <50 (additive); ≥-100 to <-50 (slightly antagonistic); <-100 (highly antagonistic)

Conclusions

- GS-9131 is a potent NRTI that is active against HIV-1 subtypes A, B, C, D, E, F, G, groups O and N, and HIV-2 with nM range EC_{50} in vitro
  - Low cytotoxicity in multiple cell types and good selectivity index
- GS-9131 has a favorable resistance profile and maintains activity against most NRTI-resistant HIV-1 isolates in vitro, including K65R, M184V/I, multiple TAMs, and T69-insertion
- GS-9131 resistance selection studies in vitro suggest a high resistance barrier with complex resistance pathways in RT:
  - K70E+D123N+T165I in RT with ~3-fold reduced susceptibility
  - Q151L+K70E+L74I+L187F/M with poor fitness and >50-fold resistance to GS-9131, but no cross-resistance to tenofovir
- GS-9131 (tested as GS-9131 or GS-9148) has additive to synergistic antiviral activity in combination with other ARVs
- GS-9131 is an attractive candidate for once-daily dosing in combination with other ARVs in patients with NRTI resistance and limited treatment options; the IND was filed in Dec 2016

References

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