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FAVOURABLE OUTCOME of *IN VITRO* SELECTIONS WITH NOVEL NRTI PRODRUG GS-9131

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Background

GS-9131 is an NRTI candidate for treatment of patients with resistance to other NRTIs. HIV reverse transcription is inhibited by GS-9131 by chain termination. In this study, we employed cell culture models to shed light on the ability of escape mutants to emerge under increasing drug pressure.

Methods

Cord blood mononuclear cells (CBMCs) and MT-2 cells were infected with clinical isolates and passaged in increasing concentrations of GS-9131 and tenofovir disoprixil fumarate (TDF). In CBMCs, virus growth was monitored by weekly determinations of reverse transcriptase (RT). For MT-2 cells, supernatants were collected at the peak of infection by cytopathic effect scoring. In order to identify alterations in the RT region, viral RNA was extracted from tissue culture supernatants and sequenced.

Results

After 40 weeks of sustained drug treatment, none of the CBMC viral cultures tested yielded major resistance mutations. Despite the lack of changes in the RT region associated with resistance to GS-9131 or TDF, most of the isolates were able to endure moderate to very high concentrations of the drugs, 500-20,000 -fold increase for GS-9131 and 100-20,000 -fold for TDF. The A62V and D67N secondary mutations arose in two isolates with GS-9131 and TDF. Using 3TC as a control, the M184I or V mutations rapidly arose in most viruses. Previous studies with GS-9148, for which GS-9131 is a pro-drug, were done in MT-2 cells, and some resistance patterns were identified. In our experiment using MT-2 cells, no major resistance pathways emerged through 18 weeks. One isolate did select for the L187M mutation, which was also identified in the previous study.

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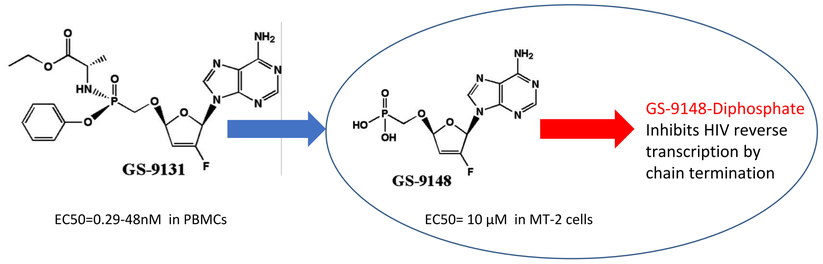
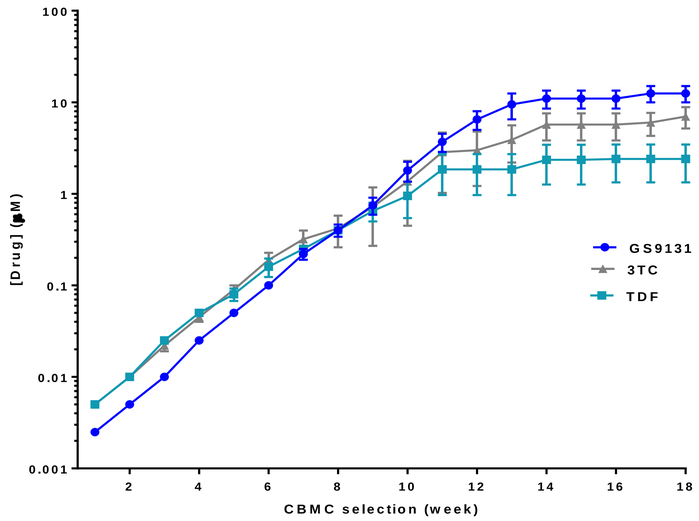
GS-9131

- ❖Broad range of activity against HIV-1 subtypes
- ❖Pro-drug of the GS-9148 with low potential for mitochondrial and renal toxicity
- ❖Maintains in vitro activity against HIV-1 viruses harboring most major NRTI resistance mutational pathways



Drug dose escalations in CBMCs in eight HIV-1 primary isolates

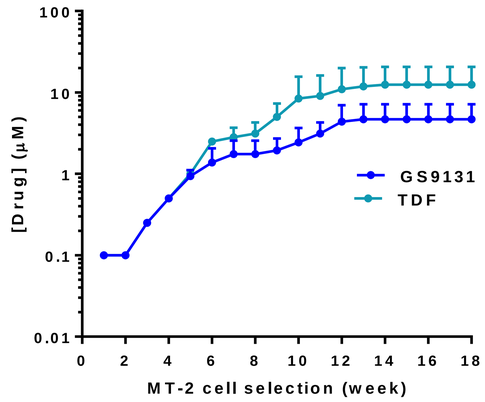


Drug dose escalations were performed in parallel with GS-9131, TDF or 3TC for 18-50 weeks. Sequencing revealed the failure to acquire resistance to GS-9131 in four subtype B and four non-B subtype isolates in CBMCs despite high dose drug escalations over the course of 50 weeks. In contrast, acquisition of M184 I or M184V arose at weeks 8-44 in the 3TC selections leading to >100x resistance.

Identification of drug resistance mutations arising in patient-derived clinical isolates grown in cord blood mononuclear in the presence of increasing concentrations of GS-9131 as compared to TDF and 3TC

Patient isolate	Viral subtype (cluster size)	GS-9131 [Drug] µM week 50	GS-9131 Resistance mutations (selection week)	TDF [Drug] µM week 50	TDF Resistance mutations (selection week)	3TC Resistance mutations (week)
14514	B (1)	1	**A62V (38,46)**	0.25	**A62V (50)**	**M184V (50)**
5326	B (4)	20	P294S (13,39)	10	P294S (21,50)	**M184I (14,23)**
14637	B (45)	5	None (21,26)	1	None (50)	**M184V (8,16)**
10249	B-K103N (44)	1	None (21,39,46)	0.25	None (50)	**M184I (44)**
6343	CRF01_AE (2)	10	None (39,46)	5	Low RT (50)	**M184V (14,24)**
4742	C (2)	5	Low RT (21)	5	None (50)	**M184V (24)**
14494	CRF02_AG (1)	0.001	**D67N (26,33)**	0.005	Lost	**D67N (27,44)**
96USSN20	CRF02_AG (D67N, T69D, K70R)	20	K388R (33,46)	20	**K219Q (24,44,50) L187F (44, 50)**	**M184V(14,24)**

Drug dose escalations in MT-2 cells infected with seven HIV-1 primary isolates



Identification of drug resistance mutations arising in patient-derived clinical isolates grown in MT-2 cells in the presence of increasing concentrations of GS-9131 as compared to TDF.

Patient-derived viral isolate	Viral Subtype (cluster size)	GS-9131 [Drug] µM	GS-9131 Resistance mutations (selection week)	TDF [Drug] µM	TDF Resistance mutations (selection week)
5326	B (4)	5	**L187M (12,16)**	25	None (16)
14637	B (45)	2.5	None (14)	5	None (18)
14969	B (32)	5	None (18)	10	**K65R** (18)
14792	B (1)	2.5	None (18)	10	None (18)
6343	CRF01_AE (2)	2.5	L228ILR (18)	5	None (18)
96USSN20	CRF02_AG	10	**K219K/Q (18)**	25	**K219K/Q**, P294A
pNL4.3	B	5	None (18)	10	None (18)

Phenotypic drug susceptibility in CBMCs of the 5326 viral variant acquiring L187M or P294S in cell culture selections with GS-9131

Patient Isolate	Acquired resistance	IC50 (µM) (fold resistance) GS-9131	TDF	3TC
5326-WT	Baseline (week 18)	**0.003**	**0.083**	**0.027**
5326-L187M	L187M (MT-2, week 18)	**0.019 (6.0x)**	**0.153 (0.5x)**	**0.064 (2.4x)**
5326 - P294S	P294S (CBMCs, week 18)	**0.001 (0.4x)**	**0.052 (0.6x)**	**0.0103 (0.4x)**

Phenotypic Profiling of HIV-1 Site-Directed Recombinants Containing L187 F/M mutations

Virus	Fold resistance relative to Wild-type virus GS-9131	TDF
L187F	**4.3**	**2.0**
L187M	**3.3**	**1.5**

Conclusion

Two methods were employed in order to obtain a better picture of the ability of GS-9131, a drug in development, to put pressure on viruses to escape. The lack of rapid emergence of drug resistance mutations or high-level resistance in emergent variants indicates that GS-9131 is a promising antiretroviral for HIV treatment, which has also been shown to be suitable for individuals harbouring NRTI mutations. Its versatility for use in combination with other drugs may provide more precise and potent options to patients with limitations due to NRTI resistance.

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