S/GSK1349572 is a Potent Next Generation HIV Integrase Inhibitor and Demonstrates a Superior Resistance Profile Substantiated with 60 Integrase Mutant Molecular Clones

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Abstract

Background: S/GSK1349572 is a potent once-daily unboosted integrase inhibitor (INI) under clinical development. Previous studies with a subset of clinically relevant IN mutant virus demonstrated limited cross resistance to other INIs *in vitro*. When virus was passaged in the presence of S/GSK1349572, highly resistant mutants were not isolated, but mutations which conferred low fold change (maximum FC=4.1) were identified within and around the integrase active site. Herein we demonstrate S/GSK1349572's profile against a large panel of clinically relevant integrase mutants.

Methods: Seventy INI-resistance associated site-directed molecular clones (SDM) were constructed from the wild-type molecular clone pNL432. Viral fitness was assessed with replication in PBMCs and/or with a HeLa-CD4 cell single round infection assay. Mutations assessed were primarily those associated with raltegravir (RAL) and elvitegravir (ELV)-resistant viruses (RVs) observed in clinical trials, and included double mutations in the 3 RAL resistance pathways (Y143C/H/R, Q148H/K/R and N155H). Novel triple-quintuple mutations identified during passage of Q148H/K/R clones in the presence of S/GSK1349572 were also included. Ten SDMs replicated poorly, and fold change (FC) in susceptibility against the remaining 60 SDMs was determined with a HeLa-CD4 cell assay.

Results: In RAL-related RVs, all 143 and 155 pathway viruses were still sensitive (FC<5) to S/GSK1349572. G140S/Q148H, a significant RAL-RV in the Q148 pathway, was still sensitive to S/GSK1349572. S/GSK1349572 showed reduced activity against E138K/Q148K (FC=19) and G140S/Q148R (FC=8.4). Addition of T97A, M154I, or V201I, to G140S/Q148H increased S/GSK1349572 resistance. Among ELV-related RVs, all viruses remained sensitive to S/GSK1349572. For the 28 single mutant SDMs, S/GSK1349572 demonstrated low FC (<5). For the 21 double mutant SDMs, S/GSK1349572 demonstrated low FC (<5) in activity against all except E138K/Q148K, G140S/Q148R, and Q148R/N155H. These INI-RVs had relatively low replication rates compared with wild-type virus.

Conclusions: S/GSK1349572 has a markedly different resistance profile, as evidenced by limited cross-resistance to RAL-resistant SDMs. All single mutant and 18/21 double mutants did not alter FC activity to S/GSK1349572 >5. These data show that S/GSK1349572 has a resistance profile distinct from RAL and ELV, and demonstrates the potential for S/GSK1349572 to have a higher genetic barrier to resistance.

Introduction

- S/GSK1349572 is the only once daily, unboosted integrase inhibitor (INI) currently in development with unprecedented antiviral activity and a superior resistance profile. [1, 2, 3]
- S/GSK1349572 has demonstrated a predictable, well-characterized exposure-response relationship and low PK variability. [3]
- S/GSK1349572 had limited cross-resistance to RAL- and ELV-resistant mutants and an improved resistance profile. [2, 4]
- In the presence of S/GSK1349572, highly resistant mutants were not isolated using HIV-1 IIIB infected MT-2 cells. Integrase region mutations selected during S/GSK1349572 passage only conferred low fold change (maximum FC=4.1). [4]

Methods

Confirmation of fold change (FC): NL-432 based site-directed mutant molecular clones (SDMs) were prepared and determined FC using HeLa-CD4 cells. [5]

Isolation of resistant mutants: HIV-1 IIIB or NL-432 based site-directed resistant molecular clones were passaged in MT-2 cells with increasing concentration of drug and analyzed the sequence of integrase region of passaged viruses. [5]

Viral fitness: As one measure of fitness, the infectivity of SDMs was assessed in single round infection assays using HeLa-CD4 cells. [6]

Results

Table 1. S/GSK1349572, RAL and ELV Mean FC Against RAL & ELV-related Single Mutation SDMs

Viruses	Mean FC		
	S/GSK1349572	Raltegravir	Elvitegravir
WT	1.0	1.0	1.0
T66A	0.26	0.61	4.1
T66I	0.26	0.51	8.0
T66K	2.3	9.6	84
E92I	1.5	2.1	8.0
E92Q	1.6	3.5	19
E92V	1.3	1.4	8.3
G118S	1.1	1.2	4.9
F121Y	0.81	6.1	36
T124A	0.95	0.82	1.2
E138K	0.97	1.0	0.93
G140S	0.86	1.1	2.7
Y143C	0.95	3.2	1.5
Y143H	0.89	1.8	1.5
Y143R	1.4	16	1.8
P145S	0.49	0.87	>350
Q146R	1.6	1.2	2.8
Q148H	0.97	13	7.3
Q148K	1.1	83	>1700
Q148R	1.2	47	240
I151L	3.6	8.4	29
S153F	1.6	1.3	2.8
S153Y	2.5	1.3	2.3
M154I	0.93	0.82	1.1
N155H	1.2	11	25
N155S	1.4	6.2	68
N155T	1.9	5.2	39
G193E	1.3	1.3	1.3

3 ≤ FC< 5 | 5 ≤ FC < 10, | 10 ≤ FC

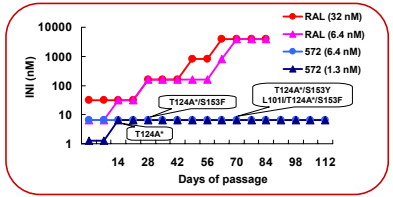
Table 2. S/GSK1349572 and RAL Mean FC Against Q148 pathway Double/Triple Mutation SDMs

Viruses	Mean FC	
	S/GSK1349572	Raltegravir
E138A/Q148R	2.6	110
E138K/Q148H	0.89	17
E138K/Q148K	19	330
E138K/Q148R	4.0	110
G140C/Q148R	4.9	200
G140S/Q148H	2.6	>130
G140S/Q148K	1.5	3.7
G140S/Q148R	8.4	200
E138A/S147G/Q148R	1.9	27

Table 3. S/GSK1349572 and RAL Mean FC Against N155 pathway and Other Double Mutation SDMs

Viruses	Mean FC	
	S/GSK1349572	Raltegravir
L74M/N155H	0.91	28
E92Q/N155H	2.5	>130
T97A/N155H	1.1	26
Y143H/N155H	1.7	38
Q148R/N155H	10	>140
N155H/G163K	1.4	23
N155H/G163R	1.1	17
N155H/D232N	1.4	20
T66I/L74M	0.35	2.0
T66I/E92Q	1.2	18
T66K/L74M	3.5	40
F121Y/T125K	0.98	11

Figure 1. *In vitro* Passage of Wild-type IIIB with S/GSK1349572 or RAL



*T124A is polymorphic and S/GSK1349572 had wild-type potency versus site-directed T124A mutants (Table 1).

Table 4. Mean FC of SDMs identified During Passage with Wild-type Virus in the Presence of S/GSK1349572

Viruses	Mean FC	
	S/GSK1349572	Raltegravir
S153F	1.6	1.3
S153Y	2.5	1.3
L101I/S153F	2.0	1.3
L101I/T124A/S153F	1.9	1.4

Figure 2. *In vitro* Passage of Q148 Mutants with S/GSK1349572[7]

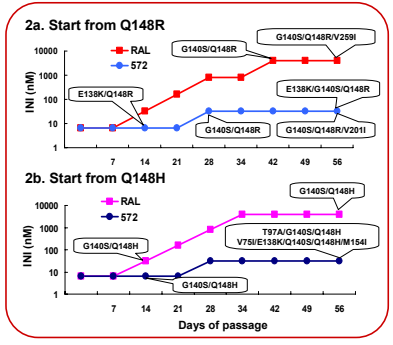


Table 5. Mean FC of SDMs identified During Passage with Q148H/R Clones in the Presence of S/GSK1349572

Amino acid substitution		Mean FC	
Start	Additional	S/GSK1349572	Raltegravir
Q148R	E138K/G140S/Q148R	8.3	>660
	G140S/Q148R/V201I	10	>660
Q148H	T97A/G140S/Q148H	13	>660
	E138K/G140S/Q148H	4.5	500
	G140S/Q148H/M154I	7.0	>660
	E138K/G140S/Q148H/M154I	8.4	>660
	V75I/E138K/G140S/Q148H/M154I	21	>660

Discussion

- S/GSK1349572 exhibited *in vitro* activity against most RAL-resistant viruses which generated in pathways of Y143, Q148, N155.
- *In vitro* passage study showed that S/GSK1349572 had a potential for higher genetic barrier to resistance when compared to RAL and selected less diverse resistant viruses with lower FC.
- Although viruses with limited cross-resistance emerged in the passage study from single mutants, S/GSK1349572 had a higher activity when compared with RAL.
- S/GSK1349572 showed reduced activity against E138K/Q148K, G140S/Q148R and Q148R/N155H, however, these INI-RVs had relatively low replication capacity compared with wild-type virus. (data not shown)
- Ten SDMs (T66I/N155S, G140A/Q148H, Y143C/N155H etc.) out of 70 IN mutants replicated poorly and the FC against INIs could not be assessed.
- Integrase amino acids T124 and L101 are polymorphic and cause no apparent decreased activity versus 572 and RAL.

Conclusions

- S/GSK1349572 demonstrates low FC (<5) in activity against all single mutants including RAL & ELV-related RVs. This is consistent with the finding that no highly resistant mutant was isolated during *in vitro* passage from the wild-type in the presence of S/GSK1349572.
- Most IN double to quintuple mutants had significantly lower FC to S/GSK1349572 compared with RAL.
- Mutants selected by S/GSK1349572 from 148H/R had at least 40-fold lower FC to S/GSK1349572 compared with RAL.
- S/GSK1349572 has a resistance profile distinct from RAL and ELV, and demonstrates the potential for a higher genetic barrier to resistance.

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