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S/GSK1349572 Integrase Inhibitor Resistance Profile

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AUTHOR CONCLUSIONS

S/GSK1349572 is a potent inhibitor of HIV integrase in vitro and in cellular HIV replication assays.

S/GSK1349572 had limited cross-resistance to RAL- and ELV-resistant mutants.

In vitro passage study showed that S/GSK1349572 leads to a less diverse resistance profile with lower fold change.

S/GSK1349572 exhibited in vitro activity against most clinical isolates obtained from patients failing RAL-based therapy.

In vitro experiments support the potential for S/GSK1349572 to have a higher genetic barrier to resistance when compared to 1st generation INIs.

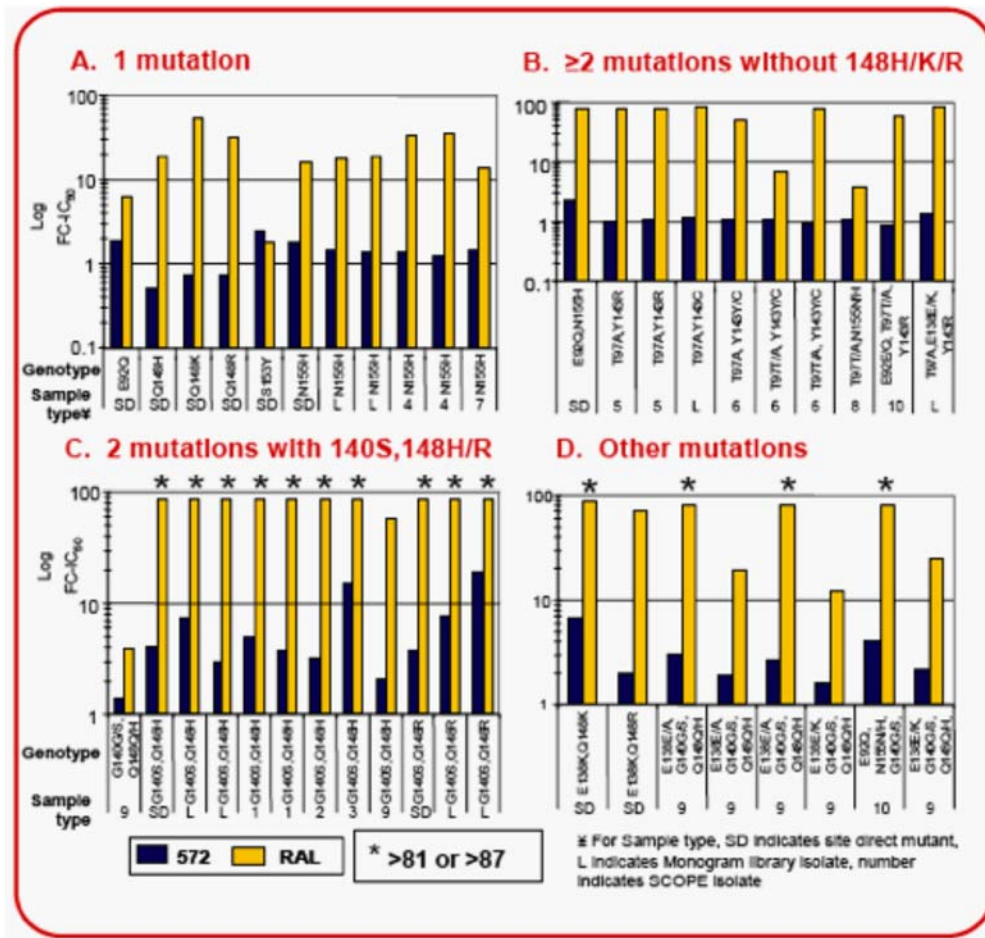
When combined with the unprecedented virologic responses observed during 10 day S/GSK1349572 monotherapy^{1,8}, these data suggest potential to treat patients with RAL resistance.

These observations need to be confirmed in clinical studies, and support further development of S/GSK1349572 for patients across the treatment spectrum.

S/GSK1349572 is a Potent Next Generation HIV Integrase Inhibitor

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Figure 3. S/GSK1349572 and RAL Fold-change IC₅₀s Against Mutated Integrase Site Directed Mutants and Clinical Isolates



- **S/GSK1349572 had greater activity against SDMs and clinical isolates examined with ≥ 2 mutations and without 148H/K/R.**
- **S/GSK1349572 had greater activity across all SDMs and clinical isolates examined with 140S, 148H/R, 138K, 148K/R or with ≥ 3 mutations than RAL.**

ABSTRACT

Objective: S/GSK1349572 was selected from a series of designed two-metal binding integrase inhibitor scaffolds. We demonstrate here its antiviral potency and resistance profile as a next generation integrase inhibitor (INI).

Methods: S/GSK1349572 activity was determined biochemically in a strand transfer assay with purified integrase enzyme and against different HIV strains and clinical isolates in various cell lines. HIV-1 IIB was passaged with S/GSK1349572 under dose escalating conditions. S/GSK1349572 and raltegravir (RAL) susceptibility of clinical isolates from patients failing RAL-based therapy was determined using Monogram Biosciences Integrase PhenoSense assay.

Results: S/GSK1349572 inhibited HIV integrase in an in vitro strand transfer assay with IC₅₀=2.7 nM. In PBMC cell assay, the antiviral IC₅₀=0.51 and IC₉₀=2.0 nM. In MT-4 antiviral assays, potency shift extrapolated to 100% human serum was 75-fold providing

PA-IC₉₀=152 nM in PBMCs. S/GSK1349572 had low nM potency against broad panels of HIV-1 isolates (clades A-G and group O) and against HIV-2 in PBMCs. When virus was passaged in the presence of S/GSK1349572, highly resistant mutants were not isolated; mutations which conferred low fold change (FC ≤ 4.1) were identified within the integrase active site. S/GSK1349572 demonstrated low FC in activity against RAL resistant site directed molecular clones and clinical isolates from RAL-failure patients. **All longitudinal RAL virologic failure samples were more susceptible (and 19/21 were >5-fold more susceptible) to S/GSK1349572 than RAL.**

Conclusions: S/GSK1349572 was a potent inhibitor of HIV integrase in biochemical assay and in cellular HIV replication assays. S/GSK1349572 had a markedly different resistance profile as evidenced by limited cross-resistance to RAL-resistant molecular clones, by its in vitro activity against clinical isolates obtained from patients failing RAL-based therapy, and in selecting different mutations with low level FC during serial passage. **These data demonstrate S/GSK1349572 had a virologic profile distinct from RAL and potential to possess a higher genetic barrier.**

INTRODUCTION

The long-standing Shionogi-GSK Joint Venture has made considerable progress in developing next-generation integrase inhibitors.

S/GSK1349572 is the only once-daily, unboosted integrase inhibitor 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

METHODS

In vitro strand transfer assay: Recombinant HIV integrase was used in an in vitro strand transfer assay which measured the amount of donor DNA covalently attached to target DNA.

Cell based antiviral & cytotoxicity assays; Antiviral & cytotoxicity assays were performed using several virus strains and multiple cell lines. Testing of S/GSK1349572 against various HIV clades and HIV-1 clinical isolates was done at Southern Research Institute in PBMCs and at Monogram Biosciences using their Integrase PhenoSense assay. The effects of protein binding on antiviral activity were tested using human serum albumin or human serum.

Passage study: Generation of virus resistant to inhibitor was performed by passage of IIIB virus in MT2 cells in the presence of S/GSK1349572. For comparison, the potency of INIs that have been studied in the clinic (raltegravir and elvitegravir) were also measured in the above assays. INI-resistant mutant HIV-1 viruses were produced from wild type virus NL-432 using site directed mutagenesis of the integrase coding region and were tested for susceptibility to INIs using infected HeLa-CD4 cells carrying a reporter β-galactosidase gene driven by HIV-1 LTR.^{5,6}

Phenotype study: Integrase resistant HIV-1 sample phenotypes were evaluated at Monogram Biosciences using the Integrase PhenoSense assay. The compounds S/GSK1349572 and RAL were tested side by side and IC₅₀ and fold-change in IC₅₀ vs. wild-type (FC-IC₅₀) were generated.

The samples included 11 site directed mutant (SDM) control HIV-1 IN sequences based on NL43 and eight clinical isolates containing IN resistance mutations from Monogram Biosciences library set. In addition, 31 clinical isolate samples were evaluated from subjects experiencing virologic failure on therapy which included RAL in the UCSF SCOPE Cohort.⁷ Altogether, 39 clinical isolate samples were examined; 30 had IN coding region mutations and 21 of those were longitudinal samples from 9 patients.

Results and Discussions

Table 1. Inhibition of Recombinant HIV Integrase and HIV Replication by S/GSK1349572

INI	Integrase, nM	Antiviral, nM			
	Strand transfer IC ₅₀	PBMC IC ₅₀	Potency Shift with 100% HS	PA IC ₅₀	PA IC ₉₀
S/GSK1349572	2.7	0.51	75	38	152

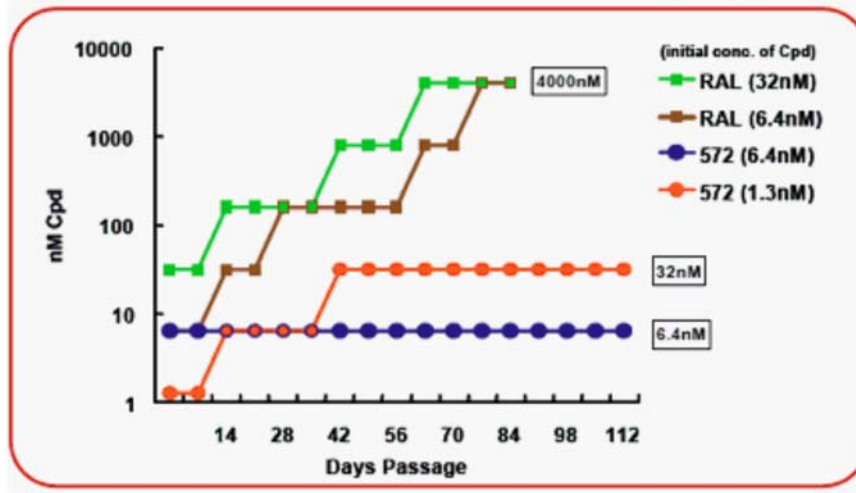
- S/GSK1349572 inhibited recombinant HIV integrase in a strand transfer assay with nanomolar potency.
- The effective IC₅₀ was determined by accounting for the antiviral IC₅₀ fold shift in presence of protein. *In vitro* antiviral assays in the presence of increasing human serum were used to calculate the 75-fold shift in IC₅₀ value of S/GSK1349572 in 100% human serum (by method of extrapolation); the protein-adjusted IC₅₀ (PA IC₅₀) for S/GSK1349572 is estimated to be 38 nM.

Table 2. Inhibition of Different HIV Clades and Clinical Isolates by S/GSK1349572

Assay	N	Mean IC ₅₀ (nM) [range]
HIV-1 Isolates in PBMCs	25	0.36 [0.02–2.14]
HIV-1 Isolates in MDMs	4	1.07 [0.37–1.98]
HIV-2 Isolates in PBMCs	3	0.25 [0.09–0.61]
HIV-1 containing Integrase coding region from clade B isolates	13	0.52 [0.41–0.60]
HIV1 IIIIB	1	0.46
HXB2	1	0.65

- Isolates examined in PBMCs included 3 each of clades A-G, and group O. There were 16 CCR5 tropic, 5 CXCR4 tropic, and 1 undetermined tropic HIV-1.
- S/GSK1349572 was determined to be highly active with broad activity against a total of 26 unique HIV-1 isolates and three HIV-2 isolates.
- S/GSK1349572 was also active against HIV-1 containing the integrase coding region from 13 clade B clinical isolates and two control lab strains (HIV-1 IIB and HXB2) in an isogenic background.

Figure 1. *In vitro* Passage Selected Virus Was Able to Replicate Under Higher Raltegravir (RAL) Concentrations than for S/GSK1349572 (572)



- HIV-1 IIIB was passaged in medium containing increasing concentrations of S/GSK1349572 or RAL.
- Viruses with RAL-resistant mutation(s) replicated in the presence of RAL at the higher concentration of 4,000nM.
- Genotypic assay identified that N155H, Q148K, or Q148R were selected during passages with RAL by day 28 or 42, and resulted in phenotypic resistance (FC=19, >23, or 8) against RAL.
- HIV was unable to replicate under an initial concentration of 32nM S/GSK1349572, and no replication was observed under 160nM during passage.
- These *in vitro* passage data demonstrate the potential for a higher genetic barrier for S/GSK1349572 when compared to RAL.

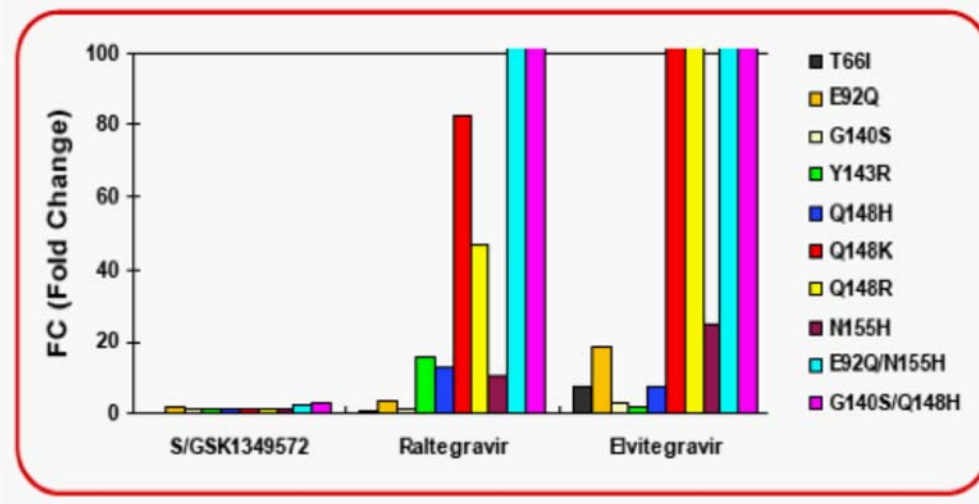
Table 3. Integrase Mutations Generated by Passage of Virus in the Presence of S/GSK1349572, Raltegravir (RAL), or Elvitegravir (ELV)

Raltegravir (84 days)	Elvitegravir (56 days)	S/GSK1349572 (56 days)
T124A Q148K* Q148R E138K/Q148K E138K/Q148R G140S/Q148R N17S/Q148K/G163R G140C/Q148K/G163R E138K/Q148K/G163R E92Q/E138K/Q148K/M154I N155H/I204T V151I/N155H T124A/V151I/N155H	T66I E92Q T124A P145S Q148K Q148R T66I/T124A T66K/T124A E92V/T124A P145S/T124A Q146L/T124A Q148R/T124A T66I/V72A/A128T T66I/E92Q/T124A T66I/T124A/Q146L	T124A T124A/S153F
(FC=6 – >138)	(FC=2 – 497)	S/GSK1349572 (84 days)
		T124A S153Y T124A/S153Y L101I/T124A/S153F
		S/GSK1349572 (112 days)
		T124A S153Y T124A/S153Y L101I/T124A/S153F (FC=1.2 – 4.1)

• Red text indicates substitutions seen in clinical trials

- T124A is polymorphic and S/GSK1349572 has wild type potency versus site directed T124A mutants.
- Highly resistant mutants with high fold change (FC>100) were isolated in the presence of RAL and ELV; many of these mutations have been observed in the clinic in patients failing RAL and ELV-based regimens.
- In the presence of S/GSK1349572, highly resistant mutants were not isolated. Multiple mutations in INI selected during S/GSK1349572 passage only conferred low fold change (maximum FC=4.1).
- Data may be consistent with higher genetic barrier of S/GSK1349572 when compared to RAL and ELV.

Figure 2. Susceptibility of INI Resistant Molecular Clones to S/GSK1349572



- Fold change (FC) of each clone was determined in comparison with the IC₅₀ value for the wild type virus NL432.
- S/GSK1349572 demonstrated low fold change in activity against RAL and ELV-resistant site directed molecular clones.
- Although cross-resistance between RAL and ELV was observed, S/GSK1349572 was active against this panel of INI-resistant mutants.

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