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Resistance Profile of the Integrase Inhibitor S/GSK-364735

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Abstract

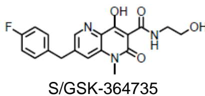
Background: To characterize the resistance profile of a new integrase inhibitor S/GSK-364735, a member of the two-metal binding naphthyridinone scaffold.

Results: With the isolation method employed, resistant mutants were first observed on Day 42; additional mutations occurred as culture continued or drug concentration increased. Q148R and F121Y were the two main pathways for S/GSK-364735 resistance, with the overall composite of mutations similar to previously reported integrase inhibitor (INI) resistant mutations. Comparison of fold resistance (FR) with those of other reported INIs against a panel of INI resistant molecular clones in general showed strong cross resistance, though highly significant differences did occur. Single round infectivity of mutant viruses relative to wild type varied from 0.1 (Q148K), 0.2 (Q148R), to 0.6 (E138K), with replication kinetics consistent with these data. In the Q148R pathway, secondary mutations showed an increase either in resistance (ex, Q148R/G140S increased 7.5-fold) or in relative infectivity (ex, Q148R/E138K increased 2-fold). Relative infectivity and replication kinetics correlated with integration activity of mutant viruses as measured by quantitative PCR analyses of viral DNA species.

Conclusions: (1) S/GSK-364735 had a resistance profile similar to other reported INIs. (2) Secondary mutations elicited examples of both increases in resistance and relative infectivity. (3) Relative infectivity and replication kinetics of mutant viruses correlated with the integration activity of that virus.

Introduction

- The HAART with HIV reverse transcriptase and protease inhibitors has resulted in significant improvement in AIDS-related morbidity and mortality. However, emerging multi-class drug resistant viruses and long-term toxicities warrant development of new classes of anti-HIV drugs.
- The Shionogi-GSK Joint Venture tested the first INI S-1360 in Phase IIa studies. The continuing collaboration has produced S/GSK-364735. We have reported its anti-HIV potency and PK profiles at ICAAC 07 (poster No.H-1047[1] & 1048[2]), and its full anti-HIV profile in a recent publication[3]. While the use of S/GSK-364735 in HIV-infected adults showed promising efficacy in early clinical trials[2], the development of this compound has been discontinued due to adverse effect in monkey long-term toxicity. Additional integrase inhibitor drug candidates are currently in development.
- We report here the resistance properties of S/GSK-364735 as determined by in vitro passage studies, fold resistance of molecular clones, and measurement of relative infectivity and replication kinetics of mutant viruses as surrogates for viral fitness.



S/GSK-364735

IC50 in strand transfer assay: 8 nM
EC50 in PBMC: 2 nM
PA-EC50 extrapolated to 100% HS: 42 nM
CCIC50 in PBMC assay: 55 μM

References

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Methods

- Isolation of resistant mutants: Passage of HIV-1 IIIB in MT-2 cells with increasing concentration of drug and sequencing the integrase gene of emerged mutants.
- Confirmation of responsive mutation and fold resistance: Preparation of site-directed resistant molecular clones, and determination of fold resistance using HeLa-CD4 cells. Mutant virus fitness assessed in single round infection assays using HeLa-CD4 cells, and replication kinetics in Jurkat cells.
- Quantitative PCR assay of viral DNA : MT-4 cells were infected with HIV-1 NL432 virus with dilutions of compounds and collected after 6 or 18 h of incubation. Samples after 6 h of incubation were prepared for total PCR to detect late RT products. Samples after 18 h of incubation were prepared for nested Alu PCR to detect integrated provirus and for 2-LTR PCR to detect 2-LTR circles. The copy number of late RT products, 2-LTR circles, and integrated provirus were determined using specific quantitative PCR methods reported by Butler[4] and Brussel[5].

Results and Discussion

Table 1. Integrase Mutations Generated by Passage of Virus in S/GSK-364735-Containing Medium

Compound	Concentration (ng/mL)	Days of culture: 13	28	42	56	70	84
S/GSK-364735	0.26	No mutation	No mutation				
	1.3	No mutation	No mutation	No mutation			
	6.4			T124A Q148R F121Y	T124A Q146R Q148R E10D/N17S/Q148R F121Y/T124A	Q146R Q148R F121Y/T124A G163R	T66K Q146R V75I/T112S/Q146P Q148R E148K/Q148R F121Y/T124A
	32			↳	Q148R	Q148R E136K/Q148R G140S/Q148R F121Y/T124A	Q95R G140S/Q148R F121Y/T124A
	160					↳	Q148R G140S/Q148R
3TC	4,000 – 20,000	M184I	M184I M184V				

- Q148R and F121Y were isolated on Day 42 in S/GSK-364735-containing medium. In the Q148R pathway, E138K or G140S were secondary mutations observed when culture continued or drug concentration increased. Resistant viruses against 3TC were isolated on Day 13 (M184I) and Day 28 (M184V).

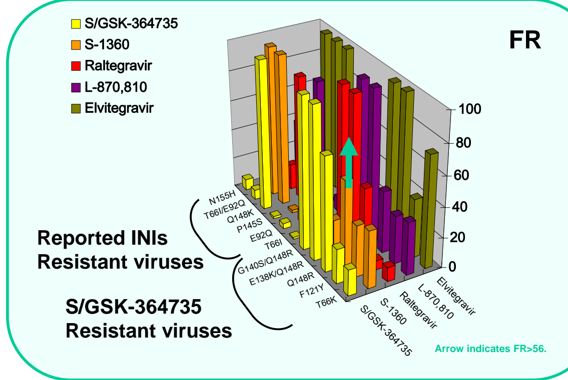
Table 2. Fold Resistance of HIV IN Mutant Molecular Clones to S/GSK-364735

Mutations	Fold resistance (Mutant EC50/WT EC50) S/GSK-364735
Wild type	1.0
T66K	17
Q95R	1.3
F121Y	23
T124A	0.97
E138K	1.1
G140S	4.9
Q146R	1.7
Q148R	75
E138K/Q148R	170
G140S/Q148R	562
V75I/T112S/Q146P	4.8

x 2.3 (Q148R → E138K/Q148R); x 7.5 (Q148R → G140S/Q148R)

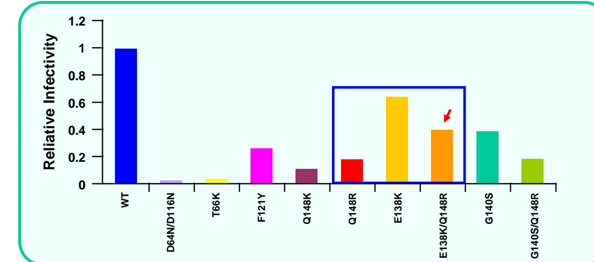
- Based on the highest fold resistance, T66K, F121Y, and Q148R were the single mutations most responsible for the reduced susceptibility to S/GSK-364735. The susceptibility of Q148R to S/GSK-364735 was further reduced by adding the secondary mutation, E138K or G140S, with the G140S effect being more pronounced.

Figure 1. Susceptibility of S/GSK-364735 Resistant Molecular Clones to Other clinical INIs



- The susceptibility of the INI-resistant viruses to these five INIs demonstrated in general high cross-resistance. However, with each resistant virus, there were examples of differences that ranged between 5- and 32-fold. These differences may or may not have clinical significance but do indicate that the 2-metal binding center has some different contact points within this set of inhibitors.

Figure 2. Relative Infectivity of S/GSK-364735 Resistant Viruses in HeLa-CD4 β-Gal Assay



- The relative infectivity of Q148R was poor and was partially improved by introduction of the secondary mutation E138K. On the other hand, infectivity was not improved with the G140S mutation. The relative infectivity of T66K was very poor, and considering its 17-fold resistance, most likely explains why it was rarely observed in passage studies.

Figure 3. Replication Kinetics of Q148R Related Mutant Viruses in Jurkat Cells

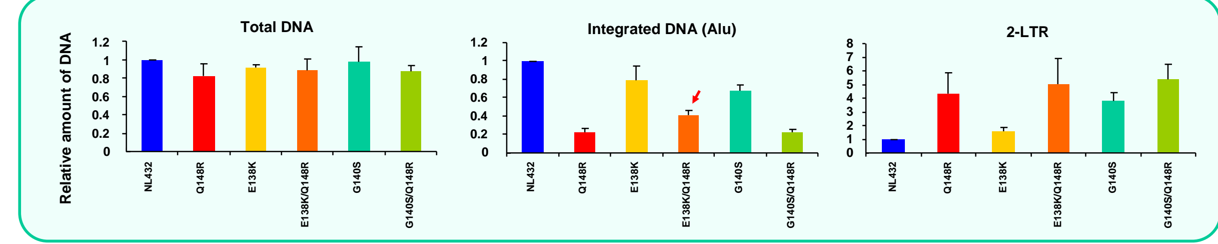
NL-432; Q148R; G140S; G140S/Q148R; E138K; E138K/Q148R

Virus production (RT): 0, 5000, 10000, 15000, 20000, 25000

Days post infection: 0, 5, 10, 15, 20

- The replication kinetics of these single and double mutants were similar to the results of HeLa-CD4 β-Gal assay – the E138K mutation improved the kinetics of the Q148R mutant virus but the G140S mutation did not.

Figure 4. Analyses of the Effects of Mutations on the Step from Reverse Transcription through Integration by Quantitative PCR



- The total DNA of all viruses were the same level, therefore reverse transcription step was not affected by these mutations of integrase region. On the other hand, the relative amount of integrated DNA of Q148R was decreased ~5-fold vs. wild-type. Furthermore, the relative amount of integrated DNA of E138K/Q148R increased ~2-fold vs. Q148R. The amount of 2-LTR circle vs. integrated DNA of these mutants were inversely correlated.

Conclusions

- Q148R and F121Y were the predominant single mutations isolated in the presence of S/GSK-364735. T66K was also isolated.
- These S/GSK-364735 resistant viruses showed strong cross resistance to other reported INIs, though differences were observed.
- In the Q148R pathway, G140S or E138K was added as a secondary mutation.
  - The fold resistance of Q148R/G140S increased versus Q148R compared to little if any change in relative infectivity/replication kinetics.
  - Relative infectivity/replication kinetics of E138K/Q148R virus improved versus Q148R compared to a small increase in fold resistance.
- The relative infectivity/replication kinetics correlated with the integration activity of mutant viruses as measured by quantitative PCR assay of viral DNA species.

Acknowledgements

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