Resistance Profile of GSK3532795

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Deceased.

Background

- GSK3532795 (formerly BMS-955176) was a second-generation MI with broad *in vitro* activity against HIV-1 subtypes B, C and AE[1-4]
- Despite comparable efficacy results as EFV in phase 2b (76%, 83%, 82% for GSK-795 60mg, 120mg and 180mg vs. 77% for EFV), unacceptable rates of gastrointestinal AEs and treatment-emergent resistance in AI468038 (Ph2b) led to the discontinuation of GSK3532795 development

Objectives

- 1) Investigate the *in vitro* resistance profile of GSK3532795 and compare to genotypic profiles seen during the AI468002 (Phase 2a) study[5]
- 2) Relate results to the underlying mechanism for MI resistance in the context of the structural model model

Methods

- Resistance selections were performed in MT-2 cells with wild-type (WT) virus (NL_{4-3}) or recombinants with *Gag* polymorphisms; resistance-inducing substitutions were introduced into NL_{4-3} (site directed changes); mutations were mapped to recently described structures of the purported region of MI binding to the immature CA/SP1 hexamer and correlated to genotypes and phenotypes observed during the 10-day monotherapy in AI468002[6]
- **Passage method**: 1 or 2x EC_{50}, increasing GSK3532795 concentration 2-fold with each passage until viral breakthrough, determine genotypes
- **Breakthrough method**: fixed 30x EC_{50} GSK3532795, measure days to viral breakthrough, determine genotypes

Results

Resistance and resistance barrier testing[6]

- **Method 1:** V362I and A364V mainly selected
  - Additional substitutions selected were A326T (not in LANL database), T332S and R286K (found in 25% of subtype B)
- **Method 2:** Days to virus breakthrough **(Table 1)**
  - GSK3532795 resistance barrier in order: WT > V370A > ΔV370
  - More rapid breakthrough was observed with a higher viral challenge and with Gag V370 polymorphic backgrounds
  - Observed substitutions: A326T, V362I and A364V
  - Additional secondary substitutions: H219Q and I333V

Table 1. Time (Days) to Virus Breakthrough and GSK3532795 Genotypes Identified: 30x EC_{50} Method

Agent and mechanism of action	WT	V370A	ΔV370	WT	V370A	ΔV370
	Lower virus challenge (1x)			Higher virus challenge (10x)		
None	7	7	7	7	7	7
3TC, NRTI	7	7	7	7	7	7
FTC, NRTI	10	10	10	7	7	7
EVG, INI	20	20	38	20	13	20
795, MI	>41	31	13	>41	17	10
TFV, NRTI	>41	>41	>41	20	20	31
RAL, INI	>41	>41	>41	17	31	>41
ATV, PRI	>41	>41	>41	>41	>41	>41

Number of days: ≤7 | 8–13 | 17–31 | >41

GSK3532795 changes at breakthrough			
Virus	**MOI**	**CA-NTD**	**CA/SP1**
WT	high	I333V	
V370A	high	A326A/T	V362I/V
		H219Q	A364V
V370A	low	H219Q/I333V	
ΔV370	not recovered		

Characteristics of mutations from Methods 1 and 2

- Selected substitutions were inserted into WT virus and examined for replication capacity (RC) and susceptibility to GSK3532795 **(Table 2)**
- Site-directed mutants from initial selections showed poor RC. RC improved in the presence of a second mutation e.g. A326T and H219Q
- A364V showed a high level of resistance and a high RC
- Secondary substitutions observed in the capsid C-terminal domain (CTD): R286K, A326T, T332S/N, I333V
- Single secondary substitutions exhibited ≤2 FC reduced GSK3532795 susceptibility; combinations with V362I or V370A, or with V362I/V370A, exhibited up to >1000 FC reduced susceptibility compared with WT

Table 2. Susceptibility and RC of Site-Directed Mutations Derived From Selected Changes

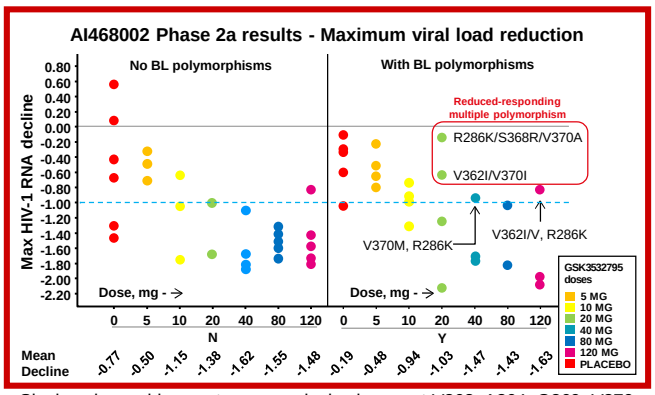
HIV-1, recombinant site-direct mutant viruses	% Subtype B	FC compared with WT	Replication capacity (%WT)
WT	50	1	100
R286K	25	3	81
A326T	0	2	96
T332S	0.5	2	61
I333V	0	2	n.d.
V370A	14	2	93
T371A	0.6	2	70
ΔT371	3.0	1	90
ΔV370	2.5	7	31
ΔV370/T371A	2.8	5	74
R286K/A326T	0	3	106
V362I doubles/triples			
V362I	11	2	68
V362I/T332S	0	6	65
V362I/A326T	0	12	93
V362I/R286K	1.2	53	41
V362I/V370A	1.6	150	76
V362I/V370A/H219Q	0	318	115
V362I/R286K/A326T	0	438	94
V362I/V370A/A326T	0	1449	108
V370 doubles/triples			
V370A/R286K	3.2	5	54
V370A/R286K/A326T	0	158	86
V370A/A326T	0	3	93
A364V	0	833	85

Black font indicates WT or polymorphic variations reported to decrease first-generation MI susceptibility and **red** indicates mutations arising under GSK3532795 selection.

Clinical response to GSK3532795 in subjects with baseline polymorphisms (AI468002 study)

- Subjects dosed 5–120 mg GSK3532795 for 10 days
- Similar to *in vitro* results, certain patient genotypes, such as double V362/V370 polymorphs were observed; such genotypes correlated with suboptimal responses **(Figure 1)**

Figure 1. Clinical Response to GSK3532795 in Subjects With Baseline Polymorphisms



Single polymorphic genotype: any single change at V362, A364, Q369, V370 or deletion of T371; double polymorphic genotypes: any two combinations of single PMs or a single PM plus L363M.

Emergent substitutions in AI468002 at Day 10

- 9/33 (27%) subjects across all doses had both genotypic *Gag* changes and change from baseline (CFB, ratio of FC-EC_{50} at Day 10 vs Day 0) >3 **(Table 3)**. 7/33 (21%) of subjects had either genotypic or phenotypic changes

Table 3. On-Treatment Genotypic and Phenotypic Changes in AI468002 at Day 10

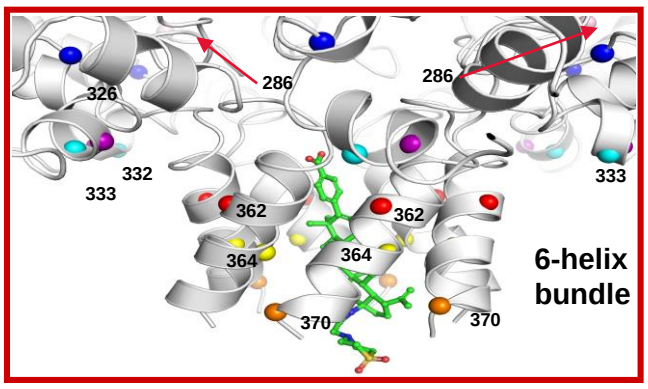
Dose (mg)	Baseline Gag polymorphism	Emergent genotypic mutation	FC-EC_{50} at Day 0/ Day 10	CFB (ratio of FC-EC_{50} at Day 0/ Day 10)	Day 11 VLR	Max viral load reduction
10	WT	A364A/V	0.63/42,2	67	-1.16	-1.76
20	WT	V370I/V	0.48/0.52	1.1	-1.59	-1.68
40	WT	V362I/V, A364A/V V370M	0.39/3.8 1.14/132	9.7 116	-0.95 -1.69	-1.09 -1.69
80	WT	V362I/V, A364V/A A364V/A	3.1/146 0.95/0.94	47 0.99	-1.31 -1.73	-1.50 -1.73
120	286K/R 286K	V362I V370A/V	6.05/>704 3.9/15.8	>116 4.1	-0.83 -1.53	-0.83 -1.57
10	V370M	V362I/V	2.5/1.6	0.62	-1.02	-1.31
20	V370A V370M	V362I/V, A364A/V A364A/V	1.7/>666 0.48/106	>633 221	-0.64 -1.94	-1.23 -2.12
40	286K, V370M 286K/R, 370A/V	Q369Q/H None (V370A)	1.6/20 68.5/496	12 7.2	-0.93 -0.64	-0.93 -1.71
80	V370M	None (A366A/V)*	2.26/2.03	1.1	-0.93	-1.04
120	286K, V362I/V V370A/V	None (V362I) None (V370X)	2.95/407 0.82/5.2	138 6.3	-0.81 -1.54	-0.83 -2.07

Red shows 9/33 (27%) subjects with **both** genotypic *Gag* changes and CFB >3. **Black** shows subjects with either genotypic or phenotypic changes only; *emergence of A366A/V; under study; parentheses indicate conversion from BL mixtures to single genotype on Day 10.

Mapping selected mutations

- Selected mutations disrupt the structure of the CA/SP1 6-helix bundle,[7] increasing access and rate of CA/SP1 cleavage **(Figure 2)**. Bound MI hypothesized to rigidify structure (bound GSK3532795 shown in green below)

Figure 2. Resistance Mutations Map to Capsid C-term Capsid and SP1



Next-generation MIs

- Next generation MIs show improved activity against all key baseline Gag polymorphisms **(Table 4)**
  - Potent against GSK3532795-resistant viruses and a highly resistant triple polymorphic variant from AI468002, suggestive of >99% clinical coverage of *Gag* polymorphisms

Table 4. Next-Generation MIs and Activity

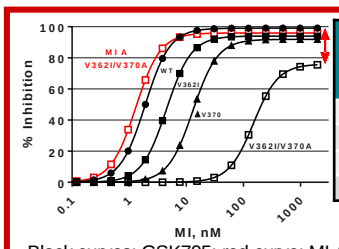
	% LANL DB	GSK3532795		MI A		MI B	
		EC_{50}, μM	FC-EC_{50}	EC_{50}, μM	FC-EC_{50}	EC_{50}, μM	FC-EC_{50}
WT	51	0.0020	1.0	0.0016	1.0	0.0024	1.0
V370A	15	0.0027	1.4	0.0019	1.2	0.0036	1.5
ΔV370	2	0.013	6.7	0.0036	2.3	0.0045	1.9
V362I/V370A	2	0.15	76	0.0013	0.8	0.0026	1.1
A326T/V362I/V370A*	0	1.5	750	0.0035	2.2	0.004	1.7
R361K/V362I/L363M†	0	1.2	601	0.0029	1.8	0.0045	1.9

*Observed in *in vitro* selections; †GSK3532795 baseline resistant genotype; not reported in the 2013 LANL DB.

Next generation MIs – higher maximal *in vitro* inhibition[4]

- Next generation MIs - higher maximal in vitro inhibition across genotypes **(Figure 3)** explains improved activity against all subtypes and polymorphisms **(Table 5)**

Figure 3 & Table 5. Next Generation MIs – Higher Maximal *in vitro* Inhibition



Virus	Maximal Percent Inhibition (MPI)[4]	
	GSK795	MI A
wt	99	99
V362I	94	99
ΔV370	92	97
V362I/V370A	76	96

MPI as described[4]

Black curves: GSK795; red curve: MI A toward V362I/V370A
Green color: MPI values of > 95% correspond to FCIC50 values of < 2 fold

Conclusions

- Emergent substitutions observed *in vitro* and in clinical studies were similar, selecting mainly for changes to A364 and V362
- Certain double Gag polymorphisms in AI468002, e.g., at 362 and 370, were associated with reduced *in vitro* susceptibility and sub-optimal clinical responses
- Secondary mutations in Gag (R286K, A326T, T332S, I333V) conferred reduced susceptibility only in the context of primary polymorphisms. Only R286K was observed clinically
- Next generation MIs exhibit complete antagonism resulting in pan-genotypic phenotypes with greatly improved activity against all single, double and triple Gag polymorphisms, including those causing significant reductions to GSK3532795 susceptibility

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