Nonclinical Pharmacology of GS-3242, a Novel Long-Acting INSTI in Clinical Development

Poster 521

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Conclusions

GS-3242 is a novel INSTI with improved antiviral potency compared to daily oral bictegravir, and a similar nonclinical virology, pharmacology and safety profile.

These data support the ongoing clinical development of GS-3242 as a long-acting INSTI for the treatment of HIV-1 infection with demonstrated preclinical and human PK indicating injectable dosing intervals of at least 4 months.

Plain Language Summary

GS-3242 is a potent and selective investigational INSTI with a favorable nonclinical profile supportive of its ongoing clinical evaluation as a component of a long-acting regimen for HIV-1 treatment.

Introduction

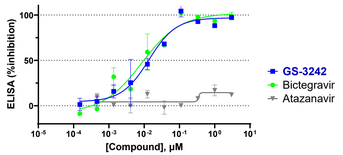
- Once daily oral single tablet regimens containing an integrase strand transfer inhibitor (INSTI) remain the standard-of-care treatment option for people with HIV (PWH).
- For some PWH, including those who experience challenges taking daily pills, the development of novel INSTIs compatible with long-acting lenacapavir dosing[1] would provide an attractive treatment option.
- GS-3242 is an investigational INSTI with a pharmacokinetic (PK) profile supportive of at least 4 months injectable dosing in both nonclinical animal species[2] and in a Phase (Ph)1a clinical study in healthy human participants[3].
- In a 10-day oral Ph1b monotherapy study (NCT07001319)[3], GS-3242 administered on Day (D)1 and D2 showed robust dose-dependent antiviral efficacy in INSTI-naive PWH with a >2-log10 mean plasma HIV-1 RNA decline by D11 without emergent genotypic or phenotypic resistance (450-mg dose).
- Herein we describe the in vitro pharmacology and nonclinical safety/toxicology profiles for GS-3242.

Methods

- HIV-1 integrase strand transfer activity was measured using a commercial ELISA assay.
- Antiviral activity against wild-type HIV-1 was measured in HIV-1BaL-infected primary human immune cells by p24 ELISA, in HIV-1IIIb-infected MT-4 cells using CellTiter-Glo, and in human peripheral blood mononuclear cells (PBMCs) infected with clinical HIV-1 and HIV-2 isolates using a radiolabeled reverse transcriptase assay. Compound cytotoxicity was assessed in primary human cells and a panel of human cell lines using CellTiter-Glo.
- Antiviral activity against INSTI-r site-directed HIV-1 mutants (HXB2 and NL4.3 strains) was measured in a 3-day MT-2 reporter assay (One-Glo) and a 5-day MT-4 cytoprotection assay (CellTiter-Glo). Drug susceptibility to HIV-1 containing known NRTI-r, NNRTI-r, PI-r or CAI-r mutations was assessed in MT-2 cells.
- In vitro selection for drug resistant mutants was performed by dose escalation in HIV-1IIIb-infected Sup-T1 cells; emergent variants were sequenced and phenotyped in MT-4 cells.
- Pairwise in vitro drug combinations were assessed in HIV-1IIIb-infected MT-2 cells and the antiviral combination effect evaluated by LOEWE model using SynergyFinder Plus.
- Safety pharmacology and toxicology profiles were evaluated in vitro and in nonclinical species (rat, monkey) following oral and subcutaneous GS-3242 administration.

Results

Figure 1. Inhibition of HIV-1 Integrase (IN) Strand Transfer Activity

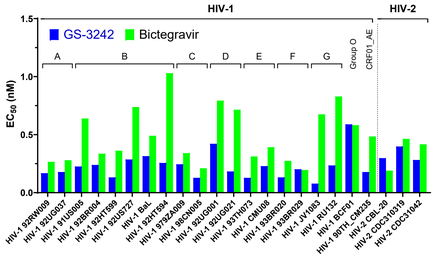


Antiviral	Class	Mean IC_{50} (nM)
GS-3242	INSTI	18.7 ± 2.4
Bictegravir	INSTI (pos control)	19.2 ± 4.9
Atazanavir	PI (neg control)	>3,000

Table 1. Antiviral Activity and Cytotoxicity of GS-3242 in MT-4 T-Cells and Primary Human Target Cells

Cell Type	Compound	EC_{50} (nM)		CC_{50} (µM)	Selectivity
MT-4 T-lymphoblastoid Cell Line	GS-3242	0.82 ± 0.56	3.3X	20.4 ± 6.0	25,100
	Bictegravir	2.71 ± 1.22		3.2 ± 0.6	1,100
CD4+ T- Lymphocytes	GS-3242	0.69 ± 0.14	2.3X	36.9 ± 8.6	53,600
	Bictegravir	1.61 ± 0.38		8.4 ± 1.8	5,200
Monocyte-derived Macrophages	GS-3242	0.23 ± 0.05	3.1X	14.3 ± 0.5	63,200
	Bictegravir	0.72 ± 0.23		34.9 ± 8.2	48,300

Figure 2. Activity of GS-3242 Against HIV Clinical Isolates in Human PBMCs



INSTI	HIV-1 EC_{50} (nM)			HIV-2 EC_{50} (nM)
	Mean	Median	Range	Mean (range)
GS-3242	0.23 ± 0.11	0.21	0.08 - 0.59	0.33 ± 0.06 (0.28 - 0.40)
Bictegravir	0.50 ± 0.24	0.44	0.20 - 1.03	0.36 ± 0.14 (0.19 - 0.46)

Figure 3. Activity of GS-3242 Against an INSTI-r Mutant Panel

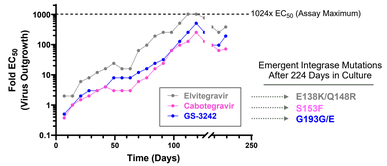
HIV-1 site-directed integrase mutants (n=15):

	E92Q	G118R	Y143R	Q148R	N155H	R263K	L74I G118R	E92Q N155H	G118R S230N	E138K Q148R	G140S Q148R	L74I E138K Q148R	L74I G140S Q148R	E138K G140S Q148H	E138D S147G N155H
GS-3242	2.1	5.6	2.3	1.3	1.7	2.6	4.6	1.0	5.3	3.0	3.2	10	16	5.0	2.5
Bictegravir	1.4	4.9	1.5	0.6	1.1	1.7	4.7	1.0	4.7	3.7	1.7	4.3	6.5	3.7	2.2
Cabotegravir	1.4	6.3	1.4	1.6	1.0	1.3	10	1.2	7.0	15	3.5	25	22	12	5.7
Elvitegravir	51	3.4	5.2	114	28	5.0	5.4	86	3.2	85	80	503	341	576	100

EC_{50} Fold-change (FC) relative to WT: < 3.0 | 3.0 - 10 | >10

Figure 4. In Vitro Resistance Selections with GS-3242

Virus Outgrowth Kinetics and Emergent Integrase Variants:



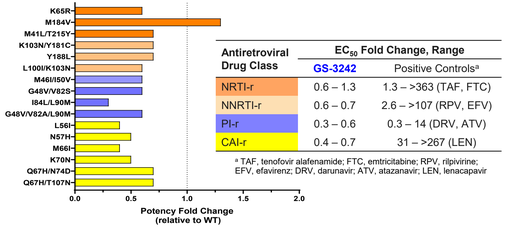
Phenotype:

Compounds	EC_{50} in nM (Fold-Change Relative to WT)			
	WT HXB2	IN-G193E	IN-S153F	IN-E138K/Q148R
GS-3242	1.6 ± 0.2 (1.0)	2.3 ± 0.3 (1.4)	2.4 ± 0.4 (1.5)	3.0 ± 0.5 (1.9)
Cabotegravir	2.6 ± 0.2 (1.0)	3.9 ± 0.4 (1.5)	4.8 ± 0.1 (1.9)	11 ± 1 (4.5)
Elvitegravir	10 ± 2 (1.0)	13 ± 1 (1.3)	18 ± 3 (2.0)	692 ± 224 (68)

Table 2. GS-3242 Shows Low Cytotoxicity In Vitro Across Human Cell Lines and Primary Human Cells

Target Cells	Human Cells	Tissue Origin	Cytotoxicity, CC_{50} (µM)	
			GS-3242	Puromycin
Immortalized Cell Lines	Huh-7	Hepatoma	>44	0.4 ± 0.1
	Gal-Hep-G2	Hepatoma, galactose-adapted	>38 ± 7	0.5 ± 0.2
	Gal-PC-3	Prostate Carcinoma, galactose-adapted	>44	0.3 ± 0.1
	MRC-5	Embryonic Lung Fibroblast	>44	0.3 ± 0.1
Primary Cells	Hepatocytes	Liver Donors (n=3)	>50	1.3 ± 0.4
	Activated PBMCs	Blood Donors (N=4), IL-2/PHA treated	14 ± 1	1.3 ± 0.5
	Unstimulated PBMCs	Blood Donors (N=4), resting	20 ± 6	1.0 ± 0.1

Figure 5. Activity of GS-3242 Against HIV-1 Mutants Resistant to Other Antiretroviral Drug Classes



Antiretroviral Drug Class	EC_{50} Fold Change, Range	
	GS-3242	Positive Controls[a]
NRTI-r	0.6 – 1.3	1.3 – >363 (TAF, FTC)
NNRTI-r	0.6 – 0.7	2.6 – >107 (RPV, EFV)
PI-r	0.3 – 0.6	0.3 – 14 (DRV, ATV)
CAI-r	0.4 – 0.7	31 – >267 (LEN)

[a] TAF, tenofovir alafenamide; FTC, emtricitabine; RPV, rilpivirine; EFV, efavirenz; DRV, darunavir; ATV, atazanavir; LEN, lenacapavir

Figure 6. GS-3242 Shows No In Vitro Antiviral Antagonism When Combined with Other Antiretrovirals

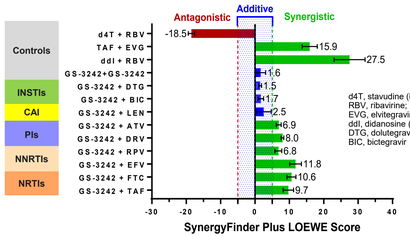


Table 3: GS-3242 Was Well Tolerated In Nonclinical Safety Studies Up to the Highest Dose Levels Tested

Safety pharmacology studies	Outcome
Safety panel (n=86 targets)	Melanocortin receptor 4 (MC4; IC_{50} 2.33 µM)
Rat neurobehavioral function	• No GS-3242 related observations • NOEL= 250 mg/kg (highest dose tested)
Monkey cardiovascular and respiratory function	• No GS-3242 related observations • NOEL= 600 mg/kg (highest dose tested)
hERG potassium channel	IC_{50} not calculable

Genetic toxicity studies	Outcome
Ames (in vitro)	Non-mutagenic
Chromosomal aberration (in vitro)	Non-mutagenic
Rat repeat dose micronucleus (in vivo)	Non-mutagenic

Repeat-dose studies	Outcome
Rat 13-week	• No GS-3242 related adversity or target organ toxicity • NOAEL= 250 mg/kg/week (highest dose tested)
Monkey 13-week	• No GS-3242 related adversity or target organ toxicity • NOAEL = 600 mg/kg/day (highest dose tested)

NOEL, no observed effect level; NOAEL, no observed adverse events level

References: 1. SUNLENCA. Prescribing information. Gilead Sciences, Inc.; 2024. 2. Subramanian M., et al. CROI. 2026, Poster Session F-02, Poster #490. 3. Gupta S., et al. CROI. 2026, Oral Presentation #174.

Disclosures: All authors are current or former employees of and own stock in Gilead Sciences, Inc.

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