Poster 539

GS-6207: A Novel, Potent and Selective First-In-Class Inhibitor of HIV-1 Capsid Function Displays Nonclinical Pharmacokinetics Supporting Long-Acting Potential in Humans

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Introduction

- Standard-of-care (SOC) for the treatment of HIV-1 infection includes the use of combination antiretroviral (ARV) therapy. Current ARV therapies are efficacious and well tolerated.
- Challenges with SOC include necessity for strong adherence to a daily dose regimen and fatigue in compliance due to long-term treatment.
- Long-acting ARVs providing less frequent dosing, for example once quarterly (Q3M) with low injection volume, are desirable.
- GS-6207, an analog of GS-CA1[1], is a novel, selective and highly potent inhibitor of HIV capsid function.
- GS-6207 safety and pharmacokinetics (PK) is currently being evaluated in human clinical trials.

Methods

- Antiviral activity was evaluated in MT-4 cells and in human peripheral blood mononuclear cells (PBMCs) acutely infected with HIV-1 (IIIb strain) and clinical HIV-1 isolates, respectively. Effective concentration resulting in 50% inhibition (EC_{50}) and Hill slopes values were determined from high resolution (40-point) dose response curves. The drug concentration resulting in 50% loss in cell viability (CC_{50}) was assessed in uninfected MT-4 cells.
- Standard *in vitro* methods were used to characterize compound lipophilicity (LogD) and aqueous solubility.
- The extent of GS-6207 (2 µM) binding to pooled plasma proteins from nonclinical species and human was assessed by equilibrium dialysis at 37 °C. Values are the mean ± standard deviation (SD) of 3 determinations.
- The relative protein binding of GS-6207 (2 µM) between human plasma and cell culture medium containing 2% (v/v) fetal bovine serum was assessed in a competitive equilibrium dialysis assay at 37 °C. Values are the mean ± SD of 5 determinations.
- Metabolic stability of [^{3}H]GS-6207 (0.25 µM) was assessed *in vitro* in Sprague-Dawley rat, beagle dog, cynomolgus & rhesus monkey, and human in cryopreserved hepatocyte suspensions over a 6-hour time course. The rates of [^{3}H]GS-6207 disappearance and appearance of radiolabeled metabolites were measured. *In vitro* half-life values were determined and then scaled to predict hepatic metabolic clearance using the well-stirred liver model[2].
- GS-6207 PK was determined following a 0.5-hour intravenous (IV) infusion at 1 mg/kg in rat, dog and monkeys. Serial blood samples were collected and the plasma concentrations were quantified. PK parameters were determined by non-compartmental analysis.
- GS-6207 PK was determined in rat and dog following subcutaneous (SC) injection of two long-acting formulations (A and B). Three animals were dosed in each group. The SC dose was administered via a syringe injection in the intra-scapular region. Factors that can affect release were evaluated – these include dose amount, dose concentration, dose volume and number of injections.
- GS-6207 concentrations in all *in vitro* and *in vivo* experiments were quantified by liquid chromatography – mass spectrometry methods.

Results

Table 1. Antiviral Activity of GS-6207 vs. Currently Marketed ARVs

	MT-4 Cell Line ± Infection with HIV-1 (IIIb strain)						
	Antiviral EC_{50} (nM)[a]	Cytotoxicity CC_{50} (µM)[a]	Selectivity Index (SI)	Hill Slope[a]	Antiviral EC_{95} (nM)[b]	Human Serum Shift	Antiviral $paEC_{95}$ (nM)[b]
GS-6207	0.10 ± 0.01	26.6 ± 14.2	140,740	3.51	0.23 ± 0.02	17.4	4.0 ± 0.4
EFV	0.79 ± 0.06	20.6 ± 4.6	14,940	3.25	2.0 ± 0.2	22.4	44 ± 3
RPV	0.57 ± 0.03	6.8 ± 1.5	11,890	3.17	1.4 ± 0.7	32.2	45 ± 2
DTG	1.34 ± 0.14	15.3 ± 5.0	7,980	2.14	5.3 ± 0.6	29.5	156 ± 16
ATV	7.23 ± 0.50	50.5 ± 8.1	4,720	3.13	18.5 ± 1.3	8.1	150 ± 11

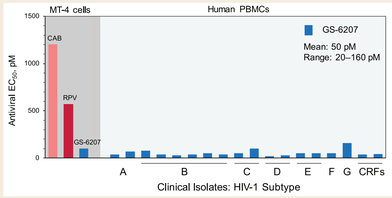
EFV, RPV = efavirenz and rilpivirine (NNRTIs, non-nucleoside reverse transcriptase inhibitors); DTG = dolutegravir (INSTI, integrase strand transfer inhibitor); ATV = atazanavir (PI, protease inhibitor); SI = CC_{50}/EC_{50} ratio

[a] EC_{50}, CC_{50} and Hill slope values (mean ± SD) obtained from at least 3 independent experiments performed in quadruplicate

[b] $EC_{95} = EC_{50} \times (95/5)^{1/Hillslope}$; $paEC_{95}$ = human serum shift X EC_{95}

- GS-6207 is more potent than currently marketed ARV drugs

Figure 1. GS-6207 Antiviral Activity Against a Panel of HIV-1 Clinical Isolates



PBMCs, peripheral blood mononuclear cells; CAB, cabotegravir (INSTI); RPV, rilpivirine (NNRTI); CRFs, circulating recombinant forms

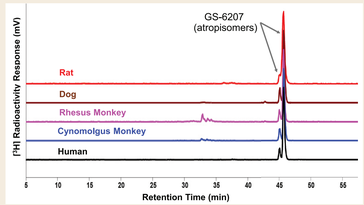
- GS-6207 is a potent inhibitor of all major HIV-1 subtypes

Table 2. Summary of GS-6207 *in Vitro* Properties

Property	Condition	Results
Aqueous Solubility (µM)	pH 2.0	< 1
	pH 7.4	< 1
Lipophilicity (LogD)	pH 7.4	3.7
Plasma Protein Binding (% free)	rat	0.13 ± 0.02
	dog	0.83 ± 0.19
	cynomolgus monkey	1.11 ± 0.22
	rhesus monkey	1.45 ± 0.18
	human	1.46 ± 0.23
Relative Protein Binding (Protein Shift)	Human plasma vs cell culture medium	17.4 ± 0.9

- GS-6207 *in vitro* properties are well-suited for an extended release parenteral formulation

Figure 2. Radiochromatogram Following a 6-hour Hepatocyte Incubation



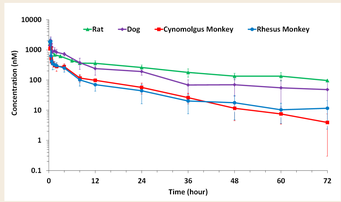
- In nonclinical species, limited turnover was observed
- Lowest turnover was observed in human

Table 3. GS-6207 *In Vitro* Rates of Metabolism in Cryopreserved Hepatocytes

Species	n	Predicted Hepatic Clearance (L/h/kg)	Predicted Hepatic Extraction (% of liver blood flow)
Rat	2	0.02	< 1
Dog	2	0.06	3
Cynomolgus Monkey	3	0.05 ± 0.01	3
Rhesus Monkey	3	0.15 ± 0.03	6
Human	3	0.01 ± 0.00	< 1

- GS-6207 predicted to have low hepatic metabolic CL in all species

Figure 3. GS-6207 Plasma PK Profiles Following IV Infusion Administration in Nonclinical Species



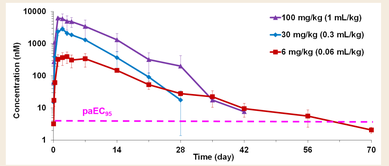
- GS-6207 half lives ($t_{1/2}$) ranged from 15 to 38 hours in nonclinical species

Table 4. GS-6207 PK Parameters Following IV Infusion Administration

Species	n	CL (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)
Rat	3	0.045 ± 0.004	2.22 ± 0.56	38.1 ± 7.9
Dog	3	0.070 ± 0.025	1.96 ± 0.91	30.2 ± 24.4
Cynomolgus Monkey	3	0.21 ± 0.06	2.86 ± 0.54	14.6 ± 5.5
Rhesus Monkey	6	0.25 ± 0.08	5.75 ± 2.24	37.7 ± 11.4

- GS-6207 demonstrated low CL (1% to 12% of hepatic blood flow) and a moderate V_{ss} in four nonclinical species

Figure 4. GS-6207 Plasma PK Profiles in Dogs Following a Single SC Dose of Formulation A at 100 mg/mL Dose Concentration



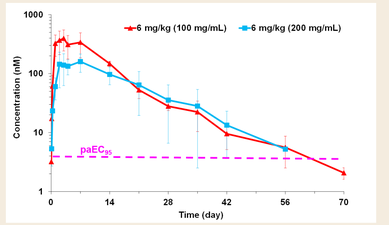
- GS-6207 displayed long-acting SC PK with a sustained slow drug release at all administered doses
- GS-6207 concentrations were quantifiable for at least 28 days
- No unintended rapid drug release (ie, dose dumping) was observed

Table 5. GS-6207 Plasma PK Parameters in Dogs Following a Single SC Dose of Formulation A at 100 mg/mL Dose Concentration

Dose (mg/kg)	AUC_{inf} (µM·h)	C_{max} (µM)	T_{max} (h)	$t_{1/2}$ (h)
6	129 ± 42	0.410 ± 0.141	40 ± 28	322 ± 51
30	497 ± 51	2.960 ± 0.295	40 ± 14	66 ± 5
100	1357 ± 500	7.243 ± 1.279	32 ± 14	94 ± 59

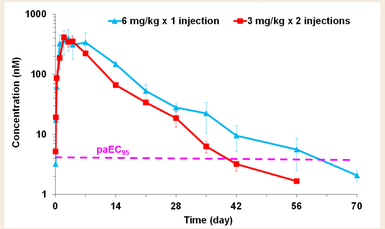
- Observed half-lives following SC administration were extended relative to those observed from IV administration
- Exposure (AUC_{inf} and C_{max}) increases were approximately proportional to dose

Figure 5. GS-6207 Plasma PK in Dogs Following a Single SC Dose of Formulation A at Different Dose Concentrations



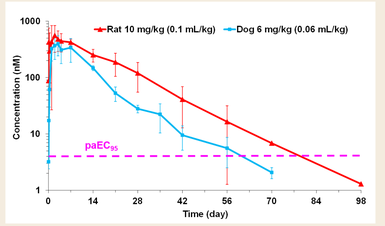
- Lower mean C_{max} and AUC_{inf} at higher dose concentration

Figure 6. GS-6207 Plasma PK in Dogs Following a Single SC Dose of Formulation A at 100 mg/mL Dose Concentration with One or Two Injections



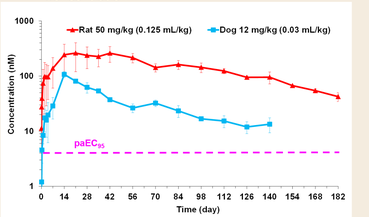
- Mean C_{max} is similar and mean AUC_{inf} is lower with two injections

Figure 7. GS-6207 Plasma PK in Rats and Dogs Following a Single SC Dose of Formulation A at 100 mg/mL Dose Concentration



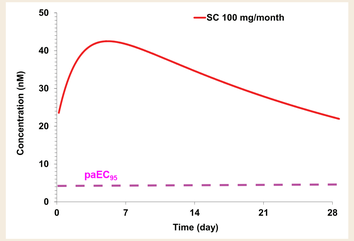
- Formulation A supports long-acting administration, potentially monthly or longer, in human

Figure 8. GS-6207 Plasma PK in Rats and Dogs Following a Single SC Dose of Formulation B at 400 mg/mL Dose Concentration



- Formulation B supports long-acting administration, potentially Q3M, in human

Figure 9. Simulated Steady-State Human Plasma PK Following Monthly SC Doses of Formulation A



- Predicted 100 mg monthly SC dose in human

Conclusions

- The anti-HIV activity, *in vitro* metabolism and nonclinical PK profiles of GS-6207 demonstrate its potential as a first-in-class, long-acting antiretroviral agent.
  - First-in-class picomolar inhibitor of HIV capsid function.
  - High potential to be clinically effective against a broad range of HIV-1 strains.
  - Demonstrates low aqueous solubility and high *in vitro* metabolic stability in human hepatocytes.
  - Exhibits low *in vivo* CL and moderate V_{SS} in nonclinical species.
  - Sustained drug release observed at all administered doses following a single SC administration. No unintended rapid drug release was observed.
  - Displays long-acting nonclinical pharmacokinetics from extended release formulation that supports potentially Q3M administration in human.
- GS-6207 is currently being evaluated in Phase 1 safety and PK clinical studies in healthy human volunteers.

References

1. Tse WC, et al. 2017 CROI Conference; Seattle, WA. Abstract # 38.
2. Pang KS and Rowland M, J Pharmacokinet Biopharm 1977; 5:655-680.

Acknowledgments

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