Preclinical Characterization of GS-5894, a Potent NNRTI with Once-Weekly Oral Dosing Potential

Poster #636

GS-5894

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Conclusions

- GS-5894 is a novel and potent NNRTI with an improved resistance profile compared to other NNRTIs
- GS-5894 demonstrates a low CL and long MRT when dosed in dogs
- Given the low predicted metabolic CL, GS-5894 shows potential as a component of a novel once-weekly oral regimen for HIV-1 treatment

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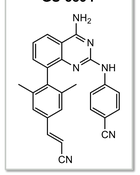
Background

- Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a clinically validated class of HIV-1 antiretrovirals
- NNRTIs bind an allosteric binding pocket near the polymerase active site of HIV-1 reverse transcriptase (RT) to prevent viral replication
- In the clinic, NNRTIs are prescribed as part of a highly active antiretroviral regimen containing one or more nucleoside reverse transcriptase inhibitors (NRTIs) and/or an integrase strand transfer inhibitor (INSTI)
- Common resistance-associated mutations (RAMs) selected by NNRTIs include K103N and Y181C within the binding pocket of HIV-1 RT
- Although all currently approved oral NNRTIs require at least once-daily administration, their physiochemical properties (low solubility, high logD) make them good candidates as a component of a long-acting regimen
- Here we describe GS-5894, a novel and potent NNRTI with an improved resistance and metabolic profile supportive of once-weekly (QW) oral dosing for the treatment of HIV-1 infection

GS-5894

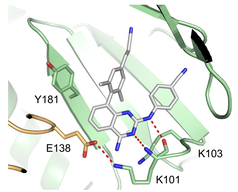


Methods

- Drug half-maximal inhibitory concentration (IC_{50}) values against recombinant HIV-1 RT enzyme were measured using a biochemical assay
- Antiviral activity resulting in 50% inhibition (EC_{50}) and 50% loss in cell viability (CC_{50}) was evaluated in:
  - Cultured human T-cell lines (MT-4, MT-2) acutely infected with HIV-1_{IIIB}
  - Primary human CD4+ T-cells activated with phytohemagglutinin (PHA) and human interleukin-2 (IL-2) and in monocyte-derived macrophages acutely infected with HIV-1_{BaL}
- HIV-1 cross-resistance was assessed against a panel of 32 HIV-1 reporter viruses containing NNRTI resistance-associated mutations at Monogram Biosciences (South San Francisco, CA)
- Mutations that emerged under selective drug pressure were selected by dose-escalation in MT-2 cells infected with HIV-1_{IIIb} and identified by population sequencing
- Level of drug resistance was expressed as a mean fold-change value calculated for each drug from the ratio of EC_{50} for the selected virus (or site-direct mutant) over the EC_{50} of the WT (or parental input) control virus
- Compound binding to rat, dog and human plasma was measured by equilibrium dialysis (EQDS). Predicted clearance (CL) was measured by metabolic stability in hepatocytes.
- In vivo pharmacokinetic (PK) studies were conducted in rat and dog following GS-5894 oral (PO) and intravenous (IV) administration. Measured PK parameters included the apparent volume of distribution at steady state (Vss), bioavailability (F%), and mean residence time (MRT).

Results

GS-5894 Directly Binds to and is a Potent Inhibitor of HIV-1 RT



GS-5894 / HIV-1 RT co-crystal structure

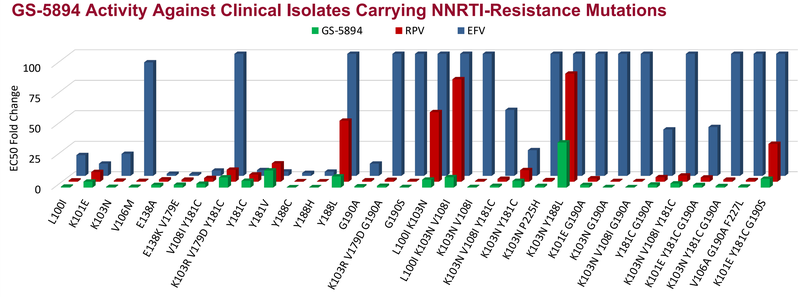
	HIV-1 RT IC_{50} (nM)
GS-5894	5.6 ± 2.9
Rilpivirine (RPV, NNRTI)	2.6 ± 1.2
Efavirenz (EFV, NNRTI)	4.1 ± 0.7
Atazanavir (ATV, PI)	>200

Inhibition of HIV-1 RT enzymatic activity

GS-5894 Activity and Selectivity in Human Cells

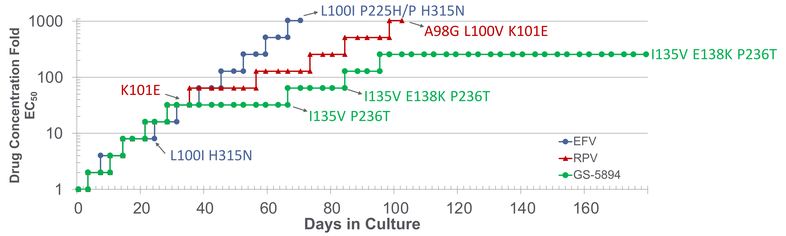
	MT-4 T-cell line			CD4+ T-lymphocytes			Macrophages		
	EC_{50} (nM)	CC_{50} (μM)	TI (CC_{50}/EC_{50})	EC_{50} (nM)	CC_{50} (μM)	TI (CC_{50}/EC_{50})	EC_{50} (nM)	CC_{50} (μM)	TI (CC_{50}/EC_{50})
GS-5894	2.7 ± 0.3	20.2 ± 10.6	7,600	1.7 ± 0.6	18.4 ± 3.3	11,200	1.5 ± 0.8	>100	>66,200
RPV	0.7 ± 0.3	6.5 ± 1.5	8,900	0.9 ± 0.8	7.3 ± 1.1	8,110	0.3 ± 0.1	>100	>400,000

GS-5894 Activity Against Clinical Isolates Carrying NNRTI-Resistance Mutations



- GS-5894 has an improved resistance profile against clinical isolates from NNRTI- experienced patients compared to control NNRTIs EFV and RPV

Dose Escalating Resistance Selection with GS-5894



Selection Drug	Fold EC_{50} Reached	HIV-1 RT Mutations	Fold Change EC_{50} from WT		
			GS-5894	EFV	RPV
GS-5894	32	I135V, P236T	1.3	0.6	1.0
	256	I135V, **E138K**, P236T	12.8	5.5	14.5
EFV	1024	**L100I**, **P225H/P**, H315N	1.6	>37	3.1
RPV	1024	**A98G**, **L100V**, **K101E**	2.7	>37	47

Known NNRTI resistance-associated mutations in boldface

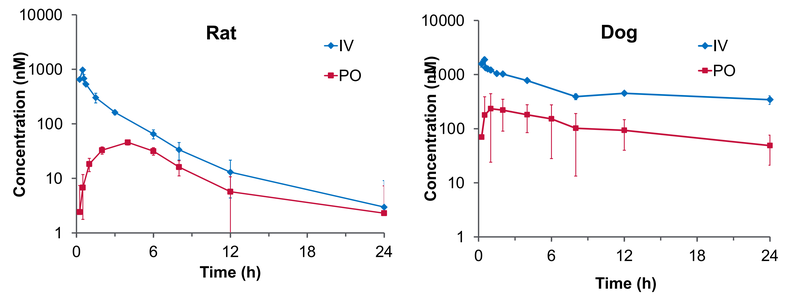
- GS-5894 selections progressed more slowly relative to control NNRTIs RPV and EFV

GS-5894 Plasma Binding and Predicted Metabolic Stability

	GS-5894		
	Rat	Dog	Human
Plasma binding (% free)	0.07	0.04	0.06
Hepatocyte predicted CL (L/h/kg)	1.39	0.50	0.17

- GS-5894 is tightly bound to plasma across species
- GS-5894 is predicted to exhibit low human hepatic metabolic CL

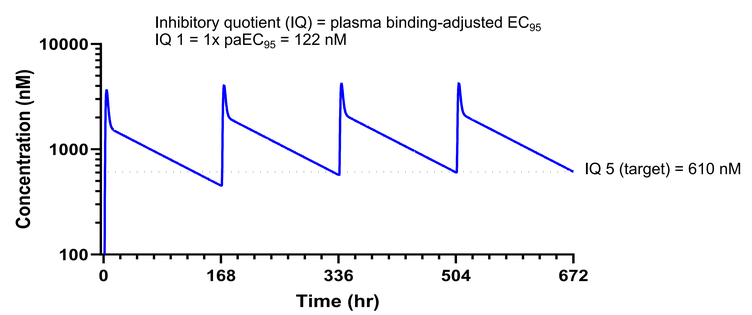
GS-5894 Pharmacokinetic Profile in Rat and Dog



	Rat	Dog
CL (L/h/kg)	1.5 ± 0.1	0.11 ± 0.02
V_{ss} (L/kg)	4.4 ± 0.9	2.6 ± 0.2
F (%)	34.4 ± 17.4	30.9 ± 17.6
MRT (h)	2.9 ± 0.8	23.4 ± 5.3

- Dog in vivo clearance is lower than predicted by hepatocyte stability

GS-5894 Human Dose Prediction



- Using a hybrid compartmental/allometric model, the predicted QW human dose is <600 mg GS-5894 to achieve an IQ ≥ 5 at steady state trough concentration