Novel Non-Catalytic Site Integrase Inhibitor with Improved Resistance Profile

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Conclusions

- GS-9822 is a novel, potent NCINI with a higher barrier to resistance relative to early prototype NCINIs, including GS-9695.
- GS-9822 retains potency against mutant viruses resistant to other classes of antiretroviral agents, including protease and strand transfer integrase inhibitors.
- GS-9822 induces a shift in CCD helix that appears to be a result of trying to accommodate the oxetanyl piperidine moiety with shift induced by T174I mutation.
- Projected once-daily oral dosing makes GS-9822 suitable for combination with other ARVs.
- A unique and difficult-to-monitor urothelial toxicity observed in cynomolgus monkeys poses a formidable challenge for further development of GS-9822.

Background

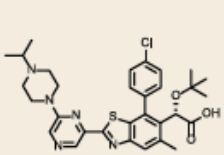
- Non-catalytic site integrase inhibitors (NCINIs) are a promising class of novel antiretrovirals (ARV). Here we describe the search for an NCINI with the potential for low-dose, unboosted once-daily oral dosing, potency against NCINI binding-pocket variants, a high barrier to resistance, and a favorable safety profile.
- Lead optimization resulted in the identification of the development candidate GS-9695, which contained the benzothiazole core. Further profiling revealed a low barrier to resistance development and difficult-to-monitor urothelial cell toxicity in monkey with an unknown mechanism of action.
- Further optimization efforts led to the discovery of GS-9822, a structurally differentiated development candidate also containing the benzothiazole core.
  GS-9822 was evaluated for potency, resistance, DMPK and toxicological profile.

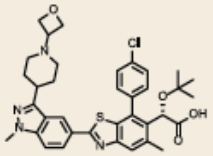
Methods

- Antiviral potency and cytotoxicity were assessed in MT-4 and MT-2 T-cell lines.
- Resistance associated mutations were identified through dose-escalation resistance selection.[1,2]
- Barrier to resistance was evaluated in a viral breakthrough assay at fixed drug concentrations equivalent to actual or projected human plasma concentrations at 24 hours.[3]
- Metabolic stability with pooled hepatic microsomal fractions and NADPH/UDP-glucuronic acid co-factors was determined using the in vitro half-life method. In vitro half- life values were scaled to predicted hepatic metabolic clearance using the well-stirred liver model.
- Protein binding was determined by equilibrium dialysis with a semipermeable membrane separating two half-cells.[4]
- Interactions of NCINIs with WT and T174I mutant catalytic core domains of integrase were elucidated by X-ray crystallography.
- Animal PK: test compounds were dosed in rats, dogs or monkeys via 30-min IV infusion or oral administration. Non-compartmental pharmacokinetic analysis was performed on the plasma concentration-time data to generate the PK parameters.
- Pre-clinical toxicology was assessed for GS-9822. Rats were dosed at 20, 60, and 200 mg/kg for 14 days and cynomolgus monkeys were dosed at 20, 60, and 200 mg/kg for 7 days.

Results

Characterization of GS-9695 and GS-9822



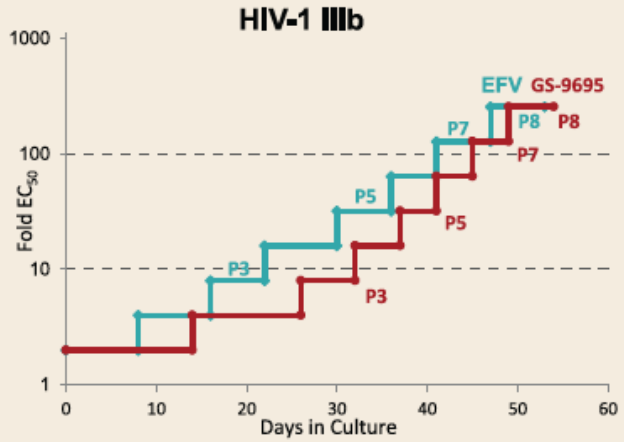


	Dolutegravir	GS-9695 1st Generation	GS-9822 2nd Generation
Wild Type EC_{50} (nM)	1.5 ± 0.3	1.2 ± 0.2	3.0 ± 0.9
Protein Adj. EC_{95} (nM)	179 ± 19	40 ± 7.5	168 ± 56
T174I fold shift	1.2	361	26
T174I/A128T/A124T fold shift	1.6	>20,000	57
Average polymorph fold shift (range)	0.8 (0.4 – 1.0)	1.7 (0.2 – 4.7)	0.8 (0.4 – 1.3)
Average EC_{50} of clinical isolates[1] (nM) (range)	0.6 ± 0.3 (0.2 – 1.3)	1.4 ± 0.8 (0.6 – 2.9)	0.7 ± 0.7 (0.13 – 3.1)
Average fold shift of INSTI mutants (range)	2.8 (0.6 – 6)	0.6 (0.5 – 0.8)	1.2 (0.94 – 1.6)
Average fold shift of PI mutants (range)	1.2 (1.0 – 1.4)	0.6 (0.4 – 1.0)	0.8 (0.43 – 1.7)
MT4 CC_{50} (µM)	14.7 ± 1.9	7.1 ± 1.4	3.0 ± 0.9
Solubility, pH 2 / 7 (µg/mL)	75 / 123	1 / 5[2]	2300 / 200[3]
Pred. human hepatic CL (L/h/kg)	0.16	0.02	0.09
Projected human $t_{½}$ (h)	12 – 14 (actual)	~17	~10
Projected human dose (mg)	50 (actual)	200	250

[1]Number of clinical isolates ≥ 14; [2]Crystalline parent; [3]Crystalline HCl salt

Results

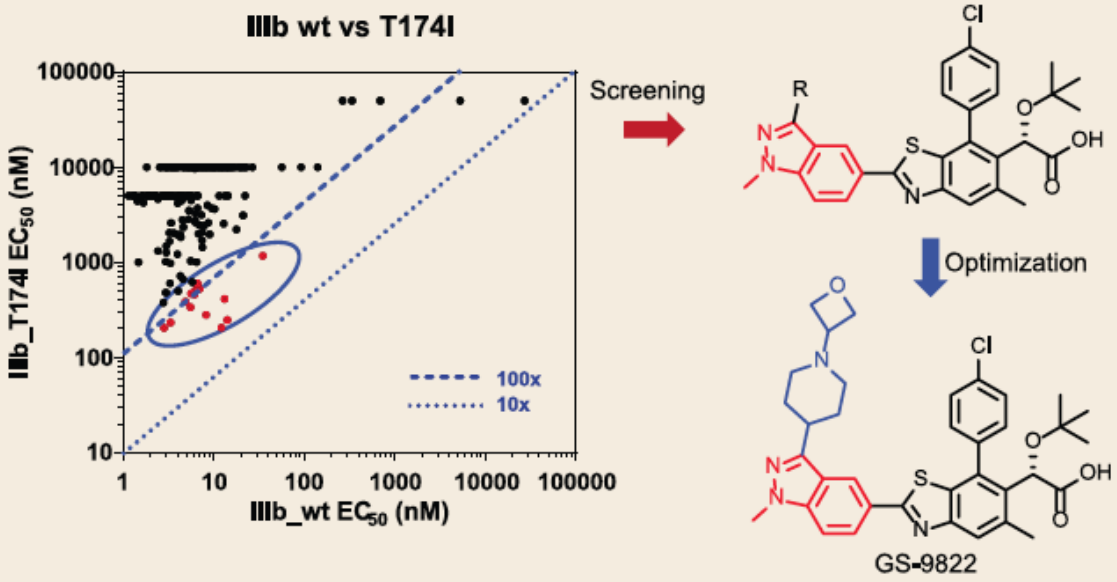
GS-9695 Resistance Selection



Passage	Conc (nM)	Variation from WT IIIb
P1	4 (2-fold EC_{50})	D10E, A128T
P2	8	A124T, T125S, A128T
P3	16	D10E, A124T, A128T
P5	64	D10E, A124T, A128T
P7	256	D10E, A124T, A128T, T174I
P8	512	D10E, A124T, A128T, T174I

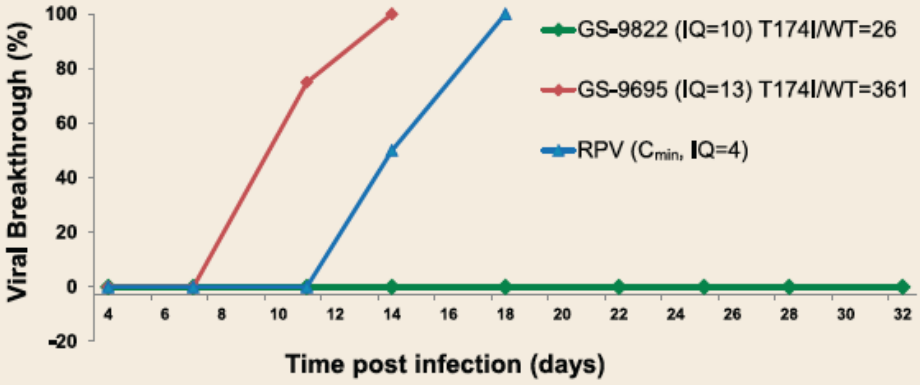
- Polymorphic variant A128T observed initially in passage 1
- T174I identified as the resistance associated mutation
- GS-9695 lost >20,000-fold in potency against P8 virus relative to WT

Identification of Series with Greater Residual Potency versus Mutant Virus



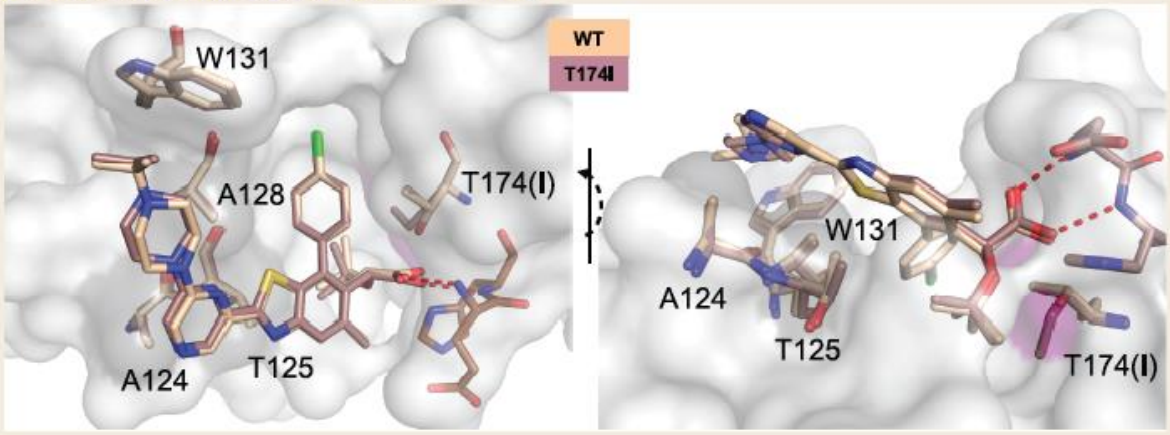
- Screened broad selection of 300 NCINI compounds using P8 mutant virus
  - Derived from GS-9695 dose escalation resistance selection study
  - Harbors T174I mutation plus polymorphic variants A124T and A128T
  - Identified 15 compounds <100 fold shift that contained the 5-indazole moiety

GS-9822 Displays Higher Barrier of Resistance



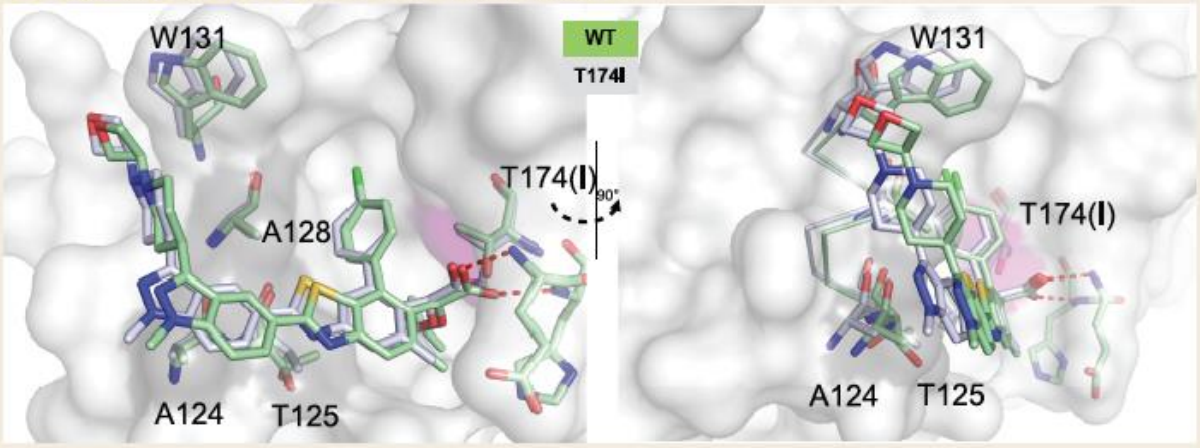
- Drug concentrations equivalent to target (or actual) clinical C_{min}
  - Inhibition quotient (IQ) = fold over protein adjusted EC_{95}
- Similar viral breakthrough patterns observed with IIIb virus (MT2, shown above) or with BaL virus (PBMC)
- Lower T174I mutant shift translates to improved performance in the viral breakthrough assay

T174I Mutation Disrupts GS-9695 Binding



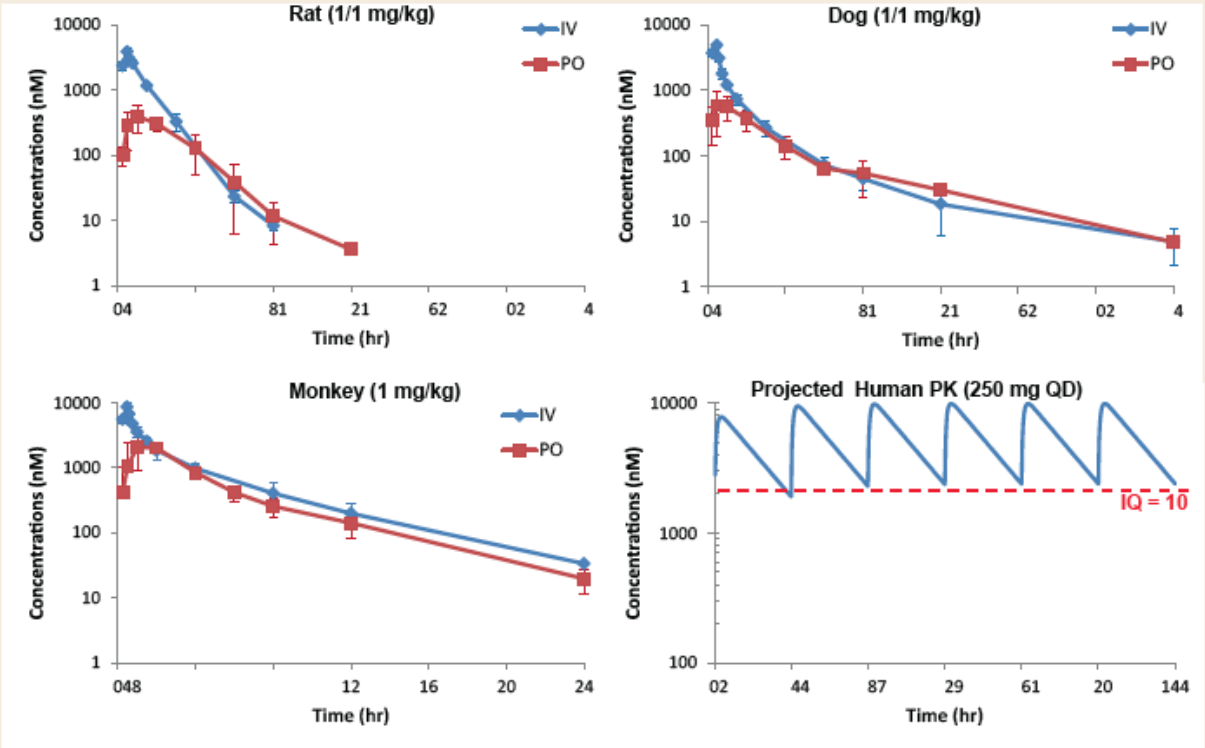
- T1741 mutation results in a steric clash with the *tert*-butyl ether
  - slightly displaces GS-9695
  - disrupts key interactions of the isopropyl piperazine with W131.

GS-9822 Induces a Protein Shift in T174I Mutant

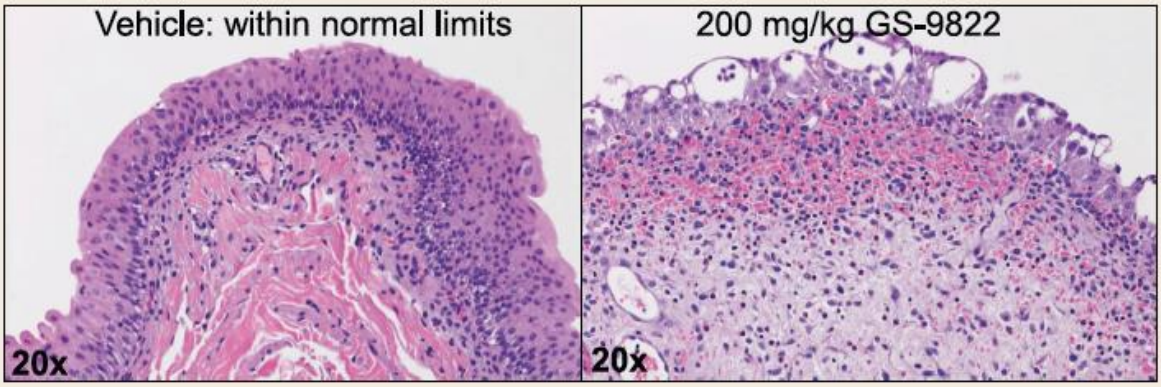


- GS-9822 induces a shift in CCD helix from residues 125 to 131
- Protein shifts to maintain oxetanyl piperidine interaction with W131

GS-9822 Preclinical and Projected PK Profiles



GS-9822 7-day Monkey Toxicology Study



- GS-9822 dosed at 20, 60, and 200 mg/kg
- Urothelial cell vacuolation in urinary bladder (60 and 200 mg/kg) and kidney and ureter (200 mg/kg); similar findings observed with GS-9695
  - urinalysis showed increase in urine protein, blood reactions, urobilinogen (250%), red cells observed (200 mg/kg)
- GS-9822 and GS-9695 have structurally distinct 2-position substitutions on shared benzothiazole core; no direct evidence yet associating benzothiazole core with toxicological findings

References

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