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Antiviral Activity of Bictegravir (GS-9883), a Potent Next Generation HIV-1 Integrase Strand Transfer Inhibitor

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Abstract

Antiviral Activity of Bictegravir (GS-9883), a Potent Next Generation HIV-1 Integrase Strand Transfer Inhibitor

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Background: GS-9883 is a potent once-daily unboosted integrase strand transfer inhibitor currently in clinical development in combination with tenofovir alafenamide (TAF) and emtricitabine (FTC) for the treatment of HIV-1 infection.

Methods: The inhibitory activity of GS-9883 was tested against wild-type HIV-1 integrase enzyme. Antiviral potency and cytotoxicity were assessed in MT-2 and MT-4 T-cell lines and primary human CD4+ T-cells and macrophages. Inhibition of integration was assessed by qPCR of 2-LTR circles and integration junctions. A panel of 14 HIV-1 and one HIV-2 clinical isolates was used for antiviral profiling. Antiviral activity of GS-9883 was also tested against several non-HIV viruses. Cytotoxicity of GS-9883 was also tested in four non-target cell lines (Huh-7, HepG2, PC-3, MRC-5) and human primary hepatocytes. Antiviral activity of GS-9883 in pairwise combination with antiretrovirals was also assessed.

Results: GS-9883 inhibited HIV-1 integrase enzyme strand transfer activity (IC_{50} = 7.5 ± 0.3 nM). Treatment with GS-9883 increased abortive 2-LTR circles and decreased viral-host DNA integration junctions in infected cells. GS-9883 exhibited high potency and selectivity in HIV-1 assays using lymphoblastoid T-cell lines, primary human CD4+T cells, and macrophages with EC_{50} range of 1.5 to 6.6 nM and selectivity indices of 1500 to 8700. GS-9883 was highly potent against all tested HIV-1 subtypes and HIV-2 in human PBMCs (mean EC_{50} = 0.81 nM range of <0.05 to 1.71 nM), and showed no activity against HBV, HCV, Influenza, HRV or RSV. GS-9883 had low cytotoxicity in multiple human cell lines (CC_{50} range from 35 to >44 µM) and in primary human hepatocytes (CC_{50} >100 µM). Highly synergistic *in vitro* antiviral effect was observed for combinations of GS-9883 with TAF, FTC or darunavir.

Conclusions: GS-9883 is a novel, potent and selective HIV integrase strand transfer inhibitor. GS-9883 was highly synergistic in combinations with TAF, FTC or darunavir. These data support the clinical investigation of GS-9883 for the treatment of HIV-1 infection.

Introduction

- Integrase strand transfer inhibitors (INSTIs) are a class of antiretroviral drugs for the treatment of HIV-1 infection and inhibit HIV-1 replication by blocking the strand transfer step of viral DNA integration into the host genome.
- The first two INSTIs, raltegravir (RAL) dosed twice-daily and elvitegravir (EVG) dosed-once daily with boosting, are approved for clinical use as components of combination therapy. However, RAL and EVG have overlapping resistance profiles such that viruses resistant to one drug are cross-resistant to the other drug, which ultimately precludes the sequential use of these two INSTIs.
- Dolutegravir (DTG) is also an INSTI and is dosed once-daily without boosting and exhibits a higher barrier to resistance development. However, due to limited absorption above 50 mg, twice-daily dosing is required in patients with past INSTI use, with INSTI resistance, or suspected INSTI resistance. Twice-daily dosing is also required when co-administered with cytochrome P450 (CYP) and/or uridine diphosphate glucuronosyl-transferase (UGT) inducers.
- We aimed to engineer a novel INSTI with once-daily dosing potential, improved tolerability, and high efficacy against INSTI-associated resistance.
- Bictegravir (BIC; GS-9883) is a novel potent once-daily unboosted INSTI currently in clinical development in combination with tenofovir alafenamide (TAF) and emtricitabine (FTC) in a single tablet regimen for the treatment of HIV-1 infection. In this study, we report the virologic characterization of BIC.

Methods

- The enzymatic inhibitory activity of BIC (GS-9883) was tested against purified wild-type HIV-1 integrase enzyme using homogeneous time-resolved FRET-based 3'-processing and strand transfer assays.
- Antiviral potency and cytotoxicity were assessed in MT-2 and MT-4 T-cell lines and primary human CD4+ T-cells and macrophages.
- Inhibition of chromosomal HIV-1 integration was assessed by qPCR of 2-LTR circles and integration junctions in MT-2 cells infected with HIV-1 (IIIb).

Methods (continued)

- A panel of 14 HIV-1 and one HIV-2 clinical isolates from the collection of Southern Research Institute was used for antiviral profiling in a PBMC-based antiviral assay using the quantitative reverse transcriptase endpoint activity through contract service with the Southern Research Institute (Frederick, MD).
- Antiviral activity of BIC was also tested against several non-HIV viruses.
- Cytotoxicity of BIC was tested in four non-target cell lines (Huh-7, HepG2, PC-3, MRC-5) and human primary hepatocytes.
- Antiviral activity of BIC in pairwise combination with other antiretrovirals was assessed. The data were analyzed for the effect of pairwise combination using MacSynergy™ II software.

Results

Table 1. Bictegravir (BIC) Exhibits Potent Antiretroviral Activity in Human T-cell lines

Compound	MT-2 Cells[a]			MT-4 Cells					
	EC_{50} (nM)	CC_{50} (nM)	Selectivity	EC_{50} (nM)[b]	CC_{50} (nM)[a]	Selectivity	Hill Slope[b]	EC_{95} (nM)[b]	EQDS Shift[d] (Fold-change)[b]	$PAEC_{95}$[d] (nM)
BIC	1.5 ± 0.2	10,300 ± 2500	6,867	1.9 ± 0.6	3,700 ± 93	1,926	2.1 ± 0.3	8.3	43.6 ± 7.7	361
DTG	1.5 ± 0.2	5,350 ± 200	3,567	1.7 ± 0.2	15,000 ± 1900	8,634	2.1 ± 0.3	7.4	27.5	2.4

[a] EC_{50} and CC_{50} values represent the mean ± SD of at least 4 independent determinations in triplicate. Test compounds were 3-fold serially diluted. [b] EC_{50} values and Hill slopes represent the mean ± SD of 8 independent determinations in quadruplicate. Test compounds were 1.24-fold serially diluted. [c] Values represent mean ± SD of 3 independent determinations for BIC and mean of two independent determinations for DTG. [d] Protein shift based on equilibrium dialysis (EQDS) of compound between culture medium and human serum. [e] EC_{95} is calculated from EC_{50} and Hill slope, *n*, using the equation: $EC_{95} = EC_{50} \times (95/5)^{(1/n)}$. [f] $PAEC_{95}$ = protein adjusted EC_{95}.

Table 2. Antiretroviral Activity of Bictegravir (BIC) in Human Primary T-Cells and Macrophages

Compound	Human CD4+ T-Lymphocytes[a]			Human Macrophages[a]		
	EC_{50} (nM)	CC_{50} (nM)	Selectivity	EC_{50} (nM)	CC_{50} (nM)	Selectivity
BIC	1.5 ± 0.3	13,000 ± 4,000	8,700	6.6 ± 4.1	29,800 ± 7,700	4,500
DTG	1.0 ± 0.3	52,000 ± 8,500	52,000	3.1 ± 2.5	24,900 ± 1,200	8,000

[a] EC_{50} and CC_{50} values represent the mean ± SD of triplicate measurements in four independent donors.

Table 3. Antiretroviral Activity of Bictegravir (BIC) against Diverse Clinical Isolates of HIV

HIV-1 Subtype	HIV-1 Isolate	Genbank Accession #	EC_{50} (nM)[a]		
			BIC[b]	DTG[c]	AZT
A	92RW016	AF009409	0.74	0.35	2.61
A	92UG037	AB253428	1.71	0.93	10.9
B	Ba-L	AY713409	0.35	0.29	3.39
B	89BZ_167	AY173956	0.88	1.13	2.65
B	91US001	AY173952	0.87	0.84	5.61
B	91US004	AY173955	0.82	0.94	2.74
C	93IN905	AF067158	0.15	0.15	1.61
C	98US_MSC5016	AY444801	1.4	0.92	10.3
D	98UG_57128	AF484502	0.31	0.67	0.55
D	99UG_A07412M1	AF484477	1.06	0.93	9.99
E	96TH_M02138	AY713424	0.8	0.69	5.79
E	96TH_NI1046	AY713421	0.28	0.3	2.6
F	93BR020	AF005494	1.13	0.37	8.72
G	01CM1475MV	AY371138	< 0.05	0.09	0.98
HIV-2	CDC 310319	AY965902	1.11	2.13	4.1

[a] EC_{50} values represent the mean of triplicate measurements in human PBMCs. [b] BIC EC_{50} range: <0.05 - 1.71 nM against HIV-1. [c] DTG EC_{50} range: 0.09 - 1.13 nM against HIV-1.

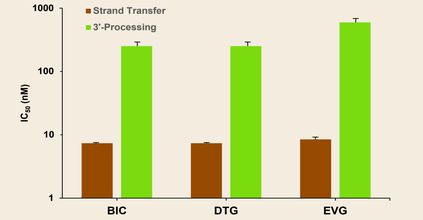
Results (continued)

Table 4. Bictegravir (BIC) is not Cytotoxic in Non-Target Cell Lines and Primary Hepatocytes

Compound	CC_{50} in Cell Lines (µM)[a]				CC_{50} in Primary Human Hepatocytes (µM)[b]	
	Huh7	HepG2	PC2	MRC5		
	Human hepatoma	Human hepatoma	Human prostate cancer	Normal human fibroblasts	Donor 1	Donor 2
BIC	43.6	34.6	> 44	> 44	> 100	> 100
DTG	> 44	43.3	> 44	30.7	> 100	> 100
Puromycin	0.72	1.68	0.62	0.36	1.2	0.83

[a] CC_{50} values represent the mean of three independent runs in triplicates.
[b] CC_{50} values were obtained from 2 donors with each assay performed in duplicate.

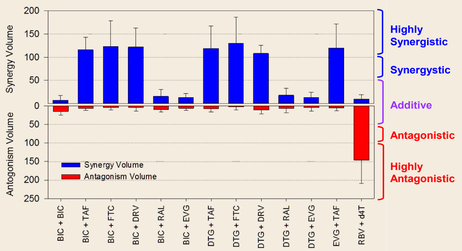
Figure 1. Bictegravir (BIC) is a Strand Transfer Inhibitor of HIV-1 Integrase



IC_{50} (nM)[a]	BIC	DTG	EVG
Strand Transfer	7.5 ± 0.3	7.4 ± 0.6	8.4 ± 0.7
3'-Processing	241 ± 51	232 ± 33	556 ± 40

[a] The data represent the mean ± SD of 3 independent experiments performed in triplicates.

Figure 2. Bictegravir (BIC) is Synergistic with TAF, FTC, and DRV



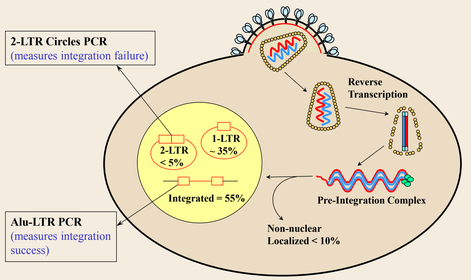
[a] EVG = elvitegravir, RBV = ribavirin, d4T = stavudine, DTG = dolutegravir, TAF = tenofovir alafenamide, FTC = emtricitabine, DRV = darunavir.
[b] The effect of pairwise drug combination was analyzed by the MacSynergyTM II software. The results were expressed as the mean synergy/antagonism volumes (nM².%) calculated at the 95% confidence interval from at least 3 independent experiments done in triplicates.

Table 5. Bictegravir (BIC) is an Authentic Inhibitor of HIV-1 Integration in HIV-1 (IIIb) Infected MT-2 Cells

Compound	Drug Concentration (nM)[a]	Integration Failure	Assessment of Direct Effect on Integration	
		2-LTR Circles (fold-change)[b]	Late-RT (fold-change)[b]	Alu-LTR (fold-change)[b]
DMSO	—	1	1	1
BIC	28	3.3	0.9	0.01
DTG	31	4.5	0.8	0.02
EFV[c]	17	0.1	0.2	0.08
DRV[c]	57	1.1	0.9	0.8

[a] Compounds were applied to HIV-1 (IIIb) infected MT-2 cultures at ~20-fold their respective antiviral EC50 value.
[b] The quantitative PCR data of HIV target sequences were normalized against the corresponding quantitative PCR data for the globin gene from the same sample and represent the mean of two independent experiments. For 2-LTR circles PCR and Alu-LTR PCR, refer to Figure 3. Fold-change is relative to the DMSO control.
[c] EFV = efavirenz (an HIV reverse transcriptase inhibitor), DRV = darunavir (an HIV protease inhibitor).

Figure 3. Distribution of HIV-1 DNA Species in an Infected Cell



Adapted from Zennou et al., 2000, Cell 101:173-185

Conclusions

- Bictegravir is a novel specific inhibitor of HIV-1 integrase strand transfer activity and an authentic inhibitor of integration in HIV-1 infected target cells.
- Bictegravir is a potent and selective inhibitor of HIV-1 replication in lymphoblastoid T-cell lines, primary human CD4+T cells, and macrophages with EC_{50} values ranging from 1.5 to 6.6 nM and selectivity indices ranging from 1,926 to 8,700.
- Bictegravir is active against diverse subtypes of wild-type HIV-1 clinical isolates and HIV-2 with a mean EC_{50} of 0.75 nM and a range of 0.05 – 1.71 nM.
- The protein adjusted EC_{95} of bictegravir is 361 nM, with a steady-state clinical trough-concentration of 6.9 µM and an IQ of ~19 in human subjects administered 75 mg once daily under fasted conditions.
- Bictegravir has low cytotoxicity in multiple non-target human cell lines and in primary human hepatocytes.
- Bictegravir is a specific inhibitor of HIV, with no measurable antiviral activity against non-HIV viruses, including HBV, HCV, Influenza A and B, HRV and RSV (data not shown).
- Bictegravir exhibited synergistic *in vitro* antiviral effects when combined with antiretrovirals from other classes, including TAF, FTC, and DRV.
- In conclusion, this virologic profile supports further clinical development of bictegravir as a novel potent once-daily unboosted integrase strand transfer inhibitor in a single tablet regimen combination with FTC and TAF.