Poster
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Absence of Naturally Existing Resistance Against The HIV-1 Capsid Inhibitor GS-6207 in HIV-1 Primary Isolates

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Introduction

- HIV capsid (CA, p24) is a product of HIV gag processing by HIV protease which plays a key role in the HIV life cycle (Figure 1)
- GS-6207 is a first-in-class HIV capsid (CA) inhibitor (Figure 2) with a multi-stage mode of action and picomolar potency (Figure 3)[1]
- GS-6207 binds to the interface between two capsid monomers and prevents CA-mediated nuclear entry of viral DNA, HIV assembly, and proper capsid formation (Figure 3)[1]
- GS-6207 physicochemical properties make GS-6207 suitable as a long acting injectable agent: picomolar antiviral potency, low predicted clearance, and low aqueous solubility[1]
- In clinical studies, a single subcutaneous dose of GS-6207 sustained measurable concentrations in HIV-negative participants for at least 24 weeks and demonstrated potent antiviral activity in people living with HIV (PLWH) over 10 days (up to 2.2 $\log_{10}$ decline in HIV-1 RNA)[2,3]
- As a first-in-class inhibitor, GS-6207 exhibits a non-overlapping *in vitro* resistance profile relative to existing antiretroviral agents
- *In vitro* dose escalation studies identified variants in the CA (p24) portion of gag–L56I, M66I, Q67H/Y, K70N, N74D/S, and T107N–associated with reduced susceptibility to GS-6207[4]
- Natural HIV gag polymorphisms found in viral isolates could be linked to loss of potency, as is the case for maturation inhibitors (MI) such as Bevirimat which target the final gag cleavage step prior to CA (p24) release
- Here, we studied the antiviral activity of GS-6207 in HIV-1 primary isolates from people living with HIV (PLWH) in the context of naturally occurring gag polymorphisms

Figure 1: HIV-1 Capsid Core Formation

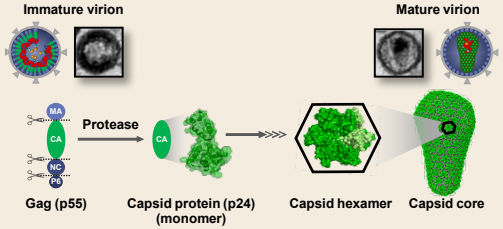


Figure 2: Structure of GS-6207

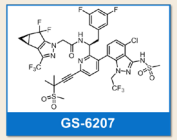
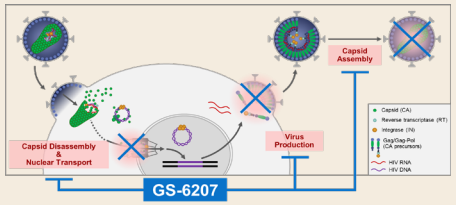


Figure 3: GS-6207 Mode of Action



EC_{50}, half-maximal effective concentration determined in peripheral blood mononuclear cells using wild-type HIV-1 patient isolates with various HIV-1 subtypes.

Methods

- Plasma samples from treatment-experienced (TE) and treatment-naïve (TN) PLWH from past Gilead clinical studies (TE: GS-99-907, GS-US-183-0105/0145; TN: GS-01-934, GS-US-292-0104) with diverse resistance profiles were used as starting material (Table 1)
- The HIV-1 gag-protease fragment from these plasma samples (51 in total; 36 TE, 15 TN) were amplified by PCR, and the unique SfoI and XmaI sites were used to clone the PCR products into the HIV-1 molecular clone pXXLAI using In-Fusion cloning (Takara, Mountain View, CA, USA)
- HIV-1 constructs were transfected into 293T cells and viral isolates were harvested after 48 hours
- Susceptibility (EC_{50}) of the HIV-1 isolates to GS-6207 and control drugs was measured in a 5-day multi-cycle antiviral assay in MT-2 cells and compared to wild-type (WT)

Results

Table 1: Genotypic Characteristics of Clinical Samples (n=51)

Sample Type	Sample #	Number of RAMs per sample for each class					
		NRTI[a]	NNRTI[b]	PI[c]	INSTI[d]	MI[e]	CAI[f]
TE	1	8	4	5	-	2	-
	2	8	4	4	-	-	-
	3	3	-	4	-	2	-
	4	6	-	3	-	-	-
	5	5	-	3	-	-	-
	6	3	-	3	-	1	-
	7	-	1	-	-	-	-
	8	-	-	-	-	-	-
	9	7	-	8	-	3	-
	10	9	2	8	-	3	-
	11	9	4	7	-	1	-
	12	8	4	6	-	-	-
	13	6	1	5	-	1	-
	14	3	1	7	-	-	-
	15	7	-	5	-	-	-
	16	1	4	3	-	1	-
	17	8	1	3	-	-	-
	18	4	2	3	-	1	-
	19	7	1	3	-	1	-
	20	8	6	4	-	-	-
	21	3	3	2	-	-	-
	22	2	3	3	-	1	-
	23	7	3	3	-	1	-
	24	4	2	2	-	-	-
	25	4	3	3	-	1	-
	26	3	1	3	-	-	-
	27	1	1	4	-	3	-
	28	3	1	5	-	2	-
	29	5	1	3	-	1	-
	30	4	1	5	-	-	-
	31	5	2	3	-	1	-
	32	4	3	3	-	1	-
	33	2	2	2	-	-	-
	34	-	-	4	-	1	-
	35	-	-	4	-	-	-
	36	-	-	2	-	-	-
TN	37	-	-	-	-	-	-
	38	-	-	-	-	2	-
	39	-	-	-	-	1	-
	40	-	1	-	-	1	-
	41	-	-	-	-	1	-
	42	-	-	-	-	2	-
	43	-	-	-	-	1	-
	44	-	-	-	-	1	-
	45	-	-	-	-	1	-
	46	-	1	-	-	1	-
	47	-	-	-	-	-	-
	48	-	-	-	-	-	-
	49	-	2	-	-	-	-
	50	-	-	-	-	-	-
	51	1	2	-	-	-	-

[a] NRTI-R mutations: M41L, E44D, A62V, K65N/R, D67N, T69D/N, T69 insertion, K70E/R, L74I/V, V75I, F77L, Y115F, F116Y, V118I, Q151M, M184I/V, L210W, T215F/Y, and K219E/N/Q/R in RT[5]
[b] NNRTI-R mutations: V90I, A98G, L100I, K101E/H/P, K103N/S, V106A/I/M, V108I, E138A/G/K/Q/R, V179D/F/L/T, Y181C/I/V, Y188C/H/L, G190A/E/Q/S, H221Y, P225H, F227C, and M230I/L in RT[5]
[c] PI-R mutations (primary): D30N, V32I, L33F, M46I/L, I47A/V, G48V, I50L/V, I54L/M, Q58E, T74P, L76V, V82A/F/I/L/S/T, I84V, N88S, and L90M in PR[5]
[d] INSTI-R mutations: T66A/I/K, E92Q/G, T97A, Y143C/H/R, S147G, Q148H/K/R, and N155H/S in IN[5]
[e] Primary MI-R mutations: V362I and any change at residues 369, 370, 371 in gag[6,7]
[f] Primary CAI-R mutations: L56I, M66I, Q67H, K70N, N74D/S, and T107N in capsid (corresponding to gag residues 188, 198, 199, 202, 206, and 239)[4]
CAI = capsid inhibitor; MI= maturation inhibitor; NNRTI = nonnucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PR = protease; -R = resistant; RAM = resistance associated mutation; RT = reverse transcriptase; TE = treatment-experienced; TN = treatment-naïve.
All samples were from HIV-1 integrase treatment-naïve PLWH

Results, cont'd

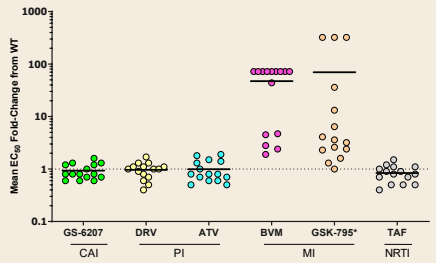
- In viruses from treatment-naïve PLWH, the HIV capsid inhibitor (CAI) GS-6207 showed high potency (average EC_{50} of 88 pM compared to WT EC_{50} of 95 pM), with minimal variability across all 15 isolates (Figure 4, Table 2)
- In viruses from treatment-experienced PLWH, GS-6207 also displayed high potency (average EC_{50} of 89 pM) with minimal variability across all 36 isolates (Figure 5, Table 2)
- Resistance to the protease inhibitors (PI) darunavir (DRV) and atazanavir (ATV) was high in TE isolates with PI resistance mutation (Figure 5), with mean fold-change EC_{50} above WT of 21 and 34, respectively (compared to 1.0 for both drugs in TN isolates) (Table 2)
- Maturation inhibitors (MI) such as bevirimat (BVM) and GSK-3532795 (GSK-795) showed significantly reduced potency in both TN and TE isolates, reflecting the occurrence of naturally existing gag polymorphisms known to affect activity of the MI class
- Resistance to the RT inhibitor (NRTI) control displayed limited variation from wild-type, reflecting the wild-type sequence in reverse transcriptase in these HIV isolates
- Overall, phenotypic resistance (Table 2) correlated with presence or absence of genotypic resistance mutations (PI, MI, CAI) (Table 1)

Table 2: Drug Susceptibility Summary

Isolate Type	Mean Drug Susceptibilities (EC_{50} Fold-Change from Wild-Type control, range)					
	GS-6207 (CAI)	ATV (PI)	DRV (PI)	BVM (MI)	GSK-795* (MI)	TAF (NRTI)
Treatment-Naïve (n=15)	**0.9** 0.6 – 1.6	**1.0** 0.5 – 1.9	**1.0** 0.4 – 1.7	**>47** 1.9 – >72	**>69** 1.0 – >322	**0.8** 0.4 – 1.5
Treatment-Experienced (n=36)	**0.9** 0.4 – 1.8	**34** 0.5 – 65	**21** 0.5 – 110	**>44** 0.6 – >72	**>91** 0.5 – >322	**0.8** 0.3 – 1.4

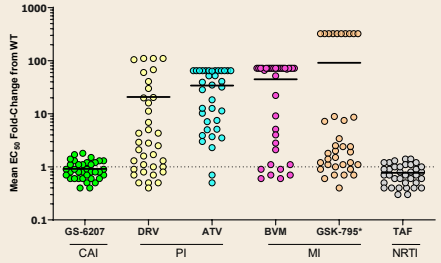
(*) GSK-3532795/BMS-955176
CAI: capsid inhibitor; PI: protease inhibitor; MI: maturation inhibitor; NRTI: nucleotide reverse transcriptase inhibitor

Figure 4: Drug Susceptibilities in Treatment-Naïve HIV-1 Isolates (n=15)



Each dot represents a single isolate. Drugs tested are represented by a different color. The black line indicates the mean EC_{50} fold-change for each drug.
(*) GSK-3532795/BMS-955176
CAI: capsid inhibitor; PI: protease inhibitor; MI: maturation inhibitor; NRTI: nucleotide reverse transcriptase inhibitor

Figure 5: Drug Susceptibilities in Treatment-Experienced HIV-1 Isolates (n=36)



Each dot represents a single isolate. Drugs tested are represented by a different color. The black line indicates the mean EC_{50} fold-change for each drug.
(*) GSK-3532795/BMS-955176
CAI: capsid inhibitor; PI: protease inhibitor; MI: maturation inhibitor; NRTI: nucleotide reverse transcriptase inhibitor

Conclusions

- The presence of naturally occurring polymorphisms in gag (including CA) and/or protease mutations in the viral isolates did not affect the high potency of GS-6207
- These observations underscore the absence of naturally occurring gag polymorphisms conferring resistance against GS-6207, in contrast to maturation inhibitors
- This confirms that the mode of action of GS-6207 is distinct from that of maturation inhibitors
- Viral isolates from TN and a wide variety of TE PLWH were equally susceptible to GS-6207, underlining GS-6207's potential for treatment in all PLWH regardless of their ART history

References

1. Yant et al: GS-6207, a Potent And Selective First-in-class Long-acting HIV-1 Capsid Inhibitor, CROI 2019, Seattle, WA, 4–7 March 2019 (Poster 1504)
2. Sager et al: Safety and PK of Subcutaneous GS-6207, a Novel HIV-1 Capsid Inhibitor, CROI 2019, Seattle, WA, 4–7 March 2019 (Oral 13)
3. Daar et al: Safety and Antiviral Activity Over 10 Days Following a Single Dose of Subcutaneous GS-6207, a First-in-Class, Long-Acting HIV Capsid Inhibitor in People Living With HIV, IAS 2019, Mexico City, Mexico, 21-24 July 2019 (Poster LBPEB13)
4. Yant et al: In Vitro Resistance Profile of GS-6207, a First-in-Class Picomolar HIV Capsid Inhibitor in Clinical Development as a Novel Long-Acting Antiretroviral Agent, IAS 2019, Mexico City, Mexico, 21-24 July 2019 (Poster TUPEA075)
5. Margot et al: Rare emergence of drug resistance in HIV-1 treatment-naïve patients receiving elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide for 144 weeks, 2018, J. Clin. Vir., 103;37-42
6. Margot et al: Phenotypic susceptibility to Bevirimat in isolates from HIV-1-infected patients without prior exposure to Bevirimat, 2010, Antimicrob. Agents Chemother., 54(6);2345-53
7. McCallister et al: HIV-1 gag polymorphisms determine treatment response to bevirimat (PA-457), DRW 2008, Sitges, Spain, 10-14 June 2008

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