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1 **Frequency of capsid substitutions associated with GS-6207 *in vitro* resistance in HIV-1**
2 **from antiretroviral-naïve and -experienced patients**

3

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21

22 **Short running title:** Resistance to HIV capsid inhibitors

23

24

25

26 **Abstract**

27 **Background:** GS-6207 is a first-in-class HIV capsid inhibitor, targeting several functions of
28 HIV capsid in the viral cycle, including viral particles assembly, capsid formation and nuclear
29 entry. GS-6207 has demonstrated picomolar potency *in vitro*, activity confirmed by high
30 potency in Phase 1 clinical study, with a long acting antiretroviral profile with potential
31 dosing every 6 months. *In vitro* resistance selections previously conducted with increasing
32 doses of GS-6207 have identified capsid variants with reduced susceptibility to GS-6207.

33 **Objectives:** We have studied the prevalence of capsid mutations associated with *in vitro*
34 resistance to GS-6207 in people living with HIV (PLWH).

35 **Patients and methods:** Plasma samples from ART-naïve or ART-experienced PLWH,
36 including PI-experienced, were sequenced, and analyzed for the presence of capsid variants
37 identified during *in vitro* resistance selections: L56I, M66I, Q67H, K70N, N74D, N74S and
38 T107N.

39 **Results:** Among the samples from the 1500 patients studied, none of the seven GS-6207
40 resistance mutations identified during *in vitro* selection experiments was detected, regardless
41 of HIV subtype or PLWH treatment history.

42 **Conclusions:** Out of the seven HIV capsid substitutions previously selected *in vitro* and
43 shown to confer phenotypic resistance to GS-6207, none of those seven mutations was
44 observed in this large dataset, suggesting that neither PLWH with previous PI failure nor
45 PLWH with emergence of PI resistance mutations are anticipated to impact GS-6207 activity
46 in these diverse HIV infected populations.

47

48 **Introduction**

49 The HIV capsid protein (commonly known as p24) is generated upon cleavage of the HIV
50 gag polyprotein by HIV protease. It provides multiple essential functions throughout the viral
51 replication cycle, making it an attractive target for antiviral intervention. ¹ GS-6207 is a first-
52 in-class HIV capsid inhibitor with a unique, multi-stage mechanism of action, including the
53 inhibition of HIV assembly, proper viral capsid formation, and nuclear entry of viral DNA. *In*
54 *vitro* characterization of the HIV capsid inhibitor GS-6207 revealed the high potency of the
55 compound, with a reported EC₅₀ as low as 100 pM with antiviral activity against all major
56 HIV-1 subtypes. ² The picomolar potency of GS-6207 along with its unique physicochemical
57 properties make it a prime candidate for a long acting agent. These long acting properties
58 were recently demonstrated in Phase 1a clinical study ³ and showed that GS-6207 had the
59 potential to be dosed every 6 months. ⁴ Subsequently, a Phase 1b proof-of-concept clinical
60 study showed that single sub-cutaneous (SC) doses of GS-6207 ranging from 50 to 450 mg
61 resulted in potent antiviral activity in people living with HIV (PLWH), with mean maximum
62 HIV-1 RNA declines ranging from 1.8 to 2.2 log₁₀ HIV copies/mL over 10 days. ⁵ As a first
63 in class compound, the HIV capsid inhibitor GS-6207 exhibits a unique *in vitro* resistance
64 profile relative to existing antiretroviral agents. *In vitro* dose escalation and viral
65 breakthrough selections performed with GS-6207 in T-cell lines and PBMCs infected with
66 clonal and clinical HIV-1 isolates, respectively, identified the HIV CA variants L56I, M66I,
67 Q67H, K70N, N74D, N74S and T107N (alone and in different combinations), with Q67H and
68 N74D being the most predominantly observed variants. ⁶ All the GS-6207-selected variants
69 showed reduced susceptibility to GS-6207 and all but Q67H showed reduced infectivity in T-
70 cell lines and impaired replication capacity in primary human CD4+ T-cells. ⁶

71 Genetic variations in gag can naturally occur depending on the HIV subtypes, immune
72 pressure (CTL epitopes), and prior use of HIV protease (PR) inhibitors, as the gag polyprotein

73 is the substrate for PR. Here, we studied the prevalence of CA mutations ⁶ previously
74 identified to be associated with *in vitro* resistance to GS-6207 in antiretroviral treatment
75 (ART)-naïve or -experienced people living with HIV (PLWH), including people with prior
76 use of PIs.

77

78 **Materials and methods**

79 Study population

80 Samples were from patients from the laboratories of Pitié-Salpêtrière and Bichat-Claude
81 Bernard hospitals, Paris, France. Patients were followed according to the local standard of
82 care and resistance testing was performed following the French national recommendations:
83 systematic resistance testing in any new HIV-1 diagnostic and in any ART failure (defined by
84 the occurrence of 2 consecutive plasma HIV-1 viral load > 50 copies/mL). Plasma samples
85 were from 1500 subjects: ART-Naïve, ART-Experienced with not any PI use in their
86 therapeutic history and ART-Experienced with at least one PI failure episode in their
87 treatment history.

88

89 Ethics

90 The research was conducted in accordance with the Declaration of Helsinki and national and
91 institutional standards. All the patients gave their written informed consent to have their
92 medical chart recorded in the electronic medical record system Nadis® (www.dataids.org;
93 CNIL number: 770134, 30 October 2001).

94

95 Sequencing

96 Sanger sequencing method was used to sequence HIV gag and protease using as previously
97 described. ⁷⁻⁹ Sequence information was used to evaluate the presence of mutations

98 previously identified in GS-6207 *in vitro*-selections (L56I, M66I, Q67H, K70N, N74D, N74S,
99 and T107N in CA).⁶ We studied these substitutions in 3 large groups of PLWH: ART-naïve,
100 ART-experienced without PI treatment experience, and ART-experienced with previous PI
101 failure with or without major PI resistance mutations. PI resistance mutations were classified
102 according to IAS-USA list of mutations (www.iasusa.org).¹⁰

103

104 **Results**

105 Among plasma samples of the subjects studied, ART-Naïve (n=500), ART-Experienced
106 without PI use (n=500), and ART-Experienced with history of PI failure (n=500), the most
107 prevalent HIV-1 subtypes were B and CRF 02_AG. These results matched the expected
108 subtypes, based of the geographic origin of the PLWH in this analysis. The other subtypes
109 observed were in accordance with the HIV-1 epidemiology in West Africa (Table 1). The
110 RAMs detected in these groups of patients were analyzed for resistance related to PI. In the
111 ART-Naïve group and in the ART-Experienced without PI use group 0.6% and 0.4% of major
112 PI RAMs were found respectively, however majority (52.8%) of ART-Experienced patients
113 with history of PI failure had at least 1 major PI resistance mutation (Table 2). None of the
114 seven GS-6207 resistance mutations identified during *in vitro* selection experiments (L56I,
115 M66I, Q67H, K70N, N74D, N74S, and T107N) was detected among either the ART-Naïve
116 (500) or the ART-Experienced patients (1000) studied, regardless of HIV-1 subtype or history
117 of PI failure in the therapeutic history. In contrast, maturation inhibitor RAMs in gag¹¹ were
118 present, with V362I found in 6.4% of ART-Naïve, 5.8% of ART-Experienced without PI use
119 and 9.6% of ART-Experienced with history of PI failure, and V370A found in 9.8% of ART-
120 Naïve, 8.4% of ART-Experienced without PI use and 17% of ART-Experienced with history
121 of PI failure. Similarly, major PI-RAMs (Table 2) were present in the PI-experienced group

122 (52.8%). Overall, the highly conserved region of HIV capsid identified as the binding site for
123 GS-6207 displayed very low variability.

124 **Discussion**

125 The genotypic analyses conducted here are contributing to document the absence of naturally
126 occurring resistance mutations against GS-6207 in different populations of PLWH. The
127 subtype distribution captured by this large number of samples analyzed covered a wide range
128 of HIV geographical distribution, representative of many subtypes circulating throughout the
129 world. This suggests that GS-6207 has the potential to be active *in vivo* regardless of HIV-1
130 subtypes, including complex recombination forms. This observation is similar to previous
131 observations made by Perrier *et al.*⁷ for a related inhibitor of the CAI class, GS-CA-1.¹²
132 However, the novelty of this study was genotyping of patients who were ART-experienced
133 with the PI-experienced subset showing a high frequency (52.8%) of acquired PI resistance
134 mutations/polymorphisms. Furthermore, the analysis of these 3 cohorts, of ART-naïve and
135 experienced PLWH (n=1500 overall), also showed that previous PI failure and emergence of
136 PI resistance mutations did not lead to the presence of potential resistance to GS-6207 in
137 HIV-1 CA. Although the lack of pre-existing resistance mutations could be anticipated for a
138 first in class such as GS-6207, these results suggest that the HIV CA inhibitor GS-6207 has a
139 very low likelihood of pre-existing resistance mutations in the PLWH population. As a first-
140 in-class compound, the resistance profile GS-6207 has yet to be fully established. Limitations
141 of our study include the fact that the reported *in vitro* drug selections with GS-6207 were only
142 performed for 100 days. Given the potency of capsid inhibitors, resistance may require more
143 extended time to develop. Additionally, the use of ultra-deep sequencing. could have
144 characterize these mutations even further. Overall, these data showing the absence of natural
145 variability at potential RAM positions, suggests that GS-6207 has the potential to be effective
146 regardless of treatment history or prior PI use.

147

148

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156

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160

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- 196

197 **Table 1. Distribution of HIV-1 subtypes among studied patients**

HIV-1 Subtype	ARV-Naïve	ARV-Experienced	ARV-Experienced
Distribution, %	(n=500)	no PI use	PI failure history
(n)		(n=500)	(n=500)
B	37% (185)	42% (210)	56% (280)
CRF02_AG	46% (230)	48% (240)	37% (185)
F1	4.6% (23)	2.4% (12)	-
CRF06	4.4% (22)	3.8% (19)	3.4% (17)
A1	2.8% (14)	-	-
D	2.2% (11)	2.2% (11)	1.6% (8)
Other non-B	3.0% (15)	1.6% (8)	1.0% (5)

198 ARV, antiretroviral; PI, protease inhibitor

199

200 **Table 2. Prevalence of HIV-1 sequences with PI and GS-6207 resistance-associated**
 201 **mutations**

Resistance Mutations	ARV-Naïve (n=500)	ARV-Experienced no PI use (n=500)	ARV-Experienced PI failure history (n=500)
Number of Major PI Resistance Mutations, % (n)			
0	99.4% (497)	99.6% (498)	47.2% (236)
1	0.6% (3)	0.4% (2)	22.4% (112)
2	-	-	16.4% (82)
3	-	-	10.4% (52)
4	-	-	3.6% (18)
GS-6207 Resistance Mutations (<i>in vitro</i> selected)			
L56I	-	-	-
M66I	-	-	-
Q67H	-	-	-
K70N	-	-	-
N74D	-	-	-
N74S	-	-	-
T107N	-	-	-

202 PI, protease inhibitor; ARV, antiretroviral; List of major PI resistance mutations according to IAS-USA list of
 203 mutations: D30N, V32I, M46I/L, I47A/V, G48V, I50L/V, I54L/M/V, Q58E, T74P, L76V, V82A/F/L/T/S,
 204 N83D, I84V, N88S, L90M.

205