Susceptibility of NRTI-resistant HIV-2 isolates to a new NRTI, GS-9131

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BACKGROUND

Management of HIV-2 infection is hampered by the limited number of active antiretroviral drugs and the quick acquisition of drug resistance-associated mutations (DRAMs). Only a few mutations are required to acquire class-wide resistance to Nucleoside Reverse Transcriptase Inhibitors (NRTI), Protease Inhibitors (PI) and Integrase Strand Transfer Inhibitors (INSTI). This has led to the description of a worrying rise of multi-drug resistant HIV-2-viruses in Europe and West Africa. Thus, there is still a strong need for new drugs effective on HIV-2, especially for patients infected by multi-drug resistant viruses [Gottlieb et al, 2018].

GS-9131 is the prodrug of GS-9148 (also known as phosphonomethoxy-2'-fluoro-2',3'-dideoxydidehydroadenosine) and has been recently described as a new NRTI with low potential for mitochondrial toxicity and renal accumulation [Ray et al, 2008], which demonstrated its in vitro efficacy against wild-type (WT) and NRTI-resistant HIV-1 isolates, except those harboring the Q151M complex [White et al, 2017]. Until now, activity of GS-9131 on HIV-2 has only been determined for a single HIV-2 isolate, bearing no mutation in the RT region [White et al, 2017]. GS-9131 was active on this strain, with an IC_{50} of 21nM, in the range of those observed for HIV-1 wild-type isolates. However, no data were available on NRTI-resistant HIV-2 isolates.

OBJECTIVE

To determine the antiviral efficacy of a new NRTI on multi-drug resistant isolates harboring various patterns of NRTI-resistance.

METHODS

- Phenotypic susceptibility to GS-9131 and zidovudine (ZDV) was assessed for:
  - 13 HIV-2 isolates (8 group A and 5 group B)
  - ROD and BRU, the references strains of HIV-1 and HIV-2, respectively
- Using a modified version of the ANRS PBMC assay (Brun-Vézinet et al., 1992):
  - Viruses previously isolated from plasma of viremic patients at time of virological failure were cultured with 6 10-fold dilutions of the drug, ranging from 6250 to 0.002nM, or without drug.
  - At days 3 or 4, viral replication was assessed on the supernatant, using Biocentric HIV-2 RNA® kit.
  - The 50% inhibitory concentration (IC_{50}) was determined using the sigmoidal dose-response (Prism v5.0)
- GS-9131 was kindly provided by Gilead Sciences for this study. Pure powder of zidovudine for research use only was purchased (Sigma-Aldrich, Germany).
- All but one of the thirteen HIV-2 isolates exhibited DRAMs in the reverse transcriptase gene (Table), with mutations representative of what is observed among patients harboring multi-drug resistant viruses in the French ANRS CO5 HIV-2 cohort (n=53, Figure 1)

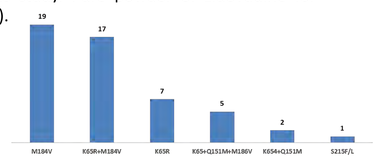

Figure 1: Distribution of NRTI-associated mutations among patients with multi-drug resistant viruses in the French ANRS CO5 HIV-2 cohort.
A multi-drug resistant virus is defined by the presence of DRAMs to at least 2 classes of antiretroviral drugs. The distribution of the NRTI-associated mutations in 53 patients with multi-drug resistant viruses is described in this table.

RESULTS

- Our phenotypic susceptibility assay was validated by two controls:
  - The susceptibility of HIV-1 reference strain, BRU, to both GS-9131 and zidovudine with our protocol (IC_{50} = 6.4 and 2 nM, respectively)
  - The simultaneous determination of the phenotypic susceptibility to zidovudine.
- Susceptibility to zidovudine:
  - As expected, wild-type and single mutants (K65R or M184V) HIV-2 isolates exhibited IC_{50} below 10nM for zidovudine (ranging from 1 to 5 nM) (Table). Antiretroviral activity decreased for the double mutants (M184V+S215Y and K65R+M184V, IC_{50} of 38 and 48 nM, respectively).
  - Al the isolates harboring the Q151M mutation were highly resistant to zidovudine, with IC_{50} higher than 420 nM for 6 isolates (Table).
- Susceptibility to GS-9131 (Table):
- **GS-9131** exhibited a **potent activity against both wild-type HIV-2 isolates** (IC_{50} = 3.4 and 4.4 nM),
- The presence of K65R mutation or M184V mutation slightly increased the IC_{50} for GS-9131 (12.0 to 27.0 nM, and 16.6 nM, respectively) (Figure 2).
- GS-9131 had a **lower activity on** two **isolates that presented a combination of 2 major NRTI mutations** (K65R+M184V and M184V+S215Y, IC_{50} = 108 and 134 nM, respectively).
- The 7 **isolates harboring a Q151M mutation**, regardless of the associated DRAMs, **were highly resistant** to both GS-9131 and ZDV (IC_{50} ranging from 178 to over 6250 nM).

		NRTI resistance-associated mutations		IC_{50} (nM) [fold-change]	
Isolate number	**HIV group**	**Major mutations**	**Other NRTI-associated mutations**	**GS-9131**	**Zidovudine**
BRU	HIV-1 M	Wild-type	-	6.4	2
ROD	HIV-2 A	Wild-type	-	4.4	2
#01	HIV-2 B	Wild-type	-	3.4 [0.8]	1 [0.5]
#02	HIV-2 B	K65R	N69S+V111I+K223R	12 [2.7]	5 [2.5]
#03	HIV-2 B	K65R	-	**27 [6.1]**	4 [2]
#04	HIV-2 A	M184V	S215T	**17 [3.8]**	1 [0.5]
#05	HIV-2 B	M184V+S215Y	-	**134 [31]**	**38 [19]**
#06	HIV-2 A	K65R+M184V	N69T+V111I	**108 [25]**	**261 [131]**
#07	HIV-2 A	Q151M	-	**178 [41]**	**40 [20]**
#08	HIV-2 A	Q151M	-	**777 [177]**	**>6250**
#09	HIV-2 A	Q151M+184V	-	**243 [55]**	**>6250**
#10	HIV-2 A	K65R+Q151M+M184V	N69T+K70R+V111I	**3500 [795]**	**2090 [1045]**
#11	HIV-2 A	Q151M+M184V+S215F	V111I+Y115F	**>6250**	**>6250**
#12	HIV-2 B	Q151M	N69T+V111I	**>6250**	**420 [210]**
#13	HIV-2 A	Q151M	-	**>6250**	**>6250**

Table: Phenotypic susceptibility of HIV-2 isolates to GS-9131.
Phenotypic susceptibility to GS-9131 and zidovudine was determined using a modified version of the ANRS PBMC assay [12]. 50% inhibitory concentrations (IC50) were determined using Prism 5.0 software with the sigmoidal dose-response. Phenotypic resistance to an antiretroviral was defined by an IC50 fold-change greater than 3 (indicated in bold), compared to the wild-type HIV-2 reference strain, ROD.

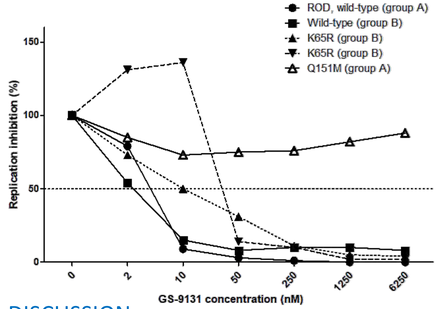

Figure 2: Antiviral activity of GS-9131 against HIV-2 clinical isolates.

Inhibition curves for 5 HIV-2 clinical isolates, including the ROD reference, are represented in this figure. HIV-2 replication is a mean of 4 wells and is expressed as a percentage of the replication in the control wells containing no drug. The continuous lines with filled symbols correspond to the two wild-type HIV-2 isolates. The dashed lines with filled symbols correspond to the 2 HIV-2 clinical isolates harboring a K65R mutation and the continuous line with empty symbol correspond to an isolate with a Q151M mutation. The mutation profile and the HIV-2 group of each isolate are indicated in the legend.

DISCUSSION

- The IC_{50} obtained for WT HIV-2 isolates were in the same range as those previously observed for HIV- 1 (i.e., 0.28-48nM; White et al, 2017). Isolates harboring K65R or M184V mutations had slightly higher IC_{50} that remained in the range observed for HIV-1 wild-type isolates.
- The presence of a Q151M mutation rendered all HIV-2 isolates resistant to GS-9131. This mutation was frequently selected at virological failure of a 3 NRTI-regimen. However, nowadays, the most encountered NRTI mutations in HIV-2-infected patients are K65R and/or M184V (Figure 1).
- Thus, GS-9131 may offer an interesting, new therapeutic opportunity for persons living with HIV-2, either at initiation of antiretroviral therapy or for second-line regimens, due to its more favorable tolerance profile than zidovudine and its maintained activity on K65R and M184V single mutants.

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