GENOTYPIC AND PHENOTYPIC SUSCEPTIBILITY TO FOSTEMSAVIR IN MULTIDRUG-RESISTANT HIV-1

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BACKGROUND

Fostemsavir (FTR) is a prodrug of the HIV-1 attachment inhibitor Temsavir (TMR), currently under investigation for the treatment of highly experienced HIV-1 infected patients with limited treatment options. TMR binds gp120 blocking its conformational change leading to CD4 interaction, thus preventing the exposure of V3 domain and the binding to coreceptors CCR5 and CXCR4[1]. Data from previous studies indicated a wide range of susceptibility to TMR in a panel of representative clones from different HIV-1 subtypes, where CRF01_AE and group O strains showed natural resistance to TMR[2].

OBJECTIVES

This study aims to characterize the env genotypic profile and the phenotypic susceptibility to TMR in a panel of samples collected from patients harboring multidrug resistant HIV-1 enrolled in the Italian PRESTIGIO Registry (www.registroprestigio.com) and potentially candidate to FTR treatment.

MATERIALS AND METHODS

The PRESTIGIO Registry includes patients with genotypic resistance to NRTI, NNRTI and PI plus either genotypic resistance to INSTI or virological failure to an INSTI regimen without an integrase genotype. Plasma samples from 24 PRESTIGIO patients were used for Sanger sequencing of the gp120 region to detect TMR RAMs (L116P, A204D/V, S375H/I/M/N, M426L, M434I, M475I, V506M). Viral tropism and susceptibility to TMR were assessed through a home-made phenotypic assay involving pseudotyped viruses expressing patient derived Env protein. PCR amplicons including the whole env gene were fused with the CMV promoter and used to create pseudoviral particles together with an env-defective vector expressing the luciferase reporter (NL4-3.Luc.R-E- plasmid) as previously described with minor modifications[3,4].

Viral tropism of pseudotyped viruses was assessed by infection of U87-CCR5 or CXCR4 expressing cell lines in presence of the coreceptor antagonists maraviroc (MVC) and AMD 3100, respectively. According to the viral tropism, pseudotyped viruses were used to infect U87-CXCR4 and/or U87-CCR5 cells in presence of serial dilutions of TMR (range 10 µM – 5.12 pM) and luciferase activity was measured after 72 hours to determine IC_{50} values. Fold change (FC) values were calculated using IC_{50} values obtained with NL4-3 or AD8 wild-type virus depending on the viral tropism.

REFERENCES

1) Langley et al., Proteins 2015; 2) Nowicka-Sans et al., Antimicrob Agents Chemother 2012; 3) Lin et al., J Virol Methods 2010; 4) Vicenti et al., J Clin Virol 2019

RESULTS

Patients' characteristics

Plasma samples were collected from treatment experienced patients with a median time since HIV-1 diagnosis of 27 years and a median time on ART of 26 years. Eighteen (75%) patients were males and 11/24 (46%) reported a previous AIDS event. At sample collection, 17 (71%) patients were receiving a salvage therapy including >3 drugs and half of the patients were receiving entry inhibitors (maraviroc and/or enfuvirtide). Data on table 1 are described as median (interquartile range 25th; 75th) or number of cases (percentage).

Table 1 — Patients' characteristics

Gender, male	18 (75%)
Age, years	55 (52; 61)
Time since HIV-1 diagnosis, years	27 (24; 30)
Time on ART, years	26 (23; 27)
Previous AIDS events	11 (46%)
Nadir CD4, cells/µl	59 (4; 131)
At sample collection:	
HIV-RNA, log_{10} copies/ml	3.87 (3.1; 4.98)
CD4, cells/µl	242 (137; 387)
CD4 %	12.6 (6.0; 21.9)
CD8, cells/µl	1089 (808; 1631)
CD8 %	59.7 (41.7; 66.0)
CD4/CD8 ratio	0.2 (0.1; 0.4)
Number of drugs included in ART:	
3	7 (29%)
4	12 (50%)
5	3 (13%)
6	2 (8%)
Exposure to entry inhibitors:	
Maraviroc	6 (25%)
Enfuvirtide	2 (8%)
Maraviroc + enfuvirtide	4 (17%)
Total	12 (50%)

CONCLUSIONS

TMR RAMs (one S375N and two M426L) were detected in 3/23 samples and the polymorphic RAM M426L was associated with variable reduction of TMR susceptibility. Except for viruses harboring M426L, the susceptibility to TMR was comparable to wild-type strains in all the samples, irrespective of coreceptor usage or exposure to other entry inhibitors.

Sequencing of gp120 region

Amplification of the gp120 coding region was successful in all samples, while the sequence was not obtained in patient 15 due to the presence of mixed virus populations. By querying the COMET HIV-1 subtyping tool, all the sequences were assigned to subtype B. TMR RAMs were detected in only 3/23 (13%) sequences (M426L in patients 3 and 9, S375N in patient 6), while other polymorphisms at TMR RAM sites were found in 7 sequences (Table 2).

Table 2 — TMR RAMs and other polymorphisms at RAM sites

	gp120 codon	116	204	375	426	434	475	506
	Consensus B	L	A	S	M	M	M	V
	TMR RAMs	P	D/V	H/I/M/N	L	I	I	M
Patient ID	1	-	-	-	R	-	-	-
	2	-	-	-	-	-	-	-
	3	-	-	-	**L**	-	-	-
	4	-	-	T	-	-	-	-
	6	-	-	**N**	-	-	-	-
	7	-	S	-	-	-	-	-
	9	-	-	-	**L**	-	-	-
	12	-	-	T	R	-	-	-
	14	-	-	T	-	-	-	-
	21	-	-	-	R	-	-	-

Legend. TMR RAMs are in bold; "-" indicate consensus amino acid at TMR RAM sites.

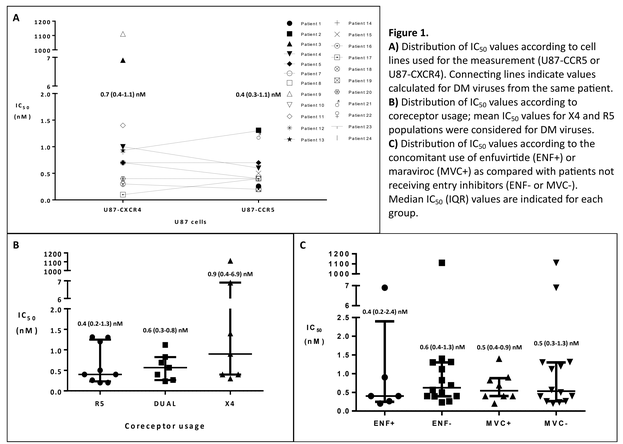

Figure 1.
A) Distribution of IC_{50} values according to cell lines used for the measurement (U87-CCR5 or U87-CXCR4). Connecting lines indicate values calculated for DM viruses from the same patient.
B) Distribution of IC_{50} values according to coreceptor usage; mean IC_{50} values for X4 and R5 populations were considered for DM viruses.
C) Distribution of IC_{50} values according to the concomitant use of enfuvirtide (ENF+) or maraviroc (MVC+) as compared with patients not receiving entry inhibitors (ENF- or MVC-).
Median IC_{50} (IQR) values are indicated for each group.

Viral tropism and susceptibility to TMR

Infectious pseudotyped viruses were generated for all but one sample (patient 6) and viral tropism was X4, R5, and dual-mixed (DM) in 7, 9 and 7 out of 23 cases, respectively.

Viral tropism was in agreement with predicted coreceptor usage based on V3 domain sequence calculated by the Geno2Pheno coreceptor algorithm (10% False Positive Rate cut-off) in 18/23 cases (78%).

Median IC_{50} to TMR was 0.5 nM (0.3-1.2). The reference wild-type viruses NL4-3 (X4), AD8 (R5) had mean IC_{50} of 1.1±0.6 nM and 1.3±0.7 nM, respectively, while the two samples harboring RAM M426L (both X4-tropic) had mean IC_{50} of 6.9±2.9 nM and 1110.6±798.2 nM, resulting in FC values of 6.2 and 1009, respectively (Table 3). According to viral tropism, median IC_{50} values were 0.9 nM (0.4-6.9), 0.4 nM (0.3-1.3) and 0.6 nM (0.3-0.8) for X4, R5 and DM viruses, respectively. Concomitant use of MVC or enfuvirtide also did not impact TMR IC_{50} values (Figure 1).

Table 3 — Determination of viral tropism and susceptibility to TMR

Patient ID	FPR %	Tropism	IC_{50} X4 ± SD (nM)	FC X4	IC_{50} R5 ± SD (nM)	FC R5
1	66.6	R5	/		0.3 ± 0.2	0.2
2	31.4	R5	/		1.3 ± 0.6	1.0
3	1.8	X4	6.9 ± 2.9	6.2	/	
4	10.8	DM	1.0 ± 0.1	0.9	0.6 ± 0.5	0.5
5	0.1	DM	0.7 ± 0.2	0.6	0.7 ± 0.1	0.5
6	0.4	NA*	/		/	
7	6.7	DM	0.7 ± 0.2	0.6	0.4 ± 0.1	0.3
8	24.7	R5	/		0.4 ± 0.1	0.3
9	0.2	X4	1110.6 ± 798.2	1009.6	/	
10	30.1	R5	/		0.4 ± 0.4	0.3
11	5.7	X4	1.4 ± 0.4	1.3	/	
12	4.4	R5	/		1.3 ± 0.7	1.0
13	50.2	DM	0.9 ± 0.1	0.8	1.3 ± 0.6	1.0
14	18.3	X4	0.9 ± 0.4	0.8	/	
15	NA	R5	/		0.5 ± 0.1	0.4
16	7.4	DM	0.3 ± 0.3	0.3	0.2 ± 0.1	0.2
17	25.3	DM	0.1 ± 0.1	0.1	0.4 ± 0.1	0.3
18	16.4	R5	/		0.2 ± 0.1	0.2
19	65.2	R5	/		0.2 ± 0.1	0.2
20	0.1	X4	0.4 ± 0.2	0.4	/	
21	97.8	R5	/		1.2 ± 0.5	0.9
22	0.5	X4	0.3 ± 0.1	0.3	/	
23	4	DM	0.4 ± 0.1	0.4	0.4 ± 0.1	0.3
24	1.7	X4	0.4 ± 0.2	0.4	/	
NL4-3	0.5	X4	1.1 ± 0.6	1.0	/	
AD8	35.3	R5	/		1.3 ± 0.7	1.0

Legend. NA: not available (pseudotyped virus not infectious)