Poster 950

No Selection of X4 Viruses by Maraviroc in Cell Reservoirs in R5X4 HIV Infections

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BACKGROUND

- ✓ **The CCR5 antagonist maraviroc, used in combination with antiretroviral drugs in HIV-infected patients, is only active against CCR5-using viruses.**
- ✓ **However, some patients were given maraviroc although they were infected by R5X4 dual-mixed viruses. This was to assess the immunological benefit of maraviroc therapy.**
- ✓ **In the MARIMUNO study, patients with undetectable plasma HIV RNA received a 24-week maraviroc supplement to an efficient antiretroviral therapy.**
- ✓ **Since the positive selection of CXCR4-using viruses in cell reservoirs may influence any response to later treatment, we investigated how the frequency of CXCR4-using variants in R5X4 dual-mixed virus populations responded to maraviroc selection pressure using ultra-deep sequencing (UDS).**

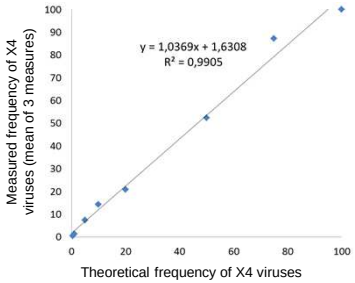

Figure 1. Sensitivity of X4 variants detection with optimized PCR amplification.
X4 (LAI) and R5 (JR-CSF) virus clones were mixed in proportions of 0:100, 0.5:99.5, 1:99, 5:95, 10:90, 20:80, 50:50, 75:25 and 100:0 and adjusted to a total input of 300 DNA copies. The measured frequencies of X4 viruses by ultra-deep pyrosequencing was compared to the theoretical frequencies of X4 viruses.

A 415-long nucleotide fragment encompassing the V3 env region was generated by nested PCR using optimized amplification steps for accurate representation of HIV-1 quasispecies. The first amplification was performed in several replicates (2 - 8) to ensure that at least 200 copies had been amplified, according to the initial HIV DNA load in the PBMCs (Fig 1).

Table 1. Within-run reproducibility of UDS for determining X4 variants frequency

DNA sample	Result 1	Result 2	Result 3	Mean	Standard deviaion	CV (%)
1	38.50%	34.70%	46.50%	39.90%	0.06	15
2	42.40%	41.60%	40.30%	41.40%	0.11	3
3	76.70%	74.40%	72.9%%	74.70%	0.19	3

The within-run reproducibility was estimated from repeated measures of 3 samples (Table 1). The coefficient of variation ranged from 3% to 15%.

METHODS

- ✓ **We explored 22 patients from the MARIMUNO study infected with R5X4 dual-mixed viruses according to the recombinant virus assay Toulouse Tropism Test.**
- ✓ **The frequency of CXCR4-using variants was determined in peripheral blood mononuclear cells (PBMCs) before maraviroc intensification (week 0) and after 24 weeks of maraviroc (week 24).**
- ✓ **UDS was performed on a 454 GS Junior system. The sequence reads of the V3 env regions were analyzed with PyroVir software (Inserm-Transfert) developed to provide a fast and automated position-specific process for inferring HIV-1 tropism from V3 env 454 ultra-deep pyrosequencing data.**

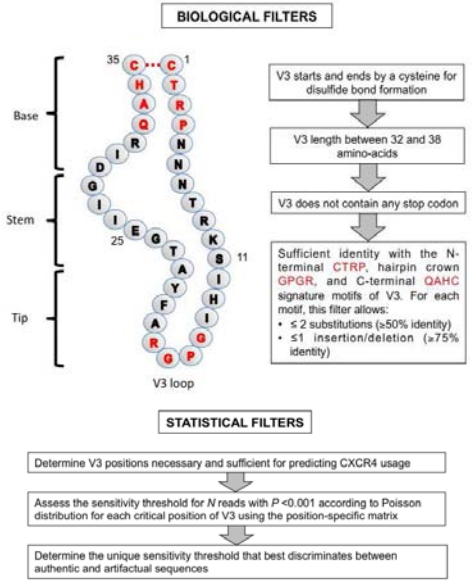

Figure 2. PyroVir flowchart

The sequence reads of the V3 env regions were quantified with GS AVA software Version 2.5p1 (Roche Diagnostics), aligned with the BaL (GenBank AY426110) consensus sequence and analysed with PyroVir software (PyroVir, IDDN FR.001.160011.000.S.P.2012.000.31230, Inserm-Transfert) developed to provide a fast and automated position-specific process for inferring HIV-1 tropism from V3 env 454 ultra-deep pyrosequencing data (Jeanne et al, Scientific Reports, 2015; 5:16944). Specific genotypic algorithms validated for HIV-1 subtypes B and non-B are embedded in the PyroVir software.

Table 2. Ultra-deep sequencing of the V3 *env* region from 22 R5X4 infected patients given maraviroc for 24 weeks

		Before maraviroc intensification (week 0)				After 24 weeks of maraviroc intensification (week 24)				
Patient	HIV-1 subtype	HIV DNA[1]	Total V3 amino acid variants	X4 amino acid variants	Percentage of X4 variants	HIV DNA[1]	Total V3 amino acid variants	X4 amino acid variants	Percentage of X4 variants	X4% W24-W0[2]
1	B	2.9	6	4	66	3	7	5	72	6
2	D	2.8	6	4	76	2.3	8	7	85	9
3	B	2.7	5	3	79	2.4	8	6	92	13
4	B	2.5	2	1	19	2.6	7	4	51	32
5	B	2.7	5	2	23	2.5	5	3	15	-8
6	B	1.6	10	3	36	2.6	7	2	34	-2
7	B	3.3	4	3	68	3	9	6	67	-1
8	B	2.3	2	1	44	3	5	3	43	-1
9	B	2	7	4	90	2.3	6	3	83	-7
10	B	2.4	6	1	3	2.8	2	0	<1.2	-3
11	B	2.2	11	3	17	2.3	8	2	10	-7
12	B	2.3	4	2	44	2.5	7	3	42	-2
13	B	2.8	9	6	86	2.8	10	7	81	-5
14	B	2.8	5	2	65	3	11	2	37	-28
15	A1	<1.7	8	6	90	0	9	7	80	-10
16	B	2.9	10	5	45	2.7	3	2	33	-12
17	B	2.1	8	4	41	2.8	4	1	26	-15
18	B	2.5	8	7	91	3	8	5	65	-26
19	B	2.6	10	7	76	2.6	4	3	54	-22
20	B	3	5	3	86	2.7	10	6	56	-30
21	B	2.3	1	1	100	2.4	4	3	27	-73
22	CRF02	2.3	24	12	59	2.1	19	7	42	-17
Mean		2.4	7	4	59	2.5	7	4	52	-10

[1]Total HIV DNA in PBMCs: log copies/106 cells; [2]Difference between the X4 percentages at weeks 24 and 0

- ➢ **The mean total HIV-1 DNA before maraviroc intensification was 2.4 log copies/106 cells; it was 2.5 log copies/106 cells 24 weeks later (Wilcoxon rank test, P=0.3).**
- ➢ **UDS with the PyroVir genotypic algorithm detected CXCR4-using viruses in the 22 R5X4 infected patients at week 0 with a mean frequency of 59% (range: 3-100%) and in 21/22 patients at week 24 (mean frequency= 52%; range: 10-92%).**
- ➢ **We found no correlation between the HIV DNA concentration in PBMCs and the number of CXCR4-using variants or their frequency.**
- ➢ **The frequency of CXCR4-using variants did not increase between weeks 0 and 24 except in patient 4 whose increase was 32%.**

CONCLUSION: A 24-week course of a CCR5 antagonist does not select CXCR4-using viruses in the PBMCs of patients on suppressive therapy infected with R5X4 dual-mixed viruses. These results indicate little or no residual HIV replication that could be subjected to selection pressure.

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