Lack of effect of Maraviroc intensification on blood and gut reservoir

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Abstract

We show that intensification of treatment with maraviroc in patients chronically infected with HIV-1 receiving successful long-term ART was not associated with improvements in HIV related morbidity, HIV reservoir, microbial translocation, immune activation or immune exhaustion in either gut or peripheral blood. The measurement of reservoir in both gut and blood longitudinally contributes to a paucity of data in the area.

Background

Despite the huge success of antiretroviral therapy (ART), increased morbidity compared with HIV uninfected individuals continues [1], particularly in those with a blunted CD4 response to ART [2]. A cure for HIV remains elusive and this is in part due to an inaccessible long-lived pool of latently infected cell; the viral reservoir, which is focused in the gut-associated lymphoid tissue [3].

Intensification of ART in individuals with suppressed plasma viremia has not consistently reduced viral reservoir or improved immune function [4,5]. The C-C chemokine receptor type 5 (CCR5) entry inhibitor, maraviroc, is an attractive option as CCR5 recpetors are most numerous in the gut and the drug penetrates tissue well [6]. Studies suggest a possible role in increasing CD4 count [7,8] and reducing immune activation [5,9]. The gut has been less investigated [4], with no consensus on microbial translocation [5,11] or immune activation [11]].

We investigated the effect of maraviroc intensification on gut immune function and viral reservoir.

Methods

Study Design and recruitment

A prospective study whereby 10 individuals with chronic HIV infection with sustained virological suppression on ARV for >12 months and a CD4+ T cell count below 500 cells/mm^3 were intensified with a 24-week course of standard dose maraviroc. Blood samples were collected at week 0, 4, 12 and 24. At baseline and week 24, individuals underwent flexible sigmoidoscopy, in which eight-ten rectal biopsies were taken and fixed overnight in 10% normal buffered formalin at 4°C.

Laboratory Tests

Bacterial translocation

Plasma bacterial 16s DNA was quantified by real time PCR [11]. Plasma sCD14 was quantified using the Quantikine Human sCD14 Immunoassay (R&D Systems, Minnesota, USA), according to manufacturer's instructions. All samples were run in duplicate.

HIV Reservoir and residual Viraemia

Low copy viral load was measured with an internally controlled ultrasensitive quantitative real-time RT-PCR able to detect 3 copies/ml. Purified peripheral blood CD4 T cells were analyzed by qPCR for HIV-1 DNA (Total and Integrated) and cell-associated HIV-1 RNA unspliced transcripts (CA-RNA) as reported elsewhere [12].

For preparation of the gut biopsies for HIV reservoir quantitation, a commercial kit was used (Qiagen, Cat. No. 56404), and the manufacturer's protocol modified as follows to ensure maximum yield of DNA. Biopsy sections were removed from paraffin blocks using a scalpel and placed into 1.5ml micro centrifuge tubes. Samples were washed repeatedly with xylene and ethanol until the precipitate had disappeared, then dried at room temperature. Biopsy sections were incubated at 37°C and DNA extracted using a commercial kit (QIAAMP DNA FFPE Tissue Extraction Kit, ID 56404, Qiagen (Hilden, Germany)). The resulting DNA was eluted into 200µl and analysed by qPCR using the same assays for peripheral blood CD4 cells detailed above.

Immune activation and lymphocyte subsets

PBMC were stained with the anchor markers (CD3- VioBlue, CD4(VIT4)-VioGreen, CD8-APC) and a Live/Dead marker Near IR- APC-Cy7 plus either an activation panel (CD25(3G10)-PE, CD38-PE-Vio770, CD69-FITC, Anti-HLA-DR-PerCP) or an exhaustion panel (TIGIT-PE, TIM-3-FITC, LAG-3-PerCPeF710, PD1-PE-Cy7). Cells were run on a MACSQuant and analysed with FlowJo software v10 (Miltenyi Biotec).

Immunohistochemistry

Rectal biopsy sections were stained for CD4 (goat polyclonal, Novus Biologicals) and CD8 antibodies (rabbit polyclonal, Abcam). Images were analysed in ImageJ and the distribution of cells determined as described by Milano et al [13].

Statistics

For each outcome measure, the results at baseline and week 24 were compared using the paired T-test. Wilcoxon rank test was used to assess the impact of tropism on each parameter.

Results:

Patient characteristics

The median age was 46 years. All patients were male with subtype B virus and were receiving two nucleoside reverse transcriptase inhibitors (NRTI) combined with either a non-nucleoside reverse transcriptase inhibitor (n=5) or protease inhibitor (n=5). The mean CD4 T cell count was 321 cells/uL (SD 112), CD4:CD8 0.51 (SD 0.27) and nadir CD4 117 cells/mL (SD 135). All patients maintained an HIV viral load <50 copies/ml throughout the study. Individual plots of viral reservoir, microbial

translocation and immune activation are shown in table 1 and mean changes in parameters between week 0 and 24 are shown in figure 1.

Baseline to week 24:

- **Clinical outcome / T-cell subsets composition in blood and gut**

Between baseline and week 24 in the blood, there was a non-significant increase of 48.22 CD4 T cells/mL (p=0.275) and 39.23 CD8 T cells/mL (p=0.672) whilst CD4:CD8 did not change. This was accompanied by a non-significant reduction in gut CD4 (-0.80 p= 0.087) and gut CD8 T cells (-5.59 p=0.052). Changes in blood CD4 and CD8 lymphocytes did not correlate with changes in these cells in the gut.

- **Bacterial translocation**

Between baseline and week 24 there was no significant change in 16s DNA copies (-0.60;p=0.797) or sCD14a (+59.35 p=0.637).

- **HIV reservoir**

Neither HIV RNA, HIV DNA (blood or gut) or cellular HIV RNA changed significantly between baseline and week 24 or between baseline and week 12. At baseline gut HIV DNA levels (mean 101806 copies/million cells) were significantly higher than blood (3669 copies/million CD4 T cells) (p=0.017).

- **T-cell activation and exhaustion**

The level of activation of CD4 + T cells and CD8+ T cells showed non- significant increases from baseline to week 24 in 6 parameters (including CD8+CD38+HLADR;p=0.677) with only %CD8+HLADR (+0.61 p=0.048) increasing significantly. These findings were the same when analysed from baseline to week 12 with only %CD8+ HLADR+ showing a significant change (+0.47; p= 0.001).

Eight out of 10 immune exhaustion markers increased from baseline to week 24 but only CD8% Tigit reached significance (+6.07; p=0.006). This was supported by analysis of week 0 to week 12 (% CD8 Tigit (mean increase +5.82; p=0.004).

Tropism:

Six out 10 individuals had R5 virus. Tropism (R5 v X4 or X4/R5) did not correlate with changes in reservoir, immune activation, immune exhaustion or microbial translocation (all P values > 0.05).

Discussion:

In this pilot study, intensification of treatment with maraviroc in patients chronically infected with HIV-1 receiving successful long-term ART with a blunted CD4 immune response was not associated with any overall improvements in surrogate markers of HIV related morbidity (CD4 T cell count or CD4:CD8 ratio), microbial translocation, immune activation or immune exhaustion in either gut or peripheral blood. The isolated significant results in the absence of a broad class-wide effect on T cell exhaustion or activation are potentially interesting but should not be over-interpreted. Furthermore, reservoir did not decline longitudinally either gut or blood. The large gut HIV reservoir supports interventions targeting this compartment; however, the large intra-individual variation means that sample sizes may need to be large. The lack of impact of tropism on the effect of maraviroc on immunological function or reservoir size is novel but limited due to small sample size.

Overall, the study shows no role for maraviroc intensification to improve clinical or immunological outcomes or to provide complete viral inhibition. The null effect may also reflect that these individuals had more resistant immune dysfunction than intensification with antiretroviral therapy could repair or the short duration of follow up. The study is limited by the small sample size and lack of control group but the detailed analysis particularly of the gut further diminishes the investigation of maraviroc as an agent of intensification.

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Table 1: baseline characteristics

Baselines	Mean (SD) / Count (%) N=10
Age	44.5(6.9)
Gender: Male	10(100%)
Ethnicity: White	5(50%)
Black	3(30%)
Oriental	1(10%)
Other	1(10%)
BMI	25(2.8)
HBV+	1(10%)
HBV-	9(90%)
HCV+	0(0%)
HCV-	10(100%)
CD4	320.6(112.3)
Nadir CD4	117.4(134.8)
Tropism X4: yes	2(20%)
Tropism R5: yes	6(60%)
Tropism mixed: yes	4(40%)

Table: Adverse event (MT trial)

Patient ID	Event name	AE status	Serious AE	Concomitant Medication	AE intensity	AE study drug action	AE outcome	Relationship to Study Drug
6	Headache	New AE	No	No	Mild	None	Resolved no sequela	Possibly
6	Diarrhoea	New AE	No	No	Mild	None	Resolved no sequela	Possibly
7	rectal spotting post baseline biopsy	New AE	No	No	Mild	None	Resolved no sequela	Not related
8	bursitis	New AE	No	Yes	Mild	None	Resolved no sequela	Not related
8	tooth infection	New AE	No	Yes	Mild	None	Resolved no sequela	Not related
10	Severe chest pain	New AE	Yes	Yes	Severe	None	Resolved with sequela	Not related
10	pain on palpation at sterno-costo-clavicular joint associated with signs of sternochondritis	New AE	No	Yes	Moderate	None	Not resolved/ ongoing	Not related
10	eye infection in the left eye	New AE	No	Yes	Moderate	None	Resolved no sequela	Not related
12	Skin Irritation associated with fungal skin infection	New AE	No	Yes	Mild	None	Not resolved/ ongoing	Not related
12	Gonorrhea throat	New AE	No		Mild	None		Not related
12	Syphillis (re-infection)	New AE	No		Mild	None		Not related

Figure 1. Consort Flow Diagram

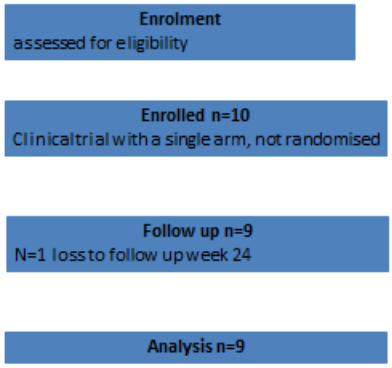

Figure 1: Individual change in key parameters between week 0 and week 24

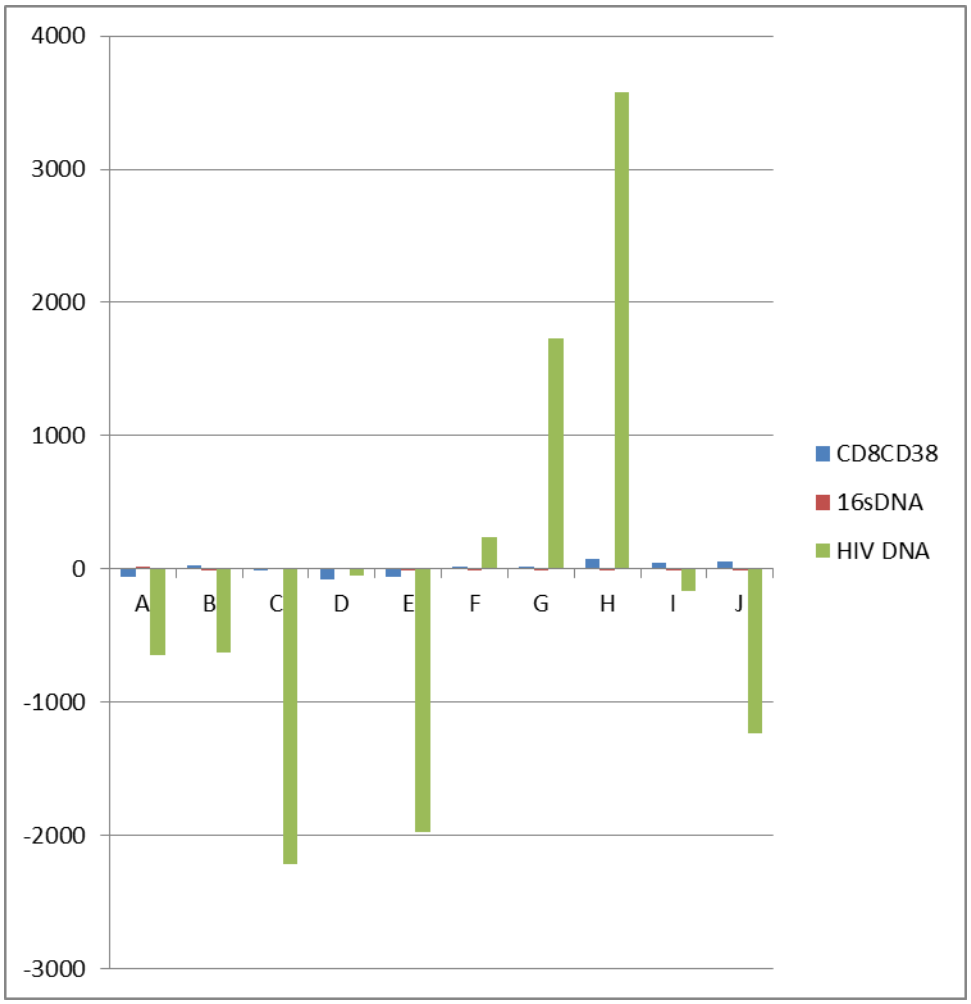

Table 2: Mean changes in parameters between week 0 and 24

Outcome variable	Mean (SD) Week 0 (visit 2) N=10	Mean (SD) Week 24 (visit 5) N=9	Mean difference (week 24 - week 0) N=9	P.value
Microbial translocation				
16s DNA copies/ul	7.95(3.7)	7.69(6.73)	-0.60	0.7972
scd14 ng/mL	1781.56(355.16)	1783.02(438.14)	59.35	0.6368
Clinical outcome markers				
CD4 cells/ml	334.4(131.64)	407.56(154.63)	48.22	0.2751
CD4%	24.39(8.77)	24.39(7.02)	-1.29	0.3349
CD4:CD8 ratio	0.51(0.27)	0.54(0.21)	0	1
Reservoir				
HIV DNA copies/ CD4 T cell	3669.41(1902.92)	3181.33(3484.3)	-343.76	0.5594
Cell HIV RNA copies/ 18s RNA copies	2755.62(4400.75)	991.18(1130.88)	-1295.49	0.5279
Low copy plasma HIV RNA/ml	16.1(14.96)	38.33(38.12)	21.78	0.1424
Immune Exhaustion				
% CD8 lag3	0.91(1.08)	0.44(0.22)	-0.52	0.2669
% CD8pd1	0.27(0.23)	0.55(0.41)	0.27	0.1762
% CD8 tigit	47.23(13.79)	52.08(16.23)	6.07	0.0059
% CD8 tim3	0.1(0.09)	0.11(0.1)	0	0.9191
% CD4 lag3	0.24(0.21)	0.26(0.2)	0.08	0.3693
% CD4 pd1	1.01(1.08)	1.64(1.24)	0.98	0.0596
% CD4 tim3	0.32(0.35)	0.26(0.2)	-0.01	0.9074
% CD4 tigit	26.56(10.01)	25.3(10.36)	1.63	0.5215
Immune activation				
% CD4CD25	1.73(0.89)	2.19(2.07)	0.58	0.4762
% CD4CD38	0.95(0.52)	1.15(0.94)	0.37	0.2302
% CD4CD69	1.4(1.15)	1.29(0.89)	0.21	0.3268
% CD4 HLA DR	4.74(2.5)	5.08(3.01)	1.32	0.163
% CD8CD38	45.67(27.47)	31.59(34.97)	-16.37	0.3669
% CD8CD25	2.04(1.3)	1.77(1.77)	-0.63	0.3275

% CD8CD69	5.7(4.34)	3.66(1.82)	-2.16	0.326
% CD8 HLA DR	0.82(0.59)	1.22(0.9)	0.61	0.0482
% CD8CD38 HLA DR	0.82(0.59)	1.23(0.84)	0.58	0.0326
Gut tissue reservoir				
HIV DNA copies/ million cells	101805.93(117858.81)	51468.83(109191.98)	-44062.84	0.4738
Gut cell composition				
Total cell number	93.9(22.33)	93.59(28.52)	1.26	0.9006
CD4 cell number	22.9(12.52)	16.41(9.98)	-8.04	0.0873
CD8 cell number	11.5(5.79)	6.26(4.57)	-5.59	0.0522
Proportion of CD4 cells	0.25(0.16)	0.18(0.12)	-0.09	0.0644
Proportion of CD8n cells	0.12(0.05)	0.08(0.05)	-0.05	0.0898