HIV Reservoir Changes in Resting CD4 Subsets in the IL7 plus ART Intensification Eramune01 Study

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ABSTRACT: # 977

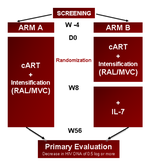
Background:
The ERAMUNE01 trial showed that a year of MVC+RAL intensification alone or with 3 IL7 injections in treated chronic HIV+ patients did not decrease the blood cell associated HIV-DNA. IL7 induced a massive but transient CD4 T cells increase affecting mainly the CM subset, that gradually decreased over 12 months.
To further understand the effects of IL7 we evaluated the distribution and characteristics of viral reservoir among the distinct resting CD4 T cell subsets.

Methodology:
We sorted resting CD25-69-DR-CD4+ N, CM, TM, EM CD4 T subsets at D0 and W56 from RAL+MVC and RAL+MVC+IL7 arms (6+6 pts). In each sorted sample: 1) HIV DNA was quantified per 10^6 subset cells, reported to absolute subset count and analysed for subset contribution to total HIV reservoir, using nonparametric tests; 2) HIV inducibility was tested ***in vitro*** with or without IL7 or antiCD3+CD28+IL2+IL7 D0 stimulation, amounts of HIV RNA released in supernatants normalized for HIV DNA content 3) Human genome-wideTranscriptome was analysed in al sorted subsets at D0 and W56 using the Illumina microarray Platform 4) Unspliced HIV RNA transcripts were detected using qPCR (GAG gene) in purified total CD4 T cells at D0, W8, W12, 28 and 56.

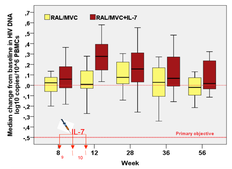
Results:
At D0 reservoir distribution did not significantly differ among sorted resting CD4 subsets despite a higher contribution of CM than EM in RAL+MVC+IL7 (***p 0.01***). At W56 no significant cell HIV-DNA changes from baseline were observed in any subset from any arm, except for the TM HIV-DNA raw and absolute values which increased at W56 in RAL+MVC+IL7 (***p 0.03***). However both the CM and TM delta raw (HIV DNA/10^6 CM, ***p<0.02***), absolute values (HIV DNA/CM/mL, ***p<0.01***) and the CM fold changes (***p 0.04***) from baseline increased more in RAL+MVC+IL7 than in RAL+MVC. Thus the RAL+MVC+IL7 reservoir distribution became skewed towards an increased TM compared to N raw values (***p 0.02***) and an increase in CM and TM compared to EM absolute values (***p 0.04***). In vitro IL7 alone induced HIV production in CM and TM from both arms (5/8 CM and 7/8 TM) while antiCD3+CD28+IL2+IL7 stimuli induced HIV production in all tested sorted samples at D0 and in CM at W56. Finally no increase in HIV RNA transcripts was observed ex vivo 2 to 46 weeks after IL7 infusions. Similarly no transcriptome changes were observed in any resting CD4 T cell subset between D0 and W56 in both arms.

Conclusions:
A year long strategy combining a cycle of IL7 injections plus MVC+RAL intensification did not show major changes in HIV reservoir nor in HIV transcriptional activity among resting CD4 T subsets, despite the IL7 ability to trigger HIV production ***in vitro,*** except an increase in transitional-memory CD4 T cells with intermediate lifespan. The reservoir distribution remained skewed towards both TM and TCM, contrasting with the predominant expansion of the long-lived (CM) central-memory CD4 T cells at the expenses of short-lived and senescent EM cells, suggesting those changes do not simply reflect homeostatic proliferation induced by IL7.

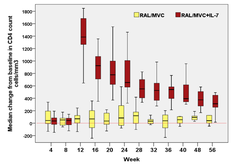
BACKGROUND



STUDY DESIGN



No decrease in cell associated HIV-DNA in PBMCs from both arms



IL7 induced a massive CD4 T cell increase, mainly among TCM

We then assessed whether IL7 therapy did perturb the distribution of HIV reservoir and provoke transcriptional changes among resting CD4 T cell subsets

METHODS

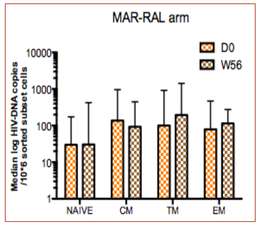
Cell sorting of resting CD25-CD69-HLADR-CD3+CD4+ subsets: N, CM, TM, EM at D0 and W56 among RAL+MVC arm (6 pts) and RAL+MVC+IL7 arm (6 pts)

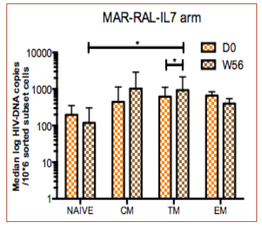
Reservoir Distribution and Contribution	• HIV DNA/10^6 subset cells • HIV DNA/subset cell%/10^6 CD4 • HIV DNA /subset cells/mm3
Reservoir Inducibility	• At D6, D10, D13 HIV RNA quantified in culture supernatants and normalised for HIV DNA of each subset. • 3 different culture conditions: 1. unstimulated cells; 2. IL7 alone; 3. antiCD3+CD28+IL7+IL2+
Statistical Analysis	• Mann Whitney test for comparisons between arms (RAL-MAR vs RAL-MAR-IL7) • Wilcoxon signed rank test for comparison among arms (D0 vs W56) • Kruskal Wallis test for comparison between subsets • p value < 0.05 was considered significant
Human Transcriptome	• Human genome wide microarray Platform Lumina • Data files uploaded into Ingenuity Pathway Analysis (IPA) software. • Right tailed Fischer's exact test was used to calculate p value • Z-score to infer activation (> 2) or inhibition (< -2) of predicted transcriptional regulators

Unspliced HIV RNA transcripts from total CD4 detected by qPCR (GAG gene) at D0, W8, W12, W56 among RAL+MAR arm (6 pts) and RAL+MVC+IL7 arm (13 pts)

RESULTS

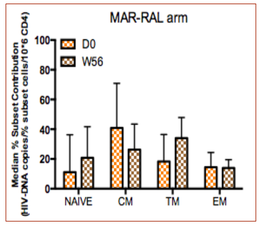
Reservoir Distribution (HIV DNA/10*6 cells subset)

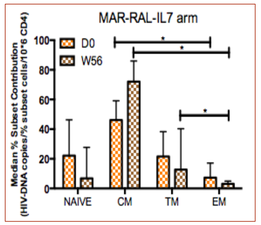




The Reservoir distribution (raw values: HIV-DNA copies/10*6 sorted subset cells) among arms did not change in RAL-MAR arm, while it increased in among the MAR-RAL-IL7 TM cells (p 0.03) that became significantly higher than in N (p 0.02).
Comparing the 2 arms, the CM HIV-DNA W56-D0 delta was significantly higher in RAL-MAR-IL7 compared to RAL-MAR (p 0.02, data not shown).

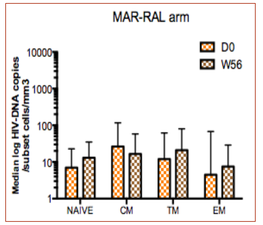
Relative subset Contribution to the HIV reservoir (HIV DNA/cell subset%/10*6 CD4)

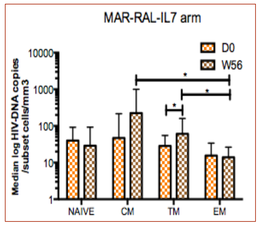




Relative reservoir contribution of various subsets (% contribution subset/10*6 CD4) did not significantly change over time.
In the RAL-MAR-IL7 arm : higher contribution of CM than EM both at D0 and W56 (p<0.01) and higher TM contribution than EM at W56 (p0.01), with a higher W56-D0 delta in CM (p 0.01) and lower fold change EM (p 0.02) contributions than in the MAR-RAL arm (data not shown).

Absolute subset Contribution to the HIV Reservoir (HIV DNA/cell subset count/mm3)

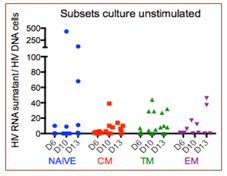


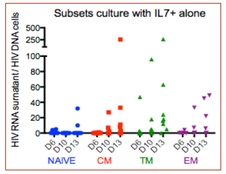


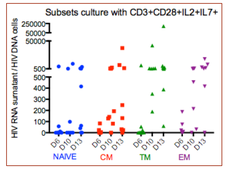
Absolute reservoir contribution values (HIV-DNA cp/subset cells/mm3) among arms did not change in RAL-MAR arm, but significantly increased in TM (p 0.03) with a trend in CM (p 0.06) in the MAR-RAL-IL7 arm. At W56 CM and TM contributed more than EM to the HIV reservoir (p 0.02, p 0.04 respectively).
In RAL-MAR-IL7, the CM and TM W56-D0 delta were significantly higher than in RAL-MAR (p 0.008, p 0.03 respectively, not shown).

In vitro viral Inducibility: Cell Subset Culture

13-days cell culture of sorted subsets (N, CM, TM, EM at D0; CM at W56, data not shown)





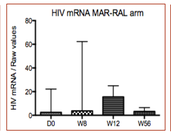


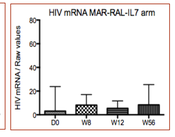
Inducibility of the HIV Reservoirs:
•HIV reservoirs were inducible in all resting CD4 subsets, with a very low spontaneous production in all subsets despite in vivo ART.
•IL-7 induced a low level HIV production at D13 mostly from memory CD4 subsets.

Ex vivo HIV mRNA transcripts

Unspliced HIV RNA transcripts from purified total CD4+ T cells:

IL7 did not induce any significant change in mRNA transcription levels over time.



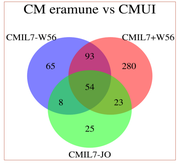


Human Genome-wide Transcriptome

- Direct comparisons between subsets did not find any differentially expressed gene among and between arms (FDR 0.05)
- Comparing sorted D0 and W56 CD4+ T CM with uninfected CD4+ T CM from healthy donors we observed:
  - no difference in RAL-MAR arm (data not shown)
  - at W56 in RAL-MAR-IL7 CM cells, the Z-score predicted significantly higher activated pathways than of healthy control (Table)

Distribution of differentially-expressed genes (FDR<0.05)

comparison between TCM from the two Eramune arms , both at D0 and W56 and from uninfected control donors



Immunovirological pathways differentially expressed in TCM from the RAL-MAR-IL7 ARM

	D0 vs CM uninfected	W56 vs CM uninfected
differentiation of lymphocytes	2,19	2,40
transcription	2,15	2,10
transcription of RNA	2,07	1,99
viral infection	0,82	2,51
cell death of immune cells	2,14	0,81
adhesion of blood cells		2,85
protein kinase cascade		2,83
adhesion of immune cells		2,67
I-kappaB kinase/NF-kappaB cascade		2,45
activation of T lymphocytes		2,43
cell death of APCs		2,42
immune response of cells		2,41
activation of leukocytes		2,40
cell movement		2,24
T cell development		2,22
apoptosis of APCs		2,20
cell death of macrophages		2,18
orientation of microtubule organising centre		2,16

CONCLUSIONS

HIV reservoirs studies in the distinct major subsets of resting CD4+ T-cells from chronically HIV-infected patients:
- After a year long intensification with RAL+MAR: No changes.
- After a year long intensification plus 3 IL7 injections, in the MAR-RAL-IL7 arm:
  - ★ cell-associated HIV-DNA significantly increased only among resting CD4+ TTM cells, with a non significant trend in CD4+ TCM, although the TCM-associated HIV-DNA at W56 was significantly higher in the MAR-RAL-IL7 arm compared to the RAL-MAR arm
  - ★ at W56 a major contribution of both CM and TM compared to EM, both in proportions and absolute values, was observed.

Transcriptome analysis. No changes in:
- HIV unspliced transcripts in total CD4 T cells, over time
- human transcriptional profiling in any resting CD4 subset from both arms between D0 and W56, despite a persistent IL7 effect on CD4 TCM compared to TCM from healthy donors. Further analysis are ongoing to assess whether these transcriptomic changes are also detectable in the other subsets.

Altogether, these findings suggest that IL7 induces some long term changes in the redistribution of the HIV reservoirs and CD4 T cell compartments, that does not simply reflect homeostatic proliferation triggered by IL7.

The EraMune-01 Study Group

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