Effects of Quadruple First-Line ART on Mucosal Immunity

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CROI 2015

POSTER SESSION P-J1
ABSTRACT #547

INTRODUCTION

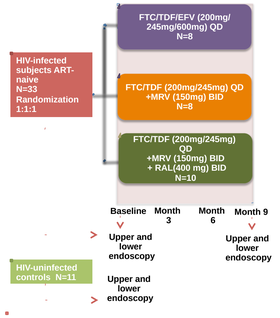
•A widely accepted conceptual model of HIV immunopathogenesis recognizes how the gut might serve as a sanctuary for HIV replication and a portal of systemic inflammation, likely contributing to residual morbidity observed in most cohorts of treated HIV-infected individuals (1-3).
•Although data on levels of antiretroviral drugs in lymphatic tissues (the gut and lymph nodes) are limited, it has been shown that suboptimal concentrations are achieved for some agents within these sites to fully suppress HIV replication, where virus production persists despite otherwise effective ART. (4,5)
•We hypothesized that the restoration of mucosal immune abnormalities following ART initiation could be affected by initiating ART with a combined CCR5 and integrase inhibitors-based regimen.

METHODS

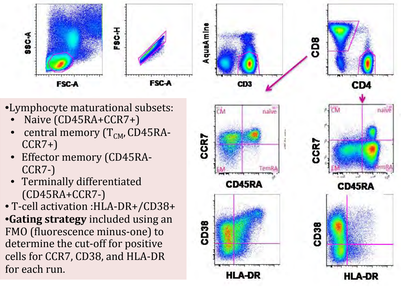
•Peripheral blood, duodenal and rectal biopsies were obtained from 11 HIV- controls and 33 ART nave HIV+ subjects participating in a randomized clinical trial comparing three different ART initiation strategies, at baseline and after 9 months of treatment.

•Tissue was digested into single-cell suspensions for immunofluorescence analysis and flow-citometry measurements.

Study Design



Flow citometry analysis on peripheral and mucosal mononuclear cells



•Lymphocyte maturational subsets:
- Naive (CD45RA+CCR7+)
- central memory (T_{CM}, CD45RA-CCR7+)
- Effector memory (CD45RA-CCR7-)
- Terminally differentiated (CD45RA+CCR7-)

• T-cell activation :HLA-DR+/CD38+
•**Gating strategy** included using an FMO (fluorescence minus-one) to determine the cut-off for positive cells for CCR7, CD38, and HLA-DR for each run.

- **Immunofluorescence (IFA) analysis:** tissue was stained using polyclonal anti-CD3 rabbit and monoclonal anti-CD4 or CD8 mouse serum. Binding of CD3 and CD4 or CD8 receptors were detected simultaneously using Alexafluor 488-labeled polyclonal goat antirabbit IgG (and Alexafluor 568- labeled polyclonal goat antimouse IgG (Molecular Probes). The numbers of positive cells were counted by a single observer and presented as cells/mm^2 of lamina propria or intraepithelial regions (above the basement membrane) of duodenal mucosa.

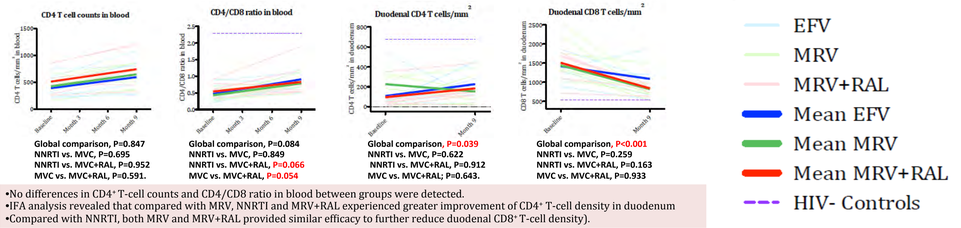
- **Assays in cryopreserved plasma:** IL-6 (R&D Systems), sCD14 (R&D Systems), hs-CRP (CardioPhase hs-CRP assay, Siemens), lipoteichoic acid (LTA, R&D Systems) and zonulin-1 (ALPCO).

- **Statistical analysis:** linear mixed models were computed to estimate the mean change of each parameter in plasma, PBMC colon and duodenum. Interaction terms were created to assess whether these changes over time differed significantly between groups.

RESULTS

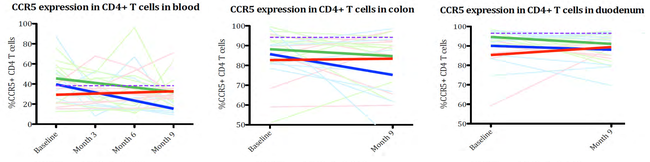
•Data were available for 26 HIV+ subjects that completed the follow-up period.
•Eight subjects received EFV, 10 MRV and 8 MVC+RAL, each in combination with FTC/TDF.
•Median age was 37 years [25-42] vs 34 [28-43] in the control group
•Median CD4 T-cell count 435 cell/mL [283-572].
•All subjects in the study achieved viral suppression.

The intervention elicited distinct effects lymphocyte subsets across compartments in duodenum but not in blood



•No differences in CD4⁺ T-cell counts and CD4/CD8 ratio in blood between groups were detected.
•IFA analysis revealed that compared with MRV, NNRTI and MRV+RAL experienced greater improvement of CD4⁺ T-cell density in duodenum
•Compared with NNRTI, both MRV and MRV+RAL provided similar efficacy to further reduce duodenal CD8⁺ T-cell density).

Combination CCR5 and integrase inhibitors-based ART elicited greater normalization of percentage of CD4⁺ mucosal T-cells expressing CCR5

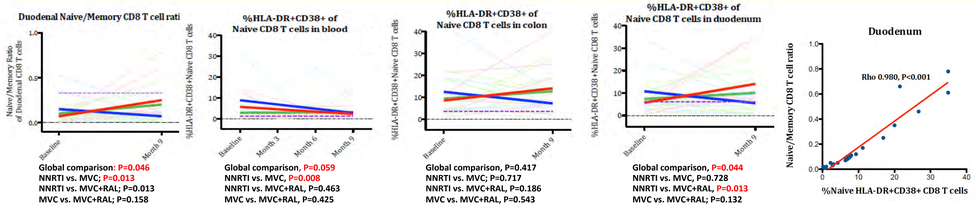


•Subjects in either the NNRTI or MRV cohorts experienced further CD4⁺ / CCR5⁺ T-cell loss in blood, colon and duodenum.

•Subjects in the MRV+RAL cohort showed improvement of these cells in all three compartments

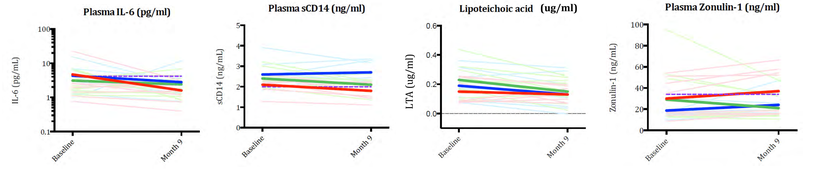
•The differences being more apparent in duodenum

Inhibition of CCR5 receptor resulted on paradoxical activation of mucosal naive CD8⁺ T cells that correlated with improvement of T-cell maturation



•With NNRTI the naïve/memory ratio still worsened during the study for both CD4⁺ and CD8⁺ T cells, but these ratios increased with MRV, and still more with MRV+RAL.
•Whereas the naive CD8⁺ T-cells with an activated phenotype decreased towards normal levels with NNRTI, these numbers increased in colon and duodenum within the MRV and MRV+RAL cohorts).
•The naive CD8⁺ activated T-cells strongly correlated with the naïve/memory CD8⁺ T-cell ratio in both tissue compartments (all Rho>0.90, P<0.001), but not in blood.

Inhibition of CCR5 receptor impacted on greater decline of sCD14 levels and quadruple ART induced greater amelioration of zonulin-1 levels



•Overall, levels of IL-6, sCD14 and LTA significantly decreased from baseline to month 9 of treatment (P=0.059, 0.038 and 0.006, respectively).

•In the grouped comparison, MVC+RAL showed the greatest decline of (P=0.039) and the greatest improvement of zonulin-1 (P=0.014).

CONCLUSIONS

•These data suggest that combined CCR5 and integrase inhibitor based combination therapy might more effectively reconstitute duodenal immunity, and decrease inflammatory markers.

•These therapeutic strategies deserve further assessment in clinical trials.

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FUNDING
This research was made possible by Grant Number UL1 RR024146 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research and by a grant from Pfizer Investigator Initiated Research Program, the UCSF/Gladstone Institute of Virology & Immunology CFAR (grant number P30 AI027763). T.S and S.SV are funded by Grants from the Spanish Ministry of Health and Innovation, and their participation on this study was also supported by Grants from the Spanish Network on AIDS Research (RIS). The results and views expressed in the poster do not represent those of Pfizer or the US Federal Government/Veterans Administration Hospital. .

ACKNOWLEDGEMENTS
The authors wish to acknowledge all the study participants who contributed to this work as well as the clinical research staff who made this research possible.