Therapeutic HIV vaccine ± TLR4 adjuvant impact on monocyte subsets in early treated youths with HIV

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BACKGROUND

Monocytes (MO) play a crucial role in restricting HIV replication and spread. After HIV acquisition, MO show increased activation and differentiation, altering subset distribution despite ART. MO enhance vaccine induced immune responses and contribute to trained immunity, boosting protection. We here investigated changes in MO phenotype and subsets composition in the phase I, proof of concept, open-label, randomized HVRRICANE (RV534) clinical trial (**Figure 1**)

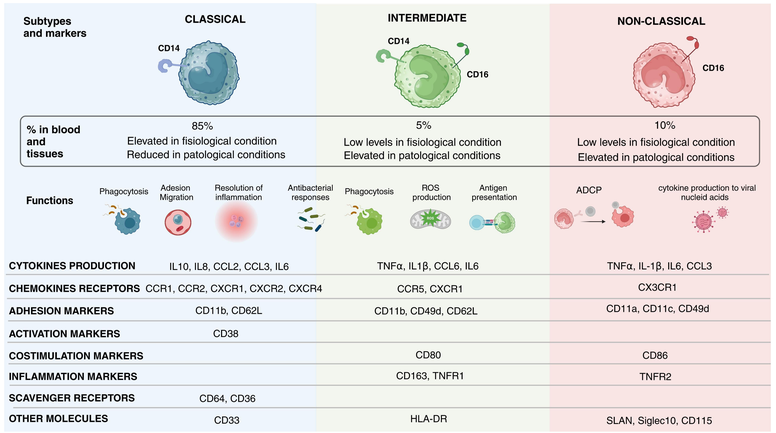
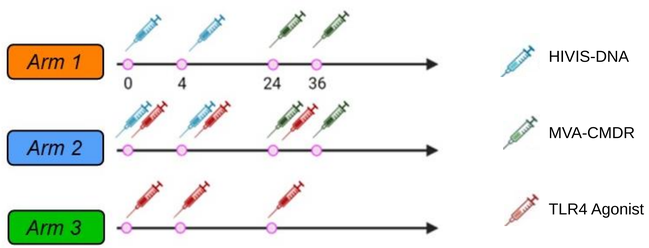


Figure 1

RV534 is the 1st pediatric combined (HIVIS DNA + MVA-CMDR) therapeutic HIV vaccine ± HPV vaccine co-administration containing the TLR4 agonist. 25 adolescents (M/F= 13/12; medina age=15 IQR:15,16) with perinatal HIV, suppressed on ART (within 6 months of age) were enrolled in South Africa and randomly assigned to three arms (**Figure 2**).

Figure 2



METHODS

Comprehensive phenotypic analysis of the MO populations was conducted in all participants across the entire cohort using a new gating strategy based on CD86, CCR2, HLA-DR, CD64, and CD33 expression markers (**Figure 3**).

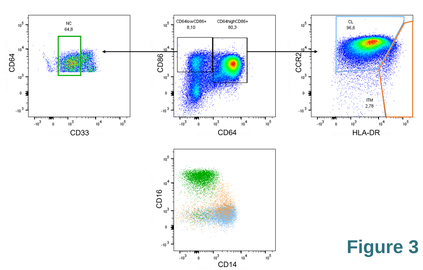


Figure 3

Data acquisition was performed using a BD Symphony A3 cell analyzer through multi-parametric approach. High dimensional analyses concatenating all participant events at baseline and weeks 16, 36, and 48 identified 8 cell clusters, analyzed via t-SNE for marker expression and temporal trends (**Figure 4**).

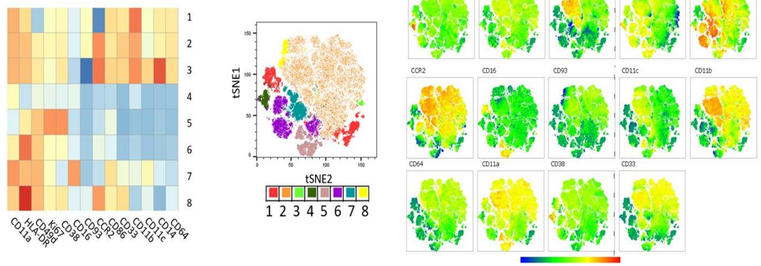


Figure 4

Therapeutic HIV vaccination triggers distinct alterations in MO subset distribution and integrin/receptor expression in early treated youths with perinatal HIV

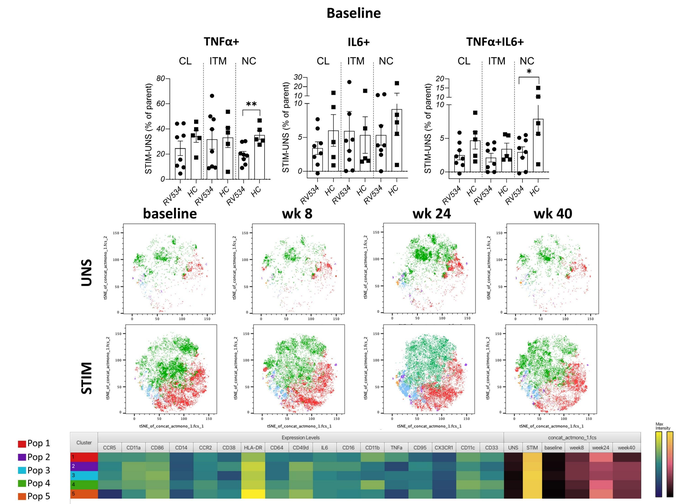
RESULTS

Classical (CL, CD86+CD64^{high}CCR2+HLADR^{low}) MO showed a significant longitudinal increase (p=0.034) only in Arm 1 (no TLR4 agonist) from a baseline median of 38.4%, peaking at wk 8 (51.6%), and remained elevated at wk 48 (44.3%).

This increase was accompanied by a waning trend in the Intermediate (ITM; CD86+CD64^{high}CCR2^{low}HLADR^{high}), from a baseline of 2.57% and reaching 1.09% at wk 48. Arm 1 also showed significant (p=0.034) changes in CD93 geometric mean fluorescence intensity (gMFI) on CL MO over time. This subset presented lower CD49d gMFI at weeks 16 (p=0.045), 40 (p=0.031) and 48 (p=0.038) in Arm 1 than to Arm 2 (with TLR4 agonist), while CD11a gMFI was higher (p=0.037) in Arm 1 at wk 40.

In contrast, Arm 3 (only TLR4 agonist) showed an increasing trend in non-classical (NC; CD86+CD64^{low}CD33^{low}) MO, confirmed by higher (p=0.043) Ki67 gMFI at wk 36 compared to Arm 1. Additionally, in the ITM subset, Arm 2 exhibited a substantial (p= 0.028) increase in CD11b gMFI, peaking at wk 48; while Arm 1 demonstrated a significant (p=0.009) decrease in CD11c gMFI in ITM MO over time.

Figure 5



We developed an affordable in vitro protocol to assess the functionality (cytokine production) of the various monocyte subsets following LPS stimulation. Preliminary data from the multi parametric longitudinal analysis of the cytokine response (8 study participants) and the baseline comparisons with an age-matched healthy control group (n = 5) are here reported.

When comparing the baseline LPS-stimulated response, the NC monocyte subset from RV534 participants showed significantly lower TNFα+ (p =0.0062) and TNFα+IL-6+ (p =0.0451) production compared to HCs (Healthy controls) (**Figure 5**).

Kruskal-Wallis test used to compare change in each endpoint at each time point between all 3 HIV vaccination arms. Mann-Whitney U test used to compare each endpoint at each time point between specific HIV vaccination arms. Each endpoint compared over all time points within each vaccination arm using Friedman's test for endpoints measured at 3 or more time points. Mann-Whitney Wilcoxon signed rank sum test and McNemar test are used for endpoints measured at 2 time points. Mann-Whitney U test used to compare the baseline comparison in the cytokines production between HC and RV534 groups.

CONCLUSIONS AND FUTURE PERSPECTIVE

Immunization with therapeutic HIV vaccines, particularly in the absence of the TLR4 agonist, reveals distinct effects on MO subset distribution and integrin/receptor expression. These findings suggest a potential positive overall impact on innate immune responses and highlight the pivotal role of CL MO in inflammation resolution. Ongoing studies are focusing on trained immunity and MO's role as immune response enhancers, and the link to viral reservoir dynamics.

ACKNOWLEDGMENTS

This study was performed by the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. MD, USA and Stellenbosch University, Cape Town, South Africa.

The study was sponsored by the Henry M. Jackson Foundation.

The study was funded by the Division of AIDS, National Institute of Allergy and Infectious Disease, NIH (Grant U01AI135941) and Fondazione PENTA for the treatment and care of children with HIV (and related diseases).The views expressed are those of the authors and should not be construed to represent the positions of the National Institutes of Health, the Department of Health and Human Services, or the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. The investigators have adhered to the policies for protection of human subjects as prescribed in AR-70-25.

We acknowledge all the collaborators and volunteers who participated in this study.

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