Lasting Immune Impacts of Age at Start of ART in Vertically HIV-Infected Adolescents

Mark Cameron[1], Stefano Rinaldi[2], Brian Richardson[1], Nicola Cotugno[3], Sion Williams[2], Suresh Pallikkuth[2], Lesley R. de Armas[2], Cheryl Cameron[1], Rajendra Pahwa[2], Paolo Palma[3], Savita Pahwa[2], for the EPIICAL Consortium*

[1] Case Western Reserve University, Cleveland, OH, USA, [2] University of Miami, Miami, FL, USA, and [3] Bambino Gesu Children's Hospital, Rome, Italy

CROI POSTER # 868

Background

Timely implementation of antiretroviral therapy (ART) in vertically HIV-infected children provides an early opportunity to restrict the establishment of infection and limit the size of reservoir. What is not fully understood is how early ART must begin, nor whether there is an effect on host immunity via delayed intervention. With the hypothesis that later ART treatment can durably impact host immune responses that associate with HIV infection, we analyzed HIV-specific CD4 T cell functionality along with RNA-Seq transcriptional assays and pathway-based bioinformatics to identify a candidate gene expression signature in young HIV-infected adults that can distinguish between their early or later start of ART after birth.

Methods

PBMCs were collected from young HIV-infected adults between 9-15 years of age enrolled at Bambino Gesù Children's Hospital. Three groups of donors (n=5-6/group) were profiled via RNA-Seq: (i) Early Treated (ET): Age at ART 0-0.5 Y, (ii) Late Treated (LT): Age at ART 1-12 Y, and (iii) HIV negative controls. Duration of ART at time of first sample (T0) in this study was approximately 3 to 15 years. All HIV-infected donors had been durably suppressed for plasma HIV. Low input libraries were generated using Roche/Kapa mRNA HyperPrep Kits and sequenced on an Illumina NextSeq 500 (75 base pair, paired-end, 40 million reads/sample). Differentially expressed genes (DEG) were determined by two-group t tests (P≤0.05) and organized into top pathways by P value (P≤0.05) via gene set variation analysis (GSVA) in R Bioconductor, with pathway annotation from mSigDB (https://software.broadinstitute.org/gsea/msigdb) or Ingenuity Pathway Analysis. Antigen-specific CD40L+ CD4 T cells were evaluated by flow cytometry for intracellular cytokines (IL2, IFNG, TNFA, IL21) following 18hrs stimulation with gp140.

Results

Start with a simple RNA-Seq contrast : Early vs Late Start of ART

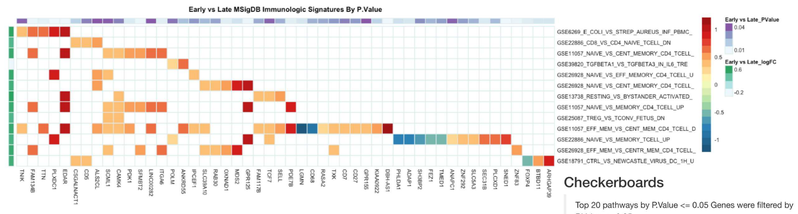
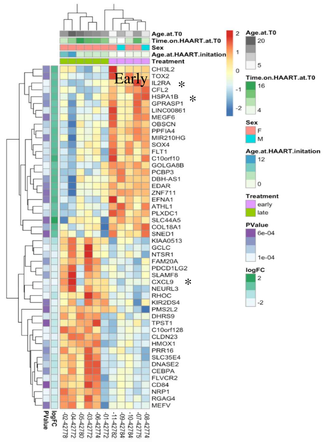


Figure 1. Pathway analysis of 1,328 differentially expressed genes by contrast of early vs late start of ART in adolescents who were vertically infected with HIV. Top enriched pathways (source: MSigDB Immunologic Signatures Database) by P value are shown vertically (P≤0.05) along with their populating genes (all P≤0.05, top row) and gene expression on the horizontal (Red = upregulated at earlier start of ART vs late, blue = downregulated). Note that the candidate T cell gene expression signature that distinguishes early vs later start of ART in PBMCs at adolescence can therefore also be found in immunophenotyping studies published at the Gene Expression Omnibus involving naïve vs effector and central memory CD4 T cell signaling.

Results

Does that tell the whole story? Next contrast : Early vs Late ART



Differentially Expressed Genes

# of DEGs with P.value <= 0.05	
Early vs Late	1328

Figure 2: A gene expression signature can be identified that distinguishes early vs later start of ART at T0. The heatmap represents the top 50 DEGs evaluated by RNAseq on vertically infected children treated with ART before 6 months (n=5, Early treated or ET) or after 1 year of age (n=6, Late treated or LT). The 50 DEG divide the 2 groups in two clusters. For this experiment, RNA was extracted from total unstimulated PBMCs.

The resulting signature appears to be potentially confounded by age at the time of the sample, which is correlated with Start and Time on ART.

Early ART : Pearson's Correlation Heatmap of Clinical and Immunophenotyping Data

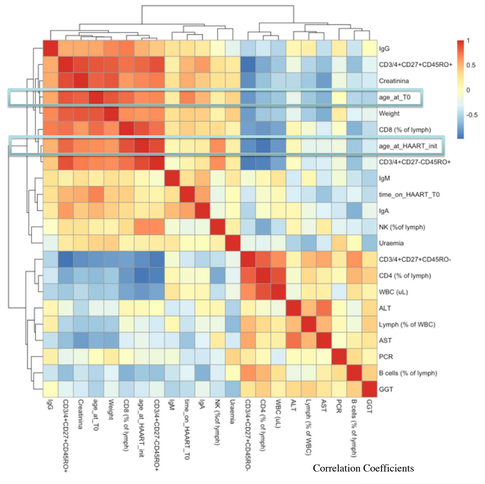
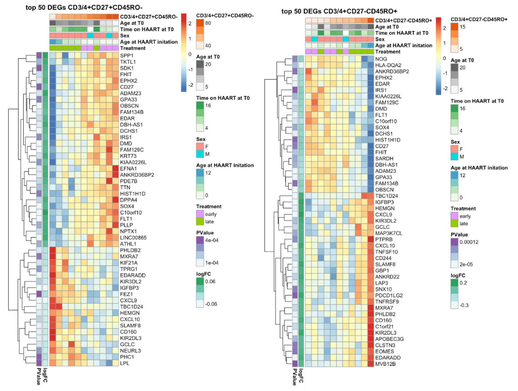


Figure 3: A Pearson's correlation heatmap of all available immunophenotyping, lab, and demographic data measurements was assembled with the purpose to show how many of the variables co-vary and where we may expect genes to overlap between the regressions. Pearson's r was calculated between each measurement including all early and late treated samples to assess their independence and subsequent impact on the analysis. The coloring represents the correlation coefficient between the two measurements, with hierarchical clustering to group measurements that correlate similarly. Much of the clinical data co-vary, therefore much of the gene expression at T0 will as well upon regression without further approaches.

Regression Analysis with Immuno-Phenotyping Data



CD4+CD27+CD45RO- (Naïve CD4 T cells)

CD4+CD27-CD45RO+ (Effector CD4 Memory)

Figure 4. With regression analysis between DEG and CD4+CD27+CD45RO+ and CD4+CD27-CD45RO- expression by immuno-phenotyping at T0, a signature emerges that correlates or anti-correlates with effector or naïve CD4+ T cells, respectively. However, age still largely correlates or anti-correlates with this signature.

How do we to separate the impact of age from the impact of time of start of ART?

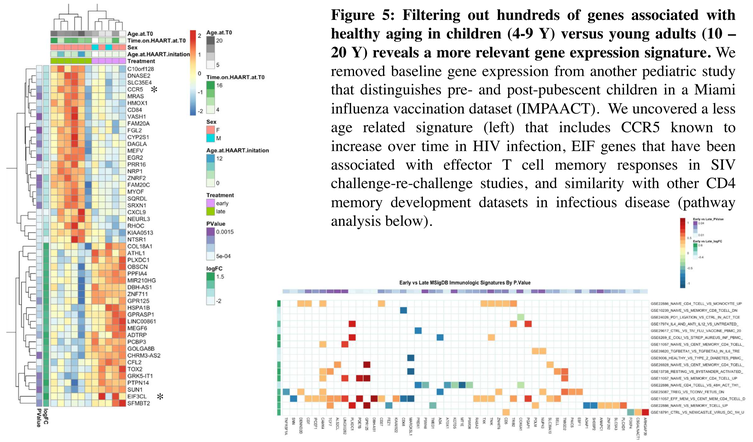
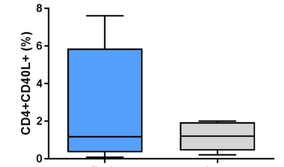


Figure 5: Filtering out hundreds of genes associated with healthy aging in children (4-9 Y) versus young adults (10 – 20 Y) reveals a more relevant gene expression signature. We removed baseline gene expression from another pediatric study that distinguishes pre- and post-pubescent children in a Miami influenza vaccination dataset (IMPAACT). We uncovered a less age related signature (left) that includes CCR5 known to increase over time in HIV infection, EIF genes that have been associated with effector T cell memory responses in SIV challenge-re-challenge studies, and similarity with other CD4 memory development datasets in infectious disease (pathway analysis below).

Assessing HIV-Specific CD4 T Cell Functionality

Frequencies of gp140 specific CD4 T cells did not differ significantly between Early and Late treated children



Early treated children show increase frequencies of gp140 specific polyfunctional CD4 T cells dominated by IL2 production

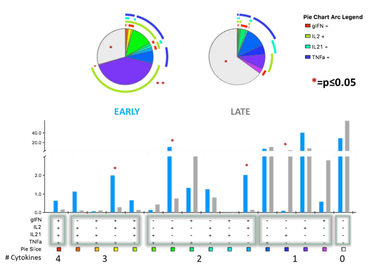


Figure 6: We analyzed HIV-specific T cell functionality by stimulating CD4 T cells from the HIV-positive individuals in this study for 18hrs with gp140. Intracellular cytokine were evaluated by flow cytometry (ICS).

CONCLUSIONS

- Our results suggest that delayed ART has a lasting impact on host T cell memory distinguishable by a candidate DEG signature and HIV-specific immune responses.
- Regression analysis revealed that different patterns of T cell signaling pathway DEG correlated with age, age of ART initiation, and % of naïve and effector memory T cells, e.g. chemokines/chemokine receptors, stress and effector molecules, and TNF superfamily signaling.
- Through pediatric age-related gene expression filtering and pathway analysis, a candidate signature of impact of start of ART was ascribed to differences mainly seen in naïve vs central and effector CD4 memory T cell datasets.
- The frequency of gp140sp CD4+ T cells was the same between ET and LT, but ET showed increased frequencies of gp140-specific polyfunctionality.
- Expanded studies that control for age across treatment groups at time of sampling are warranted to assess these profiles of host immunity in vertically HIV-infected children treated variously with ART after birth and whether they can be targeted in functional cure approaches.

ACKNOWLEDGEMENTS

We are indebted to the study participants and the support of Dr. Paolo Rossi and EPIICAL, the Cleveland and Miami CFARs, Penta Foundation and ViiV Healthcare.

* The EPIICAL Consortium: Paolo Rossi, Ilaria Pepponi, Francesca Rocchi – Children's Hospital "Bambino Gesu", Rome, Italy; Nigel Klein, Diana Gibb, Sarah Watters, Man Chan, Laura McCoy, Abdel Babiker - University College London (UCL) UK; Anne-Geneviève Marcelin, Vincent Calvez - Université Pierre et Marie Curie (UPMC) France; Maria Angeles Munoz - Servicio Madrileño de Salud - Hospital General Universitario Gregorio Marañon (SERMAS-HGUGM) Spain; Britta Wahren - Karolinska Institutet (KI) Sweden; Caroline Foster - Imperial College Healthcare NHS Trust (ICHT), London, UK; Mark Cotton- Stellenbosch University -Faculty of Medicine and Health Sciences(SU) South Africa; Merlin Robb, Jintanat Ananworanich - The Henry M. Jackson Foundation for the Advancement of Military Medicine, Maryland, USA; Polly Claiden - HIV i-Base (HIViBase) UK; Deenan Pillay - University of KwaZulu-Natal Africa Center (UKZN/AC) South Africa; Deborah Persaud - Johns Hopkins University (JHU); Rob J De Boer, Juliane Schröter, Anet J.N. Anelone - University of Utrecht (UU) Netherlands; Thanyawee Puthanakit - Thai Red Cross AIDS-Research Centre (TRCARC) Thailand; Adriana Ceci, Viviana Giannuzzi - Consorzio per Valutazioni Biologiche e Farmacologiche (CVBF) Italy; Kathrine Luzuriaga - University of Massachusetts Medical School (UMMS), Worcester, Massachusetts, USA; Nicolas Chomont - Centre de Recherche du Centre Hospitalier de l'Université de Montreal – University of Montreal (CRCHUM), Canada; Mark Cameron - Case Western Reserve University (CWRU), Cleveland, Ohio, USA; Caterina Cancrini - Università degli Studi di Roma Tor Vergata (UNITOV), Italy; Andrew Yates, Louise Kuhn - Columbia University (COLUMBIA), New York, USA; Avy Violari, Kennedy Otwombe - University of the Witwatersrand, Johannesburg (PHRU), South Africa; Stefano Rinaldi – University of Miami, Miller School of Medicine (UM), Florida, USA; Alfredo Tagarro - Hospital 12 de Octubre, Universidad Complutense, Madrid, Spain; Maria Grazia Lain, Paula Vaz – Fundação Ariel Glaser contra o SIDA Pediátrico (ARIEL), Mozambique; Elisa Lopez, Tacita Nhampossa – Fundação Manhiça, Mozambique. Special thanks to the Case Western Reserve University Genomics Core as well as Michael Cartwright, Pearline Cartwright, and Jackelyn Golden of the Applied Functional Genomics Core, Case Western Reserve University, Cleveland OH, USA.