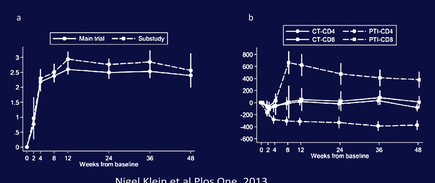
Understanding the impact of ART interruption on thymic output and TCR repertoire

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1. Introduction

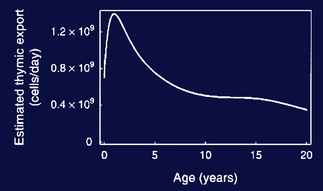
Figure 1



Nigel Klein et al Plos One. 2013

Figure 1. Antiretroviral interruptions (ART) in children with HIV cause a rapid increase in viral load (a) and a rapid reduction in CD4+ T cells (b) but with less clinical impact compared to adults and with good CD4+ T cell restoration following ART re-introduction.

Figure 2



Bains I et al J Immunol. 2009

Figure 2. T cells generated in the thymus establish the peripheral naive T cell pool during the first years of life. The thymic output then gradually declines from its peak at 1-2 years of age resulting in a reduction of T cell receptor (TCR) diversity with age.

Figure 3

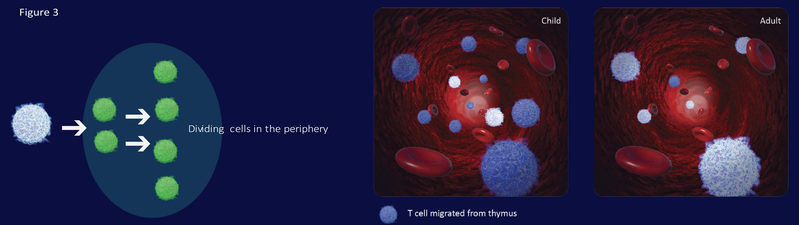


Figure 3. In contrast to adults, who predominantly reconstitute their T cells from peripheral cell expansion, children have a great capacity for immune reconstitution mainly from the thymus.

2. Aims and Hypothesis

- In this study, we have investigated the impact of ART interruption followed by ART re-introduction in children with HIV on 5 important critical immune parameters: thymic output, peripheral T cell expansion, TCR repertoire diversity, TCR clonotype sharing and TCR clonotype profiles in naïve CD4+ and memory CD8+ T cell populations.



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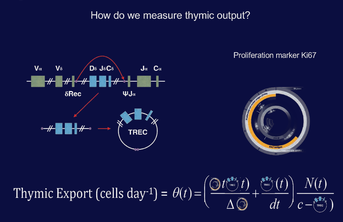
3. Methods

Table 1. Patient characteristics

	Patients with PTI	Patients with CT
No. of participants	4	4
Age in years, median (IQR)	9.5 (8.97-10)	8 (7.5-9.8)
CD4+ count/ ul at baseline, median (IQR)	965 (870-1085)	1365 (1197.5-1471)
Viral load/ml at baseline, median (IQR)	50 (50-50)	50 (50-52.5)
Sampling time points (weeks)	0,12,48,150	0,48,150

PTI: Planned treatment interruption. CT: Continous treatment. IQR: interquartile range.
Week 0: Baseline when ART was stopped. Week 12: during PTI. Week 48: ART was re-introduced. Week 150: three years after end of study

Figure 4

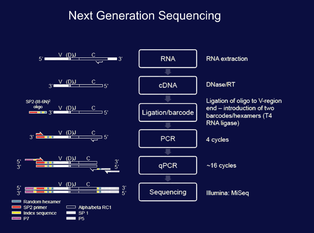


Bains et al Blood 2009 and Sandgaard et al 2013 AIDS

Thymic Output:

The method for estimating thymic output using a mathematical model explained in brief: c is a constant representing the average naive TREC content of thymic emigrants entering the peripheral naive T cell population, y(t) is the Flow Cytometry estimated Ki67 fraction of naive CD4+ T cells, Δ is the duration of Ki67 expression, τ is the qPCR estimated TREC concentration in the sorted naive CD4+ T-cell population and N(t) is the total size of the naive CD4+ T-cell population. Calculations to estimate total body CD4+,CD45RA+ T-cell numbers N(t) were performed as previously described referring to the linear relationship between blood volume and body weight.

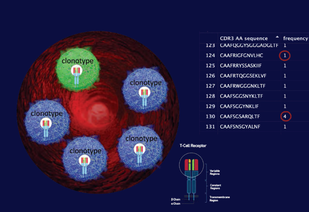
Figure 5



Next Generation Sequencing

The major site of biological interest was the complementarity determining region 3 (CDR3) of the T cell receptor encoded at the hypervariable recombination junction forming the primary contacts with the MHC-bound epitope. Library preparation was based on a protocol for quantitative TCR sequencing. Full details of the method have been recently published (Theres Oakes et al Front Immunol. 2017). Library Sequencing up to 12 final amplicon products were pooled together and loaded, at 12pM concentration, on an Illumina MiSeq, using aversion 2 chemistry 500V2 kit.

Figure 6



Data Analysis

The FASTQ files produced on the MiSeq were demultiplexed incorporating a Decombinator analysis pipeline identifying the TCR sequence reads based on the V and J gene usage, and nucleotide insertions and deletions. Decombinator classification (DCR) is defined by the identified V gene region, V region deletions, J gene region, J region deletions, and nucleotide insertions for each read demultiplexed. Following classification, the CDR3 region amino acid sequence can be translated. The DCR output is then corrected for PCR and sequencing errors initially removing any sequences with low quality barcode or sequence regions. Sequences are then organised by their barcode. For analyses, the ineq package (version 0.2.13) was used in the R environment (version 3.3.2) for computing the Gini coefficient. All manual data analyses were executed using custom scripts written in R. The Decombinator package v3.1 was executed using Python (version 2.7.3).

4. Preliminary Results

Figure 7

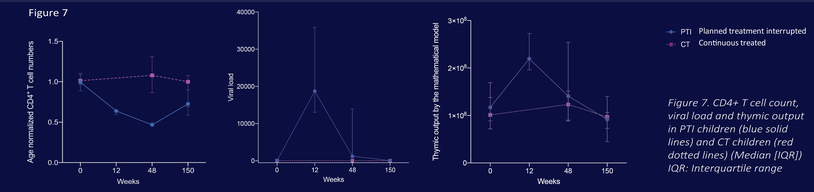
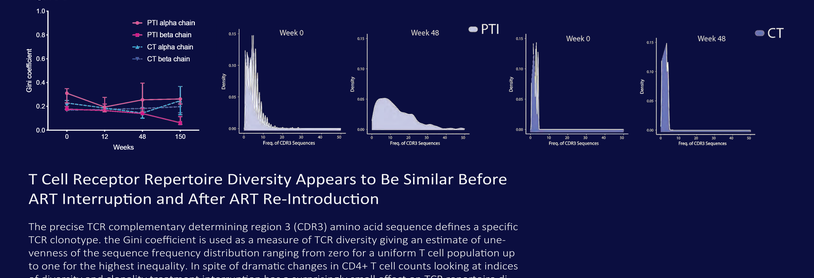


Figure 7. CD4+ T cell count, viral load and thymic output in PTI children (blue solid lines) and CT children (red dotted lines) (Median [IQR]) IQR: Interquartile range

Thymic Output Increases Rapidly During ART Interruption and Returns to Pre-Interruption Levels After ART Re-Introduction

As expected, CD4+ T cell numbers dropped during PTI and viral load went up in the PTI group. The thymic output levels in the CT patients were similar to previously published thymic output cell numbers and was well maintained throughout the study (Sandgaard et al AIDS 2014). In contrast, the PTI patients had an increase in thymic output peaking at week 12 but returning to baseline levels at 48 weeks.

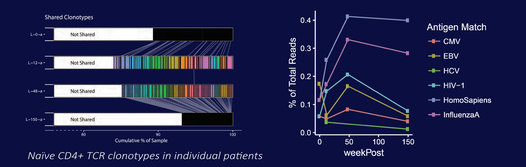
Figure 8



T Cell Receptor Repertoire Diversity Appears to Be Similar Before ART Interruption and After ART Re-Introduction

The precise TCR complementary determining region 3 (CDR3) amino acid sequence defines a specific TCR clonotype. the Gini coefficient is used as a measure of TCR diversity giving an estimate of unevenness of the sequence frequency distribution ranging from zero for a uniform T cell population up to one for the highest inequality. In spite of dramatic changes in CD4+ T cell counts looking at indices of diversity and clonality treatment interruption has a surprisingly small effect on TCR repertoire diversity. Looking in detail at the CDR3 frequency, the CT patients at week 0 and 48 looked similar. In contrast PTI patients at week 48, showed substantial clonotype expansions.

Figure 9



Naïve CD4+ TCR clonotypes in individual patients

Figure 9. Shared TCR clonotypes in an individual PTI patients between weeks 0, 12, 48 and 150 originated from the naive CD4+ T cell population. Each grey line represents a sharing of a TCR clonotype between the two corresponding weeks. Observation of color defines a specific TCR clonotype having expanded numerous times as compared to the black lines representing a unique TCR clonotype with a frequency of 1. All amino acid sequences have been color-coded alphabetically hence the same color represents the same TCR clonotype. The x-axis shows the cumulative percentage of the shared TCR clonotypes.

The Naïve T Cell Receptor Clonotypes Are Being Highly Shared In Children With HIV

The TCR clonotypes were observed to be highly shared between time points within the naïve CD4+ T cell populations in individual patients. Some specific naive CD4+ TCR clonotypes expanded during PTI compared to week 0 and week 150. The specific CDR3 sequences and their specificity against antigen epitopes is ongoing work and a preliminary figure is shown where the TCR clonotypes of one patient has been compared to an open acces database that informs on public evidence of TCR clonotypes (VDJdb).

5. Conclusions

- The shift back to normality after ART re-introduction in these critical immune parameters indicates that periods of treatment interruption in children with HIV may not have a lasting detrimental impact on their immune system.
- This may be because of a compensatory increase in thymic output during treatment interruption.
- This approach will allow us to understand in great detail how the immune system responds to HIV and ART. It will also enable the impact of novel treatment modalities.

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