



# CD4 T cell depletion, and not age, may be a driver of abnormal CD4 cell compartments in HIV-infected children initiating ART in Uganda



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## ABSTRACT

**Background:** The naïve CD4 T cell pool is maintained by thymic production of new cells, proliferation within the naïve pool and cell loss through death or differentiation to memory cells. The homeostatic mechanisms operating to maintain naïve and memory pools are not fully understood in healthy children; even less is known in HIV infection, particularly in resource-limited settings when ART is often initiated with advanced immunodeficiency.

**Methods:** 1210 ART-naïve children meeting WHO criteria for ART in Uganda/Zimbabwe were enrolled into the ARROW trial and started combination ART. 199 children in Uganda (54% girls, aged 5 months-18 years) underwent CD4 immunophenotyping at ART initiation using a combination of CD4, CD45RA and CD31.

**Results** As expected, CD4 and CD4-for-age z-score varied significantly with age at ART initiation (see table), as did the percentage of CD4 cells in the 'Recent Thymic Emigrant' (RTE, CD45RA+CD31+), Central Naïve (CN, CD45RA+CD31-) and memory (M, CD45RA-CD31-) compartments (p<0.001, 0.01, <0.001 respectively). However, multivariable modelling showed this relationship with age was predominantly the result of the lower CD4-for-age in older children (after adjusting for CD4-for-age, p(age)=0.13, 0.40, 0.70 respectively). Every 1 unit lower CD4-for-age was associated with 4.4% smaller RTE, 2.1% greater CN and 3.6% greater M subpopulations at ART initiation (p<0.001). There was no impact of sex on the CD4 subpopulations (p>0.5), but there was a trend towards children with lower weight-for-age having greater CN subpopulations (0.7% greater for every 1 unit lower weight-for-age, adjusted p=0.12).

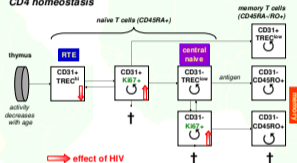
Age at ART initiation (yrs)	Children	Median CD4 (CD4-for-age)	Median %RTE	Median %CN	Median %M
0.5-2	75	778 (-2.3)	41%	11%	30%
3-6	49	458 (-3.2)	35%	11%	42%
7-12	50	256 (-5.3)	35%	14%	39%
13-18	25	215 (-7.3)	24%	19%	42%

**Conclusions:** In all agegroups, the cell proportions in these 3 CD4 compartments were lower than have been reported in healthy Caucasian children. CD4 count seems to be an important driver or consequence of lower RTEs and higher central naïve/memory populations, with a far stronger association than age alone. In children surviving without ART, there may be a shift to maintaining the CD4 cell pool through the relative expansion of naïve and memory pools at some child-specific point, possibly representing declining capacity of the thymus to keep pace with CD4 loss. The long-term consequences of this for ART response are unclear.

## BACKGROUND

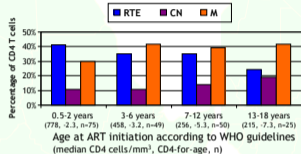
The naïve CD4 T cell pool is maintained by  
- thymic production of new cells  
- proliferation within the naïve pool  
- cell loss through death or differentiation to memory cells<sup>1</sup>.

### CD4 homeostasis



The homeostatic mechanisms operating to maintain naïve and memory pools are not fully understood in healthy children  
Even less is known in HIV infection, particularly in resource-limited settings when ART is often initiated with advanced immunodeficiency.

## RESULTS - CD4 cell subpopulations vary with age at ART initiation



CD4, CD4-for-age z-score and the percentage of CD4 cells in the 'Recent Thymic Emigrant' (RTE, CD45RA+CD31+) population decreased significantly with age at ART initiation (all p<0.001)  
The percentage of CD4 cells in the Central Naïve (CN, CD45RA+CD31-) and Memory (M, CD45RA-CD31-) populations increased significantly with age at ART initiation (p<0.01, <0.001 respectively).

However, multivariable modelling showed this relationship with age was predominantly the result of the lower CD4-for-age in older children

## METHODS

1210 ART-naïve children meeting WHO criteria for ART in Uganda/Zimbabwe were enrolled into the ARROW trial ([www.arrowtrial.org](http://www.arrowtrial.org)) and started combination ART  
199 children in Uganda (54% girls, aged 5 months-18 years) underwent CD4 immunophenotyping at ART initiation using a combination of CD4, CD45RA and CD31  
We investigated three CD4 cell subpopulations  
- CD45RA+CD31+ Recent Thymic Emigrants (RTE)  
- CD45RA-CD31- Central Naïve (CN)  
- CD45RA-CD31- Memory (M)

EDTA anti-coagulated peripheral blood from each child was incubated with anti-CD4-PerCP mAb, and with combinations of the following antibodies: anti-CD45RA-APC mAb, anti-CD45RA-FITC mAb, anti-CD45RO-FITC mAb, anti-CD31-PE mAb, anti-HLA-DR-PE mAb and Ki67-FITC.  
After incubating with the antibody cocktail, RBCs were lysed using FACSlyse and washed off with a solution of PBS containing 0.5% BSA and 0.1% sodium azide. The remaining leukocytes were fixed with 4% PFA and acquired on a flow cytometer.  
For intracellular staining the fixed leukocytes were permeabilised with saponin and then incubated with the Ki67 antibody.

<sup>1</sup> Murray JM, Kaufman GR, Hodgkin PD et al. Naïve T cells are maintained by thymic output in early ages but by proliferation without phenotypic change after age twenty. *Immunology and Cell Biology* 2003; 81:487-95.  
<sup>2</sup> Haucke S, Behl M, Fadler C et al. Age-matched lymphocyte subpopulation reference values in childhood and adolescence: application of exponential regression analysis. *Eur J Haematol* 2006; 80:532-9.

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## RESULTS - pre-ART CD4 depletion is a better predictor of CD4 cell subpopulations than age

At ART initiation	Effect (95%CI) of pre-ART factors on*		
	%RTE	%CN	%M
1 unit lower CD4-for-age	-4.4% (-5.4%, -3.5%) p<0.001	+2.1% (+1.5%, +2.7%) p<0.001	+3.6% (+2.6%, 4.6%) p<0.001
1 year older age	-0.4% (-0.2%, +1.0%) p=0.16*	+0.1% (0.4%, +0.3%) p=0.78*	-0.2% (-0.8%, +0.4%) p=0.55*
1 unit lower weight-for-age**	-0.0% (-1.5%, +1.4%) p=0.96	+0.7% (-0.2%, +1.6%) p=0.12	-0.5% (-2.0%, +0.9%) p=0.47
Girl (vs boy)	+0.6% (-3.6%, +4.9%) p=0.76	-0.7% (-1.9%, +3.4%) p=0.57	+0.0% (-4.3%, +4.3%) p=1.00

\* multivariable model including all factors shows plus centre. P values for age including only CD4-for-age and age p=0.13, p=0.40, p=0.70 respectively (as abstract)  
\*\* similar impact including height-for-age rather than weight-for-age in models

## SUMMARY

In all agegroups, the cell proportions in these 3 CD4 compartments were lower than have been reported in healthy Caucasian children<sup>2</sup>.

- this was largely due to a reduction in the RTEs and increase in CD31+ memory cells (data not shown)

Total CD4 count seems to be an important driver or consequence of lower RTEs and higher central naïve/memory populations, with a far stronger association than age alone.

In children surviving without ART, there may be a shift to maintaining the CD4 cell pool through the relative expansion of central naïve and memory pools at the expense of RTE at some child-specific timepoint. This may indicate that with time, the capacity of the thymus to keep pace with CD4 loss is diminished.

- the long-term consequences for subsequent ART response are unclear but will be investigated in this ongoing study