CD4 T cell depletion, and not age, drives reconstitution within 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 new thymic emigrants, proliferation within the naïve pool and cell loss through death or differentiation to memory cells. Drivers influencing these homeostatic mechanisms in HIV-infected children are not fully understood, particularly in resource-limited settings.

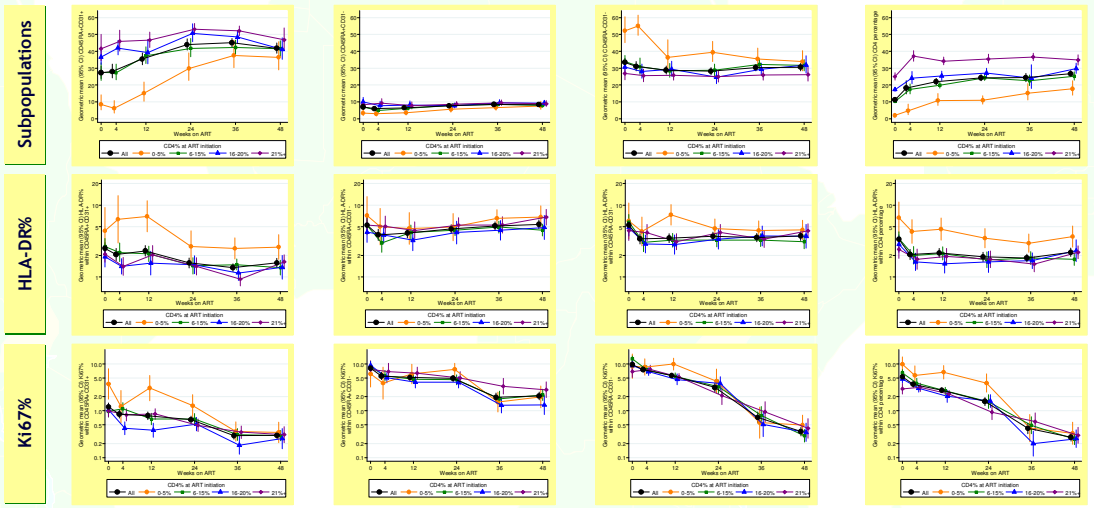
Methods: 199 ART-naive Ugandan children (54% girls, 5m-18yrs) in the ARROW trial underwent CD4 immunophenotyping at ART initiation, and 4,12,24,36 and 48 weeks later. CD4 subsets were identified by expression of CD45RA, CD31, HLA-DR and Ki67 by flow cytometry. We estimated trends over time using linear mixed models for log cell %.

Results Over 48 weeks on ART there was a marked shift from memory to naive phenotypes (Table), most substantial in those initiating ART with lowest CD4% (p<0.001). Adjusting for pre-ART CD4%, there was no independent effect of age on changes in Recent Thymic Emigrants (RTE, CD45RA+CD31+) (p=0.27), Central Naive (CD45RA+CD31-) (p=0.45) or Memory (M, CD45RA-CD31-) (p=0.72) compartments. At ART initiation, HLA-DR and Ki67 expression were lowest in RTEs, intermediate in CN, and highest in M compartments (all pairwise p<0.02). Over 48 weeks on ART, %Ki67+CD4+ T cells declined profoundly in all compartments and across all pre-ART CD4%. In contrast, declines in HLA-DR% were smaller and had mostly occurred by week 4, thereafter remaining similar.

Geometric Mean cell % (%HLA-DR+)[%Ki67+]

Pre-ART CD4%	RTE (% of CD4)		Memory (% of CD4)		CD4 (% of lymphocytes)	
	W0	W48	W0	W48	W0	W48
All (n=199)	27.2% (2.3)[1.0]	41.8% (1.6)[0.2]	33.4% (5.2)[9.5]	30.6% (3.7)[0.3]	11.4% (3.3)[5.3]	26.7% (2.2)[0.3]
0-5% (n=29)	8.4% (3.1)[2.7]	36.5% (2.6)[0.3]	52.3% (5.5)[7.6]	33.9% (4.5)[0.5]	2.2% (6.4)[9.9]	17.8% (3.6)[0.3]
6-15% (n=80)	27.4% (2.5)[0.8]	41.5% (1.3)[0.2]	34.0% (5.7)[12.9]	31.6% (3.0)[0.2]	10.3% (3.4)[6.4]	25.2% (1.8)[0.2]
16-20% (n=40)	36.6% (1.9)[1.0]	41.2% (1.4)[0.2]	30.7% (4.9)[9.3]	31.9% (3.7)[0.3]	17.3% (2.9)[4.8]	29.8% (2.3)[0.2]
21%+ (n=50)	41.6% (2.1)[1.0]	46.8% (1.6)[0.3]	26.9% (4.4)[6.8]	26.2% (4.4)[0.4]	25.0% (2.4)[3.0]	34.8% (2.2)[0.3]

Conclusions: HIV-infected children with most profound immunosuppression had highest pre-ART proliferation and activation and greatest shift from memory to naïve phenotypes after ART. ART had a sustained and dramatic effect on CD4 proliferation but only a modest impact on activation, occurring shortly after ART initiation, suggesting that non-viral drivers sustain ongoing immune activation despite ART.

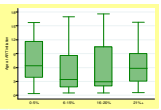


METHODS

- 1207 ART-naive children meeting WHO criteria for ART in Uganda/Zimbabwe were eligible and enrolled into the ARROW trial (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
- EDTA anti-coagulated peripheral blood from each child was incubated with the two following antibody cocktails:
  - anti-**CD4**-PerCP, anti-**CD45RA**-APC, anti-**CD31**-PE and anti-HLA-DR-FITC (marker of activation)
  - anti-**CD4**-PerCP, anti-**CD45RA**-APC, anti-**CD31**-PE and anti-Ki67-FITC (marker of proliferation)
- CD4 cell subpopulations were identified as:
  - CD45RA+CD31+ Recent Thymic Emigrants (RTE)
  - CD45RA+CD31- Central Naive (CN)
  - CD45RA-CD31- Memory (M)
- RBCs were lysed using FACSLyse and washed with a solution of PBS containing 0.2% BSA and 0.02% sodium azide. Remaining cells were fixed with 4% PFA and acquired on a FACS Calibur flow cytometer with Cellquest software
- For intracellular staining fixed cells were permeabilised with saponin and incubated with the Ki67 antibody, a marker of proliferation

RESULTS - pre-ART CD4 depletion is a better predictor than age

- The shift from memory to naive phenotypes was most substantial in those initiating ART with lowest CD4% (p<0.001)
- Although the median age varied slightly across pre-ART CD4% groups, there was a good spread of ages in each group
- Adjusting for pre-ART CD4%, there was **no independent effect of age** on changes in
  - Recent Thymic Emigrants (RTE, CD45RA+CD31+) (p=0.27)
  - Central Naive (CD45RA+CD31-) (p=0.45), or
  - Memory (M, CD45RA-CD31-) (p=0.72) compartments



CD4-depletion, and not age per se, drives CD4 reconstitution

- At ART initiation, HLA-DR and Ki67 expression were lowest in RTEs, intermediate in CN, and highest in M compartments (all pairwise p<0.02)
- Over 48 weeks on ART, %Ki67+CD4+ T cells declined strongly in all compartments and across all pre-ART CD4% (p<0.01)
- Declines in HLA-DR% were smaller and had mostly occurred by week 4, thereafter remaining similar, suggesting ongoing drivers of activation

SUMMARY

HIV-infected children with most profound immunosuppression had highest pre-ART proliferation and activation and greatest shift from memory to naïve phenotypes after ART

- Although young children have an active thymus, we did not find any evidence to support an independent contribution of age, over and above CD4 cell depletion, on CD4 reconstitution
- This may be because profound CD4 depletion is a marker for failure of the thymus to keep pace with CD4 loss through proliferation and expansion, regardless of age
- Older and younger children can reconstitute CD4 cells well, providing they have not experienced profound immunocompromise

ART had a sustained and dramatic effect on CD4 proliferation but only a modest impact on activation, occurring shortly after ART initiation, suggesting that non-viral drivers sustain ongoing immune activation despite ART. These will be investigated in future studies.

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