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Low Inducibility of the HIV Reservoir in Early ART-Treated Thai Children

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BACKGROUND

► Acute HIV-infection in newborns is characterized by a sustained high-level plasma viral load and a high risk of rapid progression to AIDS and death compared to adults[1-3]

► International guidelines recommend early initiation of antiretroviral therapy (ART) in all HIV-infected infants aged less than 1 year regardless of clinical and immunological conditions, due to the beneficial effect by limiting HIV-related symptoms[4].

► Early ART limits the size of the HIV reservoir in adults, however pediatric data are limited.

OBJECTIVE

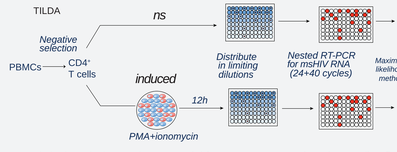
To characterize the impact of early ART initiation on the size of the HIV reservoir in vertically infected infants

METHODS

► Study cohorts: Vertically HIV-infected Thai children (N=72) who initiated ART within the first 6 months of life were enrolled. Samples prior to ART were obtained from 14 infants who received prophylactic antiretrovirals (ARV) for a median of 5 IQR [4-6] weeks (HIVNAT-209 visit 1). In addition, 58 samples were obtained from fully ART-suppressed children with median ART duration of 4 IQR [1.0-5.5] years (HIVNAT-209 visit 3 and HIVNAT-194).

► Total and integrated HIV DNA: Pellets of total PBMCs and enriched CD4⁺ T cells were digested for 12h at 55ºC using a PK lysis buffer to measure Total (5-LTR-gag primers/probe) and Integrated (5-LTR and Alu primers/probe) HIV DNA by real-time PCR. Primers specific for the human CD3 gene were used to quantify the exact number of cells. ACH-2 cells, which carry a single copy of the integrated HIV genome, were used to generate a standard curve for both assays[5].

► Inducible HIV reservoir was measured by *Tat/rev* Induced Limiting Dilution Assay (TILDA)[6]. We enriched CD4⁺ T cells from PBMC samples and quantified the frequency of cells that spontaneously produce HIV multiply-spliced RNA (no stimulation, ns) or after stimulation with PMA/iomacyn (induced,ind) for 12h in presence of antiretroviral drugs.



► Statistical analysis: continuous variables were expressed as the median [interquartile ranges] and compared using non-parametric tests (Mann-Whitney). Discrete variables were described as percentages and compared using Fisher extact test.

RESULTS

Table 1. Cohort characteristics

	Before ART HIVNAT 209 Visit 1	ART-Treated HIVNAT209 V3 HIVNAT194
	n=14	n=58
Age, months/years	2.1 [1.8-2.9]	3.9 [1.6-6]
Gender, female, n (%)	7 (50)	31 (53)
CD4 counts, cells/µl	2513 [1705-3212]	1383 [917-2125]
Detectable VL, log copies/mL	5.3 [4.4-6.0]	<50
VL <50 copies/mL, n (%)	0 (0)	58 (100)
VL <1000 copies/mL, n (%)	1 (7)	58 (100)
HIV transmission, n (%)		
In utero	7 (50)	2 (3.5)
Peripartum	3 (21)	6 (10.3)
Unknown	4 (29)	50 (86)
Prophylactic regimen		
No treatment	2 (14)	2 (3)
AZT	4 (29)	1 (2)
AZT+3TC+NVP	8 (57)	15 (26)
AZT+NVPsd	0 (0)	1 (2)
Unknown	0 (0)	39 (67)
Duration prophylactic ARV, days	34 [29-42]	44 [31-46]
Time between Prophylactiv ARV and ART initiation, weeks	NA	10 [2-14]
Age at ART initiation, months	NA	3.9 [2.8-4.7]
Time on ART, years	NA	3.7 [1.2-6]
ART regimen, n(%)		
PI-based	NA	34 (59)
NNRTI-based	NA	24 (41)

Effect of prophylactic ARV on HIV reservoir

Figure 1: Heterogeinity of newborns at visit 1.

*Prophylactic triple ARV: AZT+3TC+NVP with 1/2 standard dose of NVP



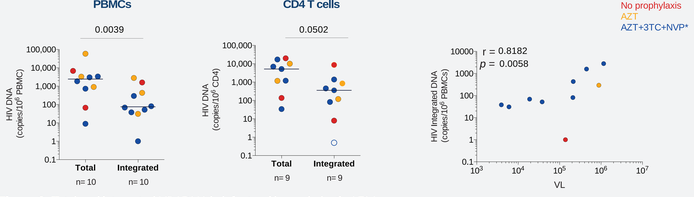


Figure 2: Total and integrated HIV DNA in infants with prophylactic ARV

► Total/integrated DNA show a 1 log difference, typical of untreated HIV infection. Prophylactic ARV seems to have a limited impact on the size of the HIV reservoir.

► Viral load strongly correlates with integrated HIV DNA, suggesting that the magnitude of HIV replication impacts the reservoir.



Figure 3: Inducible reservoir in infants with prophylactic ARV

► CD4⁺ T cells producing tat/rev msRNA were detectable in all samples.

► The frequency of CD4⁺ T cells that express ms-HIV RNA show a 1 log difference with integrated.

Prophylactic ARV in infants does not prevent the establishment of the HIV reservoir

Low inducibility of the HIV reservoir in ART treated children

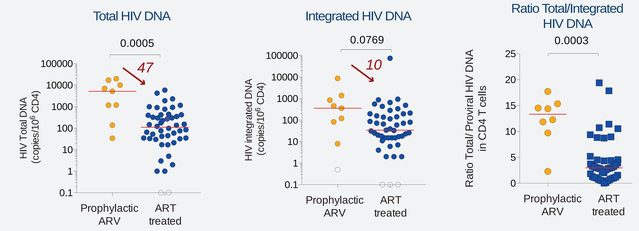


Figure 4: Total and integrated DNA before and after suppressive ART

► After ART initiation, frequencies of total and integrated HIV DNA in CD4⁺ T cells decrease, but remained detectable in almost all children tested

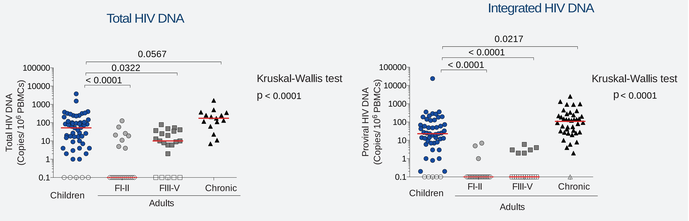
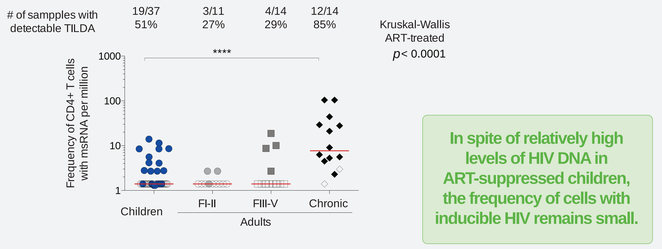


Figure 5: Comparison of total and integrated DNA in PBMCs after suppressive ART in pediatric and adult populations

► Despite prophylaxis, virologically suppressed children show higher levels of HIV DNA than early treated adults (Fiebig I-V stages).



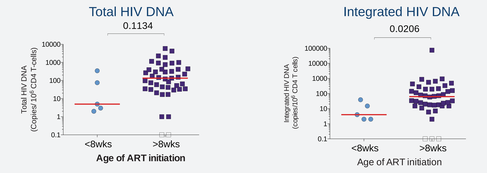
In spite of relatively high levels of HIV DNA in ART-suppressed children, the frequency of cells with inducible HIV remains small.

Figure 6: Inducible reservoir after suppressive ART in pediatric and adult populations

► The frequency of cells with inducible HIV is lower than in chronically infected adults, suggesting that the reservoir in children may be less inducible than in adults.

RESULTS

Effect of early ART on HIV reservoir



Children who initiated ART within 8 weeks of life showed reduced levels of integrated HIV DNA compared to those who started later

►Similar results were observed (significantly lower levels of total and integrated HIV DNA) when comparing children who had uninterrupted ARV (prophylactic ARV+ART) vs children with delayed ART initiation.

Figure 7: HIV reservoir in early ART-treated (<8 weeks) children

CONCLUSIONS

► Prophylactic ARV does not prevent the establishment of a large HIV DNA reservoir in infants (**HIVNAT209 V1**).

► The frequency of cells with inducible HIV remained low in virally suppressed children, despite high levels of HIV DNA, suggesting that the proviral reservoir is poorly inducible (**HIVNAT209 V3 and 194**).

► Early ART (<8 weeks) dramatically restricts the pool of cells harboring integrated HIV DNA (**HIVNAT209 V3 and 194**).

References

[1]Prendergast A *et al*, Lancet 2007; 370:68–80
[2]Mofenson LM *et al*, J Infect Dis 1997; 175:1029–38.
[3]McIntosh K *et al*. Pediatr Infect Dis J 1996; 15:1087–91.
[4]Faye A et al, Clin Infect Dis 2004; 39:1692–8.
[5]Vandergeeten C et al, J Virol 2014;88:12385–12396
[6]Procopio F et al, eBioMedicine 2015, 2 (8):872–881

Acknowledgments

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