#0805 LONG-TERM PERSISTENCE OF HIV-INFECTED CELL CLONES IN CHILDREN TREATED EARLY

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Background

Integration site analysis has shown extensive clonal expansion of HIV-1 infected cells in adults. We know that infected clones arise early, persist for many years, and that there is selection for cells with proviruses integrated in BACH2, MKL2, and STAT5B. However, little is known about the behavior of infected cells in children treated shortly after birth. We characterized clones of infected cells in a cohort of perinatally-infected and early-treated children.

Methods

PBMC samples were obtained from the Children with Early HIV Antiretroviral Therapy (CHER) cohort prior to ART and after six to nine years of continual suppression on ART. We determined the integration sites in samples taken at both time points and compared the data to integration site data from PBMC infected ex-vivo and adults who initiated ART in either early or chronic infection.

Conclusions

Although the numbers of T cells and their population dynamics are different in children and adults, the timing of the establishment of HIV-1 infected cell clones, the frequency of infected clones, and the selection for some infected clones does not appear different between children and adults, even in children who initiated ART at two months of age. Our findings indicate that clonal expansion of infected cells occurs very early after infection in children, as it does in adults, and that clonal expansion of infected cells is a major mechanism for persistence of HIV-1 despite long-term ART.

Results

Table 1. Number of Integration Sites and Detectable Clones in Children Prior to and on ART

Donor ID	Age at ART initiation (months)	Minimum number of integration sites obtained pre-ART[a]	Number of clones detected pre-ART	Oligoclonality index[b] (pre-ART)	Years on ART at 2nd timepoint	HIV DNA cps/10^6 PBMC on ART[c]	Minimum number of integration sites obtained on ART[a]	Number of clones detected on ART	Oligoclonality Index[b] (on ART)	Integration sites matches between pre-ART and on ART
ZA002	17.4	1064	27	0.085	6.87	33	113	9	0.079	✓
ZA003	1.8	1386	7	0.04	8.06	2	148	16	0.161	
ZA004#	2.7	655	4	0.024	7.92/8.76	24/--	255	25	0.223	✓
ZA005	6.0	583	2	0.027	8.04	9	77	4	0.166	✓
ZA006	9.0	486	1	0.012	7.45	47	137	20	0.313	✓
ZA007	9.9	197	4	0.07	8.24	21	85	6	0.403	✓
ZA008	2.2	1293	1	0.006	6.77	42	225	10	0.065	✓
ZA009	2.0	809	0	0.021	9.13	186	125	5	0.055	
ZA010	1.8	514	1	0.027	8.35	5	115	3	0.173	
ZA011	9.3	432	5	0.037	7.35	182	149	5	0.092	✓
ZA012	5.1	1244	3	0.01	8.6	12	431	30	0.126	✓

[a] Minimal value obtained by counting an integration from both the 5' and 3' LTR as a single integration site
[b]As described in Gillet *et al.* and Bangham
[c]Integrase cell-associated DNA (iCAD) protocol

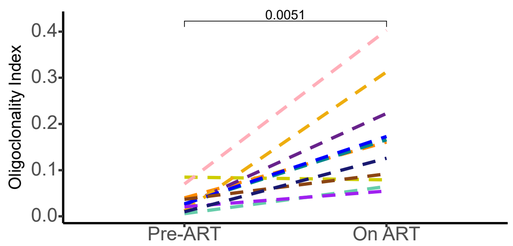


Figure 1: Comparison of clonal structures detected during uncontrolled infection and after six and nine years on ART by oligoclonality index (OCI). The OCI is an index describing the cumulative abundance of clonality within a dataset ranging from [0, 1] no clonality to a singular clone. Increased clonal structure is observed after years on ART (p= 0.0051; Wilcoxon Signed-Rank Test).

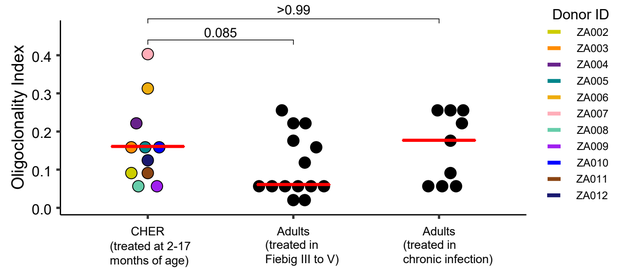


Figure 2: Comparison of clonal structures after years on ART between the CHER cohort and a cohort of adults treated early and adults treated in chronic infection. OCI on average show no difference between children and adults regardless of duration of infection before treatment.

Table 2: Analysis of enrichment of integrations into specific genes after long-term ART[a]

Gene Name	Integrations *in vivo*	Integrations *ex vivo*	Adjusted p-value[b]
STAT5B	37	562	3.3E-30
BACH2	16	132	9.6E-16
RPS10-NUDT3	3	7	0.02

[a]Data shown only for integrations into genes and for which at least one integration was detected in both libraries
[b]Adjusted p-value generated by Fisher's exact test with FDR correction

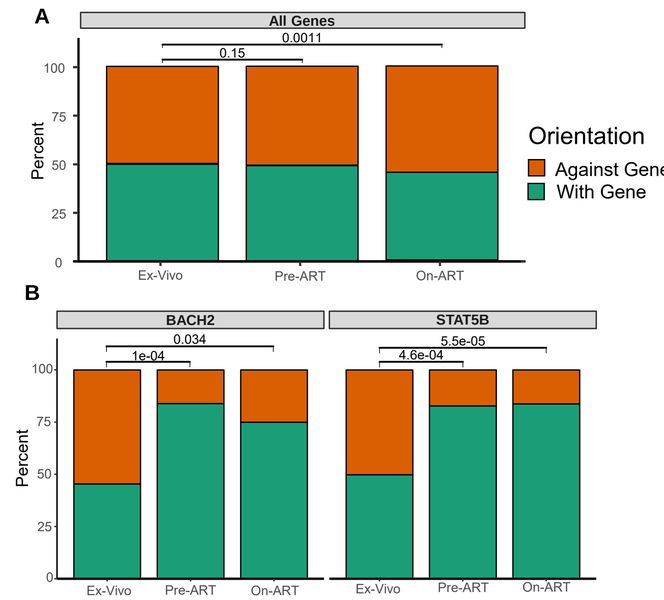


Figure 3: Analysis of orientation biases for integrations into genes. **a.** Globally, a trend for against-gene integrations is observed after many years on ART. **b.** In genes found to be enriched compared to the control library, a statistically significant bias for with-gene integrations is observed.

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