TNFα and IL-6). Furthermore, we show that HIV-infected patients have increased plasma levels of Gal-1 as compared to healthy donors ($p< 0.001$). Longitudinal analysis of plasma samples confirmed the increased serum levels of Gal-1 during cART. Interestingly, we observed a positive correlation between Gal-1 levels and HIV reservoir size, as determined by US-RNA copy number ($p< 0.01$). Finally, we show that circulating extracellular vesicle induces the secretion of Gal-1 by macrophages, suggesting that this cell type could be responsible for the increase in plasma Gal-1.

Conclusions: Gal-1 is capable of reversing HIV latency and this effect could be mediated by promotion of CD4+ T cell activation. Increased plasma levels of Gal-1 in patients and it's correlation with reservoir size suggest that Gal-1 plays an important role in reservoir dynamics and in the pathogenesis of HIV-1 infection.

Cellular and tissue reservoirs of HIV/SIV

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Cellular proliferation maintains genetically intact and defective HIV-1 over time

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Background: An understanding of the mechanisms maintaining replication-competent HIV will be needed to design eradication therapies. We examined the role of cellular proliferation in maintaining intact and defective proviruses within memory CD4+ T-cell subsets from individuals on prolonged ART.

Methods: Naïve, central (CM), transitional (TM), effector (EM), HLA-DR+ and HLA-DR- memory CD4+ T-cells were sorted from the peripheral blood of eight participants on long-term ART. Additional sequences from four participants were obtained four years later. Full-length individual proviral sequencing, which amplifies 92% of the genome, was used to characterise proviruses as intact or defective. Expansions of identical sequences (EIS) were classified as ≥2 identical sequences.

Results: At the early time-point, 1041 sequences were obtained with 4% considered intact. The proportion of intact proviruses was different across cell subsets (p< 0.001), with the highest in EM and HLA-DR+ cells. The proportion of intact and defective proviruses in an EIS was similar. When stratified by treatment duration, the proportion of all sequences in an EIS was higher in those on therapy for >14 years (n=6 participants). No intact expanded sequences were observed in participants on therapy for < 5 years (n=2 participants). Expanded intact sequences were predominantly found in EM and HLA-DR+ cells, representing 24% and 17% of all intact sequences respectively. These intact expanded sequences were observed in two participants four years later. In two participants where no intact provirus was observed, large expansions of defective sequences predominated. In one participant these sequences expanded over four years, representing 41% (28/68) and 78% (167/215) of sequences at each time-point. The expansion in the second participant was stable, with 46% (110/241) and 45% (91/202) of sequences belonging to this EIS at each time-point.

Conclusions: Cellular proliferation contributes to the expansion of both intact and defective proviruses. Expansions of defective proviruses may dilute the number of intact proviruses and lead to difficulty in their identification. Genetically identical intact proviruses are enriched in HLA-DR+ and EM cells - cells with a higher proliferation potential - and these proviruses are stable over time. This indicates that the latent HIV reservoir is maintained in these peripheral blood T-cell subsets by proliferation.

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The CARMA study – children on early suppressive therapy: total HIV-1 DNA quantitation 12 years post ART initiation

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Background: Future strategies aimed at achieving antiretroviral therapy (ART)-free HIV remission are likely to target individuals with a limited size of viral reservoir. We investigated factors associated with a low reservoir measured as total HIV-1 DNA in PBMCs in perinatally infected children (PaHIV) from 5 European centers in the EPIICAL consortium.

Methods: 40 children with PaHIV commenced ART < 2 years of age, suppressed within 12 months of start and remained suppressed (viral load, (VL) < 50 copies/ml) for at least 5 years. Total HIV-1 DNA was measured in isolated PBMCs by quantitative PCR per million PBMCs. Factors associated with total HIV-1 DNA were analyzed using generalized additive mixed models. Age and VL at ART initiation, and baseline %CD4 effects were tested including smoothing splines to test non-linear association.

Results: Of 40 perinatally infected children, 27(67.5%) female, 21(52.5%) Black/Black African, 13(32.5%) Caucasian, 10 were seronegative on 4th generation HIV antibody/antigen, median [IQR] age 12.2 [8.03;15.6] years. Total HIV-1 DNA measured at 12 [7.3;15.4] years after ART initiation was below level of detection in 5 children, with a median of 50.9 [25.3, 117.3] copies/10^6 PBMC in the remaining 35. DNA levels were positively associated with age and VL at ART initiation and baseline CD4% (Figure 18.1). While ART initiation presented a quasi-linear association (coef=0.15(0.6), p=< 0.001), the effect of VL (coef=0.35(0.16), p=0.032) is only noticeable when >6logs. The effect of baseline CD4% (coef=0.03(0.01), p=0.049) was not maintained above 40%.

Conclusions: In this early treated PaHIV cohort on sustained suppressive ART for more than a decade, total HIV-1 DNA was undetected in one eighth. Lower DNA levels were associated with younger age at ART initiation, VL < 6logs at ART initiation, and high CD4%, supporting current global guidelines of early ART initiation for all infants.

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$CD32^+CD4^+$ T cells are highly enriched in HIV DNA

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Background: CD32 was reported to mark the HIV reservoir, but several recent reports challenged this finding. We aimed to investigate the role of CD32 as a marker of the viral reservoir and to characterize the phenotype of $CD32^+CD4^+$ T cells.

Methods: Total HIV DNA and unspliced RNA was quantified in $CD32^+$ and $CD32^-$ fractions of $CD4^+$ T cells from aviremic ART-treated individuals (n=41). Fractions were obtained by magnetic sorting (negative selection to isolate $CD4^+$ T cells followed by positive selection to isolate $CD32^+CD4^+$ cells).

Results: The median frequency of $CD32^+$ cells among $CD4^+$ cells in 18 ART-treated individuals initially studied was 0.074%. Percentages of

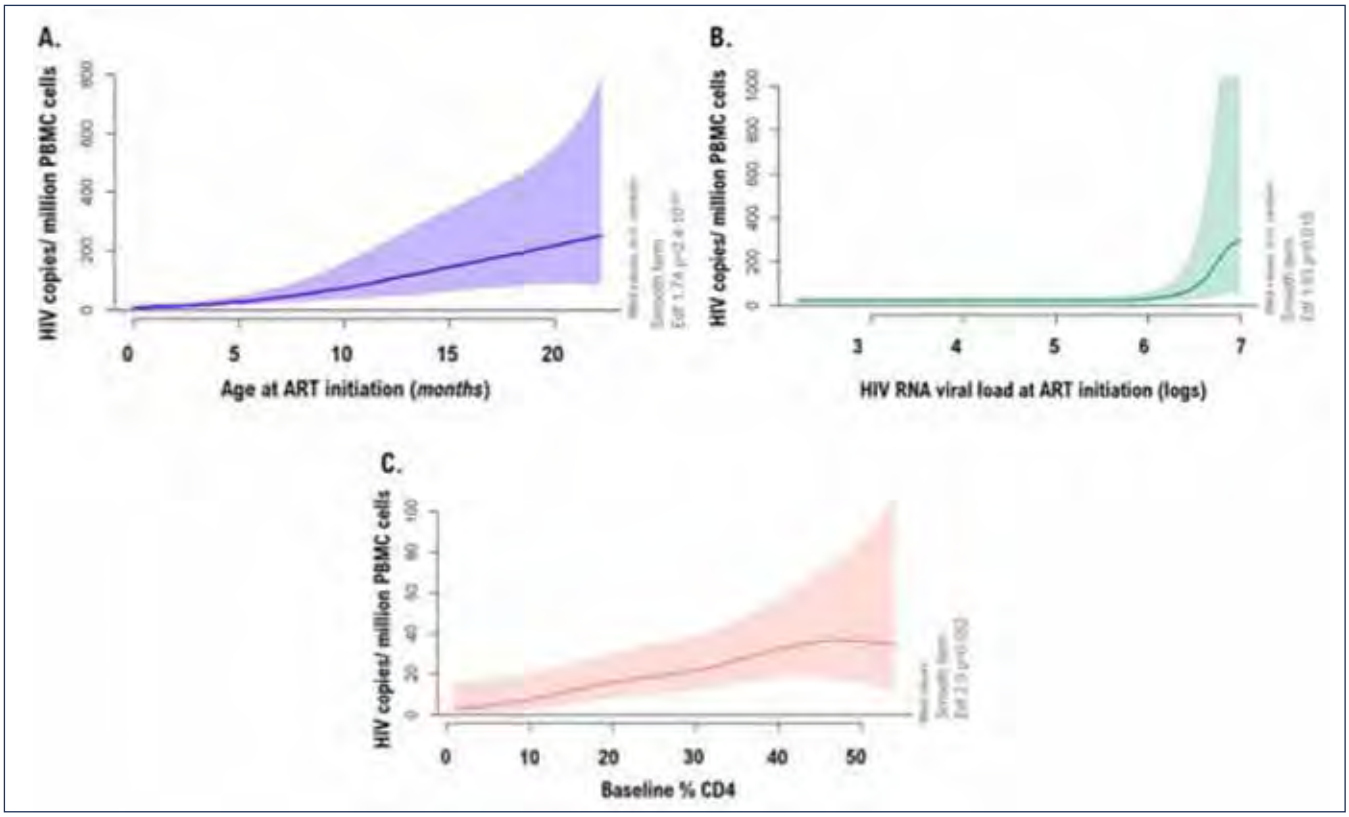

Figure 18.1. Total HIV-1 DNA by age and VL at ART initiation

$CD32^+CD4^+$ T cells positively correlated with total HIV DNA in PBMC (rho=0.51; p=0.031). $CD32^+CD4^+$ T cells demonstrated increased expression of LAG-3 and TIGIT (both p=0.016) and HLA-DR (p< 0.0001) compared with $CD32^-CD4^+$ T cells. No enrichment in HIV DNA was observed in $CD32^+CD4^+$ T cells compared with $CD32^-CD4^+$ cells in this initial set. However, the $CD32^+$ fraction was found to contain many residual non-T cells, which could have masked the enrichment in HIV DNA. Indeed, when HIV DNA was normalized to *CD3G* T-cell-specific mRNA, a significant enrichment in HIV DNA in the $CD32^+$ fraction was observed (p=0.0003). Therefore, we optimized the protocol to isolate a purer fraction of $CD32^+CD4^+$ T cells from additional 23 ART-treated individuals. An extra round of $CD4^+$ T-cell purification resulted both in a 22-fold decrease in *CD19* B-cell lineage marker mRNA level in the $CD32^+$ fraction (p< 0.0001) and in an 11-fold enrichment in HIV DNA in this fraction (p=0.0003), the latter observed even when HIV DNA was normalized to the total cell numbers. In a subset of these individuals (n=9), we performed two additional rounds of $CD32^+$ positive selection and observed a very high enrichment (mean 292-fold) for HIV DNA in the $CD32^+$ fraction. In contrast, no enrichment for HIV RNA was observed in these cells, yielding a significantly reduced HIV RNA/DNA ratio, which may indicate transcriptional latency.

Conclusions: Our results confirm that $CD32^+CD4^+$ T cells are highly enriched in HIV DNA and provide a plausible explanation for the negative results obtained by other groups.

Characterising HIV/SIV reservoirs and rebounding virus

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Kansui, an ingenol-containing herbal supplement, safely induced CD8, NK, and monocyte activation in three ART-suppressed SIVmac251-infected rhesus macaques

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Background: HIV eradication strategies aim to combine agents that reverse viral latency with therapies that boost the host immune response. We performed a non-human primate study evaluating the safety and *in vivo* biological response to an herbal supplement, *Euphorbia kansui*, containing compounds in the potent class of latency reversal agents, ingenols.

Methods: Six juvenile rhesus macaques (RM) were infected with SIVmac251 and treated with once daily subcutaneous 20 mg/kg tenofovir, 50 mg/kg emtricitabine, and 3.25 mg/kg dolutegravir for 15 weeks. Three animals were administered oral kansui, starting with a test dose, followed by a dose equivalent to that used in traditional Chinese medicine, given for an increasing number of consecutive days: (1) 5 mg/kg × 1, (2) 20 mg/kg × 1, (3) 20 mg/kg × 2 days, and (4) 20 mg/kg × 3 days. Each dose was followed by a 2-week washout period, and blood was drawn at the end of each dosing period. Markers of T-cell, NK-cell, and monocyte activation were characterized by flow cytometry, and SIV total DNA and unspliced RNA from PBMCs were quantified by real-time PCR. Data were analyzed using multivariate mixed effects regression.

Results: There were no adverse events observed at any given dose/frequency of kansui administered. There was a trend for increased activation levels in treated vs. control monkeys over time. Statistically significant kansui effects on CD8+ T (HLA-DR+CD38+; 8.5-fold increase, P=0.015), NK cell (CD3-CD8+; 6.0-fold, P=0.001), and monocyte (HLA-DR+$CD14^{hi}$CD16+CD80+; 27-fold, P=0.023) activation were observed at time point 4. Comparisons by treatment group were uninformative for SIV DNA and RNA, because 2 control animals had SIV RNA and DNA levels below the limit of detection, providing limited information on relative changes over time. Within treated animals, there was no statistically significant association between timepoint and SIV RNA or DNA.

Conclusions: Kansui was well tolerated in three ART-suppressed, SIVmac251-infected RM. Statistically significant increases in CD8+ T-cell, NK-cell, and monocyte activation markers were observed at