

Oral abstracts of the HIV Cure and Reservoir Symposium 2017

O1

Towards a block-and-lock strategy: LEDGINs hamper the establishment of a reactivation competent reservoir

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Background: Persistence of latent provirus is the main barrier towards a cure for HIV. We propose a novel strategy to reduce the HIV reservoir by drug-induced retargeting of HIV integration. A novel class of integration inhibitors, LEDGINs, inhibits the interaction between HIV integrase (IN) and the LEDGF/p75 cofactor, the main determinant of integration site selection. This results in an allosteric inhibition of HIV IN (early effect). Moreover, when present during production, progeny virus displays morphological and replication irregularities (late effect).

Methods: Integration sites were sequenced using 454 pyrosequencing. To evaluate reactivation potential, cell lines were infected with a double-reporter virus and LEDGIN was added during infection (early effect) or during production of the virus (late effect). Cells were reactivated and analyzed by FACS. In a multiple round experiment, primary activated CD4⁺ T cells were infected with wild type NL4.3 virus in the presence of LEDGIN or RAL. Cells were reactivated and virus production was measured by p24 ELISA.

Results and conclusions: LEDGIN-mediated inhibition of the LEDGF/p75-IN interaction blocks replication and relocates integration out of transcription units. This retargeting resulted in a residual reservoir that contains up to 95% of latent cells that are resistant to reactivation. In activated CD4⁺ T-cells, both LEDGIN and RAL reduced infection. The residual provirus established under LEDGIN treatment was hampered for reactivation, a phenotype not observed with RAL. Addition of LEDGINs early during acute infection does affect the formation of the latent reservoir. Bringing the majority of residual proviruses in a state of deep latency and defective for reactivation might represent an attractive approach to achieve an HIV remission.

O2

Identification of a new factor involved in DNA methylation-mediated repression of latent HIV-1

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The DNA methylation profile of the HIV-1 latent 5'LTR is heterogeneous. Previously, we reported that the DNA methylation inhibitor decitabine induces different levels of HIV-1 reactivation *in vitro* and *ex vivo*. However, the mechanism of DNA methylation-mediated HIV-1 silencing remains unclear.

To explore this mechanism, we took advantage of two latently-infected cell lines representing distinct integration sites, and showed that these two cell lines exhibited similar levels of 5'LTR CpG methylation in basal condition but different DNA demethylation extents in response to decitabine. Demethylation at CpG dinucleotides following

decitabine-induced HIV-1 reactivation occurred at specific and reproducible CpG positions that differed depending on the two cell lines studied. Interestingly, a site comprising one of this hotspot for decitabine-induced demethylation was shown to bind UHRF1, only in one of cell line. Treatment with decitabine caused a decreased *in vivo* UHRF1 recruitment to the 5'LTR. UHRF1 knockdown using RNA interference and pharmacological approach showed increased levels of HIV-1 production in latently-infected cells and of HIV-1 transcription in *ex vivo* cell cultures from cART-treated aviremic HIV+ patients, respectively.

UHRF1 has not previously been identified as a regulator of HIV latency and might thus constitute a new therapeutic target for HIV cure strategies.

O3

Heme-arginate as a latency-reversing agent for HIV-1 cure

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Current antiretroviral therapy does not lead to virus elimination in HIV-1-infected patients as HIV-1 can establish a highly stable reservoir of latently infected cells. Approaches towards HIV-1 cure involve namely attempts to reactivate and purge HIV-1 latently infected cells. Since the current strategies for latent HIV-1 reactivation do not seem to affect the size of the latent pool, it is important to search for new latency reversing agents and their combinations.

Our laboratory has demonstrated that Normosang (HA, Orphan Europe), a human hemein containing compound used to treat acute porphyria, could strongly potentiate reactivation of latent HIV-1 induced by PKC inducers in cell lines, while it inhibited HIV-1 replication in acute infection through its effect on reverse transcription. The stimulatory effects of Normosang involve a heme/iron-mediated Fenton reaction resulting in the increased redox stress (Shankaran et al., 2011; Shankaran et al., 2017).

To confirm our original hypothesis that Normosang could be used as a latency reversing agent in human, we plan to perform a study characterizing the *in vivo* effects of Normosang on reactivation of the latent HIV-1 in HIV-infected patients on combined antiretroviral therapy (cART) including an integrase inhibitor. At specific time-points after the intravenous administration of Normosang, we will quantify cell-associated HIV-1 RNA in PBMCs of HIV+ patients using 2-step semi-nested RT-qPCR. Simultaneously, levels of parameters characterizing heme and iron metabolism will be determined. Our work defines a new redox-based approach to the HIV-1 latency reversal and provides basis for new therapeutic strategies that might lead to HIV-1 cure.

O4

Early start of antiretroviral therapy (ART) during primary HIV infection (PHI) is associated with faster optimal immunological recovery: results of Italian Network of ACuTe HIV InfectiON (INACTiON) retrospective study

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Background: INACTION is a network created with the aim to study PHI. Aim of this study was to assess the benefit of early start of ART during PHI in terms of time to immunological recovery and virological suppression (VS).

Methods: 321 patients (pts) with PHI diagnosed between 2008–2014 from 20 centers were analyzed. PHI was classified according to Fiebig criteria (stage I to V). Pts who started ART within 3 months since BL were classified as early ART group (E-ART), while others as late ART group (L-ART). Optimal immunological recovery (OIR) was defined as CD4>500/mm³ and CD4/CD8>1 and CD4>30%.

Results: Characteristics of population: median age 37y, 86%M, 57.3% MSM, 66% Fiebig ≥IV, BL-median CD4 456 cells/μL, BL-median HIV-RNA 5.67 log₁₀cp/mL. During follow-up, ART was started in 99.2% pts, of which 70.6% E-ART. Pts in E-ART had more frequently symptoms, CD4+ T-cell count>500, CD4+ T cell%>30% and higher BL HIV-RNA. Pts diagnosed after 2012 were more likely to start ART early; moreover, in E-ART >3 drugs and PI and/or INSTI-based regimens were prescribed more frequently. At multivariate analysis, higher plasma HIV-RNA and PHI diagnosis between 2012–2014 vs 2008–2011 were associated with E-ART. At Cox analysis, predictors of time to VS were CD4/CD8>1 (p<0.001), HIV-RNA cp/mL (per log₁₀ increase) (p=0.003) and years of HIV diagnosis (p=0.028). E-ART had shorter time to OIR: estimated incidence at 48 weeks was 39% in E-ART vs 22.5% in L-ART (p=0.002).

Conclusions: Despite worse baseline viro-immunological conditions, PHI pts who started ART within 3 months after diagnosis achieved OIR faster than those who began later during the course of infection.

05

The expression profile of host restriction factors in different cohorts of HIV-1-infected patients

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After HIV-1 infection, several antiviral host genes are upregulated to suppress viral infectivity. These antiviral factors, defined as restriction factors, are part of a first line innate antiviral immune response. In this study, host restriction factor expression (APOBEC3G, SAMHD1, tetherin, TRIM5α, MX2, SLFN11 and PAF1) was determined in different cohorts of HIV-1 infected patients.

The expression profile of seven HIV-1 restriction factors was evaluated in 104 HIV-1 infected patients from six pre-defined cohorts: long-term-treated patients where ART is initiated during seroconversion (Early treated; N=24) or chronic infection (Late treated; N=32), long term non-progressors (LTNPs; N=17), recent ART-naïve seroconverters (Recent SRCV; N=19) and ART-naïve chronically infected patients (Chronic naïve; N=12). Patients were recruited in two clinical centers (Royal Free Hospital London and Ghent University Hospital) and an

additional group of healthy individuals (N=14) was included as a control.

In general, elevated restriction factor levels for Recent SRCV, LTNP, early treated patients and chronically infected therapy naïve patients were determined. The restriction factor levels of healthy individuals and late treated patients remain low at similar levels. For example, APOBEC3G was significantly upregulated in ART-naïve recent SRCV and long-term-treated patients that started therapy early (Early treated), in comparison to healthy individuals (p < 0.001; p < 0.001). APOBEC3G was also elevated in comparison to long-term-treated patients started on ART during chronic HIV-1 infection (Late treated) (p < 0.01, p < 0.001). APOBEC3G levels were positively correlated with CD4 count at borderline significance level (r=0.44, p=0.076).

Host restriction factor expression levels are elevated in recent seroconverters as an early antiviral host defense mechanism. LTNP are able to maintain slightly upregulated restriction factor levels, suggesting a mechanism contributing to their non progressing phenotype. For HIV-1 patients on treatment, restriction factor levels are upregulated in the early treated in comparison to the late treated HIV-1 patients. HIV-1 patients that started treatment early are able to maintain higher restriction factor levels which could contribute to a better disease control.

06

Single doses as low as 0.5 mg of the novel NRTTI MK-8591 suppress HIV for at least 7 days

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Background: MK-8591 is a nucleoside reverse transcriptase translocation inhibitor (NRTTI) in early clinical development for the treatment of HIV-1 infection. MK-8591-triphosphate (MK-8591-TP), the active phosphorylated anabolite of MK-8591, exhibits a half-life of ~150–160 hrs in human PBMCs. Here we describe antiviral efficacy and tolerability of single doses of 0.5 mg to 30 mg MK-8591 over 7 to 10 days in HIV-1 infected subjects in an ongoing Phase 1b monotherapy proof-of-concept efficacy study.

Methods: In an open-label study in HIV-1-infected subjects naïve to antiretroviral treatment (ART), subjects are being administered a single dose of MK-8591 across a range of doses. Blood samples are being collected for viral load (VL), MK-8591 PK, and MK-8591-TP PK at prespecified time points up to 7 to 10 days post-dose. Following completion of Day 7 or Day 10 procedures, subjects are being offered standard of care ART. Safety, PK, and VL data from the doses of 0.5 mg, 1 mg, 2 mg, 10 mg and 30 mg (N=6/panel) are available.

Results: Single doses of MK-8591 across the entire tested range were associated with a rapid and robust reduction in VL. At 168 hours postdose, a mean (95% CI) placebo-corrected VL reduction of 1.18 log₁₀ (0.95, 1.46) was observed for 0.5 mg. For the 30 mg dose, mean VL continued to decline through Day 10 with a mean placebo-corrected reduction of 1.57 log₁₀ (1.34, 1.85), with no evidence of recrudescence at any dose. In samples with sufficient VL for testing (14/24), no common mutant strains, including M184V/I, were detected. All doses were generally well tolerated, with a limited number of mild/moderate adverse experiences reported. MK-8591 plasma and MK-8591-TP PBMC PK were similar to previously observed data in healthy subjects.

Conclusions: MK-8591 suppressed HIV replication for at least seven days when administered as a single dose as low as 0.5 mg. The antiviral potency, human pharmacokinetics (PK), and physical properties of MK-8591 have the potential to open new paradigms for extended duration HIV treatment and prophylaxis approaches.

07

Does viral suppressive capacity in cART-treated HIV-infected patients correlate with disease parameters, viral reservoir measures or cytotoxic T cell phenotype?

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Background: A combination of potent HIV-specific immune responses and a small latent viral reservoir is likely required to achieve post-treatment control of HIV infection. Here we investigated correlations between viral suppressive capacity, phenotype of CD8+ T-cells, clinical parameters and the HIV reservoir.

Methods: Thirty-six patients on cART with suppressed viremia and six healthy donors were recruited. Total HIV-1 DNA and unspliced mRNA (usRNA) levels were measured in PBMCs. Viral suppressive capacity of CD8+ T cells and their phenotype (cytokine production, cytotoxicity and immune checkpoint markers) were determined before and after peptide stimulation. In a sub-group of 21 patients infected with HIV subtype B, viral transcriptional activity was quantified with the TILDA assay. Linear regression and student t-test were used for correlation analyses.

Results: Total HIV DNA and usRNA levels (median: 187 and 2.2 cps/million PBMCs respectively) were as expected and correlated with each other ($p < 0.05$). TILDA values ranged from 0 to 313 cells [IQR: 1.4–55.8] with detectable HIV RNA transcripts per million CD4+ T cells after stimulation. CD8+ T-cell suppressive capacity was significantly ($p < 0.01$) increased with peptide stimulation.

HIV peptide stimulation increased IFN- γ and CD107a expression in CD8+ T cells ($p < 0.0065$). Viral suppressive capacity correlated with HLA-DR expression in CD8+ T cells during co-culture ($p < 0.005$). CD8+ T cells of VIA responders had an increased IFN- γ expression between 18 and 42 hours of the CD4/CD8 co-culture. At baseline the suppressive capacity correlated with CD160/PD-1 co-expression in CD8 TEMRA ($p < 0.001$).

Conclusions: In this group of virally suppressed patients, heterogeneity in terms of immune responses and reservoir size was observed, as expected. Interestingly, T-cell exhaustion markers CD160 and PD-1 were predictive of the suppressive capacity of CD8+ T cells.

08

Integration site detection in 10 chronically infected patients

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HIV-1 infection remains incurable due to a reservoir of latently infected cells that persists despite sustained antiretroviral treatment. Recently, clonal expansion of latently infected cells has been identified as a driver of reservoir maintenance, representing a new barrier to a cure.

Therefore, much effort is being invested in unravelling the dynamics and characteristics of *in vivo* cell proliferation.

Integration site sequencing with 'Integration Site Loop Amplification (ISLA)' was performed to assess clonal expansion in a total of N=10 chronically infected individuals. Previously, reservoir persistence markers such as total HIV-1 DNA, integrated HIV-1 DNA, multiple spliced HIV-1 RNA and unspliced HIV-1 RNA were quantified by droplet digital PCR (ddPCR). In addition, quantitative viral outgrowth assay (qVOA) and *tat/rev*-induced limiting dilution assay (TILDA) were performed to measure the frequency of replication competent proviruses and the frequency of transcriptionally activatable proviruses respectively.

A total of 408 integration sites were identified from the ten subjects. Clonally expanded infected cells were identified in all but one subject, with a mean level of clonality of approximately 40% ($39 \pm 23\%$). Remarkably, one subject had a very highly expanded clone, representing over 90% of the viral reservoir. No preferential orientation of integration was observed with respect to the genome (53% reverse, 47% forward). In contrast, with respect to the genes, integration was biased towards the reverse orientation (74% reverse, 26% forward). Gene ontology testing revealed that integration of proviruses is biased towards genes that play a role in histone modification and regulation of transcription (5% level of significance, Bonferroni corrected). Furthermore, integration in oncogenes took place at a higher frequency than would be expected at random ($p=0.0095$, Fisher's exact test).

The above findings show that clonal expansion of infected cells takes place in patients under effective cART, and clones have the potential to grow large and comprise a significant portion of the reservoir. Furthermore, preferential integration into genes involved in transcription and into oncogenes might have forced the host cell to start expanding.

09

Immunovirological outcome of HIV-infected children living in a resource-limited setting of South Africa

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Introduction: Currently available combination antiretroviral therapy (cART) have transformed pediatric HIV-1 from a rapidly fatal illness to a chronic disease. However, immunovirological data collected in Africa are scarce. This study describes the long-term outcome of South African children who started cART before one (Early treated cohort : ETC) and after two years (Late treated cohort : LTC) of age in a resource limited setting (RLS) of South Africa.

Methods: 55 and 96 children were included in the ETC and the LTC respectively. Children from both cohorts were subdivided into three subgroups according to CD4+% at cART initiation (<15%, 15 to 24% and $\geq 25\%$).

Results: Children included in the ETC more often achieved normal CD4+% (100% vs 89.6% $p=0.014$) and reached higher mean CD4+% 6 years after treatment initiation. Children with 15 to 24% CD4+% at cART initiation reached higher CD4+% in the ETC. The proportion of children who experienced virological failure (>1000 cp/ml) was comparable in both cohorts. Persistent undetectable viral load (<50 cp/ml) was more frequent in the ETC ($p=0.008$). Finally, the proportion of children with detectable viral loads (50 to 1000 cp/ml) at least once after initial virological suppression was higher in the LTC ($p=0.0022$).

Conclusion: cART was highly effective both in ETC and LTC. The results of this study demonstrate that early treated children more often achieved normal CD4+%, had higher mean CD4+% and more often sustained virological suppression. These results encourage the current recommendations to initiate cART as soon as possible in RLS.

Poster abstracts of the HIV Cure and Reservoir Symposium 2017

P1

Contribution of lncRNAs in the establishment of HIV latency in central memory CD4 T Cells

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HIV cure research has been hampered by the existence of a latent viral reservoir that persists in infected individuals receiving antiretroviral therapy. To date, most of the cure research has focused on protein coding genes but recently the interest in the study of non-coding RNA has risen, as these molecules could provide insight in new therapeutical strategies.

Transcriptome profiling was performed (total RNA-Seq) in a primary HIV latency model of central memory CD4 T cells (T_{CM}) to investigate changes in lncRNA expression. Subsequently, differentially expressed mRNAs and lncRNAs were identified and a guilt-by-association analysis was implemented to infer biological roles for the lncRNAs in HIV latency.

In the primary HIV latency model, we identified 826 mRNAs (87.8%) and 115 lncRNAs (12.2%) that were significantly differentially expressed (FDR<0.05) between uninfected and latently infected T_{CM} cells. Many of these lncRNAs were associated with pathways involved in cell cycle regulation and pathways with a link to HIV latency: IL-7, PTEN, CSK and CCR5. In addition, a cluster of 17 lncRNAs was associated with the p53 pathway and corroborate earlier findings in this T_{CM} model that illustrated p53-dependent latency establishment. One of these upregulated p53-linked lncRNAs, 7SLRNA, has a characterized inhibitory role in the p53 pathway and would suit as a possible new therapeutical target.

Altogether, this study demonstrates that several lncRNAs play a role in HIV latency and can be linked to biological pathways with importance in HIV latency establishment and maintenance. Some of these lncRNAs, i.e. 7SLRNA, represent possible targets for reversing HIV latency and contribute to a HIV cure.

P2

Clinical evaluation of the next-generation sequencing (NGS) for detection of drug-resistance mutations in HIV-1

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Introduction: WHO urges action against HIV drug resistance threat. It is well known that the sensitivity of NGS is vastly superior to Sanger sequencing. The objective of this study was to evaluate the novel NGS HIV-1 drug resistance monitoring system, particularly those in low proportion.

Materials and methods: NGS analyses were performed on plasma samples from 41 HIV-1 infected patients with the *Sentosa* SQ HIV Genotyping Assay from Vela Dx. This kit was used on a semi-automated Ion Torrent-based platform. Sequences were compared to those obtained by Sanger method. Two samples were added inside and in separated runs. Quality control (QC) were added to control protease (PRO) and reverse transcriptase (RT) sequencing and one to control integrase (INT) sequencing.

Results: In the 41 patients analyzed, both methods detected 245 Drug Resistance Mutations (DRMs). The *Sentosa* SQ HIV Genotyping

Assay detected 38 additional DRMs (mutation frequency ~7%). Sanger method detected 2 additional DRMs, 39A which is not included in the list of mutations by Vela Dx because of few clinical impact and the secondary mutation 63Q. The sequences were 98,2% homologous (counting variants as mismatch) and 99,9% homologous (not counting missed variants). Duplicate in a run were 95,7%(99,9%) homologous. Duplicate in two different runs were 98%(100%) homologous. QC results were manually assessed to a score of 340/340 for detection of DRM in PRO and RT and to 100% for INT sequencing.

Conclusions: To our knowledge, this is the first clinical evaluation in Belgium employing the *Sentosa* SQ HIV Genotyping Assay. The NGS appears as a promising tool for the detection of DRM in HIV-1. Results give a higher sensitivity compared to Sanger method. Studies assessing the clinical relevance of DRM in low frequency are needed.

P3

An insertion of seven amino acids in the envelope cytoplasmic tail of human immunodeficiency virus type 2 (HIV-2) selected during disease progression enhances viral replication

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Introduction: The cytoplasmic tail (CT) of the transmembrane envelope glycoprotein (gpTM) of HIV-2 includes amino acids (aa) sequences similar to lentiviral lytic peptides (LLP) described in other lentiviruses [1,2]. Within the putative LLP-2 region, we previously observed insertions of 3 or 7 aa in sequences deduced from plasma viral RNA of symptomatic HIV-2 infected individuals [3]. Based on these observations, we reproduced the insertions in a molecular clone to assess their impact on replicative fitness and cell death *in vitro*.

Methods: Using a molecular clone of the HIV-2 ROD reference strain, site-directed mutagenesis experiments allowed the generation of plasmids with the insertion L₇₉₁TAI or L₇₉₁QRALTAI in Env protein. After transfection in HEK293T cells, the viral particles were used to infect H9 or Jurkat T cells. Viral release was quantified by RT-qPCR at three and six days post-infection. Cell viability was assessed with the percentage of living cells using a CASY cell counter. The viral infectivity were monitored using TZM-bl reporter cells.

Results: Compared to the control wild-type HIV-2 ROD virus (HIV-2 WT), the clone with a 7 aa insertion enhanced viral release thirteen times. Cell viability was 20% more impaired compared to the wild-type virus. The effect of the 3 aa insertion was milder, with a non-significant trend to enhance viral replication and cell death compared to the wild-type.

Interestingly, the insertions in the Env proteins did not induce a significant increase of viral infectivity, as revealed by the infectivity assay using TZM-bl cells.

Conclusion: A 7 aa insertion including positively charged aa in the putative LLP-2 enhances viral replication and cell death *in vitro*. The insertions in the Env CT observed *in vivo* from disease progressors may therefore be involved in the higher viral load observed in these individuals. This study may open the way to the development of laboratory diagnostic tools related to disease progression.

References

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P4

Long-term aviremia after discontinuation of antiretroviral treatment

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Background: Early ART initiation is associated with impact on HIV-1 reservoir establishment and decay with the potential for virological control post-treatment discontinuation. Underlying mechanisms of post-virological control remain unclear. We report on a clade C-infected female patient who has maintained undetectable viremia (VL) for 13 years after stopping a 6-year treatment period initiated at PHI with initial virological failure while on ART and describe her virological parameters and HIV-1 specific T cell responses.

Case report: A 23-year-old female seroconverted with a 3-week-long severe acute retroviral syndrome in October 1997. Blood parameters show a CD4 <200 cells/mm³ on 3 occasions and VL >750,000 HIV-1 copies(c)/mL (clade C) before ART initiation on 20.10.97 (AZT-3TC-indinavir 800 mg tds switched to ritonavir 600 mg bd 2 weeks later). Failure of this regimen over several weeks up to 94,000 c/mL prompted treatment intensification (double-boosted protease inhibitors). Aviremia was achieved in April 1999 on ART which was

maintained until January 2004. Following treatment discontinuation aviremia persisted without viral blips for 13 years with preservation of CD4 T cells and CD4/CD8 ratio>1. HLA genotype was not one generally associated with a favourable outcome.

At 10 years of aviremia (2014), total HIV-1 DNA, integrated HIV-1 DNA and 2-LTR circles were 148.93 (95% CI: 76.99–229.64), 134.31 (95% CI: 56.47–304.39) and 3.89 (95% CI: 0–9.15) HIV-1 copies/million PBMCs, respectively. CD4 and CD8 HIV-1 specific T cell responses showed moderately potent CD8+ T cell inhibition of a clade-matched HIV-1 isolate equivalent to that which we have observed in ART-naïve chronically infected subjects with VL set-point <10,000 HIV-1 copies/mL. Unusually broad gag-specific IFN-gamma CD4 responses were detected, targeting multiple regions of genetic vulnerability that are associated with virological control. Analysis of CSF (September 2015) showed an undetectable viral load. Her CD4 count in July 2017 was at 942 cells/mm³ with a ratio at 0.99

Conclusion: This is an unusual case of very prolonged aviremia after initial failure of treatment at the time of PHI. Intermediate levels of total and integrated HIV-1 DNA and broad HIV-1 gag-specific CD4 T cell responses, together with preserved CD8+ T cell viral inhibitory activity were noted during follow-up. Further insight should be gained into the potential role of CD4 T cells underlying virological control post-ART.