

Virological remission after antiretroviral therapy interruption in female African HIV seroconverters

Morgane Gossez¹, Genevieve Elizabeth Martin¹, Matthew Pace¹, Gita Ramjee², Anamika Premraj², Pontiano Kaleebu³, Helen Rees⁴, Jamie Inshaw⁵, Wolfgang Stöhr⁵, Jodi Meyerowitz¹, Emily Hopkins¹, Mathew Jones¹, Jacob Hurst¹, Kholoud Porter⁶, Abdel Babiker⁵, Sarah Fidler⁷, John Frater^{1,8,9} on behalf of the SPARTAC Trial Investigators

¹Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, Oxford, UK

²HIV Prevention Research Unit, South African Medical Research Council, Durban, South Africa

³Medical Research Council/Uganda Virus Research Institute, Entebbe, Uganda

⁴Wits Reproductive Health and HIV Institute of the University of the Witwatersrand in Johannesburg, South Africa

⁵MRC Clinical Trials Unit at UCL, Institute of Clinical Trials & Methodology, London, UK

⁶Institute for Global Health, University College London, London, UK

⁷Division of Medicine, Wright Fleming Institute, Imperial College, London, UK

⁸The Oxford Martin School, Oxford, UK

⁹Oxford National Institute of Health Research Biomedical Research Centre, Oxford, UK

Corresponding author:

John Frater, Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, Oxford, UK. Email: john.frater@ndm.ox.ac.uk

Abstract

Introduction

There are few data on the frequency of virological remission in African individuals after treatment with antiretroviral therapy (ART) in primary HIV infection (PHI).

Methods

We studied participants (n=82) from South Africa and Uganda in SPARTAC, the first trial of treatment interruption (TI) in African individuals with PHI randomized to deferred ART or 48 weeks of immediate ART. All were female and infected with non-B HIV subtypes, mainly C. We measured HIV DNA in CD4 T cells, CD4 count, plasma viral load (pVL), cell-associated HIV RNA and T cell activation and exhaustion. We explored associations with clinical progression and time to pVL rebound after TI (n=22). Data were compared with non-African SPARTAC participants.

Results

Pre-therapy pVL and integrated HIV DNA were lower in Africans compared with non-Africans (median 4.16 vs 4.72 log₁₀ copies/ml and 3.07 vs 3.61 log₁₀ copies/million CD4 T-cells respectively; p<0.001). Pre-ART HIV DNA in Africans was associated with clinical progression (p=0.001, HR per log₁₀ copies/million CD4 T cells increase (95% CI) 5.38 (1.95-14.79)) and time to pVL rebound (p=0.034, HR per log₁₀ copies/ml increase 4.33 (1.12-16.84)). After TI, Africans experienced longer duration of viral remission than non-Africans (p<0.001; HR 3.90 (1.75-8.71)). Five of 22 African participants (22.7%) maintained VL<400 copies/ml over a median of 188 weeks following TI.

Conclusion

We find evidence of greater probability of virological remission following TI among African participants, although we are unable to differentiate between sex, ethnicity and viral subtype. The finding warrants further investigation.

Key Words:

HIV; antiretroviral therapy; treatment interruption; post-treatment control; remission; Africa

Introduction

There is no cure for HIV infection despite effective antiretroviral therapy (ART). Latently infected CD4 T cells (1) persist despite ART and decay slowly (2). Most people living with HIV (PLHIV) experience viral reactivation soon after ART interruption. However, studies of individuals who initiate ART early in primary HIV infection (PHI) have identified more rapid clearance of the reservoir (3-5) and cases of virological remission (6-8).

As new strategies are developed to target the HIV reservoir (9), there has been an increase in the number of treatment interruption (TI) studies raising concerns over safety (10-12). An algorithm to predict individuals who might sustain undetectable plasma viral loads (pVL) after TI would therefore be of benefit. In SPARTAC (Short Pulse Antiretroviral Treatment at HIV-1 Seroconversion) (13), HIV DNA levels in CD4 T cells (14) and expression of immune checkpoint receptors PD-1, Tim-3 and Lag-3 (15) predicted time to pVL rebound after TI. Cell-associated HIV RNA (CA-RNA) (16) – a marker of a more inducible reservoir – and the frequency of viral blips on ART (17) have also been shown to predict rebound viraemia.

Most studies examining the HIV reservoir investigate European or North American participants infected with subtype B HIV. This is not representative of the global burden of disease, given that 70% of PLHIV are in Africa and 50% of prevalent HIV infections are estimated to be due to subtype C (18). Whereas non-B viral subtypes respond well to ART (19), our current understanding of the reservoir may not translate to African or non-B infected populations, especially as variation in pVL, HIV DNA and CD4 T cell count is linked to ethnicity and geography (20). There may also be differences between both sex and HIV subtypes relating to replicative fitness,

biology of transmission and disease progression (21-26). We turned to SPARTAC to analyse outcomes after TI in African participants.

Methods

Participants and trial design.

The design of the SPARTAC trial is reported elsewhere (13). SPARTAC was an international RCT of early ART, comparing 12 or 48 weeks of ART followed by treatment interruption (TI) with no immediate treatment. 366 adults within an estimated 6 months from seroconversion were recruited. All participants gave written informed consent (see Supplementary Material for details of ethics approvals, <http://links.lww.com/QAD/B378>). The primary endpoint was a composite of a CD4 T cell count of less than 350 cells/ μ L or initiation of long-term ART. Time of virological failure was defined as the first of two consecutive values of plasma viral load above 400 copies per ml. HIV RNA was measured at clinical sites by the Bayer (Chiron bDNA) assay (13). We restricted our analysis to participants who received either no ART or 48 weeks of ART. SPARTAC also recruited to a 12-week ART arm in which participants responded similarly to the untreated arm (8,13), so we have not included these participants in this sub-study.

African participants for this sub-study were women from Uganda and South-Africa, infected with non-B HIV subtypes and randomised to receive ART for 48 weeks (ART48) or no therapy. (The SPARTAC trial only recruited female participants in these countries as recruitment was linked to a vaginal microbicide study). To qualify for this analysis of TI, participants had to have either received no ART, or to have

received between 45 and 50 weeks of ART and to have been fully suppressed (pVL <400 copies/ml) at TI. Of the 82 African participants in SPARTAC who were eligible for this sub-study and had samples available, 38 were randomised to ‘deferred ART’ and 44 to the ART48 arm. Of the latter, only 22 contributed to the analysis at the point of treatment interruption (S1 Fig, <http://links.lww.com/QAD/B378>).

The data from the African participants are compared with data from subtype B-infected participants recruited in the UK, Italy, Ireland, Australia and Brazil (subsequently referred to as the ‘non-African cohort’) which is described in detail elsewhere (14), and comprised 103 participants, all male and predominantly from the UK (66.0%). The same criteria for treatment duration and undetectable pVL as in the African cohort were used for the non-African cohort. 52 non-African participants had been randomised to receive 48 weeks of ART and 51 to no immediate treatment. 44 samples were available for analyses at TI after 48 weeks of ART (Fig S1, <http://links.lww.com/QAD/B378>).

Characteristics of participants at randomisation and TI, are shown in Table 1. Due to sample availability, not all assays were performed in all participants at all time-points.

Measurement of HIV DNA.

Thawed peripheral blood mononuclear cell (PBMC) samples were enriched for CD4 T cells by negative selection (Dynabeads®Untouched™ Human CD4 T cells, Invitrogen) and CD4 T cell DNA extracted (QIAamp® DNA Blood Mini Kit, Qiagen). Cell copy number and total HIV DNA were quantified in triplicate using

albumin and HIV qPCR assays (27). The quantification of subtype C HIV DNA was validated comparing results from MJ4 and NL4-3 plasmids, containing subtype C and B HIV DNA sequences respectively. Integrated HIV DNA was measured by modified (14) *Alu-gag* nested qPCR (28). We measured total HIV DNA in African participants at both time-points (n=82 and 22 at baseline and TI, respectively) and integrated HIV DNA according to sample availability (n=54 and 22 at baseline and TI, respectively). HIV DNA data from the non-African participants were available from a previously published study (14).

Flow Cytometry

Cell surface staining was performed on thawed PBMCs from participants at baseline and TI according to sample availability (n=70/82 and 18/22, respectively). PBMCs from 10 healthy Africans were simultaneously run as controls. Two different panels of antibodies were used to explore markers of immune activation (CD25, CD69, CD38, HLA DR) and exhaustion (Lag-3, Tim-3, PD-1) (see Supplementary Material for full details, <http://links.lww.com/QAD/B378>).

Unspliced cell-associated HIV-1 RNA Transcript quantitation.

RNA was isolated from PBMCs of all 22 participants at TI, using the AllPrep DNA/RNA Mini kit (Qiagen) with the addition of an on-column double DNase digestion. The RNA assay was a modified version of those previously described (29, 30). Briefly, complementary DNA was subjected to two rounds of PCR using semi-nested primers. The HIV standard was generated *in vitro* transcribing the plasmid Sp5-NL4.3 (generously provided by the lab of D. Purcell) using the RiboMax Large Scale SP6 RNA production System (Promega). HIV RNA standards were diluted in

10ng/ μ L uninfected PBMC RNA. HIV measurements were normalized to input cellular RNA using the 18S gene with the Amplifluor Human/Mouse 18S rRNA Primer Set (FAM labelled; Millipore). The standards for this assay were derived from an HIV subtype B plasmid. Oligo dT and random hexamers were used for reverse transcription to remove any potential for subtype-specific bias. The HIV-specific component of the reaction was the same as for the HIV DNA assay, validated for subtype C amplification as above.

HLA class I phenotyping

HLA Class I type was determined to the oligo-allelic level using Dynal RELITM Reverse Sequence-Specific Oligonucleotide kits for the HLA-A, -B and -C loci (Dynal Biotech). To obtain four-digit typing, Dynal Biotech Sequence-Specific priming kits were used, in conjunction with the Sequence-Specific Oligonucleotide type.

CD8 T cell ELISpot assays.

HIV Gag-specific CD8 T cell responses were measured by IFN- γ ELISpot assays to overlapping peptides using methods described elsewhere (31, 32).

Statistical analysis.

Comparison of continuous variables was performed with Mann-Whitney, or Wilcoxon tests for matched samples. Spearman correlations were used to analyse HIV DNA, pVL and CD4 count associations. Correlations between virological,

immunological and reservoir markers were displayed through correlograms (R package “correlogram”), the order of biomarkers determined by hierarchical clustering. Associations between HIV DNA and immunological markers in the African participants were explored further with regression models. We used backward selection (exit criteria $P < 0.1$) on variables presenting with $P < 0.2$ in simple linear regression models to identify those to include in multivariable regression models. Integrated HIV DNA was not considered in these analyses because of the strong association with total HIV DNA. Association between baseline or TI markers and SPARTAC endpoints was assessed using Cox proportional hazards models. Kaplan-Meier survival analysis and log-rank tests were used to compare remission periods after TI. All plots and statistics were made on RStudio version 0.98.1103.

Results

Characteristics of African participants.

Characteristics of all participants at randomisation and TI, are shown in Table 1. For the African cohort, 82 female participants recruited from Uganda ($n=8$) and South-Africa ($n=74$) had samples available at pre-therapy baseline. All were originally randomised within a median [interquartile range, IQR] of 13.6 [10.9–16.9] weeks from the estimated date of seroconversion. All infections were with non-B HIV subtypes, mainly C (62.2%). Median [IQR] age was 25.0 [21.0–31.8] years. Heterosexual intercourse was the main mode of HIV transmission. We studied participants who had been randomly assigned to receive either deferred ART ($n=38$) or 48 weeks of ART (‘ART48’ group; $n=44$). Only 22 of the 44 ART48 participants in this sub-analysis had both received 48 full weeks of ART and suppressed viral

replication (pVL < 400 copies/ml) at the time of TI (Table 1). As a result, even though baseline pre-therapy data are included for the 44 treated participants, only data for 22 are available for analysis at TI. (Of the 22 participants excluded, 13 received less than 46 weeks of ART and 9 had a pVL > 400 copies/ml at TI).

Demographics of the African and non-African cohorts at randomisation and TI were similar except that time from the estimated date of seroconversion to randomisation was significantly longer in the African than non-African cohort with median [IQR] values of 13.6 [10.9-16.9] and 10.6 [7.1–13.6] weeks respectively (p< 0.001; Mann-Whitney).

Lower pVL and HIV DNA at baseline in African compared with non-African participants.

We compared the African cohort with participants from Europe, Australia and Brazil (Table 1; ‘non-African cohort’), reported previously (14). At pre-therapy baseline, the median [IQR] pVL in the African cohort (4.16 [3.37–4.82] log₁₀ copies/ml) was significantly lower than the non-African cohort (4.72 [4.06–5.27] log₁₀ copies/ml) (p<0.001; Mann-Whitney) (Table 1; Fig 1A). CD4 T cell counts were not significantly different between the African and non-African cohorts at either baseline or TI (Table 1; Fig 1B). Mean CD4 T cell counts improved over the 48 weeks of ART for both cohorts, although this was only statistically significant for the African participants (p<0.001; Wilcoxon signed-rank test) (Fig 1B, Fig S2A). At baseline, integrated (but not total) HIV DNA was lower in Africans compared with non-Africans (p<0.001; Mann-Whitney), while no difference in HIV DNA level was observed at TI (Table 1; Fig 1C, D). There was a significant decrease in both forms of

HIV DNA in African and non-African participants on ART ($p < 0.001$ for both; Wilcoxon signed-rank test) (Fig 1C, D, Fig S2B), the magnitude of the change in total and integrated HIV DNA being greater in the non-African cohort ($p = 0.013$ and 0.012 respectively; Wilcoxon signed-rank test).

Correlates of HIV reservoir size in African participants.

In African participants, total and integrated HIV DNA were closely correlated at randomisation and TI ($p < 0.001$; $\rho = 0.706$ and 0.843 respectively; Spearman) (Fig S3A, B), and were inversely associated with CD4 T cell count at baseline ($p < 0.001$, $\rho = -0.560$ for both forms; Spearman) (Fig 1E) and TI ($p = 0.049$, $\rho = -0.425$ for both forms; Spearman) (Fig S3C, <http://links.lww.com/QAD/B378>). HIV DNA was positively correlated with baseline pVL ($p < 0.001$; $R = 0.47$ and 0.60 for total and integrated HIV DNA respectively; Spearman) (Fig 1F).

We wished to determine how these markers associated more broadly with other virological, immunological and reservoir markers, and employed principle component analysis with correlograms (33) to represent the clustering and strength of individual associations (Fig 2). At baseline and TI, PD-1, HLA-DR and Lag-3 on both CD4 and CD8 T cells clustered together with similar positive correlations (Fig 2A, B). At baseline, there was also clustering with CD38 on CD8 T cells and Tim-3 on CD4 T cells. Association of these markers with HIV DNA was also evident at pre-therapy baseline (Fig 2A, red box), but was stronger at TI (Fig 2B, red box).

Associations between HIV DNA and immunological markers in the African participants were explored further with regression models. In the multivariable

analysis, Lag-3 expression on CD4 T cells, CD4 T cell count and pVL were independently associated with HIV DNA at baseline (Table S1, <http://links.lww.com/QAD/B378>). At TI, expression of Lag-3 and CD69 on CD4 T cells were associated with HIV DNA (Table S1, <http://links.lww.com/QAD/B378>).

HIV DNA and clinical progression.

The primary end-point in the SPARTAC trial was a composite of a CD4 T cell count decline to <350 cells/ μ l or initiation of long-term ART, and was used in this analysis as a correlate of disease progression (13). At baseline (n = 82), univariable Cox analyses identified HIV DNA (total and integrated), pVL and CD4 T cell count to be predictive of clinical progression (when adjusting for therapy), although only CD4 T cell count was independently associated with the primary endpoint in multivariable analyses (HR 0.47 per 100 cells/ μ l increase [CI 0.28-0.66]; p<0.001) (S2 Table, <http://links.lww.com/QAD/B378>). For T cell activation and exhaustion markers (when adjusted for ART duration), proportions of CD38+ and CD69+ CD4 T cells (p=0.008, HR 0.96 [0.93-0.98] and p=0.011, HR 1.47 [1.09-1.97], respectively), and Tim-3+ CD8 T cells (p=0.045, HR 0.91 [0.83-0.999]) were significantly associated with progression, although not in multivariable models (S3 Table, <http://links.lww.com/QAD/B378>). When measured at ART interruption, neither total nor integrated HIV DNA predicted progression (despite suggestive hazard ratios (HR [CI 95%]: 2.58 [0.19–34.69] and 10.2 [0.55–190.5], respectively) (S4 Table, <http://links.lww.com/QAD/B378>).

HIV DNA and time to viral rebound in African women.

Next we turned to analyses of time to viral rebound after TI, utilising data from the 22 African participants who received 48 weeks of ART and had undetectable pVL at TI. At baseline, total HIV DNA (HR 4.33 per log₁₀ increase [CI 1.12-16.84]; p=0.034) and pVL (HR 1.93 per log₁₀ increase [CI 1.17-3.17]; p=0.010) predicted time to pVL rebound in univariable models. When adjusted for pVL, CD4 T cell count or integrated HIV DNA, the association with pre-therapy total HIV DNA was no longer significant. When measured at TI, neither total (HR 3.48 per log₁₀ increase [CI 0.78-15.49]; p=0.102) nor integrated HIV DNA (HR 2.65 per log₁₀ increase [CI 0.79-8.9]; p=0.114) were associated with time to viral rebound, despite supportive hazard ratios. No activation or exhaustion marker was predictive of time to viral rebound at any time point (S5 Table, <http://links.lww.com/QAD/B378>).

Longer post-treatment remission in African women compared with non-African men.

We next compared time to pVL rebound after stopping ART in the African and non-African participants. Kaplan-Meier survival analysis revealed a significant delay in time to viral rebound in African compared to non-African participants (p<0.001; log-rank test) (Fig 3A), which was supported in multivariable Cox models adjusting for HIV DNA and CD4 T cell count (S6 Table, <http://links.lww.com/QAD/B378>). All non-African participants eventually experienced viral rebound after TI (Fig 3A), however five of the 22 African women controlled viraemia below 400 copies/ml after TI until the end of follow-up (median [IQR] 4.48 years [3.99-4.65]). In subsequent

analyses, we refer to these five individuals from the African cohort as “post-treatment controllers” (PTC), as opposed to the 17 African “non-controllers” (NC).

Comparison of post-treatment controllers and non-controllers.

Time from seroconversion to ART initiation was similar in the African PTC (median [IQR] 17.6 [17.1-17.8]) and NC groups (14.3 [13.0-17.6] weeks) ($p=0.147$; Mann-Whitney). At baseline, PTC had significantly lower pVL ($p=0.041$; Mann-Whitney) and higher CD4 T cell counts ($p=0.011$; Mann-Whitney) than NC. The median CD4 T cell count at TI was also higher in PTC ($p=0.006$; Mann-Whitney) (Fig 3B and C). A trend towards lower levels of total and integrated HIV DNA ($p=0.058$ and $p=0.085$, respectively; Mann-Whitney) in PTC was evident at baseline (Fig 3D and E). At ART interruption, no difference in total or integrated HIV DNA was found between groups (Fig 3D and E), however there was a trend for PTC to have lower levels of cell-associated RNA, with median [IQR] values of -0.36 [-0.38 - 1.38] versus 1.51 [0.90 - 2.10] \log_{10} copies/million copies of 18S RNA respectively ($P=0.153$) (Fig 3F). The only statistically significant difference between PTC and NC in regards to T cell activation and exhaustion (Fig 3G to J) in univariate analysis, was a lower proportion of PD1+ CD8 T cells in PTC at TI (Fig 3J), (although this result did not survive Bonferroni correction for multiple comparisons).

PTC maintain stable CD4 T cell counts and HIV DNA levels after TI

Measures of pVL, CD4 T cell count, and total HIV DNA during follow-up are presented for the five PTCs (Fig 4). Four PTC had subtype C HIV while the subtype

was unknown for the fifth PTC individual, as no sequence could be obtained. Three PTC experienced pVL blips after TI (maximum blips: 1,690, 5,110, 560 copies/ml), whereas two maintained undetectable viraemia throughout follow-up (Fig 4A). Two of the PTC had undetectable pVL at randomisation, prior to starting ART, and one just above the detection limit, at 459 copies/ml (Fig 4A). CD4 T cell counts increased during the 48 weeks of ART (median [IQR] 772 [639-775] cells/ μ l at baseline vs 1273 [1151-1294] cells/ μ l at TI) and then decreased after TI (although not significantly) when measured at the last documented trial time-point (962 [840-1028]/ μ l), after a median of 204 weeks (range 156 - 252) (Figure 4B and D). Total HIV DNA levels remained stable (median [IQR] values of 3.17 [3.07-3.61] at baseline, 3.09 [3.03-3.18] at TI and 2.96 [2.86-3.23] copies/million CD4 T cells at end of follow-up (Fig 4C and E)).

We looked for associations between PTC and HLA Class I – the key genetic association with HIV progression (34). Two participants with undetectable pVL prior to ART had the protective HLA-B*81:01 and made dominant responses to the HIV Gag TPQDLNTML epitope which is associated with control (35, 36); one of these also had B*35:01 (Table S7, <http://links.lww.com/QAD/B378>). In comparison, among 17 NC participants, 6 carried the disease susceptible allele HLA-B*58:02, and 4 had protective alleles HLA-B*81:01 or HLA-B*57:03.

Discussion

The SPARTAC trial offered the unique opportunity to study the outcome of a randomised treatment interruption in African women with PHI infected with non-B

HIV subtypes. To our knowledge, our study is the first to both characterise the impact of treatment interruption in African participants treated at PHI and also to compare HIV reservoir dynamics in African and non-African participants within the same RCT. Whereas interrupting ART is not recommended in current guidelines, there is increasing interest in the value of an intensively monitored TI in studies testing for virological remission or the impact of a novel intervention on the reservoir or host immunity. However, stopping ART should only be done in the context of a clinical study with a clearly defined question and under close supervision.

Our study has important confounding factors, which have been discussed previously (37), but which do not negate the difference in outcomes in the two study groups. First, we describe our cohorts as ‘African’ and ‘non-African’, although we could justifiably have described them as female and male. It has been previously documented that HIV-uninfected and infected women have higher CD4 T cell counts than men (38-42). In untreated individuals, HIV-infected women have lower pVL compared to men, including when adjusting for confounding factors (39, 43-45). HIV subtypes may also have differential characteristics, such as replication fitness and biology of transmission, which could impact disease progression and response to therapy (21, 22, 24-26). However, to date this has not been definitively proven in clinical cohorts due to confounders such as sex, ethnicity and heterogeneity in time intervals since seroconversion (46-48). Gender-matched studies will be necessary to determine whether sex or viral subtype is the major driver of our results as our non-African cohort were all male. However, the practicalities of a sex-matched study from PHI in Africa are not straightforward and a similar dataset may be hard to reproduce.

Pre-therapy African participants had lower integrated HIV DNA and pVL than non-African participants consistent with the sex difference and other studies (49). Correlations between pVL, CD4 T cell counts and HIV DNA have been previously described in African cohorts (20, 46, 50, 51), but without associated virological, immunological and clinical outcomes. HIV DNA levels pre-ART in the African participants correlated with pVL and several T cell activation and exhaustion markers (e.g. CD38, Lag-3, Tim-3). After 48 weeks of ART, Lag-3, CD69 and PD-1 expression on CD4 T cells correlated with HIV DNA. That a number of these associations existed independently supports previous data showing that immune activation in HIV-infected African individuals correlates with pVL and inversely with CD4 count (52).

In European cohorts, HIV DNA (14, 53-55) and immune activation and exhaustion (1, 56-59) have been reported as independent predictors of clinical progression. Different rates of disease progression have been associated with non-B subtypes of HIV (39, 47) and in untreated African individuals (60, 61). Consistent with these findings, we found baseline total and integrated HIV DNA, as well as several T cell activation and exhaustion markers, to be predictive of clinical progression in the African group.

Our study is one of the first to explore biomarkers of progression in Africans after ART TI. Although no marker measured at TI was significantly associated with clinical outcome despite high hazard ratios, this may be related to small sample size.

This might explain the disparity with the findings from the larger non-African SPARTAC cohort where HIV DNA (both total and integrated) predicted the primary end-point when measured at TI (14).

Even considering the variation between pVL and integrated (but not total) HIV DNA in the two cohorts at baseline, the extent of the difference in times to pVL rebound in the African and non-African cohort was unexpected. The five African individuals who did not experience pVL rebound during the course of follow up had lower pVL and higher CD4 counts than African non-controllers and lower HIV DNA levels prior to ART initiation. They also maintained higher CD4 counts at TI, and while no difference was found in HIV DNA levels, PTC had lower cell-associated RNA levels. Immune activation was similar in both groups, although there were fewer exhausted CD8 T cells in PTC based on a lower proportion of PD1+ cells. Three of the African PTC, therefore, exhibited characteristics more closely associated with T cell-mediated immune control as described in elite controllers, which again may also be impacted by the gender difference. This raises the important issue of discriminating post-treatment control from treated ‘controllers’ in these analyses, and the key value of pre-therapy measurements.

The best characterized cohort of post-treatment controllers is the VISCONTI study (6). The African PTC in our study had lower pVL and higher CD4 counts than VISCONTI PTC at baseline. Interestingly, African controllers had similar baseline levels of pVL and CD4 counts as spontaneous HIV controllers (HIC) described by Saez-Cirion *et al.* Occasional blips were recorded for some individuals in both

VISCONTI and our African controllers, and HIV DNA remained stable both on and off treatment. In VISCONTI, no over-representation of protective HLA class I alleles was described, whereas there was a high prevalence of risk alleles (6). We found the opposite picture in the African controllers, with none having disease susceptible alleles, and two making strong immune responses restricted by B*8101, a known protective allele. HIV-specific T cell immunity and subsequent escape (59) are drivers of viraemic control and rebound in untreated individuals, but are less well characterised in the context of post-treatment control. Finally, we showed that African controllers had comparable levels of immune activation to African non-controllers, whereas PTC in VISCONTI had low levels of T cell activation (6, 62).

SPARTAC provided the unique opportunity to characterise the HIV reservoir in African individuals with PHI, with the additional strength of randomised treatment interruption and natural history arms. Although it is likely that our dataset was under-powered for some of the outcomes, there was strong evidence for better virological control in the African participants after TI compared to the non-African cohort. However, the sustained control seen in five African women adds to the argument that the mechanisms driving the interaction between post-treatment remission and spontaneous control need further study.

Acknowledgements

The SPARTAC Trial Investigators: Trial Steering Committee: Independent Members- A Breckenridge (Chair), P Clayden, C Conlon, F Conradie, J Kaldor*, F Maggiolo, F Ssali, Country Principal Investigators- DA Cooper, P Kaleebu, G Ramjee, M Schechter, G Tambussi, JM Miro, J Weber. Trial Physician: S Fidler. Trial Statistician: A Babiker. Data and Safety Monitoring Committee (DSMC): T Peto (Chair), A McLaren (in memoriam), V Beral, G Chene, J Hakim. Co-ordinating Trial Centre: Medical Research Council Clinical Trials Unit, London (A Babiker, K Porter, M Thomason, F Ewings, M Gabriel, D Johnson, K Thompson, A Cursley*, K Donegan*, E Fossey*, P Kelleher*, K Lee*, B Murphy*, D Nock*). Central Immunology Laboratories and Repositories: The Peter Medawar Building for Pathogen Research, University of Oxford, UK (R Phillips, J Frater, L Ohm Laursen*, N Robinson, P Goulder, H Brown). Central Virology Laboratories and Repositories: Jefferiss Trust Laboratories, Imperial College, London, UK (M McClure, D Bonsall*, O Erlwein*, A Helander*, S Kaye, M Robinson, L Cook*, G Adcock*, P Ahmed*). Clinical Endpoint Review Committee: N Paton, S Fidler. Investigators and Staff at Participating Sites: Australia: St Vincents Hospital, Sydney (A Kelleher), Northside Clinic, Melbourne (R Moore), East Sydney Doctors, Sydney (R McFarlane), Prahran Market Clinic, Melbourne (N Roth), Taylor Square Private Clinic, Sydney (R Finlayson), The Centre Clinic, Melbourne (B Kiem Tee), Sexual Health Centre, Melbourne (T Read), AIDS Medical Unit, Brisbane (M Kelly), Burwood Rd Practice, Sydney (N Doong), Holdsworth House Medical Practice, Sydney (M Bloch), Aids Research Initiative, Sydney (C Workman). Coordinating Centre in Australia: Kirby Institute University of New South Wales, Sydney (P Grey, DA Cooper, A Kelleher, M Law). Brazil: Projeto Praca Onze, Hospital Escola Sao Francisco de Assis,

Universidade federal do Rio de Janeiro, Rio de Janeiro (M Schechter, P Gama, M Mercon*, M Barbosa de Souza, C Beppu Yoshida, JR Grangeiro da Silva, A Sampaio Amaral, D Fernandes de Aguiar, M de Fatima Melo, R Quaresma Garrido). Italy: Ospedale San Raffaele, Milan (G Tambussi, S Nozza, M Pogliaghi, S Chiappetta, L Della Torre, E Gasparotto), Ospedale Lazzaro Spallanzani, Roma (G DOffizi, C Vlassi, A Corpolongo). South Africa: Cape Town: Desmond Tutu HIV-1 Centre, Institute of Infectious Diseases, Cape Town (R Wood, J Pitt, C Orrell, F Cilliers, R Croxford, K Middelkoop, LG Bekker, C Heiberg, J Aploon, N Killa, E Fielder, T Buhler). Johannesburg: The Wits Reproductive Health and HIV-1 Institute, University of Witwatersrand, Hillbrow Health Precinct, Johannesburg (H Rees, F Venter, T Palanee), Contract Laboratory Services, Johannesburg Hospital, Johannesburg (W Stevens, C Ingram, M Majam, M Papathanasopoulos). Kwazulu-Natal: HIV-1 Prevention Unit, Medical Research Council, Durban (G Ramjee, S Gappoo, J Moodley, A Premrajh, L Zako). Uganda: Medical Research Council/Uganda Virus Research Institute, Entebbe (H Grosskurth, A Kamali, P Kaleebu, U Bahemuka, J Mugisha*, HF Njaj*). Spain: Hospital Clinic-IDIBAPS, University of Barcelona, Barcelona (JM Miro, M Lopez-Dieiguez*, C Manzardo, JA Arnaiz, T Pumarola, M Plana, M Tuset, MC Ligerio, MT Garca, T Gallart, JM Gatell). UK and Ireland: Royal Sussex County Hospital, Brighton (M Fisher, K Hobbs, N Perry, D Pao, D Maitland, L Heald), St James's Hospital, Dublin (F Mulcahy, G Courtney, S O'Dea, D Reidy), Regional Infectious Diseases Unit, Western General Hospital and Genitourinary Dept, Royal Infirmary of Edinburgh, Edinburgh (C Leen, G Scott, L Ellis, S Morris, P Simmonds), Chelsea and Westminster Hospital, London (B Gazzard, D Hawkins, C Higgs), Homerton Hospital, London (J Anderson, S Mguni), Mortimer Market Centre, London (I Williams, N De Esteban, P Pellegrino, A

Arenas-Pinto, D Cornforth*, J Turner*), North Middlesex Hospital (J Ainsworth, A Waters), Royal Free Hospital, London (M Johnson, S Kinloch, A Carroll, P Byrne, Z Cuthbertson), Barts & the London NHS Trust, London (C Orkin, J Hand, C De Souza), St Marys Hospital, London (J Weber, S Fidler, E Hamlyn, E Thomson*, J Fox*, K Legg, S Mullaney*, A Winston, S Wilson, P Ambrose), Birmingham Heartlands Hospital, Birmingham (S Taylor, G Gilleran). Imperial College Trial Secretariat: S Keeling, A Becker. Imperial College DSMC Secretariat: C Boocock. (* Left the study team before the trial ended.).

References

1. Churchill MJ, Deeks SG, Margolis DM, Siliciano RF, Swanstrom R. HIV reservoirs: what, where and how to target them. *Nat Rev Microbiol.* 2016;14(1):55-60.
2. Kandathil AJ, Sugawara S, Balagopal A. Are T cells the only HIV-1 reservoir? *Retrovirology.* 2016;13(1):86.
3. Strain MC, Little SJ, Daar ES, Havlir DV, Gunthard HF, Lam RY, et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J Infect Dis.* 2005;191(9):1410-8.
4. Jain V, Hartogensis W, Bacchetti P, Hunt PW, Hatano H, Sinclair E, et al. Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. *J Infect Dis.* 2013;208(8):1202-11.
5. Hocqueloux L, Avettand-Fenoel V, Jacquot S, Prazuck T, Legac E, Melard A, et al. Long-term antiretroviral therapy initiated during primary HIV-1 infection is key to achieving both low HIV reservoirs and normal T cell counts. *J Antimicrob Chemother.* 2013;68(5):1169-78.
6. Saez-Cirion A, Bacchus C, Hocqueloux L, Avettand-Fenoel V, Girault I, Lecuroux C, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. *PLoS Pathog.* 2013;9(3):e1003211.
7. Henrich TJ, Hanhauser E, Marty FM, Sirignano MN, Keating S, Lee TH, et al. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. *Ann Intern Med.* 2014;161(5):319-27.

8. Martin GE, Gossez M, Williams JP, Stohr W, Meyerowitz J, Leitman EM, et al. Post-treatment control or treated controllers? Viral remission in treated and untreated primary HIV infection. *AIDS*. 2017;31(4):477-84.
9. Deeks SG. HIV: Shock and kill. *Nature*. 2012;487(7408):439-40.
10. Ananworanich J, Gayet-Ageron A, Le Braz M, Prasithsirikul W, Chetchotisakd P, Kiertiburanakul S, et al. CD4-guided scheduled treatment interruptions compared with continuous therapy for patients infected with HIV-1: results of the Staccato randomised trial. *Lancet*. 2006;368(9534):459-65.
11. Danel C, Moh R, Minga A, Anzian A, Ba-Gomis O, Kanga C, et al. CD4-guided structured antiretroviral treatment interruption strategy in HIV-infected adults in west Africa (Trivacan ANRS 1269 trial): a randomised trial. *Lancet*. 2006;367(9527):1981-9.
12. Strategies for Management of Antiretroviral Therapy Study Group, El-Sadr WM, Lundgren J, Neaton JD, Gordin F, Abrams D, et al. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med*. 2006;355(22):2283-96.
13. Fidler S, Porter K, Ewings F, Frater J, Ramjee G, et al. Short-course antiretroviral therapy in primary HIV infection. *N Engl J Med*. 2013;368(3):207-17.
14. Williams JP, Hurst J, Stohr W, Robinson N, Brown H, Fisher M, et al. HIV-1 DNA predicts disease progression and post-treatment virological control. *Elife*. 2014;3:e03821.
15. Hurst J, Hoffmann M, Pace M, Williams JP, Thornhill J, Hamlyn E, et al. Immunological biomarkers predict HIV-1 viral rebound after treatment interruption. *Nat Commun*. 2015;6:8495.

16. Li JZ, Etemad B, Ahmed H, Aga E, Bosch RJ, Mellors JW, et al. The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption. *AIDS*. 2016;30(3):343-53.
17. Fidler S, Olson AD, Bucher HC, Fox J, Thornhill J, Morrison C, et al. Virological Blips and Predictors of Post Treatment Viral Control After Stopping ART Started in Primary HIV Infection. *J Acquir Immune Defic Syndr*. 2017;74(2):126-33.
18. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach - Second edition 2016 [Available from: <http://www.who.int/hiv/pub/arv/arv-2016/en/>].
19. Frater J. The impact of HIV-1 subtype on the clinical response on HAART. *J HIV Ther*. 2002;7(4):92-6.
20. Lewden C, Thiebaut R, Boufassa F, Coulibaly A, Malateste K, Seng R, et al. Comparison of early CD4 T-cell count in HIV-1 seroconverters in Cote d'Ivoire and France: the ANRS PRIMO-CI and SEROCO cohorts. *J Acquir Immune Defic Syndr*. 2010;53(2):260-5.
21. Iordanskiy S, Waltke M, Feng Y, Wood C. Subtype-associated differences in HIV-1 reverse transcription affect the viral replication. *Retrovirology*. 2010;7:85.
22. de Oliveira T, Engelbrecht S, Janse van Rensburg E, Gordon M, Bishop K, zur Megede J, et al. Variability at human immunodeficiency virus type 1 subtype C protease cleavage sites: an indication of viral fitness? *J Virol*. 2003;77(17):9422-30.
23. Ball SC, Abraha A, Collins KR, Marozsan AJ, Baird H, Quinones-Mateu ME, et al. Comparing the ex vivo fitness of CCR5-tropic human immunodeficiency virus type 1 isolates of subtypes B and C. *J Virol*. 2003;77(2):1021-38.

24. Renjifo B, Gilbert P, Chaplin B, Msamanga G, Mwakagile D, Fawzi W, et al. Preferential in-utero transmission of HIV-1 subtype C as compared to HIV-1 subtype A or D. *AIDS*. 2004;18(12):1629-36.
25. Taylor BS, Hammer SM. The challenge of HIV-1 subtype diversity. *N Engl J Med*. 2008;359(18):1965-6.
26. Lynch RM, Shen T, Gnanakaran S, Derdeyn CA. Appreciating HIV type 1 diversity: subtype differences in Env. *AIDS Res Hum Retroviruses*. 2009;25(3):237-48.
27. Jones M, Williams J, Gartner K, Phillips R, Hurst J, Frater J. Low copy target detection by Droplet Digital PCR through application of a novel open access bioinformatic pipeline, 'definetherain'. *J Virol Methods*. 2014;202:46-53.
28. Liszewski MK, Yu JJ, O'Doherty U. Detecting HIV-1 integration by repetitive-sampling Alu-gag PCR. *Methods*. 2009;47(4):254-60.
29. Lewin SR, Vesanen M, Kostrikis L, Hurley A, Duran M, Zhang L, et al. Use of real-time PCR and molecular beacons to detect virus replication in human immunodeficiency virus type 1-infected individuals on prolonged effective antiretroviral therapy. *J Virol*. 1999;73(7):6099-103.
30. Pasternak AO, Adema KW, Bakker M, Jurriaans S, Berkhout B, Cornelissen M, et al. Highly sensitive methods based on seminested real-time reverse transcription-PCR for quantitation of human immunodeficiency virus type 1 unspliced and multiply spliced RNA and proviral DNA. *J Clin Microbiol*. 2008;46(7):2206-11.
31. Kiepiela P, Leslie AJ, Honeyborne I, Ramduth D, Thobakgale C, Chetty S, et al. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. *Nature*. 2004;432(7018):769-75.

32. Frater J, Ewings F, Hurst J, Brown H, Robinson N, Fidler S, et al. HIV-1-specific CD4(+) responses in primary HIV-1 infection predict disease progression. *AIDS*. 2014;28(5):699-708.
33. Friendly M. Corrgrams: exploratory Displays for Correlation Matrices. *Am Stat*. 2002;56(4):316-24.
34. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, et al. A whole-genome association study of major determinants for host control of HIV-1. *Science*. 2007;317(5840):944-7.
35. Huang KH, Goedhals D, Carlson JM, Brockman MA, Mishra S, Brumme ZL, et al. Progression to AIDS in South Africa is associated with both reverting and compensatory viral mutations. *PLoS One*. 2011;6(4):e19018.
36. Matthews PC, Adland E, Listgarten J, Leslie A, Mkhwanazi N, Carlson JM, et al. HLA-A*7401-mediated control of HIV viremia is independent of its linkage disequilibrium with HLA-B*5703. *J Immunol*. 2011;186(10):5675-86.
37. Stohr W, Fidler S, McClure M, Weber J, Cooper D, Ramjee G, et al. Duration of HIV-1 viral suppression on cessation of antiretroviral therapy in primary infection correlates with time on therapy. *PLoS One*. 2013;8(10):e78287.
38. Kassu A, Tsegaye A, Petros B, Wolday D, Hailu E, Tilahun T, et al. Distribution of lymphocyte subsets in healthy human immunodeficiency virus-negative adult Ethiopians from two geographic locales. *Clin Diagn Lab Immunol*. 2001;8(6):1171-6.
39. Touloumi G, Pantazis N, Pillay D, Paraskevis D, Chaix ML, Bucher HC, et al. Impact of HIV-1 subtype on CD4 count at HIV seroconversion, rate of decline, and viral load set point in European seroconverter cohorts. *Clin Infect Dis*. 2013;56(6):888-97.

40. Tollerud DJ, Clark JW, Brown LM, Neuland CY, Pankiw-Trost LK, Blattner WA, et al. The influence of age, race, and gender on peripheral blood mononuclear-cell subsets in healthy nonsmokers. *J Clin Immunol*. 1989;9(3):214-22.
41. Delmas MC, Jadand C, De Vincenzi I, Deveau C, Persoz A, Sobel A, et al. Gender difference in CD4+ cell counts persist after HIV-1 infection. SEROCO Study Group. *AIDS*. 1997;11(8):1071-3.
42. CASCADE Collaboration . Differences in CD4 cell counts at seroconversion and decline among 5739 HIV-1-infected individuals with well-estimated dates of seroconversion. *J Acquir Immune Defic Syndr*. 2003;34(1):76-83.
43. Sterling TR, Lyles CM, Vlahov D, Astemborski J, Margolick JB, Quinn TC. Sex differences in longitudinal human immunodeficiency virus type 1 RNA levels among seroconverters. *J Infect Dis*. 1999;180(3):666-72.
44. Napravnik S, Poole C, Thomas JC, Eron JJ, Jr. Gender difference in HIV RNA levels: a meta-analysis of published studies. *J Acquir Immune Defic Syndr*. 2002;31(1):11-9.
45. Touloumi G, Pantazis N, Babiker AG, Walker SA, Katsarou O, Karafoulidou A, et al. Differences in HIV RNA levels before the initiation of antiretroviral therapy among 1864 individuals with known HIV-1 seroconversion dates. *AIDS*. 2004;18(12):1697-705.
46. Gray CM, Williamson C, Bredell H, Puren A, Xia X, Filter R, et al. Viral dynamics and CD4+ T cell counts in subtype C human immunodeficiency virus type 1-infected individuals from southern Africa. *AIDS Res Hum Retroviruses*. 2005;21(4):285-91.

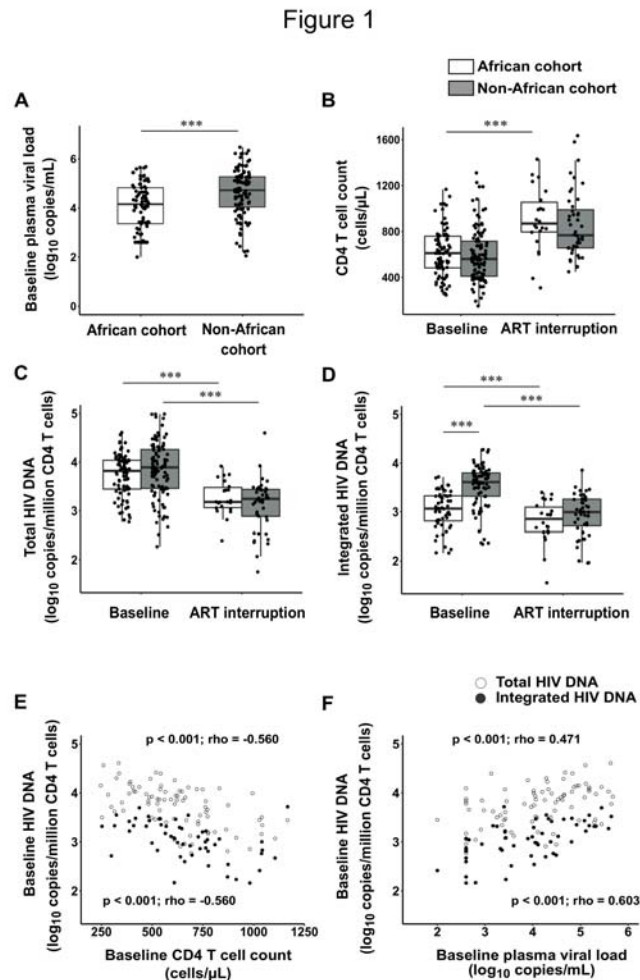
47. Vasan A, Renjifo B, Hertzmark E, Chaplin B, Msamanga G, Essex M, et al. Different rates of disease progression of HIV type 1 infection in Tanzania based on infecting subtype. *Clin Infect Dis*. 2006;42(6):843-52.
48. Klein MB, Young J, Dunn D, Ledergerber B, Sabin C, Cozzi-Lepri A, et al. The effects of HIV-1 subtype and ethnicity on the rate of CD4 cell count decline in patients naive to antiretroviral therapy: a Canadian-European collaborative retrospective cohort study. *CMAJ Open*. 2014;2(4):E318-29.
49. Rinke de Wit TF, Tsegaye A, Wolday D, Hailu B, Aklilu M, Sanders E, et al. Primary HIV-1 subtype C infection in Ethiopia. *J Acquir Immune Defic Syndr*. 2002;30(5):463-70.
50. Minga AK, Anglaret X, d' Aquin Toni T, Chaix ML, Dohoun L, Abo Y, et al. HIV-1 DNA in peripheral blood mononuclear cells is strongly associated with HIV-1 disease progression in recently infected West African adults. *J Acquir Immune Defic Syndr*. 2008;48(3):350-4.
51. Novitsky V, Woldegabriel E, Kebaabetswe L, Rossenkhan R, Mlotshwa B, Bonney C, et al. Viral load and CD4+ T-cell dynamics in primary HIV-1 subtype C infection. *J Acquir Immune Defic Syndr*. 2009;50(1):65-76.
52. Eggena MP, Barugahare B, Okello M, Mutyala S, Jones N, Ma Y, et al. T cell activation in HIV-seropositive Ugandans: differential associations with viral load, CD4+ T cell depletion, and coinfection. *J Infect Dis*. 2005;191(5):694-701.
53. Rouzioux C, Hubert JB, Burgard M, Deveau C, Goujard C, Bary M, et al. Early levels of HIV-1 DNA in peripheral blood mononuclear cells are predictive of disease progression independently of HIV-1 RNA levels and CD4+ T cell counts. *J Infect Dis*. 2005;192(1):46-55.

54. Kostrikis LG, Touloumi G, Karanickolas R, Pantazis N, Anastassopoulou C, Karafoulidou A, et al. Quantitation of human immunodeficiency virus type 1 DNA forms with the second template switch in peripheral blood cells predicts disease progression independently of plasma RNA load. *J Virol*. 2002;76(20):10099-108.
55. Goujard C, Bonarek M, Meyer L, Bonnet F, Chaix ML, Deveau C, et al. CD4 cell count and HIV DNA level are independent predictors of disease progression after primary HIV type 1 infection in untreated patients. *Clin Infect Dis*. 2006;42(5):709-15.
56. Liovat AS, Rey-Cuille MA, Lecuroux C, Jacquelin B, Girault I, Petitjean G, et al. Acute plasma biomarkers of T cell activation set-point levels and of disease progression in HIV-1 infection. *PLoS One*. 2012;7(10):e46143.
57. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, Jacobson LP, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis*. 1999;179(4):859-70.
58. Hazenberg MD, Otto SA, van Benthem BH, Roos MT, Coutinho RA, Lange JM, et al. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS*. 2003;17(13):1881-8.
59. Hoffmann M, Pantazis N, Martin GE, Hickling S, Hurst J, Meyerowitz J, et al. Exhaustion of Activated CD8 T Cells Predicts Disease Progression in Primary HIV-1 Infection. *PLoS Pathog*. 2016;12(7):e1005661.
60. Muller V, von Wyl V, Yerly S, Boni J, Klimkait T, Burgisser P, et al. African descent is associated with slower CD4 cell count decline in treatment-naive patients of the Swiss HIV Cohort Study. *AIDS*. 2009;23(10):1269-76.

61. Pantazis N, Morrison C, Amornkul PN, Lewden C, Salata RA, Minga A, et al. Differences in HIV natural history among African and non-African seroconverters in Europe and seroconverters in sub-Saharan Africa. *PLoS One*. 2012;7(3):e32369.
62. Rouzioux C, Hocqueloux L, Saez-Cirion A. Posttreatment controllers: what do they tell us? *Curr Opin HIV AIDS*. 2015;10(1):29-34.

ACCEPTED

Figure 1. Baseline and TI characteristics of reservoir-associated markers in the African and non-African cohorts.



Reservoir-associated markers were compared between the African cohort (open boxes) and non-African cohort (grey boxes) at baseline ($n = 82$ and 103 respectively) and ART interruption ($n = 22$ and 44 respectively). Plasma viral load (**A**), CD4 T cell count (**B**), total HIV DNA (**C**) and integrated HIV DNA (**D**) were studied. Correlations between HIV DNA and CD4 T cell count (**E**) and plasma viral load (**F**) were considered at baseline (open and black dots for total and integrated HIV DNA

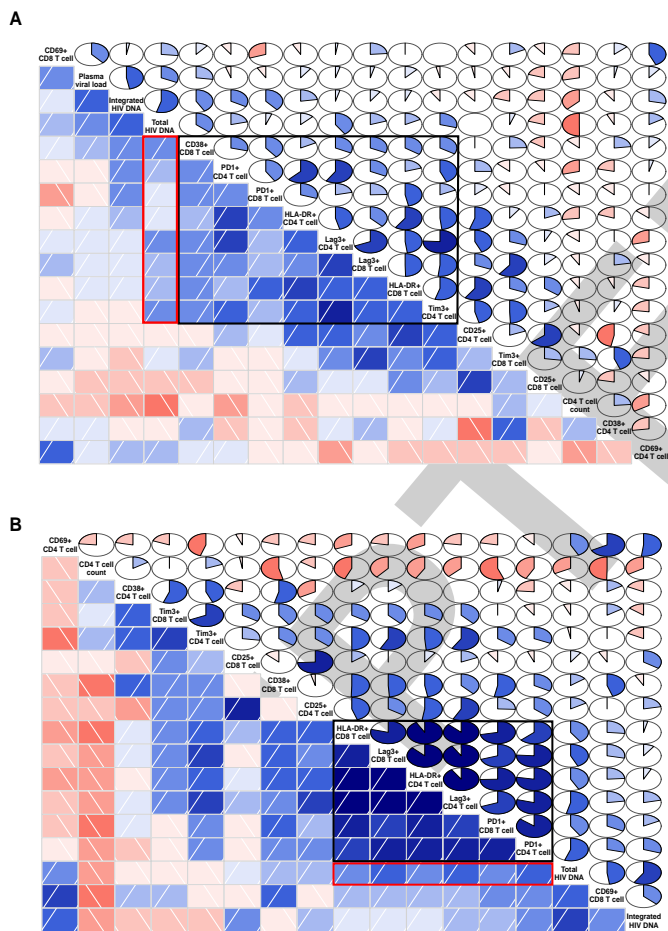
respectively). Integrated HIV DNA data at baseline were available for only 54 and 77 participants from the African and non-African cohorts respectively. Comparisons were performed using non-parametric Mann Whitney tests. When studying the evolution after 48 weeks of ART, only data from participants with results at both time points were analysed and compared by paired Wilcoxon test. Correlations were tested using Spearman correlations.

*** $p < 0.001$

ACCEPTED

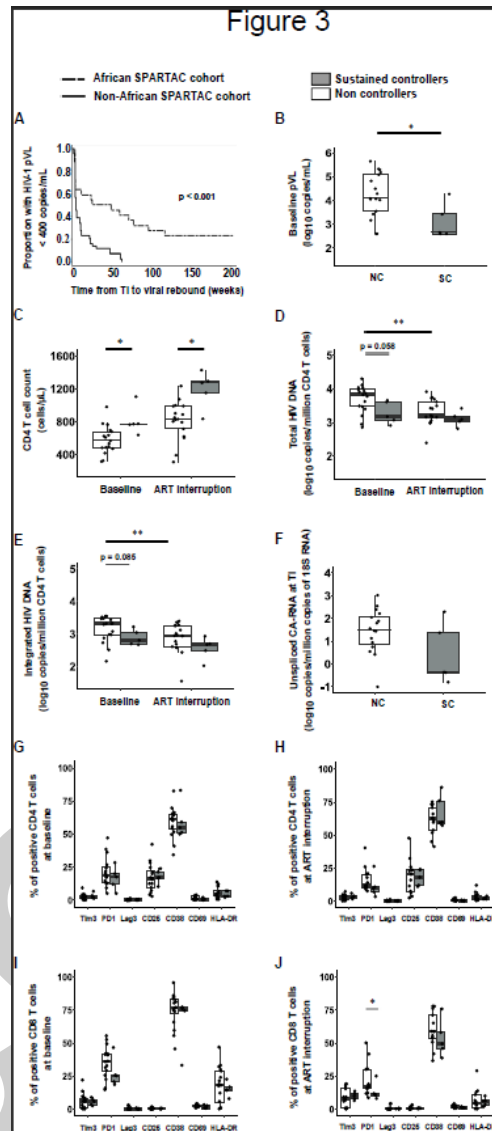
Figure 2. Association between reservoir-associated and immunological markers in the African cohort.

Figure 2



Correlograms of baseline (n = 70) (A) and ART interruption variables (n = 18) (B). Strength of associations is illustrated by pie charts and heat maps, red showing negative correlation and blue a positive correlation between parameters. Intensity of the colour and size of the pie is linked to the strength of the association. Variables were arranged by hierarchical clustering, in PC2/PC1 order. Immunological variables strongly associated together are indicated in the black box, and in red boxes if strongly associated with reservoir-associated markers.

Figure 3. Determinants of time to virological failure and sustained control in the African cohort



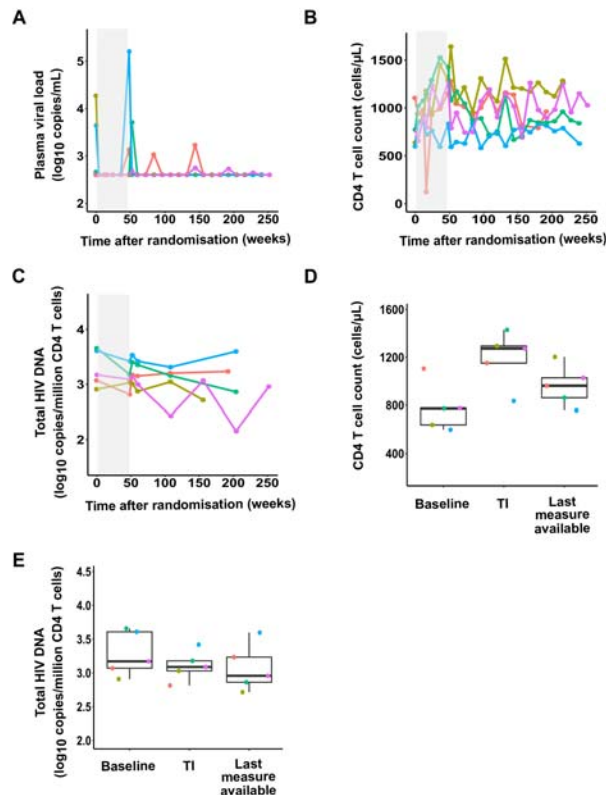
(A) Survival analyses of time to viral rebound (weeks) of participants undertaking TI after 48 weeks of ART in the African cohort (dashed line, $n = 22$) and the non-African cohort (solid line, $n = 44$). Significance was determined by log-rank test. Reservoir-associated markers were compared between the sustained controllers (SC; grey boxes; $n = 5$) and the non controllers (NC; open boxes; $n = 17$) from the African cohort:

plasma viral load (**B**) was measured at baseline, while CD4 T cell count (**C**), total HIV DNA (**D**) and integrated HIV DNA (**E**) were studied at both baseline and TI; unspliced cell-associated HIV RNA (**F**) was analysed at TI. Immunological markers were also compared in the sustained controllers (n = 5) and the non controllers (n = 16 at baseline and 13 at TI). Expression of activation and exhaustion markers was studied on CD4 and CD8 T cells at baseline (**G** and **I** respectively) and TI (**H** and **J** respectively). Comparisons between the two groups were performed using non-parametric Mann Whitney test, while comparison between time-points within a same group was done using a paired Wilcoxon test.

* $p < 0.05$, ** $p < 0.01$

Figure 4. Individual evolution of reservoir-associated markers in sustained controllers from the African cohort during the follow-up period of the trial.

Figure 4



Plasma viral load (A), CD4 T cell count (B) and total HIV DNA (C) were measured at different time-point throughout the clinical trial in the 5 sustained controllers. These individuals underwent 48 weeks of ART during primary HIV infection (grey area) then interrupted the treatment. Global evolution of CD4 T cell count (D) and total HIV DNA (E) in the group of sustained controllers was studied at pre-therapy baseline, ART interruption and last time point with samples available for total HIV DNA measurement (median of 204 weeks, min 156 - max 252 weeks).

Table 1. Participant characteristics for African and non-African participants.

	BASELINE		ART INTERRUPTION	
	Non-African (n = 103)	African (n = 82)	Non-African (n = 44)	African (n = 22)
Age (years)	35.0 [30.0 –	25.0 [21.0 –	36.0 [31.3 –	26.0 [21.3 –
Number of males:females	103:0	0:82	44:0	0:22
Country				
UK	68 (66.0%)	0	27 [61.4%]	0
Uganda	0	8 (9.8%)	0	2 [9.1%]
South Africa	0	74 (90.2%)	0	20 [90.9%]
Africa	35 (34.0%)*	0	17 [38.6%]	0
Other				
HIV-1 clade				
A1	0	2 (2.4%)	0	1 (4.5%)
B	103 (100%)	0	44 (100%)	0
C	0	51 (62.2%)	0	19 (86.5%)
D	0	3 (3.7%)	0	1 (4.5%)
Unknown	0	26 (31.7%)	0	1 (4.5%)
Time from seroconversion to randomisation (weeks)	10.6 [7.1 – 13.6]	13.6 [10.9 – 16.9]	10.9 [7.3 – 13.2]	15.5 [13.2 – 17.7]
Plasma viral load (log₁₀ copies/mL)	4.72 [4.06 – 5.27]	4.16 [3.37 – 4.82]	<400	<400
CD4 T cell count (cells/μl)	560 [410 – 715]	611 [483 – 759]	767 [657 – 989]	869 [796 – 1055]
Total HIV DNA (log₁₀ copies/million CD4 T cells)	3.89 [3.46 – 4.27]	3.82 [3.44 – 4.04]	3.24 [2.89 – 3.44]	3.18 [3.06- 3.45]
Integrated HIV DNA (log₁₀ copies/million CD4 T cells)	3.61 [3.32 – 3.80]	3.07 [2.81 – 3.33]	2.99 [2.71 – 3.26]	2.85 [2.59- 3.22]
Time from randomisation to last plasma viral load measurement (months)	50.2 [43.7 – 56.7]	48.6 [43.4 – 52.6]	53.6 [48.2 – 61.6]	50.2 [43.9 – 54.9]

Data are presented as values for categorical data or medians and interquartile ranges [Q1-Q3] in brackets for continuous variables.

* 14 from Australia, 12 from Italy, 8 from Brazil and 1 from Ireland.

ACCEPTED