

# UCSF

## UC San Francisco Previously Published Works

### Title

Impact of Allogeneic Hematopoietic Stem Cell Transplantation on the HIV Reservoir and Immune Response in 3 HIV-Infected Individuals

### Permalink

<https://escholarship.org/uc/item/71f8x986>

### Journal

J AIDS Journal of Acquired Immune Deficiency Syndromes, 75(3)

### ISSN

1525-4135

### Authors

Koelsch, Kersten K  
Rasmussen, Thomas A  
Hey-Nguyen, William J  
[et al.](#)

### Publication Date

2017-07-01

### DOI

10.1097/qai.0000000000001381

Peer reviewed



# HHS Public Access

Author manuscript

*J Acquir Immune Defic Syndr.* Author manuscript; available in PMC 2018 August 18.

Published in final edited form as:

*J Acquir Immune Defic Syndr.* 2017 July 01; 75(3): 328–337. doi:10.1097/QAI.0000000000001381.

## Impact of allogeneic hematopoietic stem cell transplantation on the HIV reservoir and immune response in three HIV infected individuals

KK Koelsch<sup>1,2,\*</sup>, TA Rasmussen<sup>3</sup>, WJ Hey-Nguyen<sup>1</sup>, C Pearson<sup>1</sup>, Y Xu<sup>1</sup>, M Bailey<sup>1</sup>, KH Marks<sup>2</sup>, SC Sasson<sup>2</sup>, MS Taylor<sup>2</sup>, R Tantau<sup>2</sup>, S Obeid<sup>2</sup>, B Milner<sup>2</sup>, CO Morrissey<sup>4,5</sup>, AN Pinto<sup>1,2</sup>, K Suzuki<sup>1,2</sup>, MP Busch<sup>6</sup>, SM Keating<sup>6</sup>, P Kaiser<sup>7,8</sup>, S Yuki<sup>7,8</sup>, JK Wong<sup>7,8</sup>, BM Hiener<sup>9</sup>, S Palmer<sup>9,10</sup>, J Zauanders<sup>1,2</sup>, JJ Post<sup>11,12,13</sup>, DJ Chan<sup>12,14</sup>, S Avery<sup>4</sup>, ST Milliken<sup>2,15</sup>, AD Kelleher<sup>1,2</sup>, SR Lewin<sup>3,4</sup>, and DA Cooper<sup>1,2</sup>

<sup>1</sup>The Kirby Institute, UNSW Medicine, UNSW Australia, NSW, Australia

<sup>2</sup>St Vincent's Hospital, Sydney, Darlinghurst, NSW, Australia

<sup>3</sup>The Peter Doherty Institute for Infection and Immunity, University of Melbourne, VIC, Australia

<sup>4</sup>The Alfred Hospital, Melbourne, VIC, Australia

<sup>5</sup>Monash University, VIC, Australia

<sup>6</sup>Blood Systems Research Institute, San Francisco, California, USA

<sup>7</sup>University of California, San Francisco, CA, USA

<sup>8</sup>Veterans Affairs Medical Centre, San Francisco, CA, USA

<sup>9</sup>Westmead Millennium Institute, Westmead, NSW, Australia

<sup>10</sup>University of Sydney, Sydney, NSW, Australia

<sup>11</sup>Prince of Wales Hospital, Randwick, NSW, Australia

<sup>12</sup>The Albion Centre, Surry Hills, NSW, Australia

<sup>13</sup>Prince of Wales Clinical School, UNSW Medicine, UNSW Australia, NSW, Australia

<sup>14</sup>School of Public Health, UNSW Medicine, UNSW Australia, NSW, Australia

<sup>15</sup>The Kinghorn Cancer Centre, Darlinghurst, NSW, Australia

### Abstract

\*Corresponding author: Dr Kersten Koelsch, MD, The Kirby Institute, UNSW Medicine, UNSW Australia, Level 6, Wallace Wurth Building, Sydney, NSW, 2052, Australia, t: +61-2-9385-0466, f: +61-2-9385-0920, m: +61 415 092 182, kkoelsch@kirby.unsw.edu.au, w: [www.kirby.unsw.edu.au](http://www.kirby.unsw.edu.au).

#### Conflict of Interest and Funding

None of the authors report a conflict of interest. This work was supported by the Australian National Health and Medical Research Council (NHMRC) through a Program Grant 1052979 (DAC, ADK, SRL) and Fellowships 1020536 (ADK) and 1063422 (JZ) and 1042654 (SRL), a grant from the St. Vincent's Clinic Foundation (KK). The Kirby Institute is funded by the Australian Government Department of Health & Ageing. SRL is funded by the Delaney AIDS Research Enterprise (DARE), National Institutes of Health (U19 AI096109) and a High Impact Research Grant from the American Foundation for AIDS Research (109226–58-RGRL).

**Background:** Allogeneic hematopoietic stem cell transplantation (HSCT) can lead to significant changes to the HIV reservoir and HIV immune responses, indicating that further characterisation of HIV infected patients undergoing HSCT is warranted.

**Methods:** We studied three patients who underwent HSCT after either reduced intensity conditioning or myeloablative conditioning regimen. We measured HIV antigens and antibodies (Ag/Ab), HIV specific CD4+ T cell responses, HIV RNA and DNA in plasma, peripheral blood mononuclear cells (PBMCs), isolated CD4+ T cells from peripheral blood and lymph node cells. The patients remained on ART throughout the follow up period.

**Results:** All patients have been in continued remission for 4–6 years post-HSCT. Analyses of HIV RNA and DNA levels showed substantial reductions in HIV reservoir related measurements in all three patients, changes in immune response varied with pronounced reductions in two patients and a less dramatic reduction in one patient. One patient experienced unexpected viral rebound 4 years after HSCT.

**Conclusion:** These three cases highlight the substantial changes to the HIV reservoir and the HIV immune response in patients undergoing allogeneic HSCT. The viral rebound observed in one patient indicates that replication competent HIV can re-emerge several years following HSCT despite these marked changes.

---

## Introduction:

Human immunodeficiency type-1 (HIV) infection persists despite antiretroviral therapy (ART). Cessation of ART is followed by viral rebound in most patients. Current knowledge suggests that rebounding virus is released from stable, long-lived reservoirs, which pose the primary obstacle towards a cure for HIV infection [1, 2]. The mechanisms underlying the establishment of HIV reservoirs in various cells and tissue compartments, and the maintenance of these reservoirs are currently under intense investigation. In parallel, new therapeutic strategies that target HIV persistence are in development, although at this stage with limited success [3].

Allogeneic hematopoietic stem cell transplantation (HSCT) is a well-established therapeutic procedure for haematological malignancies, often the approach of choice in the context of clinical relapse following conventional chemotherapy. In 2008 one patient, Timothy Brown, also widely known as the ‘Berlin patient’, received an allogeneic HSCT using donor cells with a homozygous deletion in their C-C chemokine receptor type 5 (CCR5) gene (CCR5-32/32) making them resistant to HIV infection. Remarkably, ART was ceased in this patient following HSCT and he has since not experienced viral rebound [4]. Detailed studies of plasma, cells and tissues from this patient were unable to convincingly detect any residual virus [5]. This case has fuelled hopes that, for example, gene therapeutic approaches attempting to artificially induce CCR5 deficiency in stem cells or autologous CD4+ T cells might achieve significant reductions of the HIV reservoir. However, in another six patients who received an allogeneic HSCT with CCR5-32/32 donor cells this approach resulted in either early viral rebound and/or death within the first 12 months following HSCT [6, 7].

Furthermore, a study of two patients (the ‘Boston patients’) who underwent reduced intensity conditioning (RIC) and received an allogeneic HSCT without the inclusion of

CCR5- 32/ 32 cells reported significant changes in their HIV immune response and the HIV reservoir. In both patients ART was temporarily ceased, but both experienced viral rebound requiring re-initiation of ART [8, 9]. Despite these disappointing outcomes, no other intervention in chronically infected patients has shown such a dramatic effect on the HIV reservoir, and therefore further investigations into the effects of allogeneic HSCT in the HIV infected population are warranted.

Here we report results from three patients residing in Australia who underwent allogeneic HSCT. Immune responses to HIV as well as the size of the HIV reservoir were characterised in detail in peripheral blood as well as lymphoid tissue.

## Methods:

We studied three patients (A, B and C) who underwent allogeneic HSCT with RIC (patients A and B) or myeloablative conditioning (patient C). This was an observational study, HSCT was performed because of hematological malignancies. Patient C was enrolled prospectively to study the impact of HSCT on HIV persistence prior to receiving HSCT, patients A and B were followed retrospectively. Blood (patients A, B and C) and lymphoid tissue from fine needle biopsies of peripheral (inguinal) lymph nodes (LN, patients A and B only) were collected at several time points before and after allogeneic HSCT. HIV RNA and DNA in blood and tissues, HIV immune responses and drug levels in blood, and HIV sequencing was performed by using either routine clinical laboratory assays or specific in-house assays as detailed in the supplementary methods section. All three patients provided written and informed consent to participate in the study. The study was approved by the St Vincent's Hospital, Sydney and The Alfred Hospital, Melbourne Human Research Ethics committees.

## Patient specific findings

**Patient A clinical history:** Patient A is male, diagnosed with HIV infection in 1987 at age 22. In 2006, he was first treated with chemotherapy for a plasmablastic non-Hodgkin lymphoma (NHL). The lymphoma subsequently multiply relapsed including after an autologous stem cell transplant (a poor prognostic factor), and he received an unrelated HLA matched allogeneic HSCT in April 2010 (Figure 1 patient A) with RIC (fludarabine/melphalan, FluMel). He has been on ART throughout the procedure and during follow up. Post-HSCT this patient developed moderate to severe systemic acute and chronic graft versus host disease (GVHD), prolonged lymphopenia and CMV reactivation. Other co-morbidities include steroid related osteopenia, squamous cell carcinoma (SCC) of the tongue and anal SCC. The patient had good donor engraftment post HSCT with full donor chimerism. We only have CCR5 information post-transplant on this patient. Blood samples, as well as buccal swabs, were +/- for CCR5 in this patient post-transplant.

**Patient A HIV related findings:** A pre-HSCT HIV specific serology obtained in 2008 was positive on both CMIA and western blot analysis (see Table 1 patient A). CMV serology obtained in 2010 was positive for IgG (203 IU/mL). Nearly four years after the HSCT (February 2014) the HIV specific serology was markedly changed with moderate antigen and antibody (Ag/Ab) detectability by CMIA and EIA and predominantly trace Ab detectability on the western blot. HIV RNA in plasma was undetectable at the same time-

point by standard diagnostic assay (Roche® HIV test version 2.0) and by single copy real-time PCR (Table 3). HIV DNA was absent in peripheral blood CD4+ T cells post-HSCT in early 2014 on two occasions and by three separate assays (UCSF, St Vincent's Hospital (SVH) and the Kirby Institute laboratory). HIV DNA was not quantified prior to HSCT (Figure 1 patient A). CMV IgG Ab was positive pre- and post-HSCT. There was a very strong CD4+ T cell response against CMV Ag in this patient while CD4+ T cell responses against HIV Ag were absent post-HSCT.

Surprisingly, in late 2014 this patient experienced HIV rebound with HIV plasma virus RNA levels of >10,000,000 copies/mL and immunological seroconversion, with detectable HIV Ag/Ab levels by CMIA, EIA and western blot (Figure 1 patient A, Table 1 patient A). A small increase in HIV Ab concentration was detected using the LS Vitros Anti-HIV 1+2 assay (Figure 2). HIV DNA was readily detectable in peripheral blood cells after the HIV rebound (Table 3). Sequencing analyses post-viral rebound confirmed that the rebounding virus was phylogenetically related to the pre-transplant virus (Figure 3). No antiretroviral resistance mutations were detectable by genotypic resistance testing of the rebounding virus. The patient reported strict treatment adherence, but ART drug levels were profoundly reduced at the time of viral rebound, possibly attributable to poor drug absorption due to an exacerbation of gastrointestinal GVHD or undisclosed poor adherence (Table 2).

**Patient B clinical history:** Patient B is male, diagnosed with HIV infection in 2002 at age 49. The zenith HIV RNA was 9940 copies/mL and CD4+ T cell nadir count was 270 cells/ $\mu$ L (15%). In 2004 he was diagnosed with stage 4 diffuse large B cell lymphoma with predominant hepatic involvement, which was treated successfully with chemoradiation therapy. He subsequently commenced zidovudine/lamivudine/lopinavir/ritonavir. In 2009 he was diagnosed with acute myeloid leukemia (AML) with dysplastic features (possibly secondary to prior chemo-radiotherapy), and following chemotherapy went into dysplastic clonal remission with overall poor prognosis. In 2011 he relapsed with AML and myelodysplastic syndrome (MDS) and again achieved a remission with further chemotherapy. He then underwent an unrelated, HLA matched allogeneic HSCT in May 2011 following RIC (FluMel) (Figure 1 patient B). Post-HSCT this patient only developed mild and self-limiting GVHD (macular trunk/shoulder rash). The only other significant adverse event relating to the HSCT was a prolonged lymphopenia post-transplant. Non-haematological co-morbidities include HIV associated neurocognitive disorder, a cerebral aneurysm and cerebral toxoplasmosis. The recipients CCR5 gene PCR results were +/- and he had full donor-chimerism following HSCT. He is being treated with tenofovir/emtricitabine/raltegravir since 2012.

**Patient B HIV related findings:** Pre-HSCT, HIV specific serology was positive by both CMIA assays and western blot (Table 1). Post-transplant western blot analyses showed a profound reduction in HIV antibodies. In fact, no HIV antibodies were detectable by western blot in late 2014. The LS Vitros Anti-HIV 1+2 assay showed very low and unchanging levels at two separate time points (Figure 2). Some low level reactivity was measured by the EIA assay (Genscreen 0.308 and 0.319 absorbance units (duplicates), with a cut off at 0.124 absorbance units) and CMIA assay (Architect 1.27 and 1.37 S/CO, with a cut off at 0.80).

HIV RNA in plasma was undetectable throughout the follow up post-HSCT by routine laboratory testing, and by the single copy assay in May 2014. Post-HSCT HIV DNA was undetectable in this patient in both peripheral blood CD4+ T cells (three separate laboratories, UCSF, SVH and KI), and in lymphocyte cells from peripheral lymph node fine needle biopsies (performed at KI only). HIV DNA was not quantified in this patient prior to HSCT. In addition, cell-associated unspliced (CA-US) HIV RNA was also not detectable in neither peripheral blood CD4+ T cells or lymphoid tissue (all results Figure 1 patient B) post-HSCT. His CD4+ T cell count remained stable post-transplant at 500–600 cells/ $\mu$ L.

**Patient C clinical history:** Patient C is male, diagnosed with HIV infection in 2004 at age 43 and initiated on ART (tenofovir, emtricitabine and efavirenz) in Nov 2007. His nadir CD4+ T cell count was 256/ $\mu$ L, his plasma HIV RNA levels peaked at 35,700 copies/mL prior to initiating ART. He was diagnosed with AML (FLT3+/NPM1+) in July 2012 and underwent induction chemotherapy with high-dose cytarabine/idarubicin (HiDAC) and a single course of idarubicin/cytarabine/etoposide (Little ICE) consolidation chemotherapy. At commencement of induction chemotherapy efavirenz was switched to raltegravir while continuing tenofovir/emtricitabine to minimise drug-drug interactions. Induction chemotherapy was complicated by *Candida glabrata* fungaemia, treated with caspofungin. Complete remission was achieved following induction chemotherapy, but as he was at high risk of AML relapse he underwent a myeloablative HLA-matched sibling HSCT in October 2012 and was enrolled in a prospective observational study to quantify his HIV reservoir following HSCT. The conditioning regimen included cyclophosphamide and total body irradiation; GVHD prophylaxis was with cyclosporin and methotrexate. The HSCT was complicated by mucositis, *Clostridium difficile* diarrhoea and dapsone-induced methaemoglobinaemia. While routine chimerism analysis showed 98% donor chimerism for CD34+ cells on day 150 post-HSCT, this declined to 89.5% 180 days post-HSCT. As a result immunosuppressive therapy was discontinued, precipitating mild oral and cutaneous (biopsy proven) GVHD. Both resolved with topical corticosteroid treatment. Repeat testing on day 210 post-HSCT showed 100% donor chimerism in all cell subsets. Both the donor and the recipient were CCR5 +/- as assessed by PCR genotyping of recipient cells pre- and post-transplant.

**Patient C HIV related findings:** Patient C had a CD4+ T cell count of 168 cells/ $\mu$ L (43%) at the time of transplant but this increased steadily, reaching 371 cells/ $\mu$ L (29%) 12 months post-HSCT (Figure 1 patient C). The level of HIV DNA in PBMCs immediately prior to HSCT was 15 copies/ $10^6$  cells and 4 copies/ $10^6$  cells 6 months after HSCT, where CA HIV DNA was not detected in one of 2 replicates. CA HIV DNA was not detected at 3 and 9 months following HSCT (quantification limit was 14 and 18 copies/ $10^6$  cells, respectively). CA HIV DNA was detected pre-HSCT (39 copies/ $10^6$  cells) in isolated CD4+ T cells, but was beneath the lower limit of detection in CD4+ T cells obtained 12 and 36 months post-HSCT. Levels of CA-US RNA were analysed in PBMCs immediately prior to and 3, 6 and 9 months post-HSCT. CA-US RNA was detected at 1 copy per million cell equivalents pre-HSCT and again at 9 months post-HSCT at 1 copy per million cell equivalents. At 3 and 6 months post-HSCT CA-US RNA was not detected. In isolated CD4+ T cells, the pre-HSCT CA-US RNA level was 199 copies per million cell equivalents. CA-

US RNA could not be detected in CD4+ T cells obtained 12 and 36 months post-HSCT. After having achieved ART-induced viral suppression in 2007, plasma HIV RNA remained below 20 copies/mL until HSCT except for one measurement of 29 copies/mL in 2012. Furthermore, plasma HIV RNA was below the limit of detection of the clinical assay (AmpliPrep/COBAS®TaqMan® HIV test version 2.0; limit of detection 20 copies/mL) when analysed immediately prior to HSCT and 3, 6, 9, 12 and 36 months post-HSCT. Similarly, plasma HIV RNA remained below the limit of detection of the single copy assay (<0.3 or <0.5 per mL of plasma) pre-HSCT and 6 and 12 months post-HSCT. The results of serological analyses for patient C did, overall, not indicate any change in antibody quantity in the 12 months following HSCT (Table 1 patient C). Thus, HIV specific serology was positive with both the Liaison XL Ag/Ab assay and western blot analyses prior to HSCT and when analysed 6 and 12 months post-HSCT. However, HIV-Ab concentration measured by the LS Vitros Anti-HIV 1+2 assay showed a moderate decrease 12 months following HSCT (Figure 2).

## Discussion:

In this study, we assessed the HIV specific immune responses and virological correlates of HIV persistence in three patients who underwent allogeneic HSCT in Australia. In two of three patients (patients B and C) we have been unable to measure any virus post-transplant in plasma, CD4+ T cells (B and C) and LN tissue (B only), despite applying a range of sensitive assays. Additionally, in patients A and B we detected a remarkable reduction of HIV serological markers (Ags and Abs). These cases confirm the ability of allogeneic HSCT to profoundly reduce the HIV reservoir size below the sensitivity of PCR-based detection methods and prompt the question of: under what circumstances one might consider suggesting an analytic treatment interruption (ATI) to definitively assess the extent of HIV suppression or possible eradication.

While all three patients remained on ART throughout the transplant procedure and follow up period, one patient (A) experienced HIV rebound together with a repeat HIV seroconversion despite very low HIV antibodies and undetectable HIV RNA and DNA prior to viral rebound. Sequencing of the rebounding viral RNA and available pre-transplant RNA strongly suggested relatedness of these respective viral strains, indicating that a superinfection was unlikely. The patient reported strict drug adherence, but antiretroviral drug levels were low in this patient prior to viral rebound, possibly explained by poor drug absorption or non-disclosed poor adherence.

Patient B also continued ART and experienced an even more pronounced reduction in HIV specific antibodies post-HSCT, no detectable HIV RNA in plasma by either routine as well as single copy real-time PCR assays, and no detectable CA-HIV DNA or CA-US HIV RNA in both peripheral blood CD4+ T cells or lymphoid tissue.

In patient C, levels of CA-US HIV RNA and HIV DNA were low but consistently detectable before HSCT. Conversely, apart from 6 months following HSCT where HIV DNA was detected, no CA or plasma virus has been detected during 36 months of follow up after HSCT despite applying sensitive detection methods. Similar to the Berlin patient, patient C

underwent myeloablative conditioning prior to HSCT, but most likely did not receive CCR5 deficient donor cells. Patient C patient was not interested in undergoing treatment interruption. Therefore, we discussed with him and agreed that there was therefore no indication to perform leukapheresis or tissue biopsies as even if these were both negative, he would still not be interested in discontinuing ART, given the current available knowledge of risks of an ATI in this setting [10]. In addition to Timothy Brown, we note only few other cases of myeloablative allogeneic HSCT in an HIV patient who subsequently went on to discontinue ART. One patient from Essen, Germany, who stopped ART prior to myeloablation and CCR5- 32/ 32 HSCT but experienced viral rebound 3 weeks post-HSCT [6] This patient had pre-existing X4 virus minority variants. Also, at the recent AIDS 2016 conference in Durban other cases of SCT from CCR5- 32/ 32 were presented [11].

There are a number of limitations to our study. Firstly, patients A and B were only studied retrospectively, therefore information on the donors are unavailable, as well as baseline measurements of the HIV reservoir in these patients. Similarly, we do not have results for viral tropism studies on our patients, but given their CCR5 genotypes we believe that this information would not add significantly to the interpretation of the study results. Also, for all three patients, we did not perform more extensive blood sampling such as leukapheresis or gut-biopsies, or have donor CCR5 genotyping results; however, these are available for all three recipients. For patients A and B inguinal lymph node biopsies have been performed. There are also limitations in regard to the detectability of HIV-1 DNA in blood specimen, or biopsies, using any type of real time PCR or other assays. The frequencies of CD4+ T cells carrying HIV DNA can vary widely from 1 copy per  $10^6$  cells to 500–1000 copies per  $10^6$  cells, and these numbers depend on the patient samples analysed and on the assays used (for an overview see [12]). Therefore, we cannot rule out residual HIV DNA in our patients definitively. In fact, it is, based on our current knowledge regarding HIV reservoirs, conceivable that some HIV DNA may persist in these patients, in particular in tissues. Nevertheless, compared with other interventions described to date, the effects on HSCT on the HIV reservoir size measured here are remarkable.

Persisting HIV reservoirs despite ART are the prime obstacle towards eradication. Hopes that HIV eradication, or a functional cure, might be attainable were fuelled by the report of the Berlin Patient who underwent allogeneic HSCT with CCR5- 32/ 32 donor cells [5]. Reports from the two Boston patients [8, 9, 13] who underwent allogeneic HSCT without homozygous CCR5 deletions demonstrated that the success obtained in the Berlin patient could not be repeated in these patients. The question as to which was the key factor leading to the success in the Berlin patient, i.e. changes associated with transplantation of CCR5- 32/ 32 donor cells, the pre-transplant conditioning regimen (which was more intense for the Berlin patient than it was for either the Boston patients, or the patients described here), the degree of GVHD, or indeed a combination of these factors, is not definitively resolved.

To date, there are only very few other published cases of allogeneic HSCTs involving donor cells carrying the CCR5- 32/ 32 deletion, and none of these were able to repeat the curative success of the Berlin patient [6, 7]. Further studies of HIV infected patients undergoing HSCTs will be required to advance our understanding of the effects of transplantations with CCR5- 32/ 32 donor cells, but also CCR5 wild-type or heterozygous



deletions. It will be important to assess the effects of less intense pre-transplant conditioning regimens and thus higher survival rates, and it will be important to further explore gene-therapeutic approaches targeting the expression of CCR5. Continuation of ART in order to prevent the establishment of HIV infection in the transplanted stem cells appears to be a safe practice [14]. Whether interventions to reduce the degree of acute GVHD and/or peri-transplant treatment with e.g. CCR5 inhibitors may be beneficial by reducing the HIV reservoir size will also need to be tested. Finally, questions relating to possible co-receptor switching have not been definitively resolved and will also require further exploration [4, 7] [15].

Taken together, the cases described here highlight the significant changes to both the HIV specific immune responses, and/or the total HIV burden reflected by measures of both plasma HIV RNA and CA-HIV RNA and DNA in peripheral blood and tissue, following allogeneic-HSCTs. However, the viral rebound observed in patient A also show that, despite these considerable changes, HIV can rapidly re-emerge years after the transplant procedure, and, together with the two Boston patients, strongly suggest that eradication, or even a reduction of the HIV reservoir to levels allowing for a 'functional cure' via allogeneic HSCT, remains a very rare event.

Finally, the viral rebound observed in patient A in our study also confirms that the current measures of the HIV reservoir are quite likely to have a poor predictive value for possible viral rebound in absolute terms, in particular in patients with extremely small HIV reservoirs who have minimal HIV-specific T-cell responses, underlining that there is an urgent need to identify better markers predicting possible viral rebound, possibly via ATIs in the context of studies investigating curative therapeutic approaches for HIV infection.

## Supplementary methods:

### Molecular and serological analyses:

HIV RNA in plasma was measured by either the COBAS® AmpliCor HIV Monitor test version 1.5 (Roche Diagnostics, Castle Hill, NSW, Australia, detection limit 50 copies/mL), the COBAS® AmpliPrep/COBAS®TaqMan® HIV test version 2.0 (Roche Diagnostics, Castle Hill, NSW, Australia, detection limit 20 copies/mL), or by a real-time PCR assay with single copy sensitivity as previously described [16].

HIV antibodies and p24 antigen (Ag/Ab) were measured by 4<sup>th</sup> generation chemiluminescence microparticle immunoassay (CMIA, Architect HIV Ag/Ab Combo assay, Abbott Diagnostics, North Ryde, NSW, Australia), and the presence of HIV antibodies was confirmed by enzyme immunoassay (EIA, Genscreen™ HIV/2 Version 2, Bio-Rad Laboratories, Gladesville, NSW, Australia). The presence of HIV p24 antigen was also confirmed using a separate enzyme immunoassay (Genscreen™ HIV P24 antigen EIA, Bio-Rad Laboratories, Gladesville, NSW, Australia). Western Blot analyses were performed by: New LAV Blot I, (Bio-Rad Laboratories, Gladesville, NSW, Australia). A lower sensitivity (LS) modification of the Vitros Anti-HIV 1+2 assay (Ortho-Clinical Diagnostics, Rochester, USA) was used to determine HIV-Ab concentration [17].

**Cytomegalovirus (CMV) antibodies (IgG)** were measured by CMIA Abbott ARCHITECT i2000 SR immunoassay analyser (Abbott Diagnostics, North Ryde, NSW, Australia).

**HIV DNA and RNA** in cells from peripheral blood CD4+ T cells was measured in four different laboratories:

Sydney laboratory at St Vincent's Hospital, Sydney (HIV DNA only): Roche AMPLICOR HIV DNA test, version 1.5 (Roche Diagnostics, Castle Hill, NSW, Australia)

University of California, San Francisco (UCSF): Genomic DNA and total RNA was extracted using Trizol reagent (Ambion, Foster City, USA) according to the manufacturer's protocol. HIV DNA was quantified using a Taqman assay specific for the U5 region of the HIV 5'LTR [18]. Briefly, PCR was performed using Taqman Gene Expression Mastermix (Life Technologies, Carlsbad, USA) and primers F (gcctcaataaagcttgccctga), R (ggcgccactgctagaga) and fluorescent probe (FAM-ccagagtcacacaacagacgggcaca-TAMRA) at a final concentration of 200nM each in a total volume of 50µl. Amplification was performed as follows: 10minutes at 95°C, then 60 cycles of 15seconds at 95°C and 1minute at 59°C. Samples were measured in triplicates of up to 1µg of total DNA. Cell-associated HIV RNA was quantified with a one-step RT-qPCR assay using the Taqman RNA-to-Ct 1-Step Kit (Life Technologies, Carlsbad, USA) and the same primers and fluorescent probe as for HIV DNA quantification[18]. Reverse transcription was performed at 48°C for 30minutes. Otherwise the reaction conditions were similar to the ones mentioned for qPCR.

Kirby Institute, UNSW Medicine: Cell-associated (CA) DNA and RNA were extracted using the Trizol Reagent (Life Technologies, Carlsbad, USA), using the back extraction buffer modification for DNA. Two real time qPCR, specific to the *pol* [19] or *gag* [16] gene were used to quantify total levels of CA-HIV DNA. HIV DNA copies were calculated by comparison with a set of plasmid (pNL4-3) standards, and normalized for DNA input by the measurement of β-actin using the Taqman β-actin Detection Reagents Kit (Life Technologies, Carlsbad, USA).

Residual DNA was removed from extracted RNA by treatment with DNase I (Promega, Madison, USA). CA-unspliced (CA-US) HIV RNA was quantified by reverse-transcription (RT)-qPCR [16]. HIV RNA copies were determined by comparison with a set of plasmid (pNL4-3) standards and normalized for RNA input by quantification of GAPDH by RT-qPCR (primers; forward TCG ACA GTC AGC CGC ATC TT and reverse GGC AAC AAT ATC CAC TTT ACC AG; and probe FAM-AAG GTC GGA GTC AAC GGA TTT GGT CGT-BHQ1).

**Doherty Institute, The University of Melbourne, Melbourne**—Levels of CA HIV DNA and CA-US HIV RNA were analysed in peripheral blood mononuclear cells (PBMC) and isolated CD4+ T cells using a minimum input of 10 million PBMCs per time point assayed. For quantification of CA-US RNA, a semi-nested quantitative PCR was performed in quadruplicate as previously described [20]. Quantification of cell-associated HIV DNA was performed with a single round quantitative PCR in duplicate as previously described [21].

**CCR5 gene PCR:**

Presence of mutations in the CCR5 gene were determined by PCR by two assays.

**CCR5 PCR assay I (patients A and B)**

Extracted genomic DNA from both patients and control DNA was amplified by PCR using the following primers: Forward: CCR5F:

5'TTCATTACACCTGCAGCTCTC3', Reverse: CCR5R:

5'TCACAGCCCTGTGCCTCTTC3'. The cycling conditions were: 95°C for 2minutes, then 94°C for 20seconds, 61°C for 30seconds, 72°C for 45seconds over 45 cycles followed by 72°C for 10minutes. The resulting PCR products were visualised on a 2.5% agarose gel.

**CCR5 PCR assay II (patient C only):**

Genomic DNA from both patient and control samples was extracted using a Qiagen All prep RNA/DNA Minikit and amplified by PCR. Positive controls consisted of CCR5 wild-type DNA, CCR5- 32 homozygous DNA and CCR5- 32 heterozygous DNA. Negative control was nuclease free water. PCR reaction mix consisted of AmpliTaq Gold polymerase and 10uM CCR5 sense and antisense primers. 2uL of sample each per reaction. PCR products were visualised on a 2% agarose gel.

**HIV sequence analysis**

Protease/reverse transcriptase regions were analyzed with either a commercial drug resistance assay (TRUGENE HIV Genotyping Kit, Visible Genetics, Inc., Toronto, Canada), according to the manufacturer's instructions, or an in-house drug resistance assay. The in-house assay, including the integrase region, consists of two reaction steps. In the initial reaction step, the extracted RNA from plasma was amplified by one step RT-PCR with Platinum Taq High Fidelity PCR kit (Invitrogen, Mt Waverley, VIC, Australia) for 30 minutes at 52°C, 2 minutes at 94°C, and 50 cycles of 15seconds at 94°C, 30seconds at 55°C, 1 min 30 seconds at 68°C using a forward primer: 5' - TGA TGA CAG CAT GYC ARG GAG T -3' and a reverse primer: 5' - CTG CTA TTA ADT CTT TTG CTG GG -3'. In the second step, a nested PCR was performed with VELOCITY DNA Polymerase PCR kit (Bioline, Alexandria, NSW, Australia) using a forward primer: 5' - GAA GGA CAC CAA ATG AAA GAY TG -3' and a reverse primer: 5' - GTA TGT CAT TGA CAG TCC AGC -3' for 2minutes at 98°C and 40 cycles of 30seconds at 98°C, 30seconds at 55°C, 1minute 30seconds at 72°C. The amplified DNA was subjected to a standard BigDye sequence analysis with 7 sequencing primes: Primer-1: 5' - GAA GGA CAC CAA ATG AAA GAY TG -3', Primer-2: 5' - ATT GTT TAA CYT TTG GGC CAT CC -3', Primer-3: 5' - TAG GAC CTA CAC CTG TCA ACA TAA TTG G -3', Primer-4: 5' - CCA AAA GTT AAA CAA TGG CCA TTG ACA GA -3', Primer-5: 5' - TCT AAA AGG CTC TAA GAT TTT TGT CAT GC -3', Primer-6: 5' - GCT TCC ACA GGG ATG GAA AGG -3', and Primer-7: 5' - CAG CAC TAT AGG CTG TAC TGT CCA -3'.

Phylogenetic analyses were undertaken 18 local control individual sequences (i.s)- obtained from recent laboratory confirmed seroconverters; a subtype B reference sequence (B.FR. 83.HXB2\_LAI\_IIIIB\_BRU\_K03455); and four patient A sequences. Local control multiple sequence (m.s)- obtained from same individual within calendar year to demonstrate

inpatient sequence variability. Sequence alignment was performed using Geneious® 8.1.7, and manually edited using Bioedit Version 7.2.5. Phylogenetic inference –using Geneious® 8.1.7 using Tanura-Nei Neighbor-Joining method with bootstrap value of 100, and 95% support threshold.

**Antigen-specific CD4+ T cells** were measured using the CD25/CD134 upregulation assay, as previously described [22, 23]. Briefly, PBMC at  $1 \times 10^6$ /ml in Iscove's Modified Dulbecco's medium (Life Technologies) containing 10% human AB serum (Sigma) were cultured for 44–48 hours in the presence of (i) no antigen (negative control); (ii) SEB (1µg/mL; Sigma; positive control); (iii) CMV lysate (1/500 dilution; Grade III purified; Meridian) and (iv) HIV Gag peptide pool (123 peptides from HXB2 sequence; each at 2µg/mL; AIDS Reagent Program). At the end of the incubation, cells were stained with CD3-PerCP-Cy5.5, CD4-Alexa Fluor700, CD25-APC, and CD134-PE (BD Biosciences) and analysed on a four-laser LSR II flow cytometer (BD Biosciences). Antigen-specific CD4+ T cells were identified as CD3+CD4+CD25+CD134+, using gates set on the negative and positive controls, respectively, as previously described [22].

### Lopinavir drug levels

Lopinavir was assayed using reverse phase high performance liquid chromatography (HPLC) with ultraviolet detection on a C18 analytical column (Shimadzu Prominence, Shimadzu, Rydalmere, NSW, Australia). Samples, standards and controls were prepared by liquid/liquid extraction. The method has been validated to ISO 17025 criteria.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

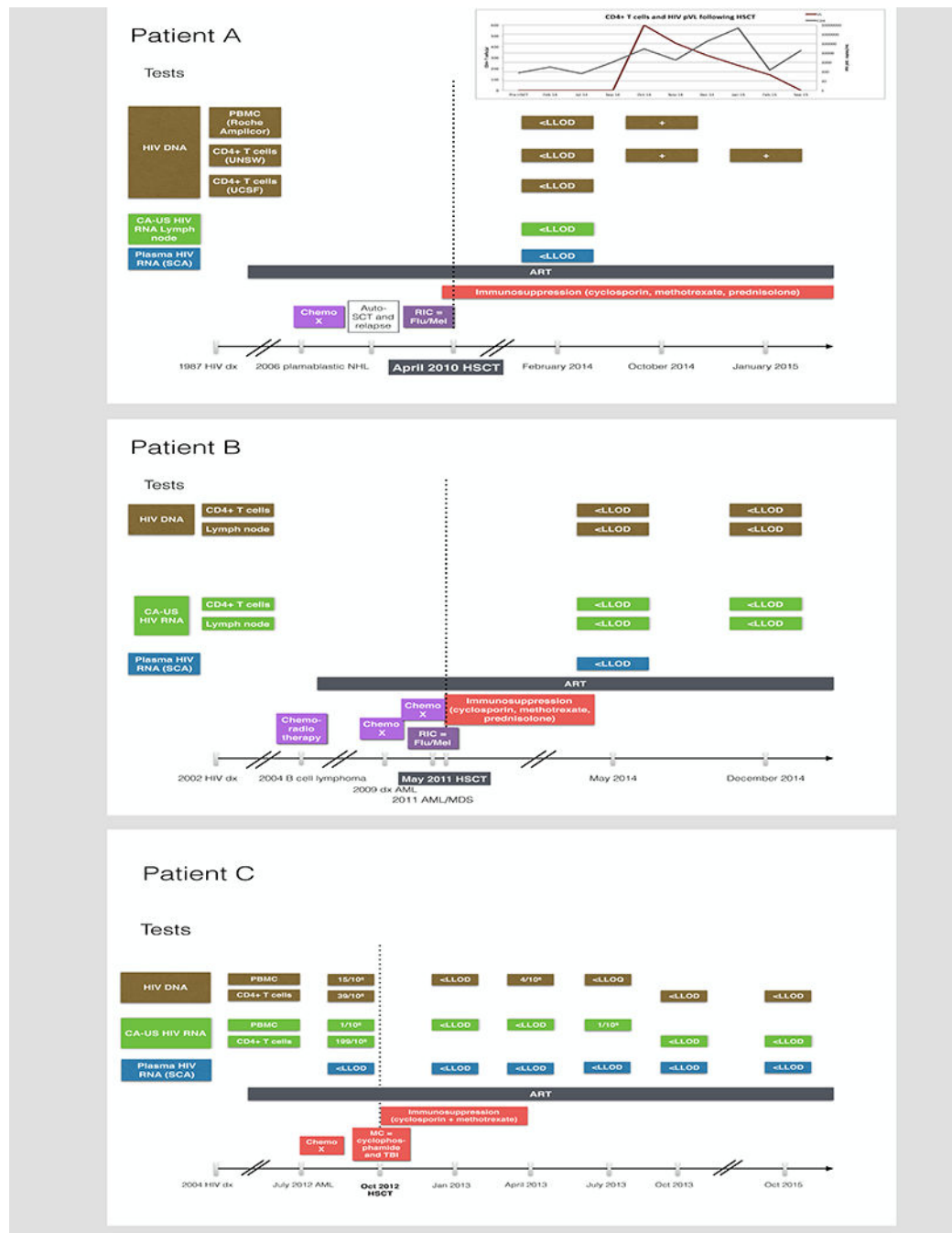
### Acknowledgements:

We are grateful to the patients for their generous participation in this study. We would also like to thank Karen MacRae and Annabel Horne for their support in coordinating the study visits, Kim Grassi, Bertha Fsadni, Beth Catlett, Melanie Lograsso, Kate Merlin and Ajantha Rhodes for laboratory support; Tracey Barrett for administrative support. Drs Nenad Mecasic and Christina Chang for assistance in the protocol development and ethics application to The Alfred Hospital; and Julia Stout and Jennifer Audsley for administrative and ethics support of the HIV Cure Program at the Alfred Hospital and Doherty Institute.

### References:

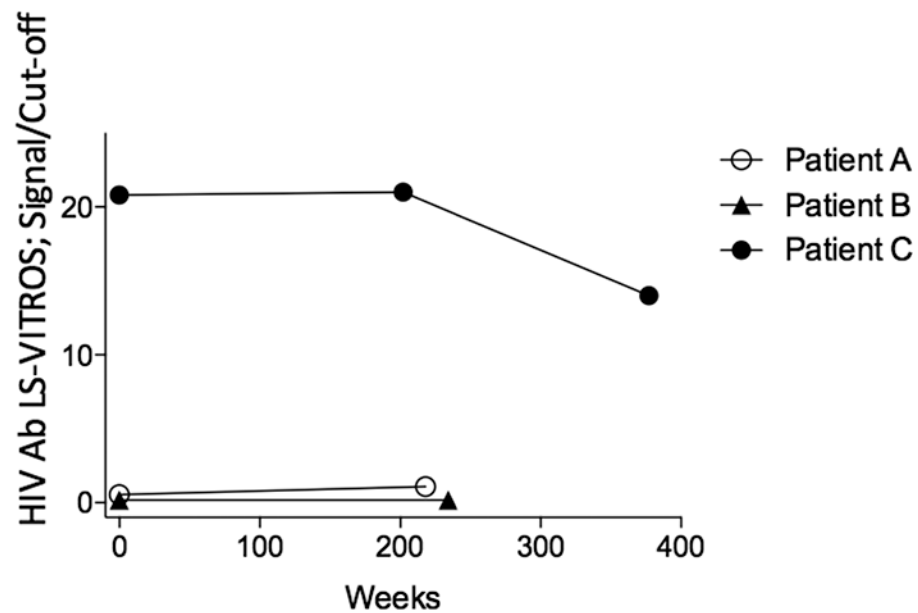
1. Richman DD , Margolis DM , Delaney M , Greene WC , Hazuda D , Pomerantz RJ . The challenge of finding a cure for HIV infection. *Science* 2009; 323:1304–7. [PubMed: 19265012]
2. Kent SJ , Reece JC , Petravic J , et al. The search for an HIV cure: tackling latent infection. *Lancet Infect Dis* 2013; 13:614–21. [PubMed: 23481675]
3. Spivak AM , Planelles V . HIV-1 Eradication: Early Trials (and Tribulations). *Trends Mol Med* 2016; 22:10–27. [PubMed: 26691297]
4. Hutter G , Nowak D , Mossner M , et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 2009; 360:692–8. [PubMed: 19213682]
5. Yukl SA , Boritz E , Busch M , et al. Challenges in detecting HIV persistence during potentially curative interventions: a study of the Berlin patient. *PLoS Pathog* 2013; 9:e1003347. [PubMed: 23671416]

6. Kordelas L , Verheyen J , Beelen DW , et al. Shift of HIV tropism in stem-cell transplantation with CCR5 Delta32 mutation. *N Engl J Med* 2014; 371:880–2.
7. Hutter G More on shift of HIV tropism in stem-cell transplantation with CCR5 delta32/delta32 mutation. *N Engl J Med* 2014; 371:2437–8.
8. Henrich TJ , Hanhauser E , Marty FM , et al. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. *Ann Intern Med* 2014; 161:319–27. [PubMed: 25047577]
9. Henrich TJ , Hu Z , Li JZ , et al. Long-term reduction in peripheral blood HIV type 1 reservoirs following reduced-intensity conditioning allogeneic stem cell transplantation. *J Infect Dis* 2013; 207:1694–702. [PubMed: 23460751]
10. Sugarman JL SR ; Henrich T and Rasmussen T Ethical Considerations in Analytic Treatment Interruptions Following Hematopoietic Stem Cell Transplantation. *Lancet HIV* 2016; In press.
11. Wensing AM ; Diez-Martin DL ; Huetter GK,J ; Kwon ; Nijhuis M ; Saez-Cirion A ; Rocha V ; Salgado M ; Schulze zur Wiesch J ; Stam A ; Martinez-Picado J Allogeneic stem cell transplantation in HIV-1-infected individuals; the EPISTEM consortium. In: *AIDS 2016 (Durban, South Africa)*.
12. Eriksson S , Graf EH , Dahl V , et al. Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. *PLoS Pathog* 2013; 9:e1003174. [PubMed: 23459007]
13. Henrich TJ , Hanhauser E , Hu Z , et al. Viremic control and viral coreceptor usage in two HIV-1-infected persons homozygous for CCR5 Delta32. *AIDS* 2015; 29:867–76. [PubMed: 25730507]
14. Johnston C , Harrington R , Jain R , Schiffer J , Kiem HP , Woolfrey A . Safety and Efficacy of Combination Antiretroviral Therapy in Human Immunodeficiency Virus-Infected Adults Undergoing Autologous or Allogeneic Hematopoietic Cell Transplantation for Hematologic Malignancies. *Biol Blood Marrow Transplant* 2015.
15. Symons J , Vandekerckhove L , Hutter G , et al. Dependence on the CCR5 coreceptor for viral replication explains the lack of rebound of CXCR4-predicted HIV variants in the Berlin patient. *Clin Infect Dis* 2014; 59:596–600. [PubMed: 24759824]
16. Palmer S , Wiegand AP , Maldarelli F , et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 2003; 41:4531–6. [PubMed: 14532178]
17. Keating SM , Hanson D , Lebedeva M , et al. Lower-sensitivity and avidity modifications of the vitros anti-HIV 1+2 assay for detection of recent HIV infections and incidence estimation. *J Clin Microbiol* 2012; 50:3968–76. [PubMed: 23035182]
18. Yukl SA , Shergill AK , Ho T , et al. The distribution of HIV DNA and RNA in cell subsets differs in gut and blood of HIV-positive patients on ART: implications for viral persistence. *J Infect Dis* 2013; 208:1212–20. [PubMed: 23852128]
19. Althaus CF , Gianella S , Rieder P , et al. Rational design of HIV-1 fluorescent hydrolysis probes considering phylogenetic variation and probe performance. *J Virol Methods* 2010; 165:151–60. [PubMed: 20116399]
20. Elliott JH , Wightman F , Solomon A , et al. Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. *PLoS Pathog* 2014; 10:e1004473. [PubMed: 25393648]
21. Lewin SR , Murray JM , Solomon A , et al. Virologic determinants of success after structured treatment interruptions of antiretrovirals in acute HIV-1 infection. *J Acquir Immune Defic Syndr* 2008; 47:140–47. [PubMed: 18300365]
22. Zaunders JJ , Munier ML , Seddiki N , et al. High levels of human antigen-specific CD4+ T cells in peripheral blood revealed by stimulated coexpression of CD25 and CD134 (OX40). *J Immunol* 2009; 183:2827–36. [PubMed: 19635903]
23. Seddiki N , Cook L , Hsu DC , et al. Human antigen-specific CD4(+) CD25(+) CD134(+) CD39(+) T cells are enriched for regulatory T cells and comprise a substantial proportion of recall responses. *Eur J Immunol* 2014; 44:1644–61. [PubMed: 24752698]



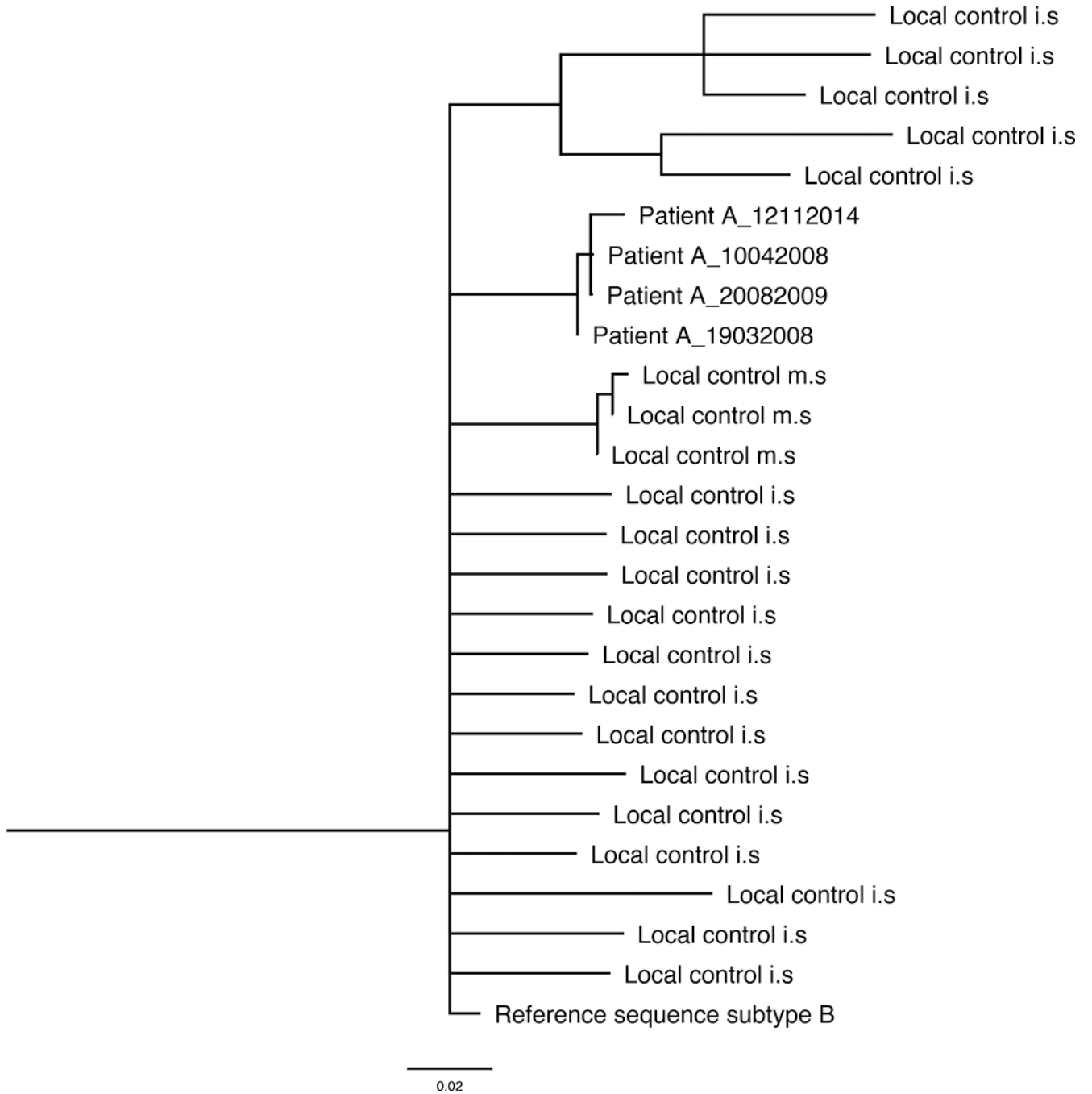
**Figure 1.** Timelines of the clinical histories and test results for patients A, B and C. LLOD = lower limit of detection, MC = myeloablative conditioning. Values given for patient C indicate HIV DNA or RNA copies /  $10^6$  cells.

## HIV-1 antibody concentration changes over time



**Figure 2.**

Results of the LS-VITROS Ab assay for patients A, B and C. For patient A, the Ab concentrations were very low at the first measured time point 4 years post-transplant, but subsequently increased after the patient experienced viral rebound at second measurement 31 weeks later. For patient B the Ab concentrations were measured at two time points, first time point 3 years, second time point 3 years and 7.5 months after transplant. At both time points the Ab concentrations were in the very low range. For patient C, Ab concentrations are decreasing between months 6 and 12 post transplantation.



**Figure 3.** Phylogenetic analysis of pre- and post HSCT sequences from the protease and reverse transcriptase regions, amplified from plasma RNA samples from patient A. The phylogenetic tree shows clustering of patient A sequences with bootstrap value of 100 and genetic distances of <0.012. Local control multiple sequence (m.s) and individual sequences (i.s) are shown for comparison.



**Table 1**

Serologies patient A, Serologies patient B, Serologies patient C

HIV serologies patient A	Sep 2008 (pre-SCT)	Feb 2014 (post-SCT)	Oct 2014 (post-SCT)	Nov 2014 (post-SCT)
HIV Ag/Ab CMIA	Reactive	Reactive	Reactive	Reactive
p24 Ag Qual	Reactive	Negative	Detected	Negative
p18 HIV WB	1+	Trace	Trace	No band
p24 HIV WB	2+	Trace	2+	3+
p34 HIV WB	2+	Trace	1+	1+
p40 HIV WB	1+	No band	2+	1+
gp41–45 HIV WB	3+	Trace	1+	2+
p53 HIV WB	2+	Trace	3+	3+
p55 HIV WB	3+	Trace	2+	3+
p68 HIV WB	2+	Trace	3+	3+
gp120 HIV WB	3+	Trace	Trace	No band
gp160 HIV WB	3+	2+	1+	1+

HIV serologies patient B	2002 (pre-SCT)	Feb 2012 (post-SCT)	Dec 2014 (post-SCT)
HIV Ag/Ab CMIA	Reactive	Reactive	Reactive
p24 Ag Qual		p24 Ag Qual Negative	Negative
p17 HIV WB	3+	p18 HIV WB 1+	No band
p24 HIV WB	3+	P24HIV WB 3+	No band
p31 HIV WB	3+	p34 HIV WB p40 3+	No band
gp41 HIV WB	3+	gp41–45 HIV WB 2+	No band
p51 HIV WB	3+	p53 HIV WB 2+	No band
p55 HIV WB	3+	p55 HIV WB 3+	No band
p66 HIV WB	3+	p68 HIV WB 3+	No band
gp120 HIV WB	3+	gp120 HIV WB 2+	No band
gp160 HIV WB	3+	gp160 HIV WB 2+	No band

HIV-1 serology patient C	Oct 2012 (pre-HSCT)	Apr-13 (6 months post-HSCT)	Oct 2013 (12 months post-HSCT)
HIV 1/2 Ag/Ab Liaison XL	Reactive	Reactive	Reactive
p24 Ag Qual	4+	4+	4+
gp41 HIV WB	4+	4+	4+
p51 HIV WB	4+	4+	4+
p66 HIV WB	4+	4+	4+
gp120 HIV WB	4+	4+	4+
gp160 HIV WB	4+	4+	4+
LS-VITROS	20.8	21.2	14

**Table 2**

ART plasma levels patient A

	July 2014	September 2014	October 2014	November 2014	December 2014
Lopinavir/r (µg/L)	9162	Not detected	Not detected	1316	1089

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript