ID# MOPDA0105

HIV rebound and meningoencephalitis following ART interruption after allogeneic hematopoietic stem cell transplant: an investigation of the source of HIV rebound

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INTRODUCTION

Antiretroviral therapy (ART) can control HIV-1 viremia, reducing it to clinically undetectable levels and leading to immune function and clinical recovery. Despite virologic control, if ART is interrupted, HIV-1 viremia universally rebounds within a few weeks.[1, 5] It is thought that the source of rebound viremia originates from the HIV-1 latent reservoir in resting memory CD4+ T cells.[1] In HIV-1-infected individuals, this latent reservoir poses the greatest known barrier to cure.[5]

The only case of HIV cure was reported in the 'Berlin Patient' who received an allogeneic hematopoietic stem cell transplant (alloHSCT) for treatment of acute myelogenous leukemia (AML). In this unique case, his donor cells were protected and rendered resistant to HIV-1 infection by the specific genetic polymorphism CCR5Δ32, resulting in lack of CCR5 expression, one of the main HIV-1 coreceptors.[1, 2]

There is interest in the alloHSCT with CCR5 wild-type donors as another strategy which may lead to reduction or eradication of HIV-1 reservoirs. Components of alloHSCT that may reduce reservoirs include cytotoxic therapy and allogeneic or graft versus host (GVH) effects, the process where recipient hematopoietic-derived cells are recognized by donor CD8+ T cells and are killed. If ART is continued during alloHSCT, it should protect donor hematopoietic-derived cells from infection and result in a reduction or elimination of HIV-1.[1] AlloHSCT with uninterrupted ART is being investigated as a component of HIV-1 eradication strategies.

Proof-of-concept for this was provided by the reports of the 'Boston patients' who received alloHSCT from homozygous wild-type CCR5 donor cells as treatment for refractory lymphoma resulting in the disappearance of HIV-1 in peripheral blood.[3] However, after analytical ART interruption, viral rebound occurred in both patients; both developed symptoms of acute retroviral syndrome and one had concomitant meningitis.[4] The source of HIV-1 rebound in these cases was not clear.

In a pilot clinical trial at Johns Hopkins, we are evaluating whether alloHSCT with optimized ART reduces HIV-1 reservoirs in HIV-1-infected patients who require alloHSCT for a standard clinical indication (see abstract #TUPEB298 for detailed clinical data).[6] Optimized ART includes: 1) avoidance of ritonavir-boosted regimens to minimize drug interactions, 2) ART changes for organ dysfunction, and 3) subcutaneous enfurvirtide (ENF) during post-transplant cyclophosphamide and if oral ART was not tolerated.[6]

Here, we present the case of an HIV-1-infected patient enrolled in our study who received alloHSCT for AML and experienced acute retroviral syndrome and meningoencephalitis after self-discontinuing ART post-alloHSCT.

CLINICAL HISTORY

This is a 39-year-old African American male with HIV-1 infection and multiply-relapsed AML.

I: Oncology

He was diagnosed with AML in 2004 and was initially treated with daunorubicin and high-dose cytarabine. He had a complicated oncologic history, with multiple relapses due to incomplete follow up. In 2013, the patient had residual leukemia and remission was achieved with a chemotherapy regimen of mytoxantrone, etoposide, and cytarabine and he was referred for alloHSCT.

II: HIV-1 Infection

The patient was first diagnosed with HIV-1 infection concurrently with AML in 2004, presumably during chronic phase of infection. The patient's HIV-1 infection history has been complicated due to his multiple AML relapses, being lost to follow-up, and lack of ART adherence (Fig. 1a). Genotyping assays revealed the patient had developed the K103N and M184V drug resistance mutations (Celera Diagnostics ViroSeq v.2.8 assay) and a Monogram Trofile demonstrated R5 tropic virus prior to alloHSCT.

The patient's ART regimen prior to alloHSCT was abacavir/lamivudine, raltegravir, and ritonavir-boosted darunavir. For his optimized ART regimen, ritonavir-boosted darunavir was replaced with the CCR5 antagonist, maraviroc.

III: AlloHSCT Course

On December 27th, 2013, the patient received a reduced-intensity conditioning matched-related alloHSCT without complication. Per protocol, he received subcutaneous injection of enfuvirtide on day 3 post-alloHSCT without adverse events. Engraftment (ANC > 500 µL/cc for 3 days) was achieved by day 20. On day 28, donor chimerism in peripheral blood was >95% (Table1).

Table 1 Donor chimerism determined clinically by microsatellite PCR analysis at the Johns Hopkins Medical Laboratories. Formal limit of detection of 5%. (ND Not determined)

	Donor Chimerism (%)		
Time post-alloHSCT (days)	Peripheral blood	CD3+ T cells	Bone Marrow
28	> 95	89	ND
54	ND	ND	> 95
105	95	93	ND
147	> 95	> 95	ND
234	ND	ND	> 95
430	> 95	ND	ND

On day 84 post-alloHSCT, his HIV-1 viral load (VL) was <20 c/mL, where after he began missing clinic appointments and declining research blood draws. By day 100, he developed fevers with no clear etiology; an HIV-1 VL test was recommended but not done. Fevers persisted with a change in mental status. At 146 days post-alloHSCT, the patient was admitted into the hospital and a lumbar puncture was performed. Cerebrospinal fluid (CSF) analysis revealed a white blood cell count 28 [range 0-3 cells/µL]), Protein 150 [range 15-45 mg/dL], and normal glucose. CSF fluid was sent for microbiology studies and was negative for: VZV, EBV, CMV, HSV, JCV, cryptococcal, VDRL, West Nile, and parvovirus. Blood tests were negative for histoplasma, syphilis, Hepatitis B and C, adenovirus; and negative for bacterial, fungal, and AFB stains. Aspartate transaminase (AST) and alanine transaminase (ALT) were 282 [range 5-40 U/L] and 286 [range 7-56 U/L], respectively. HIV-1 VL was 25,518 c/mL in peripheral blood. HIV-1 RNA was measured in CSF (Abbott assay, Quinn lab) at 17,000 c/mL (Fig. 1b). A clinical genotyping assay was performed and demonstrated wild-type virus (Celera Diagnostics ViroSeq v.2.8 assay). During the hospital stay, the patient began having decreased cognitive function and was placed on broad spectrum antibiotics. Due to elevated liver enzyme levels and poor mental status, his oral ART regimen was temporarily discontinued for 6 days. Subcutaneous ENF was initiated. After 3 days his mental status and liver enzymes improved and was placed back on his oral ART regimen. He was discharged at day 159 and his HIV-1 VL was 23 c/mL on day 190 post-alloHSCT.

Over the next three months, the patient was admitted on two occasions – one for liver dysfunction believed to be drug-induced liver injury (dapsone) and another for acute pancreatitis from which he recovered. Day 410 post-alloHSCT, he was admitted for liver failure, due to non-adherence to tacrolimus, with AST 2675 [range 5-40 U/L], ALT 1894 [range 7-56 U/L], total bilirubin 19 [range 0.0-1.2 mg/dL], and INR 2.4 [range 0.9-1.1]. At 450 days post-alloHSCT, the patient passed away from liver failure, possibly GVHD. Autopsy was declined.

HIV-1 INFECTION CLINICAL HISTORY

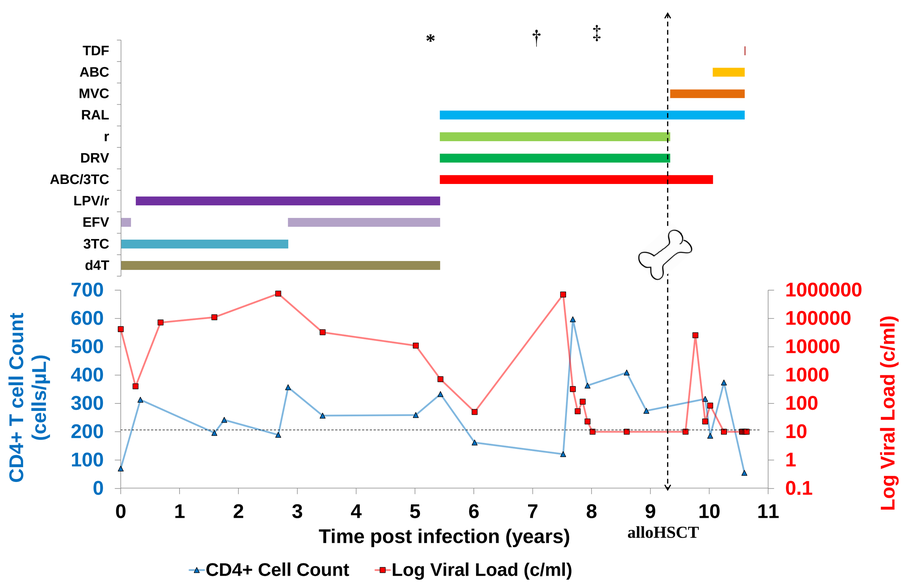

Fig. 1a. HIV-1 Infection clinical course of patient first diagnosed in 2004 concurrently with AML.

d4T (stavudine), 3TC (lamivudine), EFV (efavirenz), LPV/r (lopinavir/ritonavir), ABC/3TC (abacavir/lamivudine), DRV (darunavir), r (ritonavir), RAL (raltegravir), MVC (maraviroc), ABC (abacavir), TDF (tenofovir)

*** Genotype assay: K103M**
† Genotype assay: K103N and M184V
‡ Trofile: R5

ALLOHSCT COURSE HISTORY

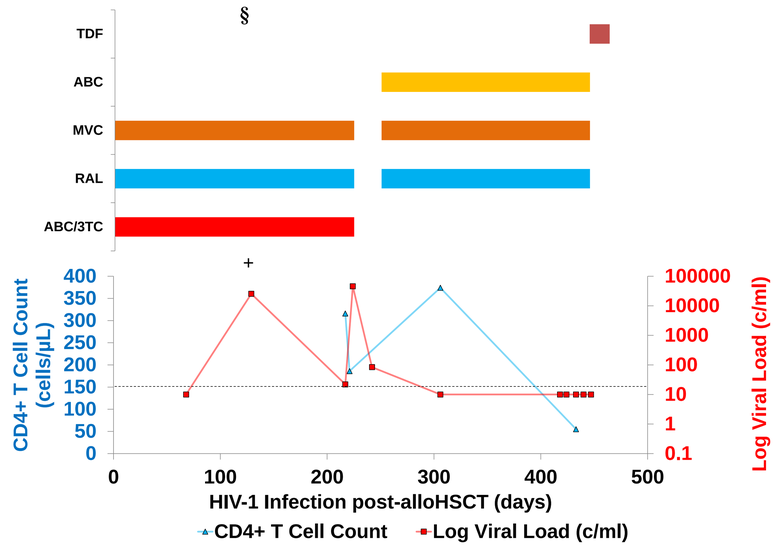

Fig. 1b. HIV-1 Infection clinical history post-alloHSCT. At the time of alloHSCT, CD4+ T cell count was 409 cells/µL and viral load was <20 c/mL. At viral rebound, measured viral load was 25,518 c/mL and 17,000 c/mL in the peripheral blood and CSF, respectively.

ABC/3TC (abacavir/lamivudine), RAL (raltegravir), MVC (maraviroc), ABC (abacavir), TDF (tenofovir)

§ Genotype assay: No drug resistance mutations (wild-type)
+ Viral Rebound

METHODS

Resting memory CD4+ T cells obtained 100 and 25 days prior to alloHSCT were used in a limiting-dilution viral outgrowth assay (VOA) in which each well that demonstrates viral growth contains a single replication-competent viral clone. Peripheral blood mononuclear cells (PBMCs) were isolated using density centrifugation from whole blood. Resting memory CD4+ T cells ($CD4^+$, $CD69^-$, $CD25^-$, and $HLA\text{-}DR^-$) were enriched using magnetic microbeads through negative depletion (Miltenyi Biotec). Purity of resting CD4+ T cells were verified by flow cytometry (purity >90%). The resting CD4+ T cells are plated in limited dilution with healthy donor γ-irradiated PBMCs. The cells are subjected to maximum *in vitro* activation by the potent mitogen, phytohaemagglutinin (PHA). Replication of viruses released in the supernatant are amplified by the addition of MOLT-4/CCR5 cell line for targets. Viral outgrowth is measured by detection of p24 by ELISA in supernatant at day 14-21.

RNA was extracted from VOA p24 positive supernatants, and collected cerebrospinal fluid CSF and plasma at viral rebound. The RT *pol* region of the virus from p24 positive VOA supernatants was sequenced using Sanger Sequencing. Rebound virus from blood and CSF was also analyzed using next-generation sequencing (Roche 454).

Final sequence alignment was generated using Multiple Alignment using Fast Fourier Transform (MAFFT) with minimal manual adjustments with high gap penalty. Best model of evolution for maximum likelihood (ML) analysis was determined in jModelTest using the Akaike Information Criterion (AIC). ML analysis was carried out natively in PAUP*v.4b using the model parameters generated in jModelTest with bootstrapping of 100 pseudoreplicates.

RESULTS

Before alloHSCT, 31 sequences were isolated from the VOA. At rebound, 14,645 and 5,003 sequence reads were obtained from CSF and blood respectively; and were combined into consensus sequences using a cut-off of >0.2% of total sequence reads. This generated 55 sequences from CSF and 92 from peripheral blood.

A distinct sub-group branch of sequences (9/31) isolated from the VOA with harbored drug resistance mutations (M184V and/or K103N) did not group within the rebound virus clade (**Fig. 2**). The rebound virus was mainly comprised of wild-type sequences and only differed by 1 to 4 nucleotides with high sequence similarity between CSF and peripheral blood (**Fig. 2**). A small portion of rebound virus in the CSF (12/55 sequences) and peripheral blood plasma (1/92 sequences) had the M184I mutation (**Fig.2**).

A total of 9/31 (29%) of independent VOA sequences from both pre-alloHSCT time-points intermixed with the peripheral blood and CSF viral rebound sequences in a monophyletic clade with high sequence homology **(Fig. 2; star symbol).** Of the independent VOA sequences, 6/22 and 1/12 sequences obtained from the first and second pre-alloHSCT time-points, respectively, were identical; with the other two viral clones having 1 -3 nucleotide differences (**Fig.2**).

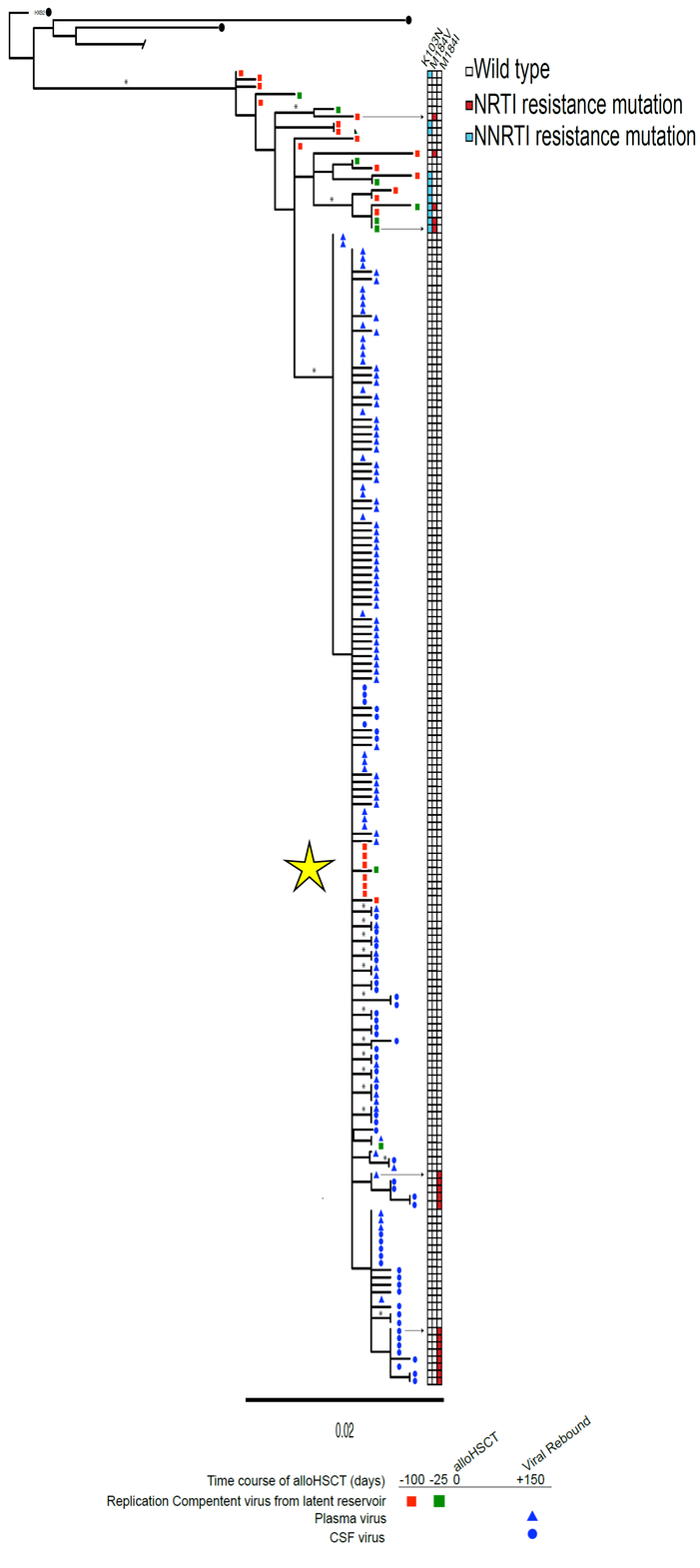

Fig. 2. Maximum likelihood tree (-ln L=3553.1264) with bootstrap values reported on branches (* >50%). The GTR+G model of evolution was selected using Akaike Information Criterion in jModelTest. Likelihood analysis were conducted with heuristic search with random sequence addition and nearest-neighbor interchange (NNI) branch swapping.

DISCUSSION

We present the case of an HIV-infected individual with acute myelogenous leukemia who underwent alloHSCT. After self-interrupting ART post-alloHSCT the patient experienced a severe acute retroviral syndrome with meningoencephalitis concurrent with HIV-1 viral rebound in peripheral blood and CSF. Donor chimerism was greater than 95% measured in both peripheral blood and bone marrow prior to ART interruption. The virus at the time of rebound was identical to pre-alloHSCT replication-competent virus from resting memory CD4+ T cells. This virus was present at multiple time-points pre-alloHSCT and comprised nearly 1/3 of the replication-competent HIV-1 reservoir sampled. This virus was also present in the CSF at the time of rebound. It can be inferred from the phylogenetic analysis that the source of rebound virus is consistent from resting memory CD4+ T cells.

Potential explanations for viral rebound with ART interruption in this case may be a result of persistent recipient cells due to an incomplete donor chimerism at a relatively early point post-alloHSCT. Alternatively, the allogeneic effect/GVH effect may not completely replace the host hematopoietic-derived cells regardless of time post-alloHSCT. In addition, incomplete adherence to ART may have allowed for infection of donor cells.

Further studies on the degree and time course of recipient cell replacement by donor cells are needed. In addition, strategies to prevent acute retroviral syndrome after alloHSCT are warranted.

CONCLUSION

Interruption of ART post-alloHSCT can lead to a severe clinical acute retroviral syndrome including meningoencephalitis which may be due to lack of HIV-1 immunity in naïve donor cells.

This unique case suggests that recipient cells persist at early time points after alloHSCT and that a single predominant viral population latent in resting memory CD4+ T cells can re-establish infection.

ACKNOWLEDGEMENTS

This work was supported by amfAR, The Foundation for AIDS Research (Mathilde Krim Fellow 108707-54-RKRL), the Johns Hopkins Center for AIDS Research (P30AI094189), NIH grant K23CA177321-01A1, Sidney Kimmel Comprehensive Cancer Center Program Project Grant (P30CA006973) and the Howard Hughes Medical Institute.

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