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Optimized ART and alloBMT to reduce HIV reservoirs

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INTRODUCTION

In HIV-infected (HIV+) individuals with high-risk hematologic malignancies, allogeneic bone marrow transplantation (alloBMT) is a life-saving treatment. Components of alloBMT include cytotoxic therapy and allogeneic or graft-versus-host (GVH) effects.

Maintaining antiretroviral therapy (ART) during alloBMT is a challenge, often requiring an interruption of ART. These can be due to drug interactions between protease inhibitors and immunosuppressants, intermittent organ dysfunction, and chemotherapy-associated mucositits and vomiting, thereby compromising oral ART delivery.

AlloBMT may provide an opportunity to eradicate the HIV latent reservoir (LR) if ART is maintained to prevent infection of donor cells during the replacement of the hematopoietic system. Here we report the results from a clinical trial investigating the feasibility of continuing optimized ART without interruption. We also report the impact of alloBMT on the size of the LR and the potential on HIV cure.

METHODS

HIV+ adults with a clinical indication for alloBMT were eligible. Optimized ART was achieved to include: the avoidance of protease inhibitors to minimize drug interactions, ART changes to avoid organ dysfunction, and subcutaneous injection of enfurvirtide (ENF) during post-transplant cyclophosphamide and if oral ART was not tolerated.

The HIV LR within resting memory CD4+ T cells (CD4+/CD69-/CD25-/HLA-DR-) was measured prior to transplant and then every 12 weeks post-transplant by the quantitative viral outgrowth assay (qVOA) using the MOLT-4/CCR5 cell line. Viral outgrowth was detected by enzyme-linked immunosorbent assay for HIV p24 antigen in culture supernatant. Frequency of cells with infectious virus was determined by limiting-dilution maximum likelihood based statistics and expressed as infectious units per million cells (IUPM).

HIV proviral DNA measurements were detected using digital droplet PCR. DNA was extracted from PBMCs and digested with BSAJ-1. Primers and probes for HIV pol and RPP30 were used. The ddPCR HIV probe was a 3' minor groove binder-non-fluorescent quencher with 6FAM 5' dye and RPP30 had the same quencher but with a VIC 5' dye.

For phylogenetic analysis, RNA from p24+ qVOA supernatants and DNA from PBMCs were sequenced using Sanger and Next-generation methods for the RT-region of *pol.* Maximum likelihood analysis with bootstrapping was carried out.

RESULTS

Participant	1†	2	3†*	4	5†	6	7
Age	34	53	38	50	51	46	50
Sex	Male	Male	Male	Male	Male	Male	Male
Race	Black	White	Black	Black	White	Hispanic	White
Cancer	HL	DLBCL	AML#	AML*	DLBCL	HL	AML[F]
Conditioning Type	NMA	NMA	NMA	MAC	NMA	NMA	NMA
Donor type	MUD	MUD	MRD	MRD	Haplo	Haplo	MMUD
PBMC Donor Chimerism	>95%	87%	>95%	73%	>95%	>95%	>95%
CD3+ Donor Chimerism	>95%	73%	>95%	<5%	>95%	>95%	>95%
Survival	Died at week 49	Alive, week 213	Died at week 64	Alive, week 168	Died at week 67	Alive, week 122	Alive, week 98
Oncology outcomes	Liver failure	Cancer free	Liver failure, possible GVHD	Cancer free	Renal failure	Cancer free	Cancer free

Table 1. Seven patient with chronic HIV infection and hematologic malignancy received alloBMT and ART. Cancer: HL (Hodgkin lymphoma), DLBCL (Diffuse large B-cell lymphoma), AML (acute myeloid leukemia), AML[#] (multiply relapsed), AML* (biphenotypic acute myeloid leukemia), AML[F] (Flt3 AML). Donor type: MUD (Match-unrelated donor), MRD (match-related donor), Haplo (Haploidentical), MMUD (mismatched-unrelated donor). Conditioning-Type: Non-myeloablative (NMA), Myeloablative-conditioning (MAC). † Participant has died * Taken off study due to lack of compliance.

Participant	1†	2	3†*	4	5†	6	7
Baseline HIV plasma RNA (c/ml)	79	<20	<20	<20	<20	<20	<20
Baseline CD4 count (cells/ul)	85	231	274	57	227	547	219
HIV-Tropism	R5	R5	R5	R5/X4	R5	R5/X4	R5
ART Maintenance	70%	>95%	Poor	>95%	>90%	>98%	100%
Number of ART changes	3	1	1	3	1	2	1

Table 2. Persistence and monitoring of HIV. Maintenance of ART was monitored with electronic pill bottle caps (MEMS®), in-patient records, and self-reporting. R5: HIV strains which use the beta-chemokine receptor CCR5 for cell entry and are able to replicate in macrophages and CD4+ T-cells. R5/X4: Mixed strains which can use the chemokine receptor CCR5 or CXCR4 for entry.
† Participant has died * Taken off study due to lack of compliance.

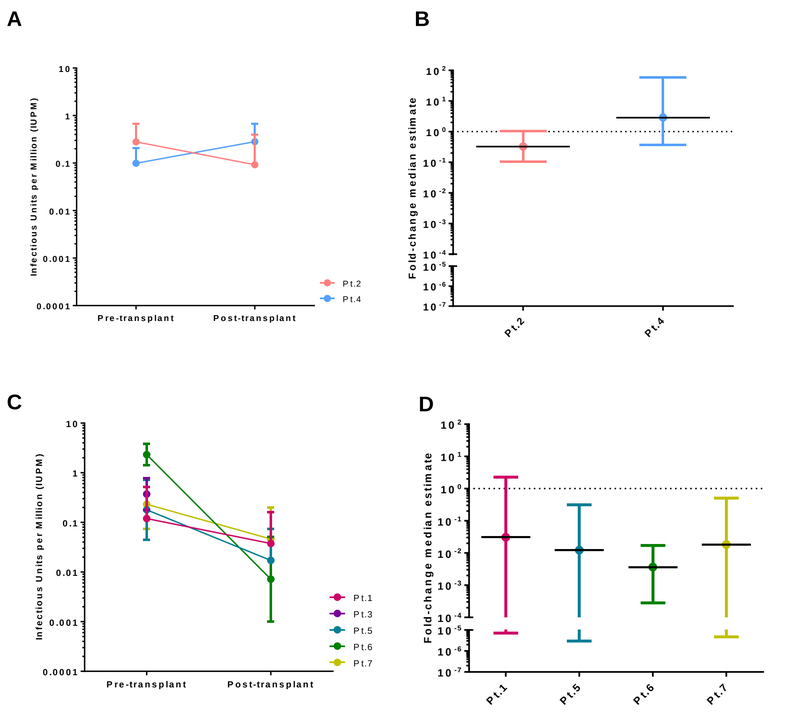

Figure 1 Measurements of HIV persistence following alloBMT. (A,B) Patients that did not achieve complete donor chimerism. **(C,D)** Patients who achieved complete donor chimerism. **(A,C)** HIV latent reservoir measurement of replication-competent virus by qVOA using limiting dilution maximum likelihood statistics expressed as a frequency of infectious units per million cells (IUPM). In cases of no viral outgrowth, the median posterior estimate was reported. **(B,D)** The fold-change median estimate pre-to post-transplant with 95% CIs using a mixed effects Bayesian model.

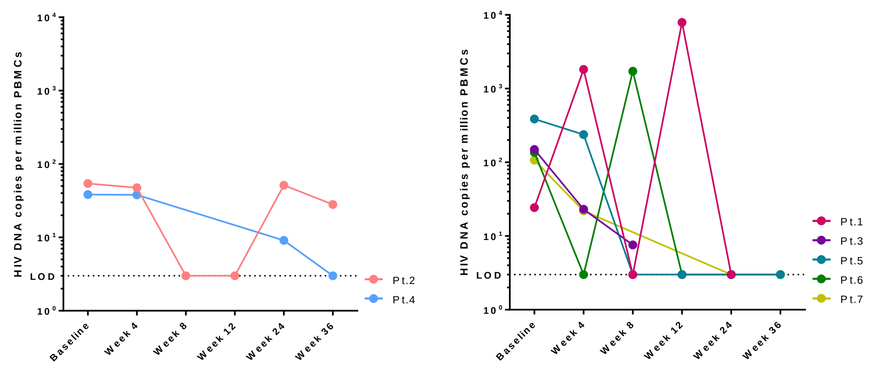

Figure 2 Proviral HIV DNA measurements of HIV persistence following alloBMT. (*Left*) Incomplete donor chimerism patients (*Right*) Complete donor chimerism patients. Proviral HIV DNA measurement of *pol*+ by ddPCR using digested DNA extracted from PBMCs in duplicate of 450,000 genomes each. The limit of detection is 3 HIV pol+ copies/10^6 PBMCs. Shapes that fall on the dotted line are below the limit of detection.

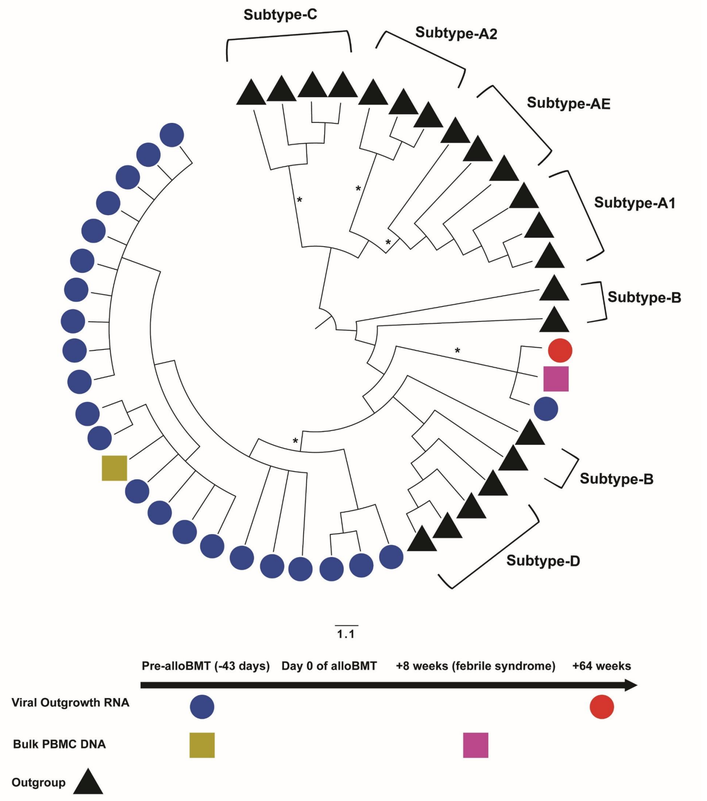

Fig. 3 Phylogenetic analysis of patient 6 using the RT-region of *pol*. Maximum likelihood tree analysis (-*lnL*=1260.1020) was carried out in PAUP* with the T92+Γ model of evolution selected with an α=0.26 and ti/tv=5.49 based on the Akaike Information Criterion. Bootstrapping of 1000 replicates was carried out with tree-bisecting-rearrangement branch swapping. The patient had no predicted drug resistance mutations or hypermutation. (*) Reported bootstrap values >75%.

RESULTS

Seven participants with HIV infection received alloBMT with optimized ART and remained suppressed (**Table 1**). There were no reported AEs related to ENF administration. All participants had successful engraftment (500 X10^3/mm^3 neutrophil recovery) and 5/7 patients achieved complete donor chimerism by week 24.

While ART was maintained through day 60, modification of ART was required in all participants (**Table 2**). Patient 3 self-interrupted ART post-transplant, and presented with acute retroviral syndrome associated HIV viral rebound with a HIV RNA of 25,518 c/ml in plasma and 17,000 c/ml in cerebral spinal fluid. ENF was initiated where he recovered and was able to re-start oral ART upon discharge.

In the two patients who did not achieve complete donor chimerism, the viral reservoir remained detectable without significant change in size by the viral outgrowth assay post-transplant (**Fig.1A,B**). In the four patients who achieved complete donor chimerism, the latent reservoir decreased **(Fig.1C,D)** with mean decrease of $_{0.51}2.11_{3.07}$. Log-reductions in the size of the latent reservoir were all greater than 1.5 in patients: Pt.1 ($_{0.35}1.51_{5.15}$), Pt.5 ($_{0.51}1.91_{5.52}$), Pt.6 ($_{1.77}2.44_{3.54}$), and in Pt.7 ($_{0.29}1.74_{5.33}$).

All participants had detectable proviral DNA in PBMCs prior to transplant (**Fig. 2**). In participants who achieved complete donor chimerism HIV proviral DNA was undetectable at week 36 post transplant. However, at early timepoints, patients 1 and 6 had transient increases in proviral DNA measurements.

In participant 6, phylogenetic studies revealed that the proviral DNA virus from week 8 matched a pre-transplant outgrowth sequence (**Fig.3**) and rare late post-transplant outgrowth sequence. In addition, at this week 8 timepoint the participant was clinically ill with CMV and possible histoplasmosis reactivation.

DISCUSSION

AlloBMT with optimized ART was safe and feasible in our cohort. In addition, ENF was well-tolerated with no adverse events. The importance of ART maintenance during alloBMT was highlighted by participant 3, who experienced life-threatening HIV viral rebound upon self-interruption of ART.

The impact of alloBMT on the size of the latent reservoir depended on the extent of donor replacement of the recipient hematopoietic system. For those who achieved full donor chimerism, there was significant log reduction in infectious virus by the outgrowth assay and disappearance of proviral DNA. However, in patient 6 despite full donor chimerism, infectious virus was identified at one timepoint using the qVOA, indicating complete viral eradication was not achieved by this approach. In this case, detection of HIV post-transplant could represent *de novo* infection of donor cells or the persistence and/or clonal expansion of a rare residual host cell.

Future strategies for potential HIV cure in the context of alloBMT might include strategies to protect donor cells such as CCR5delta32 or enhancement of donor cell activity against HIV with generation of cytolytic donor T cells and adoptive transfer technologies.

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