Adoptive Lymphocyte Transfer to an HIV-infected Progressor from an Elite Controller

Supplemental information

Enrollment Criteria

Patients 18 years or older with confirmed HIV infection were eligible as recipients if they met the following inclusion criteria: HIV plasma viral load >10,000 copies/mL on available optimized cART, to include at least 3 drugs, one of which is a non-nucleoside reverse transcriptase inhibitor, integrase inhibitor, or protease inhibitor; failure or intolerance of at least 2 previous combination antiretroviral regimens; CD4 count of <350 cells/μL; hematocrit ≥ 27%; platelets ≥ 25,000/μL; no significant underlying cardiac, renal (creatinine < 2.0 mg/dL) or hepatic (ALT< 150 U/mL) disease; and willingness to use 2 forms of contraception. Recipient exclusion criteria included: pregnancy or breastfeeding; discordant antibody status with a donor who is antibody positive for EBV, CMV, or HHV-8; malignancy requiring systemic therapy; active untreated opportunistic infection requiring systemic therapy; and current or recent (< 30 days) treatment with a systemic corticosteroid, immunosuppressive, or cytotoxic agent.

Patients 18 years or older with confirmed HIV infection for ≥ 7 years were eligible as donors if they met the following inclusion criteria: matched to at least one HLA-B allele of the potential recipient at a two-digit resolution or higher; hematocrit ≥ 30%; platelets ≥ 100,000/μL; white blood cells ≥ 3.0 x 10^9/L; and no underlying cardiac, pulmonary, renal, or hepatic disease that would preclude patient from undergoing apheresis. Initially donors were required to have no or minimal (one or two nucleotide

reverse transcriptase inhibitor drugs) prior antiretroviral therapy; CD4+ cell count ≥ 400 cells/μL; and HIV viral load < 50 copies/mL, with no recorded HIV viral load obtained after acute HIV infection > 2,500 copies/mL. After the results of the START trial became available (*3*), the protocol was amended to allow current cART as long as they had received no antiretroviral therapy for a period of at least 7 years prior to starting cART, with CD4+ cell count ≥ 400 cells/μL and HIV viral load < 50 copies/mL at the time of initiation of cART, and no recorded HIV viral load obtained after acute HIV infection > 2,500 copies/mL. Donor exclusion criteria included the following: history of AIDS-defining illness; history of malignancy other than basal cell carcinoma of the skin, or in situ carcinoma of cervix or colon; pregnant or breast-feeding; HLA homozygous, haplo-identical to recipient; and any NIH Blood Bank criteria, other than HIV positivity, for exclusion from donating blood (https://clinicalcenter.nih.gov/blooddonor/can_i_donate.html).

Sequencing of recipient HIV

For near full-length sequencing of the recipient HIV, viral RNA was isolated from 400 μL of plasma using the EZ1 Virus Mini Kit v2.0 (Qiagen), reverse transcribed using Superscript III first Strand synthesis kit (Invitrogen Life Technologies) with a gene specific primer reported previously UNIEF7 (5'-GCACTCAAGGCAAGCTTTATTGAGGCTT-3') (*51*) and stored at -80^{0}C. cDNA for 3 samples was amplified for 40 cycles using the High Fidelity PrimeSTAR GXL DNA Polymerase kit (TakaRa Clontech) with primer pair FGF60 (5'-

CAGACCCTTTTAGTCAGTGTGGAAAATC-3') and FGR95 (5'-GGTCTAACCAGAGAGACCCAGTACAG-3'), as previously reported, to generate a 9.0 kb amplicon (*52*). PCR conditions were 1 cycle of 98°C for 10 second, then 40 cycles of 98°C for 10 second and 68°C for 9 minutes, and finally a 10 minutes extension at 72°C.

One step RT/PCR was performed on 12 recipient samples to amplify the 2.6 kb polymerase gene by PrimeScript™ RT-PCR Kit Ver.2 (Takara Clonetech) according to manufacturer instruction. RT-PCR primers were (A) Forward 5'-CAGGAGCAGATGATACAG-3' and Primer (D) Reverse 5'-CCCTTCACCTTTCCAGAG-3 (*53*). RT-PCR conditions include an initial RT step at 50^0C for 50 min, then 94^0C for 2 min, followed by 45 cycles of PCR at 94^0C for 30 sec, 52^0C for 30 sec, 68^0C for 2.7 min and an extension at 68^0C for 10 min.

The Illumina Nextera XT kit was used for shotgun library preparation according to the manufacturer's instruction (Nextera XT, Illumina). In brief, one ng of purified PCR product was tagmented, followed by 12 cycles of PCR to add necessary adapters and barcode indexing sequences to the ends of the DNA fragments, and purification using Agercourt Ampure XP beads (Beckman Coulter). The final libraries were normalized, pooled and loaded on an Illumina MiSeqDX for a 250-cycle, paired-end sequencing using Miseq V2 kit.

Paired reads from recipient samples pre- and post-infusion were filtered for HIV specific sequences using Lastal (version last-460) (*54*) with HIV-1_{HXB2} (GenBank acc. no. K03455) as a reference sequence. Filtered reads were then adaptor and quality trimmed with Trimmomatic (*55*). The remaining reads were de novo assembled using

Trinity (*56*) and resulting contigs were ordered and merged using V-FAT (https://www.broadinstitute.org/viral-genomics/v-fat) with HIV-1_{HXB2} as a reference backbone to produce an initial sample-based consensus sequence. Paired reads were then aligned to this initial consensus sequence with NovoAlign (http://novocraft.com). The above steps were executed through wrappers in viral-ngs v.1.0.0 (https://github.com/broadinstitute/viral-ngs). A pileup file was then produced from the initial consensus alignment using SAMtools mpileup (*57*) from which a variant read count file was created using VarScan v.2.4.2 (*58*). A final consensus sequence was created based on the majority variant at each position.

Multiple sequence alignment was performed on the recipient consensus sequences (major variants, pre- and post-infusion) and the donor sequences using Clustal Omega (*49*) to determine overlap and major sequence differences. Paired reads from recipient samples pre- and post-infusion were analyzed using VirVarSeq (*59*) to determine minor codon frequencies in regions overlapping the donor sequences. Recipient codons that matched codons found in the donor were filtered based on their frequencies in pre- and post-infusion samples. Codons with frequencies greater than or equal to one percent in the pre-infusion samples were removed as an indication that they were already present in the recipient before infusion with those frequencies less than one percent falling below the detection limit for viral minority variants using Illumina sequencing (*60*). The remaining codons which matched the donor sequence and had frequencies greater than or equal to one percent in the post-infusion samples were retained as they could indicate donor virus present in the recipient.

Supplemental Figure

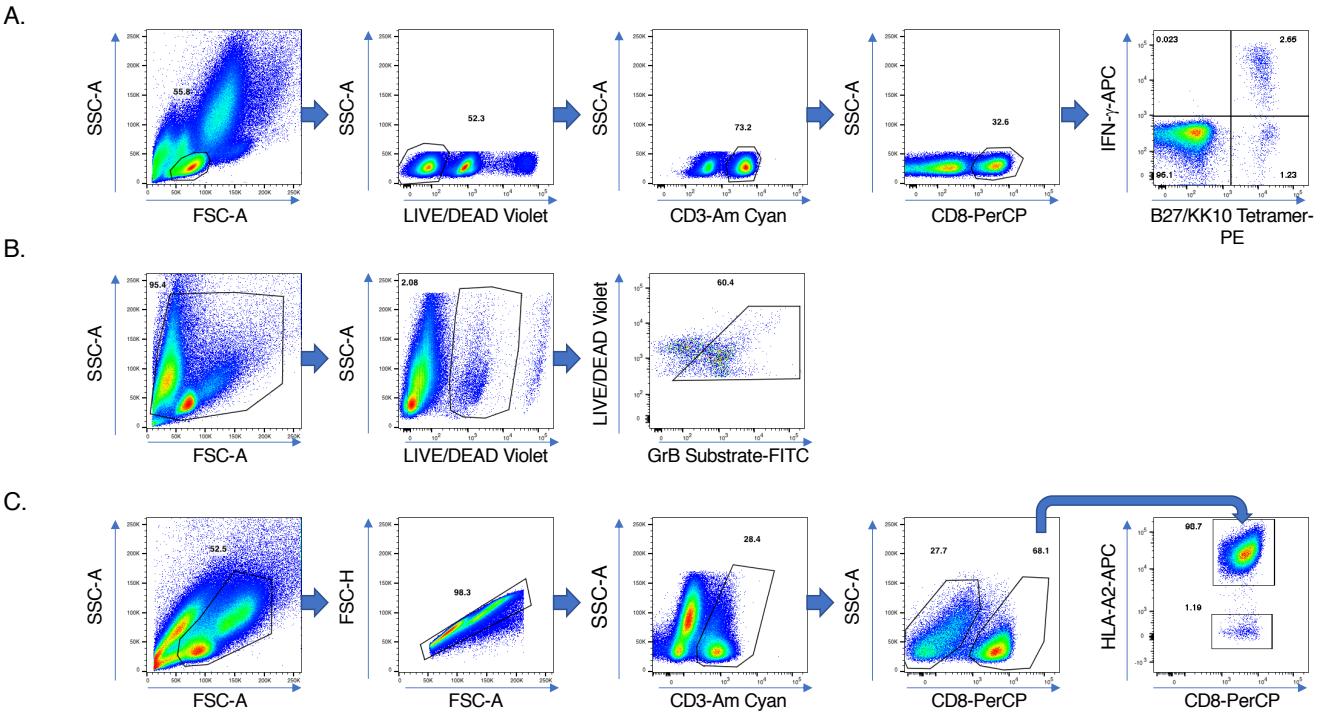

Figure S1. Main flow cytometry gating strategies. A. In experiments assessing functions of T cells following brief (<6 hour) re-stimulation, small elliptical FSC-A by SSC-A gates were drawn to identify lymphocytes by light scatter properties prior to gating on live PBMC effectors (negative by LIVE/DEAD Fixable Violet staining) bearing the CD3+ and CD8+ surface phenotype, as shown here. B. After more prolonged stimulation, live and dying large, complex lymphoblasts based on light scatter were further gated on live targets (that had been labeled with the LIVE/DEAD Fixable Violet Stain prior to co-incubation with effectors and confirmed to be CD3+CD4+ or CD3+CD4- in parallel tubes) before gating on pre-apoptotic, viable targets exhibiting increased GrB substrate fluorescence, as depicted in these representative plots from cytotoxicity experiments. C.

PBMCs recovered after adoptive transfer were gated on small and blasted mononuclear cells based on light scatter and then gated on singlet (based on FSC-H and FSC-A), CD3+CD8+ or CD3+CD8- donor or recipient cells.

Supplemental Table 1

Monoclonal Ab	Company	Catalogue number	Clone
Anti-IFN-gamma-APC	BD Biosciences	554702	B27
Anti-CD107a-PE Cy7	BD Pharmingen	561348	H4A3
Anti-p24-RD1	Beckman Coulter	6604667	KC57
Anti-CD3-AmCyan	BD Pharmingen	339186	SK7
Anti-CD8-PerCP	eBioscience	8043-0087-120	SK1
Anti-CCR7-PE Cy7	BD Pharmingen	557648	3D12
Anti-CD27-APC Cy7	BioLegend	302816	O323
Anti-perforin-BV421	BD Pharmingen	563393	dG9
Anti-granzyme B-Alexa Fluor700	BD Pharmingen	560213	GB11
Anti-Ki67-FITC	eBioscience	11-5699-42	20Raj1
Anti-human HLA A2-APC	BioLegend	343307	BB7.2