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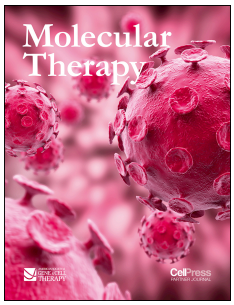

HIV-Specific T-cells Generated from Naive T-cells can Suppress HIV *in vitro*, Display Cytotoxicity, and Recognize Wide Epitope Breadths

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Short Title: Cord and Adult-derived HIV-Specific T-cells

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ABSTRACT

The Berlin Patient represents the first and only functional HIV cure achieved by hematopoietic stem cell transplant (HSCT). In subsequent efforts to replicate this result, HIV rebounded post-HSCT after withdrawal of antiretroviral therapy. Providing HIV-specific immunity through adoptive T-cell therapy may prevent HIV rebound post-HSCT by eliminating newly infected cells before they can seed systemic infection. Adoptive T-cell therapy has demonstrated success in boosting Epstein-Barr virus and cytomegalovirus-specific immunity post-HSCT, controlling viral reactivation. However, T-cell immunotherapies to boost HIV-specific immunity have been limited by single-epitope specificity and minimal persistence or efficacy *in vivo*. To improve this strategy, we sought to generate allogeneic HIV-specific T-cells from HLA-A02+ HIV-negative adult or cord blood donors. We focused on HLA-A02+ donors due to well-characterized epitope restrictions observed in HIV+ populations. We show that multi-antigen HIV-specific T-cells can be generated from naïve T-cells of both cord blood and adults using a reproducible good-manufacturing practice (GMP) grade protocol. This product lysed antigen-pulsed targets and suppressed active HIV *in vitro*. Interestingly, these cells displayed broad epitope recognition, despite lacking recognition of the common HLA-A02-restricted HIV epitope Gag SL9. This first demonstration of functional multi-antigen HIV-specific T-cells has implications for improving treatment of HIV through allogeneic HSCT.

Key Words: HIV-Specific T-cells; adoptive T-cell therapy; allogeneic transplant

INTRODUCTION

Current therapies for human immunodeficiency virus (HIV) are not curative. While antiretroviral therapy (ART) successfully suppresses active infection, it does not eradicate latent HIV reservoirs. HIV cure strategies have focused on replicating the successful allogeneic hematopoietic stem cell transplant (HSCT) strategy used for the Berlin Patient [3, 4], without success due to viral rebound of the patients' autologous viral reservoirs [5, 6]. The delayed viral rebound in the Boston patients suggests large reductions in the HIV reservoir may allow HIV-Specific T-cells to control viral rebound from the low levels of virus that persist post-transplant.

Several lines of evidence have firmly established that HIV-specific T-cell responses play a critical role in controlling HIV replication in infected individuals. First, the emergence of HIV-specific CD8+ T-cell responses following infection is temporally associated with a 10^2 - 10^3 fold reduction in viremia during acute HIV infection [7-9]. Second, in the rhesus macaque SIV infection model the depletion of CD8+ T-cells has been shown to lead to a dramatic increase in viral load and rapid progression [10]. Third, in infected humans it has been established that there are strong links between the possession of certain MHC-I alleles and clinical progression [11, 12]. Fourth, CD8+ T-cells exert immune pressure on targeted epitopes, driving the emergence of escape mutations often at a fitness cost to the virus [13, 14]. Finally, CD8+ T-cells isolated from HIV-infected individuals show a clear ability to eliminate infected cells and suppress viral

replication *in vitro* [15]. Thus, there is a strong rationale for enlisting HIV-specific T-cells to prevent viral rebound from any residual infected cells that may persist following allogeneic HSCT and achieving a functional cure.

Adoptive T-cell therapy post-HSCT has been successful in augmenting anti-viral immunity against chronic infections such as cytomegalovirus (CMV), Epstein-Barr virus (EBV) [16-19], and associated cancers, emphasizing the critical role T-cells play in preventing viral rebound. However, HIV is able to avoid immune pressures more successfully than viral counterparts due to downregulation of MHC I and CD4 on infected cells, leading to suboptimal anti-HIV CD8+ T cell responses [20]. Despite efforts to augment anti-viral immunity against HIV, T-cell therapy has shown no efficacy, likely due to infusion of single epitope-specific clones that are susceptible to immune escape [21], or the absence of CD4+ T-cells resulting in a lack of persistence of infused cells [22]. Furthermore, prevention strategies such as the HIV vaccine trial RV144 [23], have been criticized for the lack of eliciting strong T cell responses needed to achieve sustained anti-HIV immunity [24, 25]. Thus, HIV-specific T cell therapies that demonstrate the ability to persist and overcome immune escape through recognition of multiple HIV epitopes, will be critical boosting anti-HIV immunity.

The post-HSCT setting presents a unique opportunity where adoptive HIV T-cell therapy could target residual infected cells to prevent rebound from the low levels of virus remaining. Furthermore, these HIV-specific T-cells may demonstrate

better persistence compared to the previous HIV immunotherapy trials mentioned, which had no conditioning regimen. Based on the successful generation of EBV and CMV-specific T-cells from virus-naïve allogeneic donors [26-29], we sought to generate HIV-specific T-cells from HIV-seronegative, adults and cord blood naïve T-cells, in a good manufacturing practice (GMP)-compliant manner. While a closely related HIV negative donor could serve as the source of both the HSCT and the adoptively transferred T-cells, we also explored the use of unrelated cord blood donors to generate HIV-specific T-cells. There are several benefits associated with the use of cord blood for HSCT including: (1) less restrictive HLA matching requirements compared to their adult counterparts, reducing the likelihood of graft-vs-host disease (GvHD) [30], (2) rapid availability, (3) flexibility for scheduling transplantation, and (4) lower risk of relapse due to graft-vs-leukemia [30].

To develop a widely applicable form of HIV immunotherapy, we focused on HLA-A02+ donors, as this allele has one of the highest frequencies across several ethnic groups and is dominant in HIV+ individuals infected with clade B HIV [31]. Many immunodominant HIV A02-restricted CTL epitopes have been identified and well characterized in HIV+ populations [32, 33]. Here, we describe a novel approach to generating HIV-specific T-cells from HLA-A02+ HIV-naïve adults (HNA-T) and cord blood (CB-T) which demonstrate cytolytic capacity, suppress active HIV *in vitro*, and broadly recognize epitopes from HIV antigens Gag, Nef, and Pol.

RESULTS

HNA-Ts and CB-Ts are derived from the Naïve T-cell Compartment of HIV Seronegative Adult donors and Cord Blood

Based on our previous success generating HIV-specific T-cells from HIV seronegative adults (HNA-Ts) **(Supplemental Figure 1)** [34], we sought to determine if HNA-Ts were generated from the naïve T-cell compartment of healthy HIV seronegative adults, as seen in CMV [19]. Using magnetic selection, naïve T-cells were selected for CD3+CD45RA+CCR7+CD62L+ T-cells **(Supplemental Figure 2).** After the naïve selection step, T-cells derived from both the naïve compartment and non-naïve compartment were expanded in parallel using antigen presenting cells (APC) pulsed with Gag, Nef, and Pol (GNP) **(Supplemental Figure 1).**

Naïve-T-cell derived HLA-A02+ HNA-T products expanded to clinically relevant numbers (median=100.5e6 cells; range=23.8e6-195.3e6 cells) **(Figure 1A)**. IFNγ ELISPOT against GNP pepmix confirmed that HIV-specific T-cells were only detected in T-cell products derived from the naïve T-cell compartment, since T-cell products derived from non-naïve T-cells elicited insignificant levels of IFNγ in response to HIV antigens (**Figure 1A**). The naïve T-cell-derived HNA-Ts showed HIV-specificity against Gag (mean=158.83 IFNγ SFC/1e5 cells; $p<0.0001$), Pol (mean=122.87 IFNγ SFC/1e5 cells; $p=0.016$), and GNP pepmix (mean= 225.75 IFNγ SFC/1e5 cells; $p<0.001$) compared to irrelevant controls CTL alone

(mean=3.66 IFNγ SFC/1e5 cells) and Actin (mean=5.37 IFNγ SFC/1e5 cells), (two-way ANOVA).

To further confirm that HIV-specific T-cells can be derived from virus naïve donors, we repeated the approach using cord blood [30]. As shown in **Figure 1B**, CB-Ts were successfully expanded from cord blood (median=56.8e6 cells; range=25e6-132e6 cells). IFNγ ELISPOT was performed to evaluate for HIV-specificity. Increased IFNγ production was observed in response to HIV Gag (mean=136.05 IFNγ SFC/1e5 cells; p=0.023), Nef (mean=196.86 IFNγ SFC/1e5 cells; p=0.0028) and Pol (mean=233.59 IFNγ SFC/1e5 cells; p=0.0006), as well as the GNP pepmix (mean=272.73 IFNγ SFC/1e5 cells; p<0.0001), compared to T-cells alone (mean=4.182 IFNγ SFC/1e5 cells), (2way ANOVA) **(Figure 1B)**.

To evaluate whether the expansion and function of virus-naïve donor-derived HIV-specific T-cell products differed from products derived from HIV-positive adults (HPA-Ts), we also generated HPA-Ts from HIV+ HLA-A02+ donors, based on established and FDA approved (IND17562 and IND15984) protocols **(Figure 1C)** [35, 36]. HPA-T products derived from HIV+ individuals expanded to clinically relevant numbers (median=95.5e6 cells; range=65.8e6-171.72e6 cells) similar to naïve donor-derived HIV specific T-cells. Similarly, increased IFNγ production was observed in response to HIV GNP pepmix stimulation (mean=529.6 IFNγ SFC/1e5 cells; p<0.0001) compared to T-cells alone (mean=5.667 IFNγ SFC/1e5 cells), (two-way ANOVA).

HIV-specific T-cell products derived from virus naïve donors have higher CD4+ T-cell frequencies than products derived from HIV+ donors

Phenotyping analysis of HNA-Ts, CB-Ts and HPA-Ts revealed differences in the composition of the three product types **(Figures 2A-C)**. Notably, HIV-specific T-cells derived from HIV+ donors (HPA-T products) had low frequencies of CD4+ T-cells (mean=7.65%; range=0.69-25.4%) **(Figure 2C)** compared to HNA-T (mean=38.4%; range=13.4-73.6%) and CB-T (mean=23.35%; range=3.8-51.5%) products **(Figures 2A-B)**. The frequencies of CD8+ T-cells in HPA-T (mean=44.1%; range=16.9-69.6%; n=7), HNA-T (mean=26.46%; range=6.4-59.8%; n=6), and CB-T (mean=49.48%; range=13.0-73.0%; n=6) products were similar between products with higher levels of CD3neg/CD56+ NK cells (mean=17.29%; range=3.1-42.5%) observed in HPA-T products **(Figures 2A-C)**. The memory populations of all three cohorts were skewed towards an effector memory (EM) phenotype, with a smaller population of T-cells detected in all three product types that had a central memory (CM) phenotype (HPA-T-EM mean=85.71%; range=61.1-97.8%; HNA-T-EM mean=58.13%; range=41.4-77.8%; CB-T-EM mean=69.9%; range=49.5-89.6%) **(Figures 2A-C).**

HIV-specific T-cells from all sources show minimal expression of exhaustion markers

Flow analysis revealed similarly low expression levels of markers associated with exhaustion in HPA-T and HNA-T products (PD-1: HNA-T mean=10.12% vs.

HPA-T mean=10.13%; and TIM-3: HNA-T mean=7.63% vs. HPA-T mean=9.62%) **(Figure 2A and C)**. Moreover, CB-T products showed even lower levels of exhaustion marker expression, (mean: PD-1=6.8%; LAG-3=3.45%; TIM-3=3.05%; KLRG1=0.34%; CD57=1.08%) **(Figure 2B)**.

CB-T, HNA-T, and HPA-T Products Exhibit Similar Cytolytic Ability and Polyfunctionality in vitro

Since exhaustion marker expression is also associated with cell activation at low levels, we next evaluated the functionality of the HIV-specific T-cell products to ensure they all elicited antigen specific cytolytic activity and were polyfunctional. In a chromium-release cytotoxicity assay, CB-Ts were tested for their ability to lyse autologous LCL or PHA blasts pulsed with GNP pepmix **(Figure 3A)**. CB-Ts (n=2) were able to lyse LCL pulsed with GNP, with an E:T of 40:1 producing a mean specific lysis of 25.28±4.22%. CB-T also displayed lysis against unpulsed LCL (40:1 mean 13.63±1.80%). CB-Ts were manufactured using autologous LCL lines as APCs, which generated products with both HIV and EBV specificity. Similarly, HNA-T products (n=2) showed a mean lysis of 26.53±4.83% at an E:T ratio of 40:1 against autologous PHA blasts pulsed with GNP, and HPA-T products showed a mean lysis of 34.52%±3.75 at 40:1 (n=3).

To evaluate cytolytic activity against virus infected cells, CB-T were tested in a viral inhibition assay to determine whether these products suppress a laboratory strain of HIV (SF162), in an *in vitro* model of active HIV infection **(Figure 3B)**. CB-T were co-cultured at varying effector-to-target ratios with autologous CD8-

depleted PBMCs that had been infected with SF162. Supernatants were measured for p24 by ELISA, as an indicator of HIV presence on Day 7. At E:T ratios of 40:1 and 20:1, CB-Ts were able to significantly suppress HIV through Day 7 *in vitro* ($p<0.0001$, two-way ANOVA) compared to CD8-depleted HIV-infected cells alone. This was similar to the levels of HIV suppression we found in HNA-T products, as shown in **Figure 3B.**

Products from all three cohorts were also tested for product polyfunctionality in response to GNP pepmix stimulation **(Figure 3C)**. T-cells were stimulated with either actin (negative control) or GNP pepmix overnight and cell culture supernatants were tested by multiplex for levels of cytokines IL-2, IL-8, IFNγ, and TNFα. Actin-stimulated T-cell cytokine production levels were negligible (data not shown). The production of similar cytokine levels among the three cohorts in response to GNP stimulation suggests these HIV seronegative, naïve-derived T-cell products have similar polyfunctional capacity as those products generated from HIV+ individuals.

Epitope Mapping of HPA-T, HNA-T, and CB-T Products Reveals Wide Epitope Breadth in HIV Seronegative Individuals.

We previously showed [19] that CMVpp65-specific T-cells from cord blood and adult CMV seronegative donors did not recognize the expected typical peptides such as the HLA-A02-restricted peptide NLVPMVATV. To determine whether this

result was unique to CMVpp65 and NLV, we compared the peptide repertoires of HIV-specific T-cells from HLA-A02 HNA donors, CB-T donors and HIV positive donors using HIV gag peptide pools.

Products from all three donor cohorts were epitope mapped using overlapping 15-mer peptides (AIDS Reagent Program). Products were mapped using pool matrices with each pool consisting of 8-10 15-mer peptides. Cross-reactive pools containing the same 15mer peptide were confirmed by individual 15mer ELISPOTs, as previously described [34]. There were common 15-mers mapped among HNA-T **(Table 1)**, CB-T **(Table 2)**, and HPA-T **(Table 3)** products.

Based on the Los Alamos National Laboratory (LANL) Database of known HIV epitopes [37] and lists of HLA-associated selection on the HIV proteome [38], the majority of HNA-T and CB-T products recognized known epitopes in HIV+ populations. However, the commonly recognized A02-restricted epitope SLYNTVATL (SL9), was not recognized by any HIV-negative naïve-derived products using this screening approach.

HLA A2-restricted HIV-epitope specific T-cells derived from virus naïve donors do not recognize the typical HLA-A02 Gag epitope SL9, but have high functional avidity

Knowing that SL9 specific T-cells were not detected in 21/21 (10 HIV-negative adults and 11 cord donors) HLA-A02+ virus-naïve donor-derived HIV-specific T-

cell products, and given that an estimated 75% of HIV-1 infected HLA-A02+ individuals recognize the Gag-SL9 epitope [39], we next asked whether we could force the expansion of SL9-specific T-cells from these naïve donors. For this we stimulated HLA-A02+ HIV seronegative donor-derived PBMCs with APCs pulsed with the SL9 9mer alone versus the GNP pepmix **(Figure 4).** As shown in **Figure 4A** it was not possible to force Gag-SL9 specificity in any of the HIV seronegative donor-derived products (n=3) **(Figure 4A)**. However, from the same donors, we produced HNA-T and CB-T products specific for other Gag/Nef/Pol epitopes **(Figure 4B)**. In contrast, and as expected, we could successfully expand Gag-SL9 specific T-cells from an HIV+ donor using the same methodology, **(Figure 4C)**.

Since HIV-specific T-cells derived from HLA A2+ naïve donors did not recognize the expected SL9 Gag epitope and instead recognized other epitopes spanning HIV Gag, Nef, and Pol, we considered whether the avidities of these T-cell receptors for their respective peptide/MHC complexes might differ depending on the donor source. The functional avidity was determined by the EC_{50}, or the concentration of peptide used to still provide one-half the maximum magnitude of IFNγ response. Functional avidity for HPA donor OM9 Gag SL9-specific T-cells revealed a low functional avidity (EC_{50}=3.72 ng/ml) **(Figure 4D)**.

To compare this functional avidity to the HIV negative products, we used limiting dilutions of peptides to test the functional avidity of two HIV epitopes (one Gag

and one pol epitope) recognized by both HNA-T and CB-T products. As shown in **Supplemental Figure 3A**, for T-cells recognizing the Gag epitope HLVWASREL, the mean peptide concentration needed to induce a half-maximum IFNγ response (EC_{50}) was 0.1 ng/ml in the CB-T-derived donor product compared to an EC_{50}=0.009 ng/ml observed with the seronegative adult donor product. Additionally, naive donor-derived T-cells that recognized the Pol epitope KLVGKLNWA showed similar avidity irrespective of the donor source: 0.01 ng/ml (CB-T donor) versus 0.03 ng/ml (adult seronegative donor) (**Supplemental Figure 3B**) suggesting that HIV naïve donor-derived HNA-Ts and CB-Ts may have higher functional avidity for their cognate epitope, compared to HPA-T products.

DISCUSSION

This is the first description of a GMP-compliant, reproducible platform for generating HIV-specific T-cells from HIV seronegative, naïve T-cells derived from adult or cord blood donors. These HNA-Ts and CB-Ts demonstrated HIV-specificity against epitopes spanning the breadth of Gag, Nef, and Pol. Assessing functionality of these products, HNA-T and CB-Ts suppressed active HIV infection *in vitro* and lysed autologous LCL and PHA blast targets pulsed with HIV pepmix. We also observed a cytokine response dominated by production of IFNγ and TNFα in response to HIV pepmix stimulation, suggestive of a T_H1 skewed response associated with intracellular pathogens such as HIV. IL-8 was also detected, normally involved in innate immune responses such neutrophil recruitment, and is not unexpected, based on the diverse phenotypes of these HNA-T and CB-T products.

Hanley *et al*. previously demonstrated that CMV-specific T-cells can be generated from cord blood, an obligatory source of naïve T-cells [27, 28]. It was shown that HLA-A2+ CMV-specific T-cells derived from naïve cord blood recognized atypical epitopes LQT and MLN, whereas CMV-specific T-cells generated from CMV+ donors recognize the typical NLV epitope. That study demonstrated *in vivo*, that CMV-specific T-cells recognizing atypical epitopes were protective, with the presence of these T-cells correlating with an absence of CMV reactivation. Extending this model to HIV, we compared the epitope

recognition breadths of HNA-Ts, CB-Ts, and HPA-Ts together with known HIV+ epitopes from the Los Alamos National Database (LAND).

Interestingly, we were unable to generate Gag-SL9 specific T-cells from HLA-A2+ HIV seronegative donors, suggesting that naïve donor-derived T-cells recognize a different epitope repertoire from HIV+ donors. This finding is similar to that of a study of 13 HIV uninfected individuals where a Gag-vaccine did not produce Gag-SL9 responses [39] and even forced-expression failed to produce Gag SL9-specific T-cells from healthy adult or cord donors. Nevertheless our approach was able to produce SL9-specific T-cells from the HIV+ individuals. Other investigators have also succeeded in generating SL9-specific T-cells from healthy cord blood, suggesting the type of APC used for manufacturing may play a role in determine T-cell specificity [40]. Gag SL9-specific T-cells are commonly identified in chronically infected HIV+ individuals, but not during acute infection, demonstrating the HIV epitope repertoire and resulting T-cell responses change over the course of HIV infection [41-43]. In chronic HIV infection, HIV+ individuals may be unable to control viral load due to the accumulation of escape mutations in targeted epitopes [13, 14], T-cell exhaustion [44-46], or the presence of cytotoxic T lymphocytes (CTLs) that recognize immunodominant epitopes, such as Gag SL9 (SLYNTVATL), a response negatively associated with viral load in HIV progression, but limited to chronic infection [43, 47-49]. This has significant implications for developing non-exhausted T-cell products that target epitopes associated with multiple stages of HIV infection and progression. Importantly,

HNA-Ts and CB-Ts display a wide breadth of specificity across Gag, Nef, and Pol antigens, offering extensive coverage and may be critical for preventing immune escape.

Despite this advantage, one concern with the infusion of HNA-T and CB-T products is the presence of a notable CD4+ population with the potential to be infected by the recipient's virus. To address this concern, we are currently investigating two strategies to render HIV-specific T-cells resistant to HIV infection: gene modification and selection of donors naturally resistant to HIV. Gene modification approaches have shown promise in the HIV field. Several groups have shown chimeric antigen receptors (CARs) can target conserved HIV epitopes [50]. Other groups have employed Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology and Zinc Finger Nucleases (ZFN) to target HIV co-receptor CCR5 through disruption of the host genome, to prevent viral entry [51, 52]. Applying these gene modification strategies to our HIV-specific T-cells could produce a potent cell product with the desired bi-functionality – cytotoxicity and resistance to infection.

We are also exploring generating HIV-specific T-cells from HIV negative homozygous Delta32 CCR5 donors, who possess natural resistance to R5-tropic HIV strains [53]. The National Marrow Donor Program (NMDP) and the German Cord Blood Bank (DKMS) have typed homozygous Delta32 CCR5 cord units, providing a platform for rapid availability and clinical translation [54].

Lastly, it is important to note that demonstrating the persistence of these HIV-specific T cells will be critical to produce durable, long-term anti-HIV immunity, especially if ART interruption is considered. The use of other virus-specific T cells against EBV, CMV, and adenovirus in a post-transplant setting have demonstrated durability during this period of immune suppression, with few patients experiencing recurring viral infection or progression [55]. Translating this to the HIV setting, it is critical that HIV-Specific T cells persist to produce a durable long-term anti-HIV response, particularly during immune reconstitution post-HSCT where low levels of residual reservoir virus may rebound if left unchecked.

Ultimately, we envision multiple platforms for using HIV-specific T-cells in the allogeneic setting. First, HIV+ individuals with hematologic malignancies who receive an allogeneic-HSCT, could receive HIV-specific T-cells from the same stem cell transplant donor, to help control viral rebound during immune reconstitution. Second, HIV-specific T-cells derived from HIV seronegative sources could be used in combination with latency reversing agents to “shock-and-kill” infected cells and reactivate latent infection for subsequent targeting by T-cells [56-58]. The broad epitope recognition of HNA-Ts and CB-Ts may be critical in this setting, as they can recognize multiple HIV epitopes, reducing the chance of immune escape. These potential applications for HIV-specific T-cells from HIV seronegative donors set the stage for future cell therapy trials to

validate the efficacy of adoptive T-cell therapy in HIV. Currently we are also exploring safety and efficacy of autologous HIV-specific T-cells generated using the same approach in HIV positive individuals (NCT02208167; NCT03212989).

In summary, we show that HIV-specific T-cells can be generated from the naïve T-cell compartment of HIV seronegative adults and cord blood. These products have wide epitope recognition, suppress HIV *in vitro*, and demonstrate cytolytic abilities. This has important implications for HIV positive individuals undergoing stem cell transplantation for malignant disease where the HSCT donor can serve both as a source of hematopoietic stem cells and for the generation of HIV-specific T-cells.

METHODS

Isolation of Peripheral Blood Mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from HIV negative and HIV+ donors on ART, with acute or chronic HIV infection (University of North Carolina, Chapel Hill, NC; University of Texas MD Anderson Cancer Center, Houston, TX; and The George Washington University, Washington D.C.). All donations were obtained under informed consent approved at each institution. PBMCs were diluted 1:4 to 1:2 (blood: 1X PBS) and layered on top of 10-15 mL of Lymphocyte Separation Medium (MP Biomedicals, CA). Blood was spun for 30 minutes at 600 RPM at Room Temperature (RT). PBMCs were harvested from the lymphocyte layer and washed three times with 1X PBS prior to counting.

Generation of Dendritic Cells

PBMCs were plated for 2-hour adherence at 37 C, after which non-adherent cells were washed off and frozen. Dendritic cells (DCs) isolated from plastic adherence of PBMCs were fed with IL-4 (1000 U/mL), granulocyte macrophage colony stimulating factor (GM-CSF) (800 U/mL) for 6 days, added on Day 1 and Day 4. DCs were matured on Day 7 with IL-4 (1000 U/mL), granulocyte macrophage colony stimulating factor (GM-CSF) (800 U/mL), IL-6 (100 ng/mL), TNFα (10 ng/mL), IL- 1b (10 ng/mL; all R&D Systems, MN), and Prostaglandin E1 (1 mg/mL; Sigma-Aldrich, MO). DCs were harvested 24 to 48 hours after maturation for Stimulation 1.

411 ***Generation of Phytohemagglutinin blasts (PHAb) for HNA-T Manufacturing***

412 To generate PHAb, PBMC were stimulated with PHA-P (5 mg/mL; Sigma-Aldrich,
413 MO) in the presence of IL-2 on Day 1. PHAb were fed with IL-2 on Day 3, and
414 every 2-3 days thereafter with IL-2. PHAb were used as APCs in Stim 2 and 3,
415 for the HIV-naïve adult (HNA-T) and HIV-positive adult (HPA-T) T-cell
416 manufacturing protocols.

417

418 ***Generation of HIV-Specific T-cells from the HIV-Seronegative Adult Naïve T-***
419 ***cell Compartment (HNA-Ts)***

420 A magnetic MACS column (Miltenyi Biotech, Germany) was used to positively
421 select naïve T-cells from adult HIV negative PBMCs selecting for
422 CD3+CD45RA+CCR7+CD62L+. The negatively selected fraction was flushed
423 through the magnetic column and both the naïve and non-naïve T-cell
424 compartments were stimulated with antigen coated DCs in Stim 1, and
425 subsequently expanded according to our established protocol [34, 35]. Irradiated
426 PHAb were used as APCs in Stim 2 and 3. APCs were pulsed with HIV Gag, Nef,
427 and Pol pepmixes. These were chosen as they are more conserved compared to
428 Env, and allow targeting of multiple stages of HIV infection, as Nef is expressed
429 early while Gag and Pol are expressed later in infection [59]. These overlapping
430 HIV peptide libraries consisted of 15mers, overlapping by 11 amino acids (JPT,
431 Germany), based on consensus sequences of HIV-1. For Stim 3, modified K562
432 cells were added, expressing co-stimulatory molecules 41-BBL, CD80, CD83,
433 and CD86 (gift of Dr. Clio Rooney, Baylor, TX) to aid in expansion. The ratios of

cells cultured were: Stim 1 (1:10, T:DC), Stim 2 (1:4, T:PHAb), Stim 3 (1:1:4, T:PHAb:K562).

Generation of Autologous Lymphoblastoid Cell Lines (LCLs) for CB-T Manufacturing

Autologous LCL lines were generated from cord blood mononuclear cells (CBMCs), to serve as antigen-presenting cells for the generation of cord blood-derived HIV-specific T-cells (CB-Ts). 5e6 PBMCs were pelleted and resuspended in 200 ul of live B95-8 EBV (produced by infected marmoset cells). This B95-8-CBMC mixture was then resuspended in 2 mLs of cRPMI containing cyclosporine A (1 ug/ml). On a 96-well plate, 5 wells were plated at 200 ul each of the viral-cell suspension. The remaining 1 mL was diluted to 2 mL with cRPMI containing cyclosporine, and plated in 10 wells of 200 ul each. The cells were monitored weekly, and as the wells became confluent, cells were expanded into 24-well plates, T25 flasks, and ultimately T75 flasks. The generation of autologous LCL lines required from 1-2 months.

Generation of Cord blood-derived HIV-Specific T-cells (CB-Ts)

The protocol for the generation of CB-Ts is similar to that of the HNA-Ts [34, 35] with several key differences: (1) K562 were not used to expand CB-Ts T-cells in the 3rd stimulation; (2) At Stim 1, IL-7, IL-12, and either IL-15 OR IL-21 was used to assist in proliferation and expansion of T-cells; (3) autologous lymphoblastoid cell lines (LCLs) replaced PHA blasts as the antigen presenting cells (APCs) for

457 the second and third stimulation at a LCL: T-cells ratio of 1:1 **(Supplemental**
458 **Figure 1)**.
459
460 ***IFNγ ELISPOT Assay and Epitope Mapping***
461 No peptide or the irrelevant peptide, actin (JPT, Germany), was used as negative
462 control. Staphylococcus enterotoxin B (SEB) was used as a positive control
463 (Sigma-Aldrich, MO). T-cells were plated at 1e5/well on IFNγ coated ELISPOT
464 plates (Millipore, NJ). Positive responses are defined as having more than
465 double the spot forming cells (SFC) obtained in the negative controls, with a
466 minimum of 50 IFNγ SFC/1e5 cells/well. For epitope mapping, the 15mer
467 peptides overlapping by 11 amino acids spanning the consensus region of the
468 Gag, Nef, and Pol antigens were pooled and used according to previously
469 published matrices [34]. Using the matrices, cross-reactive pools were analyzed
470 for common 15mer epitopes and these 15mer epitopes were then individually
471 tested on ELISPOT to confirm epitope specificity.
472
473 ***HLA Epitope Specificity: IFNγ ELISPOT***
474 Based on epitope mapping ELISPOTs, expanded HIV-specific T-cells were
475 tested for HLA specificity to Gag, Nef, Pol, or individual peptides. For HLA
476 blocking, $1x10^5$ cells/well were treated with monoclonal mouse anti-human HLA
477 Class I or HLA Class II antibody (Dako, Agilent, CA) in a 96-well round bottom
478 plate for 1 hour at 37℃. Treated cells were transferred to ELISPOT plate,
479 stimulated with peptide, and developed as previously described.

480

481 ***Flow Cytometry Phenotyping and Exhaustion Panels***

482 Flow for phenotyping and exhaustion panels were run on the MACSQUANT
483 Analyzer (Miltenyi Biotech, Germany) with analysis done with FlowJo software
484 (FlowJo LLC, OR). For the phenotyping, the following antibodies were used: anti-
485 CD3, CD4, CD8, CD45RA, CD45RO, CD56, CD16, and CD62L (Miltenyi Biotech,
486 Germany). For Exhaustion phenotyping, the following antibodies were used: anti-
487 CD3, PD-1, LAG-3, TIM-3, KLRG1, and CD57. For isotype controls, the
488 recommended isotype for each of the previous antibodies was purchased and
489 used. 1e6 cells were stained per condition, incubated for 30 mins at 4 C, washed
490 twice with FACS buffer (2-5% FBS/1X PBS), and run on the MACSQUANT.

491

492 ***Multiplex Assay***

493 To assess polyfunctionality of T-cell products, a multiplex assay was run using
494 the Bio-plex Pro Human 17-plex Cytokine Assay kit (Biorad, CA). HPA-T, HNA-T,
495 and CB-T products were thawed overnight with IL-2 (50 U/mL) on Day 1. On Day
496 2, T-cells were washed and plated at 1e6 cells/well with 1 ul of corresponding
497 pepmix: actin, GNP, or SEB. On Day 3, supernatants were harvested from the
498 wells and plated on the multiplex plate. The multiplex protocol provided by Biorad
499 was followed for the 17-plex kit, and the plate was analyzed for concentrations of
500 cytokines, based on the standard curves produced.

501

502 ***Viral Inhibition Assay***

CD8-depleted PBMCs were activated in IL-2 (50U/mL) and PHA (2 µg /mL) before being infected with HIV laboratory strain SF162. Infected target cells were co-cultured for 5 days with expanded HIV-specific T-cells or unexpanded CD8 T-cells that were isolated using magnetic beads (Miltenyi Biotech, Germany) added at 1:2 E:T and 20:1 E:T. The following conditions were used as controls: uninfected CD8-depleted PBMCs, infected CD8-depleted PBMCs alone, antiretrovirals (ARVs), ARVs + expanded HIV-specific T-cells, and expanded CMV- and EBV-specific T-cells. Day 5 supernatants were measured for HIV-1 gag p24 concentration by ELISA (ABL, Rockville, MD). The ARVs used in combination were Indinavir and Raltegravir (Selleck Chemicals, TX).

Cytotoxicity Assay

The cytolytic activity of HPA-T products was determined with a chromium 51-release assay. Autologous PHAb targets were pulsed with nothing (negative control) or Gag/Nef/Pol pepmix and incubated with Chromium 51 for 1 hour. Targets were then washed 3 times and co-cultured with autologous HPA-Ts at Effector-to-Target ratios of 40:1, 20:1, 10:1, and 5:1. Targets alone were plated as a spontaneous release control. Targets mixed with 1% Triton X (Sigma-Aldrich, MO) were plated as a maximum release control. Targets were co-cultured with effectors for 4-5 hours at 37 C. Plates were spun and supernatant was collected onto a Luma plate (Perkin Elmer, MA). Plate was left overnight, and Cr51 release was measured the next morning in a MicroBeta2 counter. Specific lysis % was measured as (Experimental Release-Spontaneous

526 Release)/(Maximum Release-Spontaneous Release) x 100.

527

528 ***Functional Avidity***

529 Functional avidity of HPA-T, HNA-T, and CB-T products were tested using
530 limiting dilutions of individual peptides (NIH AIDS Reagent Program and
531 GenScript, NJ). Once our T-cell products were confirmed as specific against
532 individual 15mer peptides (AIDS Reagent Program), the individual 9/10mer
533 peptides within the 15mer sequence were ordered (GenScript, NJ) to determine
534 the exact epitope responsible. T-cells were plated on IFNγ ELISPOT at 1e5
535 cells/well with limiting dilutions of peptide to determine the EC_{50} (the
536 concentration at which T-cells produce half the maximum response to
537 stimulation). ELISPOTs were sent out for analysis for unbiased spot counting
538 (Zellnet, NJ).

539

540 ***HLA Typing***

541 Samples were sent for high-resolution HLA typing (Kashi Clinical Laboratories,
542 OR).

543

544 ***Statistical analysis***

545 Two-way ANOVA with Holm-Sidak correction was used to determine statistical
546 significance of IFN-γ release on ELISPOT in response to HIV antigens compared
547 to the negative control, as well as significance of HIV suppression in viral
548 inhibition assay. Means, medians, and ranges were provided where applicable.

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The authors have no COI relevant to this work.

AUTHOR CONTRIBUTIONS

S.P. contributed to the writing of this manuscript and formal data analysis. S.P., E.C., and S.A. conducted the experiments. S.P., C.R.Y.C., R.B.J. and C.M.B. contributed to the protocol development and experimental plans. C.R.Y.C., R.B.J., E.J.S., D.M.M., R.F.A., and C.M.B. provided expertise on experimental approaches and edited the manuscript. C.M.B. supervised the experiments, provided funding acquisition, and contributed to the writing and editing of the manuscript.

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FIGURE LEGENDS

Figure 1. ***HNA-Ts and CB-Ts are derived from the naïve compartment, expand to clinically relevant levels, and demonstrate HIV-specificity*****. A.** HNA-T generated from the naïve T-cell compartment (red) produced HIV-Specific T-cells, compared to the non-naïve compartment (blue). HNA-Ts displayed significant IFNγ secretion to GNP stimulation ($p<0.0001$) compared to negative controls CTL only and actin. **B.** CB-Ts produced significant levels of IFNγ in response to GNP stimulation compared to CTL only ($p<0.0001$). **C.** HPA-Ts were manufactured from HIV+ HLA-A02 individuals for use in comparative studies later on and demonstrated significant IFNγ secretion to GNP stimulation ($p<0.0001$).

Figure 2. ***Phenotyping of HIV-Specific T-cell products reveals differences in composition and minimal expression of exhaustion markers*****. A-B.** HNA-Ts and CB-Ts display a primarily CD3+ phenotype, comprised of substantial CD4+ and CD8+ subsets. **C.** HPA-T are comprised of primarily CD8+ T-cells and NK/NKT-cells, with a negligible CD4+ population. All three cohorts display skewed effector memory responses and low expression of exhaustion markers.

Figure 3. ***HNA-Ts and CB-Ts demonstrate cytotoxicity, suppression of active HIV infection in vitro, and secretion of multiple cytokines*****. A.** HNA-Ts and CB-Ts display specific lysis of GNP-pulsed autologous PHAb targets (red) at comparable levels to HPA-T products. CB-Ts demonstrate dual EBV-specificity by specific lysis of unpulsed autologous LCLs (green). Error bars represent the

standard deviation of triplicate values. **B.** HNA-Ts (n=4) and CB-Ts (n=4) demonstrate suppression of active HIV infection *in vitro* at E:T of 40:1 and 20:1 at Day 7. Error bars represent the standard deviation of triplicate values. **C.** HNA-T, CB-T, and HPA-T products secrete IL-2, IL-8, IFNγ, and TNFα in response to GNP stimulation, demonstrating product polyfunctionality. Error bars represent the standard deviation from the mean.

Figure 4. ***HLA-A02-restricted HIV-specific T-cells derived from virus-naïve donors do not recognize Gag SL9, despite forced expression manufacturing methods*****. A.** No SL9-specific HNA-T or CB-T products were generated using forced expression methods with SL9 9mer peptide. **B.** In comparison, GNP-specific HNA-T and CB-T were generated from the same donors as a control. **C.** SL9-specific T-cells were produced using our manufacturing methods, from an HIV positive donor (OM9), determined by IFNγ ELISPOT. **D.** SL9-specific T-cells show low functional avidity on IFNγ ELISPOT, stimulating with progressive dilutions of SL9 9mer peptide. In this figure, error bars represent the standard deviation.

Table 1. ***HIV Naïve Adult-derived A02+ HNA-T Epitope Mapping*****.** HNA-Ts were mapped with overlapping 15mers based on HIV-1 consensus sequences of Gag, Nef, and Pol. Known HLA-A02 epitopes (based on LAND) are shown in **bold** and confirmed 9mer epitopes are *italicized*, with unknown HLA restrictions.

Table 2. ***HIV Naïve Cord-derived A02+ CB-T Epitope Mapping*****.** CB-Ts were mapped with overlapping 15mers based on HIV-1 consensus sequences of Gag, Nef, and Pol. Known HLA-A02 epitopes (based on LAND) are shown in **bold** and confirmed 9mer epitopes are *italicized*, with unknown HLA restrictions.

Table 3. ***HIV Positive-derived A02+ HPA-T Epitope Mapping*****.** HPA-Ts were mapped with overlapping 15mers based on HIV-1 consensus sequences of Gag, Nef, and Pol. HPA-T#2 (OM9), is the de-identified HIV+ donor from which SL9-specific T-cells are derived from in Figure 4C and D.

Table 1. HIV-Naïve Adult-derived (HNA-T) A02+ Epitope Mapping

Product Name	HLA type		Epitope Mapping **Indicates known HLA-A02 restricted epitope,** *Indicates known epitope*		
HNA-T	**HLA Class I**	**HLA Class II**	**NEF**	**GAG**	**POL**
HNA-T #1	**A02**, 68; B07, 35; C04, 07	DRB1 01, 15; DRB5 01; DQA1 01, 01; DQB1 05, 05; DPB1 04, 04	WPAVRE**RIRRTHPAA** RE**RIRRTHPAA**EGVG GFPVRPQVPLRPMTY RPQVPLRPMTYKAAL		
HNA-T #2	**A02**, 03; B07, 50; C06, 07	DRB1 07, 15; DRB4 01; DRB5 01; DQA1 01, 02; DQB1 02, 06; DPB1 03, 04		IYKR**WIILGLNKIV**R **WIILGLNKIV**RMYSP	
HNA-T #3	**A02** known			HQAISPRTLNAWVKV	
HNA-T #4	**A02**, 32; B40, 57; C03, 06	DRB1 12, 13; DRB3 02, 03; DQA1 01, 05; DQB1 07, 06; DPB1 03, 04		SGGKLDAWEKIRLRP LDAWEKIRLRPGGKK IYKR**WIILGLNKIV**R **WIILGLNKIV**RMYSP	
HNA-T #5	**A02**, 24; B35, 51; C04, 14	DRB1 04, 11; DRB3 02; DRB4 01; DQA1 03, 05; DQB1 03, 03; DPB1 01, 14		YRLK*HLVWASREL*ER *HLVWASREL*ERFALN	MGYELHPDKWTVQPI LHPDKWTVQPIVLPE VNDIQ*KLVGKLNWA*S
HNA-T #6	**A02**, 68; B08, 35; C04, 07	DRB1 03, 11; DRB3 01, 02; DQA1 05, 05; DQB1 02, 03; DPB1 02, 03	EVLMWKFDSRLALRH	PVGEIYKRWIILGLN IYKRWIILGLNKIVR RDYVDRFFKTLRAEQ DRFFKTLRAEQATQ	VGPTPVNIIGRNLLT AYFLLKLAGRWPVKT LKLAGRWPVKTIHTD
HNA-T #7	**A02**, 01; B08, 51; C07, 15	DRB1 04, 11; DRB3 02; DRB4 01; DQA1 03, 05; DQB1 03, 03; DPB1 02, 03		GLNKIVRMYSPVSIL	THLEGK**IILVAVHVA** GK**IILVAVHVA**SGYI
HNA-T #8	**A02**, 24; B39, 51; C01, 07	DRB1 08, 11; DRB3 02; DQA1 04, 05; DQB1 03, 04; DPB1 02, 03			MASDFNLPPVVAKEI

Table 2. Cord Blood-derived (CB-T) A02+ Epitope Mapping

Product Name	HLA type		Epitope Mapping **Indicates known HLA-A02 restricted epitope,** *Indicates known epitope*		
CB-T	**HLA Class I**	**HLA Class II**	**NEF**	**GAG**	**POL**
CB-T #1	**A02**, 29; B18, 51; C05, 16	DRB1 03, 11; DRB3 02, 02; DQA1 05, 05; DQB1 02, 07; DPB1 02, 03	YPLTFGWCFKLVPV FKLVPVDPEEVEEAN		
CB-T #2	**A02**, 24; B35, 35; C01, 04	DRB1 04, 11; DRB3 02; DRB4 01; DQA1 03, 05; DQB1 03, 03; DPB1 04, 17	EEEVGFPVRPQVPLR GFPVRPQVPLRPMTY HTQGYFPDWQNYTPG YFPDWQNYTPGPGIR Class I definitely	*HLVWASREL*ERFALN IVRMYSPVSILDIRQ RQGPKEPFRDYVDRF Class I definitely	KMIGGIGGFIKVRQY VNDIQ*KLVGKLNWA*S KLPIQKETWEAWWTE WQATWIPEWEFVNTP WIPEWEFVNTPPLVK
CB-T #3	**A02**, 01; B27, 49; C01, 07	DRB1 01, 01; DQA1 01, 01; DQB1 05, 05; DPB1 04, 04		HQAISPRTLNAWVKV	KMIGGIGGFIKVRQY QKETWEAWWTEYWQA
CB-T #4	**A02**, 30; B15, 53; C02, 04	DRB1 01, 11; DRB3 02; DQA1 01, 01; DQB1 05, 06; DPB1 01, 02	DEEREVLMWKFDSRL	RDYVDRFFKTLRAEQ DRFFKTLRAEQATQ	AYFLLKLAGRWPVKT LKLAGRWPVKTIHTD
CB-T #5	**A02**, 68; B35, 51; C04, 08	DRB1 14, 14; DRB3 01, 01; DQA1 05, 05; DQB1 03, 03; DPB1 04, 04		RDYVDRFFKTLRAEQ	VGPTPVNIIGRNLLT KVYLAWVPAHKGIGG
CB-T #6	**A02**, 33; B35, 51; C04, 16	DRB1 13, 15; DRB3 03; DQA1 01, 01; DQB1 06, 06; DPB1 01, 131			KMIGGIGGFIKVRQY
CB-T #7	**A02**, 68; B15, 39; C07, 07	DRB1 04, 07; DRB4 01, 01; DQA1 02, 03; DQB1 02, 03; DPB1 04, 14		Nef-specific: Individual peptides not determined	Pol-specific: Individual peptides not determined
CB-T #8	**A02**, 24; B13, 42; C06, 17	DRB1 10, 11; DRB3 02; DQA1 01, 01; DQB1 05, 06; DPB1 02, 04	RPQVPLRPMTYKAAL PLRPMTYKAALDLSH LWVYHTQGYFPDWQN WQNYTPGPGIRYPLT TPGPGIRYPLTFGW	HQAISPRTLNAWVKV	YNVLPQGWKGSPAIF NTPPLVKLWYQLEKE EQVDKLVSAGIRKVL KLVSAGIRKVLFLDG

Table 3. HIV-positive derived (HPA-T) A02+ Epitope Mapping

Product Name	HLA type		Epitope Mapping **Indicates known HLA-A02 restricted epitope,** *Indicates known epitope*		
HPA-T	**HLA Class I**	**HLA Class II**	**NEF**	**GAG**	**POL**
HPA-T #1	**A02**, 03; B07, 40; C03, 07	DRB1 04, 15; DQB1 03, 06; DRB4 01; DRB5 01; DQA 01, 03	EEEVGFPVRPQVPLR GFPVRPQVPLRPMTY RPQVPLRPMTYKAAL DLSHFLKEKGGLEGL FLKEKGGLEGLIYSK	RQANFLGKIWPSNKG FLGKIWPSNKGRPGN	PQGWKGSPAIFQSSM KGSPAIFQSSMTKIL AIFQSSMTKILEPFR
HPA-T #2	**A02**, 03; B07, 52; C07, 12	DRB1 15, 15; DRB5 01, 01; DQA1 01, 01; DQB1 06, 06; DPB1 04, 04	RPQVPLRPMTYKAAL TPGPGIRYPLTFGW	VGGPGHKARVLAEAM **SLYNTVATL**	KGSPAIFQSSMTKIL AIFQSSMTKILEPFR
HPA-T #3	**A02, 02**; B35, 44; C04, 05	DRB1 01,04; DRB4 01; DQA1 01, 03; DQB1 05, 03; DPB1 04, 04	PGIRYPLTFGWCFKL YPLTFGWCFKLVPV		
HPA-T #4	**A02**, 26; B15, 44; C02, 03	DRB1 04,11; DRB3 02; DRB4 01; DQA1 03, 05; DQB1 03, 03; DPB1 02, 04	DLSHFLKEKGGLEGL FLKEKGGLEGLIYSK	ASVLSGGKLDAWEKI EKIRLRPGGKKKYRL FSALSEGATPQDLNT **WIILGLNKIV**RMYSP GLNKIVRMYSPVSIL	ANRETKLGKAGYVTD THLEGK**IILVAVHVA** GK**IILVAVHVA**SGYI
HPA-T #5	**A02**, 11; B15, 15; C08, 08	**Unknown**	RPQVPLRPMTYKAAL	GLNKIVRMYSPVSIL DRFFKTLRAEQATQ	KKKSVTVLDVGDAYF VTVLDVGDAYFSVPL
HPA-T #6	**A02**, 32; B15, 40; C02, 03;	DRB1 01, 14; DQB1 05, 05; DRB3 02; DQA 01		LDAWEKIRLRPGGKK EKIRLRPGGKKKYRL YRLKHLVWASRELER WIILGLNKIVRMYSP GLNKIVRMYSPVSIL VSILDIRQGPKEPFR	QKQITKIQNFRVYYR TKIQNFRVYYRDSRD DDCVASRQDED

Patel et al. demonstrate the ability to generate HIV-Specific T cells from HIV seronegative adults and cord blood with a Good Manufacturing Practice-compliant strategy. These immunotherapies are multi-antigen specific, display cytotoxicity, and suppress HIV *in vitro*, providing a promising platform for adoptive T cell therapy in a post-transplant setting.

Figure 1

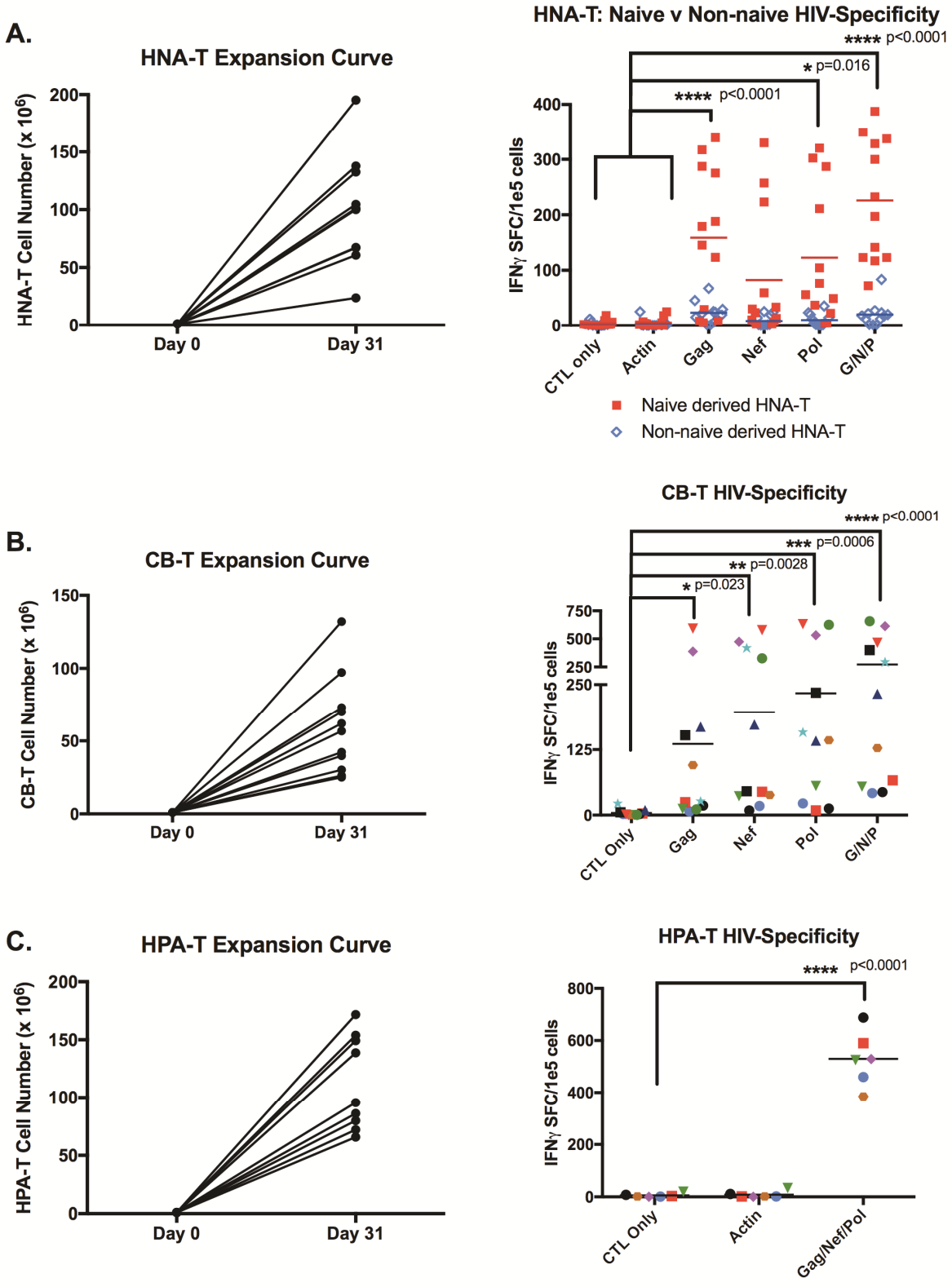

A.
HNA-T Expansion Curve
HNA-T Cell Number (x 10^6)
200
150
100
50
0
Day 0
Day 31
HNA-T: Naive v Non-naive HIV-Specificity
**** p<0.0001
* p=0.016
**** p<0.0001
IFNγ SFC/1e5 cells
400
300
200
100
0
CTL only
Actin
Gag
Nef
Pol
G/N/P
Naive derived HNA-T
Non-naive derived HNA-T
B.
CB-T Expansion Curve
CB-T Cell Number (x 10^6)
150
100
50
0
Day 0
Day 31
CB-T HIV-Specificity
**** p<0.0001
*** p=0.0006
** p=0.0028
* p=0.023
IFNγ SFC/1e5 cells
750
500
250
250
125
0
CTL Only
Gag
Nef
Pol
G/N/P
C.
HPA-T Expansion Curve
HPA-T Cell Number (x 10^6)
200
150
100
50
0
Day 0
Day 31
HPA-T HIV-Specificity
**** p<0.0001
IFNγ SFC/1e5 cells
800
600
400
200
0
CTL Only
Actin
Gag/Nef/Pol

Figure 2

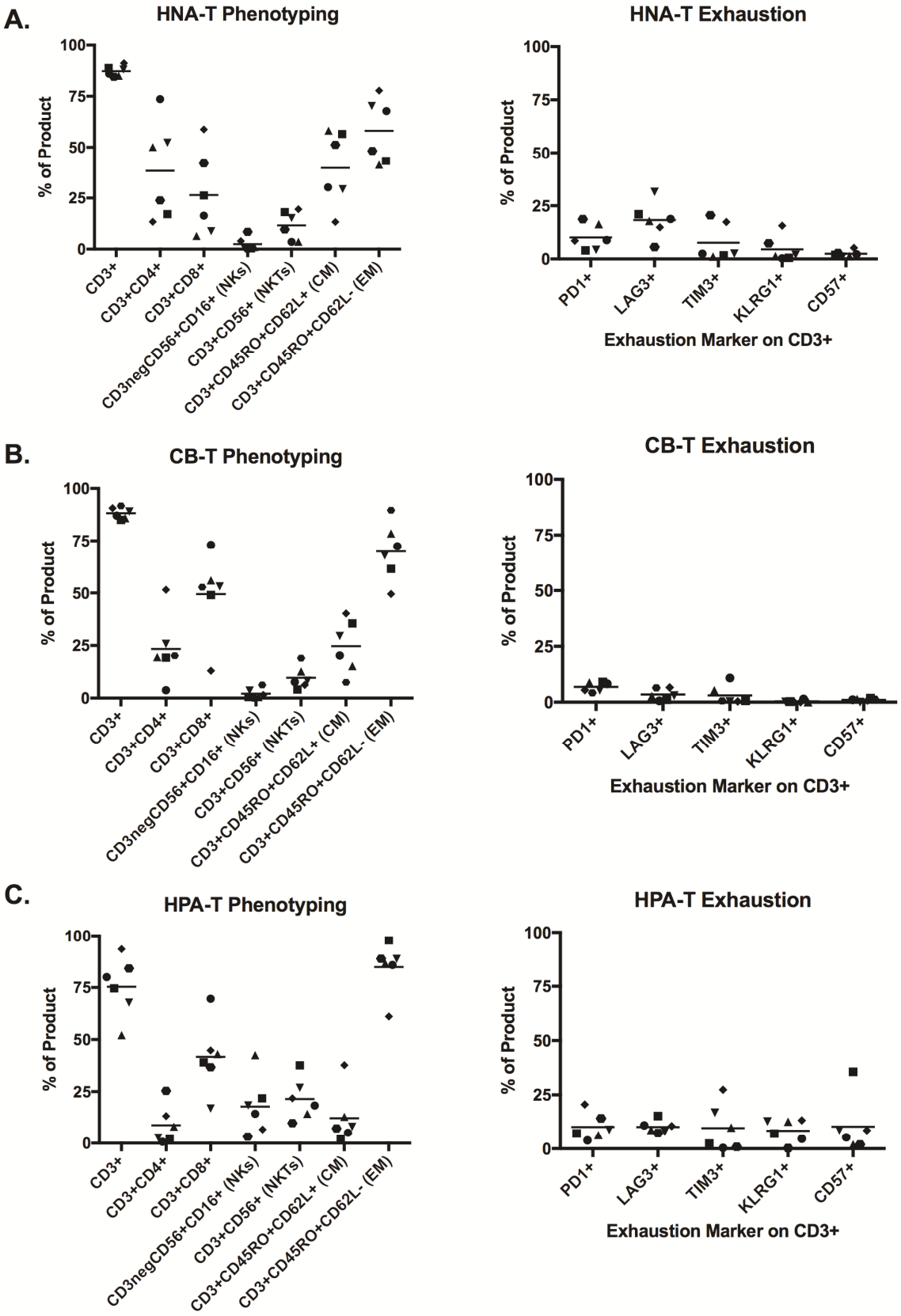

A.
HNA-T Phenotyping
% of Product
100
75
50
25
0
CD3+
CD3+CD4+
CD3+CD8+
CD3negCD56+CD16+ (NKs)
CD3+CD56+ (NKTs)
CD3+CD45RO+CD62L+ (CM)
CD3+CD45RO+CD62L- (EM)
HNA-T Exhaustion
% of Product
PD1+
LAG3+
TIM3+
KLRG1+
CD57+
Exhaustion Marker on CD3+
B.
CB-T Phenotyping
% of Product
CD3+
CD3+CD4+
CD3+CD8+
CD3negCD56+CD16+ (NKs)
CD3+CD56+ (NKTs)
CD3+CD45RO+CD62L+ (CM)
CD3+CD45RO+CD62L- (EM)
CB-T Exhaustion
% of Product
PD1+
LAG3+
TIM3+
KLRG1+
CD57+
Exhaustion Marker on CD3+
C.
HPA-T Phenotyping
% of Product
CD3+
CD3+CD4+
CD3+CD8+
CD3negCD56+CD16+ (NKs)
CD3+CD56+ (NKTs)
CD3+CD45RO+CD62L+ (CM)
CD3+CD45RO+CD62L- (EM)
HPA-T Exhaustion
% of Product
PD1+
LAG3+
TIM3+
KLRG1+
CD57+
Exhaustion Marker on CD3+

Figure 3

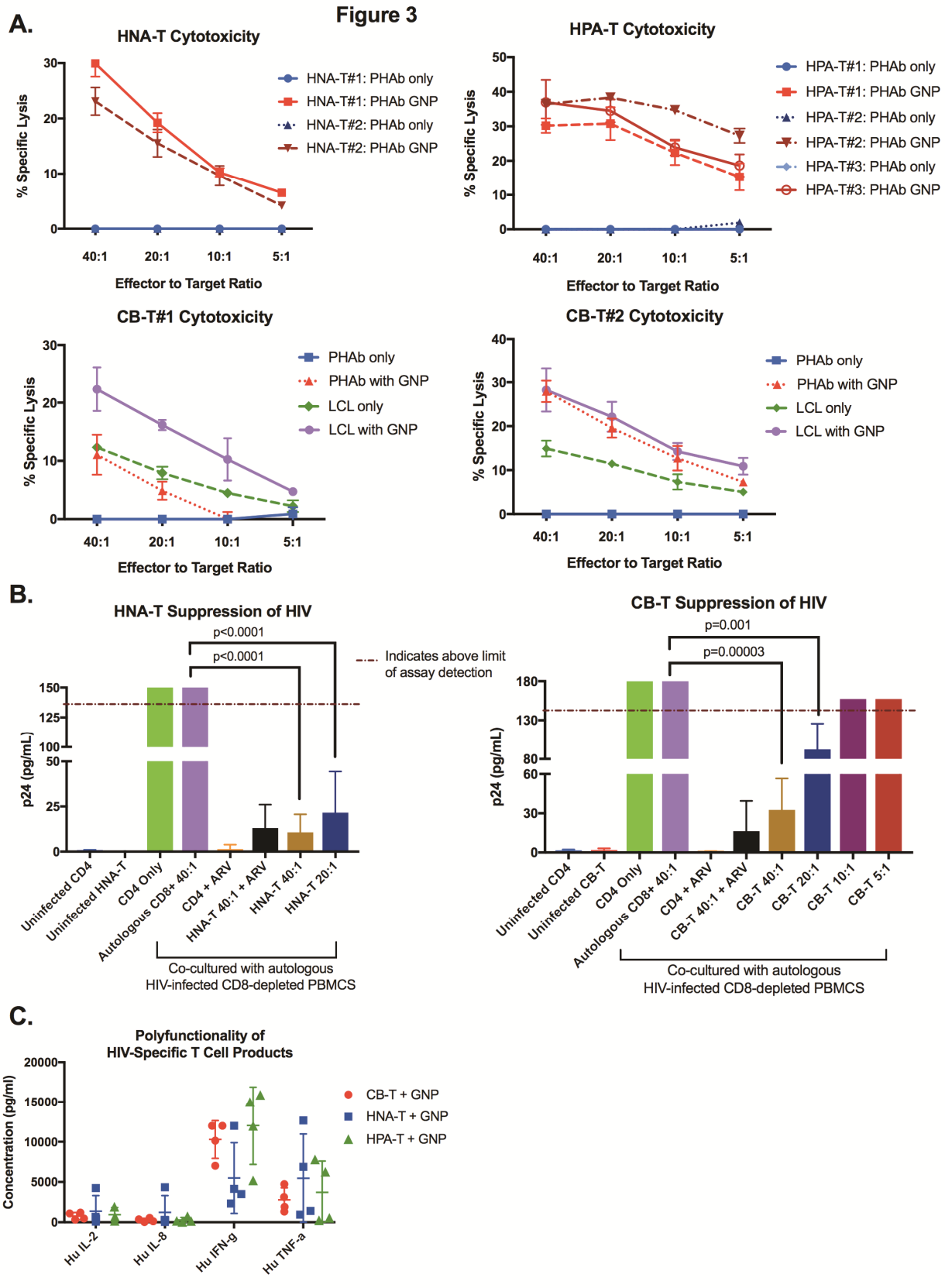
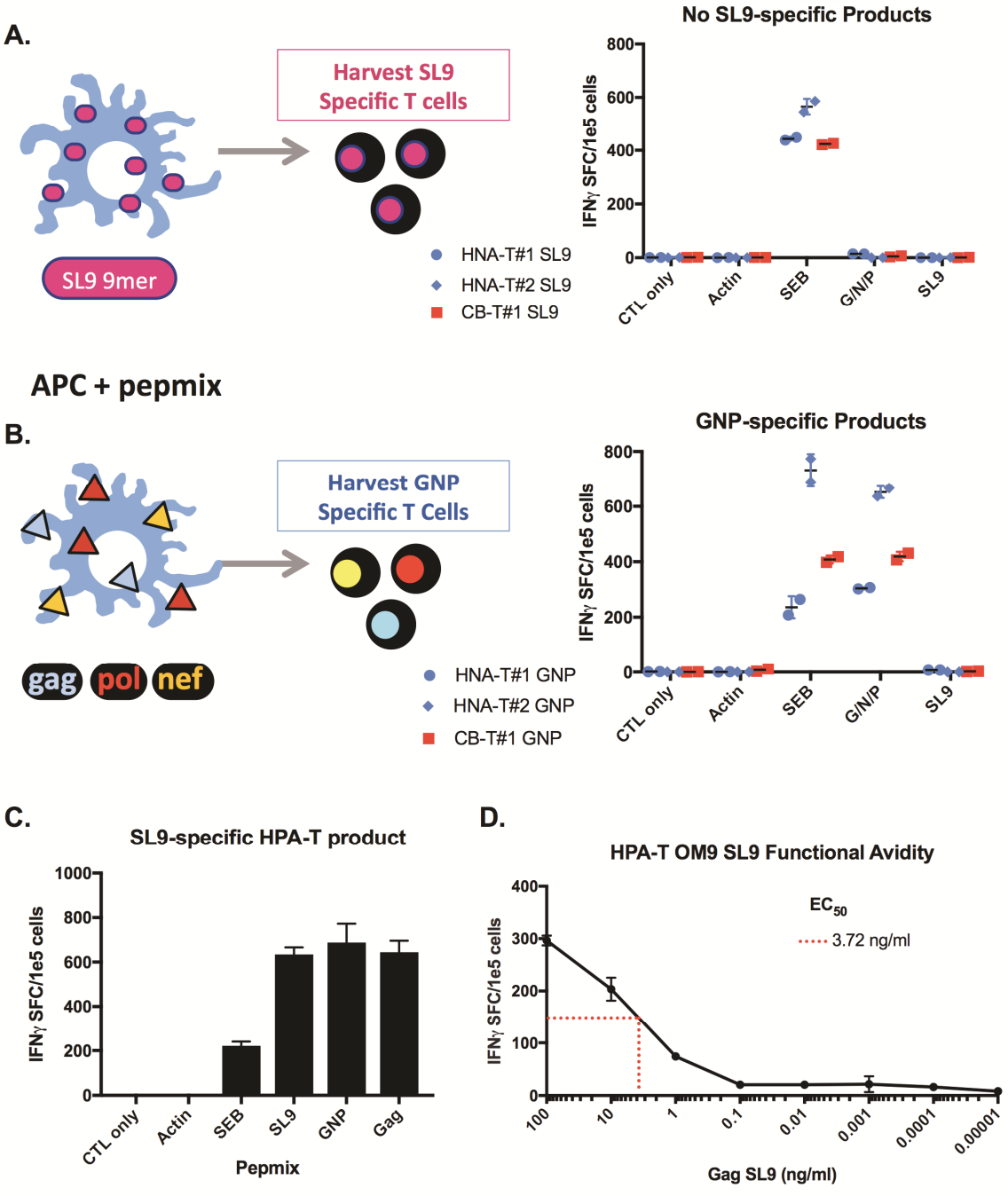
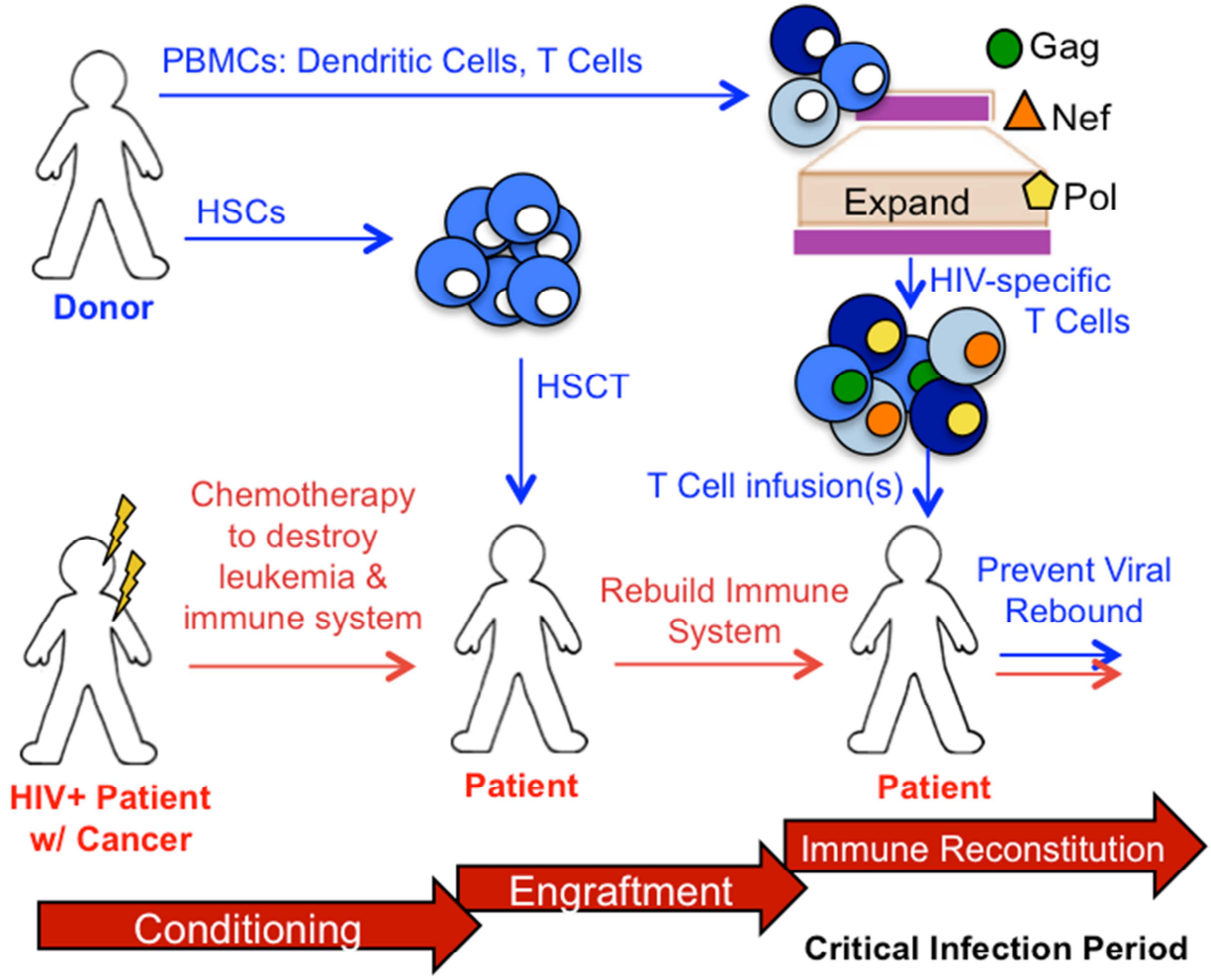

A.
HNA-T Cytotoxicity
% Specific Lysis
Effector to Target Ratio
40:1
20:1
10:1
5:1
HNA-T#1: PHAb only
HNA-T#1: PHAb GNP
HNA-T#2: PHAb only
HNA-T#2: PHAb GNP
HPA-T Cytotoxicity
HPA-T#1: PHAb only
HPA-T#1: PHAb GNP
HPA-T#2: PHAb only
HPA-T#2: PHAb GNP
HPA-T#3: PHAb only
HPA-T#3: PHAb GNP
CB-T#1 Cytotoxicity
PHAb only
PHAb with GNP
LCL only
LCL with GNP
CB-T#2 Cytotoxicity
B.
HNA-T Suppression of HIV
p<0.0001
p<0.0001
Indicates above limit of assay detection
p24 (pg/mL)
Uninfected CD4
Uninfected HNA-T
CD4 Only
Autologous CD8+ 40:1
CD4 + ARV
HNA-T 40:1 + ARV
HNA-T 40:1
HNA-T 20:1
Co-cultured with autologous HIV-infected CD8-depleted PBMCS
CB-T Suppression of HIV
p=0.001
p=0.00003
Uninfected CB-T
CB-T 40:1 + ARV
CB-T 40:1
CB-T 20:1
CB-T 10:1
CB-T 5:1
C.
Polyfunctionality of HIV-Specific T Cell Products
Concentration (pg/ml)
CB-T + GNP
HNA-T + GNP
HPA-T + GNP
Hu IL-2
Hu IL-8
Hu IFN-g
Hu TNF-a

Figure 4


A.
Harvest SL9 Specific T cells
SL9 9mer
HNA-T#1 SL9
HNA-T#2 SL9
CB-T#1 SL9
No SL9-specific Products
IFNγ SFC/1e5 cells
800
600
400
200
0
CTL only
Actin
SEB
G/N/P
SL9
APC + pepmix
B.
Harvest GNP Specific T Cells
gag
pol
nef
HNA-T#1 GNP
HNA-T#2 GNP
CB-T#1 GNP
GNP-specific Products
C.
SL9-specific HPA-T product
1000
800
600
400
200
0
GNP
Gag
Pepmix
D.
HPA-T OM9 SL9 Functional Avidity
EC50
3.72 ng/ml
300
100
100
10
1
0.1
0.01
0.001
0.0001
0.00001
Gag SL9 (ng/ml)


Post-HSCT: Adoptive HIV-Specific T Cell Therapy
PBMCs: Dendritic Cells, T Cells
Gag
Nef
Pol
Expand
HSCs
Donor
HIV-specific
T Cells
HSCT
T Cell infusion(s)
Chemotherapy
to destroy
leukemia &
immune system
Rebuild Immune
System
Prevent Viral
Rebound
HIV+ Patient
w/ Cancer
Patient
Patient
Immune Reconstitution
Engraftment
Conditioning
Critical Infection Period