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Engineered Antigen-Specific T Cells Secreting Broadly Neutralizing Antibodies: Combining Innate and Adaptive Immune Response against HIV

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While antiretroviral therapy (ART) can completely suppress viremia, it is not a cure for HIV. HIV persists as a latent reservoir of infected cells, able to evade host immunity and re-seed infection following cessation of ART. Two promising immunotherapeutic strategies to eliminate both productively infected cells and reactivated cells of the reservoir are the adoptive transfer of potent HIV-specific T cells and the passive administration of HIV-specific broadly neutralizing antibodies also capable of mediating antibody-dependent cellular cytotoxicity (ADCC). The simultaneous use of both as the basis of a single therapeutic has never been explored. We therefore sought to modify HIV-specific T cells from HIV-naive donors (to allow their use in the context of allotransplant, a promising platform for sterilizing cures) so they are able to secrete a broadly neutralizing antibody (bNAb) directed against the HIV envelope to elicit ADCC. We designed an antibody construct comprising bNAb 10-1074 heavy and light chains, fused to IgG3 Fc to elicit ADCC, with truncated cluster of differentiation 19 (CD19) as a selectable marker. HIV-specific T cells were expanded from HIV-naive donors by priming with antigen-presenting cells expressing overlapping HIV antigens in the presence of cytokines. T cells retained specificity against Gag, Nef, and Pol peptides (218.55 ± 300.14 interferon γ [IFNγ] spot-forming cells [SFC]/1 × 10^5) following transduction (38.92 ± 25.30) with the 10-1074 antibody constructs. These cells secreted 10-1074 antibodies (139.04 ± 114.42 ng/mL). The HIV-specific T cells maintained T cell function following transduction, and the secreted 10-1074 antibody bound HIV envelope (28.13% ± 19.42%) and displayed ADCC activity (10.47% ± 4.11%). Most critically, the 10-1074 antibody-secreting HIV-specific T cells displayed superior *in vitro* suppression of HIV replication. In summary, HIV-specific T cells can be engineered to produce antibodies mediating ADCC against HIV envelope-expressing cells. This combined innate/adaptive approach allows for synergy between the two immune arms, broadens the target range of the immune therapy, and provides further insight into what defines an effective anti-HIV response.

INTRODUCTION

Antiretroviral (ARV) therapy has successfully resulted in viral suppression but has not led to a cure. Without a cure, even patients with well-controlled virus are still subject to a poorer quality of life: they remain at risk for cardiovascular disease and malignancy, and they are still subject to the stigma of being HIV-positive (which in turn is associated with depression and risks for mental health problems).[1]

Recently, immune-based approaches have shown encouraging results as cure strategies for HIV. Allogeneic stem cell transplant,[2] antigen-specific T cells,[3] and the passive infusion of broadly neutralizing antibodies (bNAbs)[4] have all resulted in antiviral responses. In this study, we propose the mobilization of multiple immune responses to target the virus, combining previously promising antiviral approaches.

HIV-specific cytotoxic T cell responses have long been associated with control of viremia. In hyperacute infection, both the magnitude and rapidity of their activation negatively correlates with viral load set point.[5,6] Viral load set point, or the level of viremia that stabilizes in a patient after HIV infection,[7] has often been used to predict disease progression and as a surrogate marker for efficacy of interventions like vaccines.[8] Furthermore, T cells specific for HIV show inhibition of viral replication *in vitro*,[9,10] while loss of T cell function through exhaustion is associated with HIV disease progression.[11–15] These studies (and additional ones reviewed in Patel et al.[3]) all provide a rationale for using HIV-specific T cells as part of an HIV cure strategy.[3]

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A role for ADCC in HIV control has been suggested by the presence of increased natural killer (NK) cell activity in individuals who have been exposed to HIV but remain uninfected[16] and by the high level of ADCC in elite controllers, or patients without ARV and without detectable viral loads, compared to viremic patients.[17] However, it was the partially successful RV144 vaccine trial that emphasized the key roles of antibody mechanisms beyond neutralization. Investigators found that ADCC (primarily through NK but can also be through monocytes and neutrophils) correlates with protection.[17] Participants receiving ALVAC and AIDSVAX vaccine showed some measure of efficacy and modest benefits, with trends toward protection against HIV[18,19] despite the absence of neutralizing antibody activity—pointing to ADCC as the process responsible for the clinical effects. In fact, the presence of antibodies mediating ADCC in vaccine recipients was associated with protection against HIV.[20] As with cluster of differentiation 8+ (CD8+) T cells, there is a strong rationale for directing ADCC activity against HIV-infected cells as a component of therapies targeting HIV cure or remission (reviewed in Bunders et al.[21]).

Recent evidence supports the idea that ongoing HIV-associated immune dysfunction may limit immune-mediated clearance in ARV-treated individuals.[22,23] Several immune effector boosting approaches are currently being explored, including therapeutic vaccines,[24] passive administration of neutralizing antibodies,[25] and immune checkpoint blockade.[26] To overcome inadequate T cell immunity, peripheral blood mononuclear cells (PBMCs) can be stimulated and expanded *ex vivo* to generate HIV-specific T cells, which can then be subsequently infused into HIV patients.[27] Virus-specific T cells have shown efficacy against opportunistic infections post hematopoietic stem cell transplant.[28,29] Although cell therapy approaches using *ex vivo* expanded, but otherwise unmodified, HIV-specific T cells hold promise, we hypothesize that mobilizing an immune response capable of overcoming the daunting and complex challenge of almost completely inhibiting HIV replication in HIV-infected individuals will likely require an innovative strategy that invokes multiple arms of the immune system.

By taking advantage of advances in genetic modification of T cells[30] and antibody engineering,[31] we propose to combine both cellular and humoral immune effector mechanisms into a single therapeutic product: HIV-specific T cells that have been engineered to secrete HIV-specific bnAbs, which also elicit ADCC. In the current report, we show that this strategy mobilizes the adaptive and innate immune response to mount an anti-HIV response with the enhanced ability to suppress active viral replication.

RESULTS

Antibody Construct and Gene Modification of T Cells

We designed a retroviral vector that contains the light chain and heavy chain variable regions of the 10-1074 antibody separated by a P2A cleavage site. Both chains followed an endogenous immunoglobulin secretory signal. To determine transduction efficiency, we coupled antibody expression to expression of a truncated CD19 receptor (lacking a cytoplasmic signaling domain, which is not naturally expressed on T cells). This marker is part of the transgene, separated from the antibody by furin and 2A cleavage sites (Figure 1A). We then tested whether T cells could be modified to express these antibodies, by transducing non-specifically activated cells from healthy donors. Following gene modification with our retroviral vectors, we observed mean transduction efficiencies of 25.13% ± 6.76% (median, 27.05%; range 16.00–30.40; n = 4 donors; Figures 1B and S2A). Transduced and nontransduced products contained mixed populations of CD4+ T cells and CD8+ T cells (Figures 1C and S2B). For transduced cells, we detected a mean 148.38 ± 76.5 ng/mL of antibody in the supernatant collected after 2–3 days from T cells plated at 2.5×10^5/mL (median of 146.70 ng/mL; range, 80.70–219.40; n = 4; Figure 1D).

T Cell-Secreted Antibodies Bind to HIV Envelope Expressed on Cells

To determine whether the T cell-secreted 10-1074 antibody recognized HIV envelope, we used HeLa cells expressing Env obtained from the AIDS reagent program.[32] Using flow cytometry, we determined that our T cell-secreted antibodies (obtained from the supernatant of transduced cells) bind to envelope-expressing HeLa cells but not to non-expressing unmodified HeLa cells. Only transduced T cell supernatants bind to HeLa cells expressing Env, while non-transduced cell supernatant does not. Collecting supernatants from three different T cell cultures, we observed that binding, as measured by immunoglobulin G (IgG) expression, ranged from approximately 57%–75% (Figures 2 and S3). These results suggest that secreted 10-1074 antibody retains its ability to specifically bind HIV envelope.

HIV-Specific T Cells Can Be Modified to Secrete 10-1074 Antibodies

We then tested whether we could combine antiviral activity from HIV-specific T cells and ADCC-inducing broadly neutralizing antibodies into one platform by genetically modifying HIV-specific T cell lines. Cells that were expanded to recognize the HIV antigens Gag, Pol, and Nef were modified by our retroviral vector (Figures 3A and S4A; mean, 38.92 ± 25.30 transduction efficiency; n = 10) to secrete 10-1074 antibodies (Figure 3B; 139.04 ± 114.42 ng/mL per 2.5×10^5 cells 3 days following transduction; n = 10) into the cell supernatant. Genetic modification did not significantly alter the makeup of CD4+ versus CD8+ populations within the T cell product (Figures 3C and S4B; CD4+ T cell: 33.85% ± 22.22% nontransduced versus 36.69% ± 23.45% transduced, n = 10, not significant [ns]; CD8+ T cell: 45.94% ± 19.55% nontransduced versus 43.20% ± 17.82% transduced, n = 10, ns). Genetic modification did not significantly alter the expression of activation marker CD25 in the total T cell product, though significant reduction in CD69 was observed (Figure S5A). When CD19+ cells were compared to CD19− cells within the transduced HIV-specific T cell condition, CD19+ fraction did have increased expression of both CD25 and CD69 (Figures 3D and S5B; CD25: mean 90.45% ± 14.28% expression CD19+ versus mean 74.15% ± 19.80% expression CD19−, n = 4, ns; CD69: mean 78.13% ± 12.16% expression CD19+ versus mean 48.85% ± 19.33% expression CD19−, n = 4, p = 0.0135). Finally, we measure the expression of exhaustion markers on the genetically

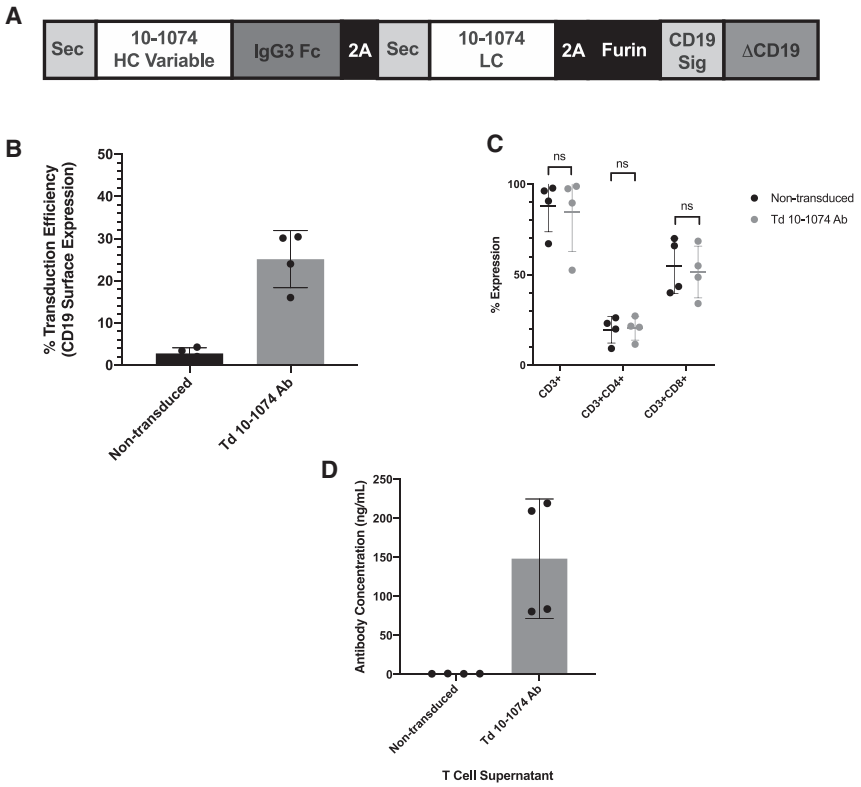

Figure 1. Antibody Construct and Gene Modification of T Cells

(A) Schematic of the transgene introduced to T cells via an Moloney murine leukemia virus (M-MLV) retroviral vector. The entire product is under the control of the constitutively active cytomegalovirus (CMV) promoter. The entire light chain variable region (LC) and heavy chain variable region (HC) of 10-1074 antibody were expressed as a single polypeptide separated by a 2A cleavage sequence. HC was attached to the Fc region. Each chain was directed to be secreted by a signal peptide. Transduction efficiency was measured by truncated CD19 (ΔCD19), separated from the LC and HC regions by a 2A cleavage peptide. (B) Mean transduction of mitogenically stimulated T cells (gray bar) was measured by co-expression of CD3 and CD19 (a product of the Ab construct) on the surface. Error bars depict standard deviation. Each peripheral blood donor is shown by a black circle; n = 4. (C) Phenotype of mitogenically stimulated T cells is shown as percent expression of lymphocytes in both nontransduced (black circles) and transduced (gray circles) cells. The long horizontal bar is the mean expression, and error bars depict standard deviation; n = 4. (D) Mean secretion of 10-1074 antibody in T cell supernatant of nontransduced (black rectangle) and transduced (gray rectangle) cells was measured by ELISA. Error bars depict standard deviation, black circles represent individual peripheral blood donors; n = 4.

modified HIV-specific T cells. Genetic modification did not significantly alter the expression of exhaustion markers lymphocyte activation gene 3 (LAG-3) and programmed cell death protein 1 (PD-1) in the total cell product (Figure S6A). Within the transduced HIV-specific T cell condition, CD19+ cells had slightly higher exhaustion levels compared to CD19− cells, but this trend did not reach significance (Figures 3E and S6B; LAG-3: mean 51.68% ± 27.27% expression CD19+ versus mean 29.48% ± 14.43% expression CD19−, n = 4, ns; PD-1: mean 47.03% ± 22.66% expression CD19+ versus mean 25.56% ± 14.29% expression CD19−, n = 4, ns). These results suggest that HIV-specific T cells can be genetically modified to secrete 10-1074 antibody without significant alteration to the cell phenotype or expression of cell surface activation and exhaustion markers.

Transduced HIV-Specific T Cells Maintain Antigen-Specific T Cell Functions

To determine whether genetic modification of HIV-specific T cells to secrete 10-1074 antibody negatively affected their T cell effector function, we compared the expansion, specificity, and secretion of cytokines and chemokines of transduced and nontransduced HIV-specific T cells. Genetic modification of these cells decreased their ability to expand (mean fold expansion of 22.96 ± 23.69 in nontransduced versus 11.75 ± 9.06 in transduced cells, ns, n = 8, measured from transduction to 7 days post stimulation 3; Figure 4A), though T cells still expanded to significant numbers in the transduced conditions. Genetically modified T cell lines retained specificity to HIV peptides Gag, Nef, and Pol (GNP), as measured by interferon γ (IFNγ) enzyme-linked immune absorbent spot (ELISpot). There was a mean of 218.55 ± 300.14 IFNγ spot-forming cells (SFCs)/1 × 10^5 cells responding to Gag/Nef/Pol peptide pools in nontransduced cells (n = 11), and a mean of 235.59 ± 331.10 IFNγ SFC/1 × 10^5 cells in transduced cells (n = 8). No statistical difference was observed in Gag/Nef/Pol response between nontransduced and transduced (p = 0.5000; Figure 4B), though response to Gag/Nef/Pol peptide pools in each were significantly different from response to Actin (p = 0.0010 for nontransduced and p = 0.0078 for transduced). We observed no significant differences in T cell cytokine secretion between transduced and non-transduced products, including: granulocyte-macrophage colony-stimulating factor (GM-CSF) (1,525.4 ± 1,374.5 pg/mL nontransduced versus 1,142.4 ± 1,030 pg/mL transduced, p = ns, n = 6), tumor necrosis factor alpha (TNF-α) (4,003.7 ± 2,777.3 pg/mL nontransduced versus 3,774 ± 2,958.8 pg/mL transduced, p = ns, n = 6), interleukin 17 (IL-17) (16.7 ± 10.4 pg/mL nontransduced versus 13 ± 10.6 pg/mL transduced, p = ns, n = 6), and the monocyte chemoattractant protein 1 (51.7 ± 37.8 nontransduced pg/mL versus 41.2 ± 38.6 pg/mL transduced, p = ns, n = 6) were observed between nontransduced and transduced T cells (Figure 4C). These results support the assertion that genetic modification of HIV-specific T cells does not alter their effector function while conferring the new functionality of antibody secretion.

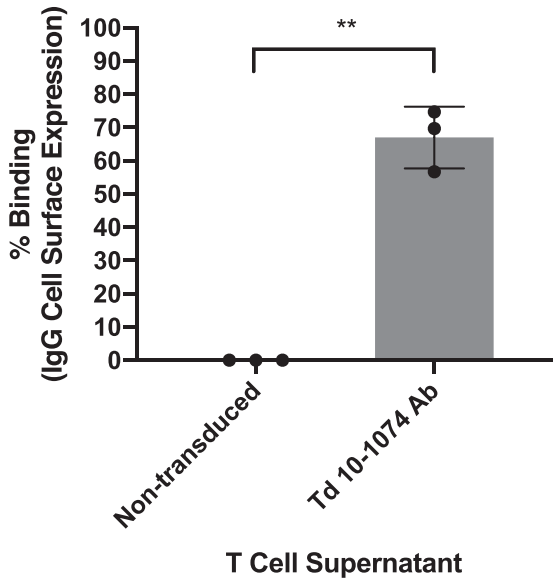

Figure 2. T Cell-Secreted Antibodies Bind to HIV Envelope Expressed on Cells

Summary of detectable bound IgG from supernatant of nontransduced (black) and transduced (gray) T cells on the surface of envelope-expressing HeLa cells; n = 3. Error bars depict standard deviation. Nontransduced and transduced cell supernatants were analyzed by paired t test, where **p = 0.0063.

T Cell-Secreted Antibodies from HIV-Specific T Cells Mediate ADCC

To determine whether 10-1074 antibody derived from HIV-specific T cells retained its ability to mediate ADCC, we first tested their ability to bind HIV envelope-expressing target cells and increase NK-mediated killing. Nontransduced HIV-specific T cell (HST) supernatant and transduced HIV-specific T cell supernatant containing T cell-secreted 10-1074 antibody were cultured in the presence of HIV envelope-expressing target cells. We observed increased expression of surface bound IgG in the transduced HST antibody containing supernatant condition compared to nontransduced HST supernatant (Figure 5A; mean 1.12% ± 0.115% binding in nontransduced supernatant versus 28.13% ± 19.42% binding in 10-1074 antibody supernatant, n = 3). Importantly, we saw no 10-1074 antibody binding to non-HIV envelope-coated target cells in either model used (Figures S7A and S7B). An increase in NK cell killing of HIV envelope-expressing target cells was measured to demonstrate the ability for T cell-secreted antibody to elicit ADCC. In 3 of 4 lines tested, we observed increased NK cell killing among a range of effector to target ratios (Figure 5B). To compare ADCC between T cell-secreted 10-1074 antibody and wild-type (WT) 10-1074 antibody, we quantified the difference in NK cell killing at 2:1 NK:target ratio. Secreted 10-1074 antibody enhanced NK cell killing at a similar magnitude to WT 10-1074 antibody (mean 10.47% ± 4.11% increase in NK cell cytotoxicity by secreted 10-1074 antibody, n = 4, versus mean 11.70% ± 2.31% increase by WT 10-1074 antibody, n = 3; WT 10-1074 Ab used at a concentration similar to the average of antibody concentrations obtained from T cell supernatants, 250 ng/mL; Figure 5C). Thus, the 10-1074 antibody produced from engineered T cells exhibits similar ability to elicit ADCC as a corresponding control (wild type, WT) 10-1074 antibody produced by 2.5×10^5 cells/mL transduced cells.

Genetic Modification of HIV-Specific T Cells to Secrete 10-1074 Antibody Increases Anti-Viral Efficacy against HIV-Infected Targets

Finally, to test whether we successfully combined innate (ADCC) and adaptive (T cell-mediated killing) immunity to HIV in a single platform, we measured the anti-viral efficacy of engineered cells against autologous HIV-infected CD4+ T cells over five-day viral inhibition assays. We compared viral inhibition of 10-1074 antibody-secreting HIV-specific T cell lines against non-transduced HIV-specific T cell lines in both the presence and absence of autologous NK cells. HIV-specific T cells alone mediate antiviral efficacy (CD4+ infected cells: 14,048.49 ± 18,265.60 pg/mL; HIV p24 versus HST: 1,604.33 ± 2,162.80 pg/mL HIV p24 when plated at 10:1 E:T ratio, n = 4, ns; Figure 6A). In 3 out of 4 donors, we observed a decrease in HIV viral load by 10-1074 transduced HST compared to non-transduced. In 1 out of 4 donors, there was no difference in HIV viral load between non-transduced and antibody-transduced T cells (Figure S8). We next sought to determine whether gene-modified HIV-specific T cell-conditioned supernatant alone has antiviral efficacy similar to WT 10-1074 antibody (a single administration of a comparable concentration, 250 ng/mL). Again, we quantified HIV p24 in the cell supernatant after 5 days. We observed no significant difference between antiviral efficacy of WT and secreted 10-1074 antibody (CD4+ HIV infected: 36,905.50 ± 33,056 pg/mL versus WT 10-1074 antibody: 9,176.28 ± 9,762.73 pg/mL versus secreted 10-1074 antibody: 6,617.88 ± 7,337.30, n = 3, ns; Figures 6B and S9), suggesting that secretion from HIV-specific T cells does not impair the ability for 10-1074 antibody to reduce HIV viremia. Next, we sought to determine the extent to which ADCC could control HIV. Nontransduced and 10-1074 antibody-transduced HIV-specific T cells were plated in the presence and absence of autologous, isolated NK cells. Again, we measured viral inhibition by the concentration of HIV p24 in the cell supernatant after five days. We observed a trend for greatest antiviral efficacy by 10-1074 antibody-secreting HIV-specific T cells in the presence of autologous NK cells (CD4+ HIV infect: 14,048.49 ± 18,265.60 pg/mL p24 versus non-transduced HST: 1,604.33 ± 2,163.80 pg/mL p24 versus transduced HST: 351.99 ± 510.76 pg/mL p24 versus non-transduced HST + autologous NK: 1,442.10 ± 2,103.00 pg/mL p24 versus transduced HST + autologous NK: 170.33 ± 232.09 pg/mL p24, n = 4; Figures 6C and S10). Of note, where non-HIV-specific T cells were used as the platform, no viral inhibition was seen in the transduced cells (CD4+ HIV infect: 67,646.83 ± 1,060.66 pg/mL p24 versus non-specific T cell: 78,004.00 ± 808.93 pg/mL p24; Figure S11), emphasizing the importance of HIV-specific T cells in control of viral-infected cells. Indeed, in these conditions we observed that there was a statistically significant increase in p24, likely as a result of reinfection of the nonspecific T cells (which contain CD4+ cells). Thus, each component of this combined innate/adaptive immune therapy approach—HIV-specific

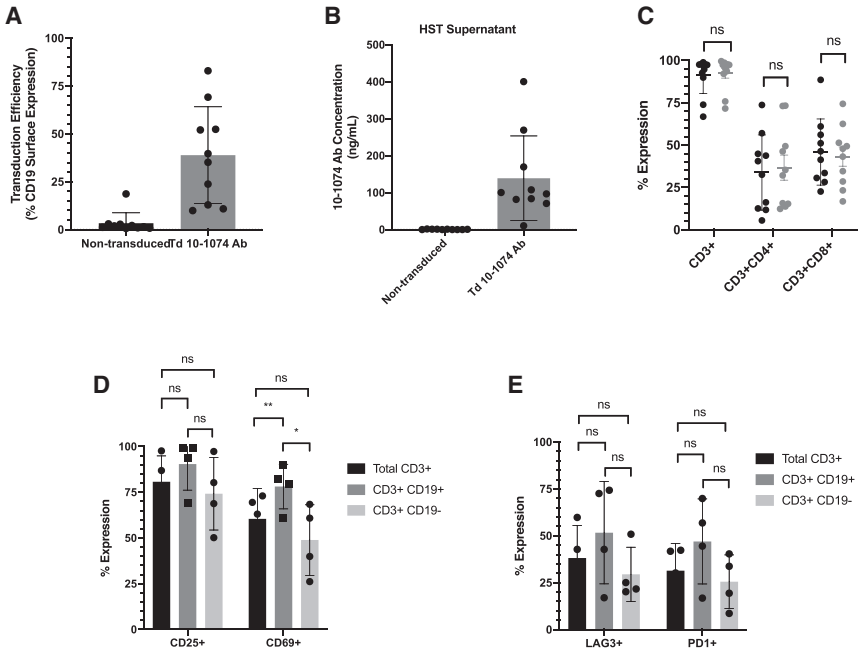

Figure 3. HIV-Specific T Cells Can Be Modified to Secrete 10-1074 Antibodies

(A) Mean transduction of HIV-specific T cells (gray bar) was measured by co-expression of CD3 and CD19 (expressed from construct, see schematic Figure 1A) on the surface. Error bars depict standard deviation, and each HIV-specific T cell line is shown as black circles; n = 10. (B) Mean concentration of 10-1074 antibody in supernatants from nontransduced (black rectangle) and transduced (gray rectangle) cells was quantified by ELISA. Error bars depict standard deviation, and each HIV-specific T cell line is shown as black circles; n = 9. (C) Phenotypes of HIV-specific T cells, shown here as percent expression of lymphocytes in both nontransduced (black circles) and transduced (gray circles) cells; n = 10. Long horizontal bars represent mean expression, and error bars depict standard deviation. ns denotes $p > 0.05$ by paired t test. (D) Mean surface expression of activation markers CD25 and CD69 of transduced HIV-specific T cells (black bar) and CD19+ (dark gray) and CD19− (light gray) fractions within the transduced T cell population were measured by flow cytometry; n = 4. Error bars depict standard deviation. Paired t tests were used for statistical analysis, where ns denotes $p > 0.05$, *$p = 0.0135$, **$p = 0.0061$. (E) Mean surface expression of exhaustion markers LAG3 and PD1 of transduced HIV-specific T cells (black bar) and CD19+ (dark gray) and CD19− (light gray) fractions within the transduced T cell population were measured by flow cytometry; n = 4. Error bars depict standard deviation. Paired t tests were used for statistical analysis, where ns denotes $p > 0.05$.

T cells, antibody, and NK cell effectors—were observed to contribute to the overall viral inhibition *in vitro*.

DISCUSSION

To our knowledge, the use of broadly neutralizing antibodies, multi-antigen HIV-specific T cells, and antibody-dependent cell cytotoxicity through NK cells as the basis of a single therapeutic platform has never been previously explored. Our results echo related findings from groups that modified hematopoietic stem cells to secrete broadly neutralizing antibodies.[33–35] In these prior publications, the antibodies were secreted by antibody-producing B cells after transduced hematopoietic stem cells differentiated. These modified B cells produced neutralizing antibodies that provided continuous amounts of HIV neutralization *in vivo*.[33,34] These antibodies were able to delay HIV viremia and persisted in circulation—detectable in the spleen, thymus, lymph node, and gut-associated lymphoid tissue in humanized mice.[34] While the modification of B cells to express antibodies is the most straightforward approach, we hypothesized that modifying T cells to secrete these antibodies could allow for spatio-temporal coordination of innate and adaptive responses to sites of HIV infection. Our group is currently pursuing T cell therapy with HIV-specific T cells, and modification to include broadly neutralizing antibodies is a natural extension of this clinical trial (NCT03485963). The feasibility of T cells secreting antibodies (or other therapeutic molecules) has been demonstrated in the cancer setting.[36] For example, T cells modified to secrete anti-programmed death-ligand 1 (PD-L1) antibodies effectively target renal cell carcinoma in a humanized mouse model.[37]

Our findings support previous reports that HIV-specific T cells do significantly inhibit HIV viremia.[3,27,38] The antigen-specific T cell generation used in these studies did not preclude the inclusion of CD4+ T cells. The addition of CD4+ T cells into our viral inhibition assay is subject to additional infection by HIV; however, we show in all donors tested that HIV-specific T cells, composed of both CD4+ T cells and CD8+ T cells, decrease HIV. Additionally, we show that the use of nonspecifically activated genetically modified T cells secreting 10-1074 antibody are unable to control viremia and result in an increase in HIV p24 in the cell supernatant. This suggests that CD4+ T cells are, in fact, infected with HIV in our viral inhibition assay, but this effect is overcome by HIV specificity in the HIV-specific T cell products.

Our findings support previous reports that genetic modification of *ex vivo* expanded antigen-specific T cells do not significantly alter their primary function.[28] Furthermore, our findings show that secretion from HIV-specific T cells does not alter the function of the antibody. In a virus inhibition assay, we observed decreased HIV p24 in the cell supernatant in our genetically modified HIV-specific T cells; however, these results did not reach significance.

Our findings also support previous reports using broadly neutralizing antibodies to mediate ADCC.[39–41] From a therapeutic standpoint, however, it is difficult to elicit production of broadly neutralizing antibodies *in vivo*,[42] and so passive immunization by direct administration of these broadly neutralizing antibodies has been attempted. Our method aims to enhance the distribution of such antibodies by combining with HIV-specific T cells, able to

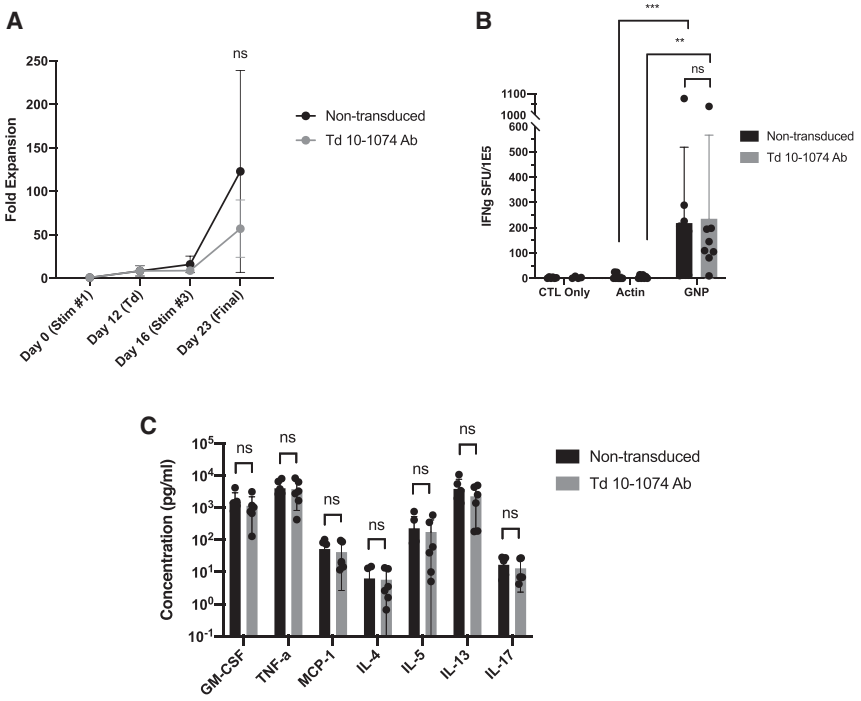

Figure 4. Transduced HIV-Specific T Cells Maintain T Cell Function

(A) Fold expansion of transduced and non-transduced HIV-specific T cells is shown 7 days following stimulation 3 (day 23). The black line shows mean fold-expansion of non-transduced cells (black circles), while the gray line shows mean fold-expansion of non-transduced cells (gray squares); n = 8. Error bars depict standard deviation. Wilcoxon signed rank tests were used for statistical analysis, where ns denotes $p > 0.05$. (B) IFNγ ELISPOT (shown as the number of SFCs per 100,000 cells) measures HIV-specific T cell specificity. Rectangles depict mean responses to media (cytotoxic T lymphocyte [CTL] only), irrelevant antigen (actin), and HIV antigens gag, nef, and pol (GNP) of non-transduced cells (black) and transduced cells (gray); n = 10. Error bars depict standard deviation. Wilcoxon signed rank tests were used for statistical analysis, where ns denotes $p > 0.05$, $^{**}p = 0.0078$, $^{***}p = 0.0010$. (C) Multiplex ELISA of T cell responses following stimulation with antigen were used to measure secretion of cytokines by nontransduced (black) and transduced (gray) HIV-specific T cells; n = 6. Circles show individual T cell donors, and error bars depict the standard deviation. Paired t tests were used for statistical analysis, where ns denotes $p > 0.05$.

home to sites of HIV reservoir (i.e., spleen and gut), allowing for concomitant migration of broadly neutralizing antibodies to sites of disease by localized secretion from HIV-specific T cells.[43]

This therapy can be readily deployed with existing approaches, especially in the context of allogeneic HSCT for HIV+ patients with malignancies. More importantly, the platform is modular and readily adaptable to combination with other modalities. For example, the HIV-specific T cells can be substituted with T cells specific for both HIV and tumor-associated antigens, and the broadly neutralizing antibodies that they secrete can be substituted with checkpoint inhibitors. Additionally, future iterations of this platform could include the use of CD8+ T cells alone, as well as a comparison of antibody secretion by CD4+ T cells and CD8+ T cells. It would also be interesting to model an *in vivo* system to test the platform's antiviral efficacy. An *in vivo* model would require the presence of NK cells in addition to infected memory cells harboring latent virus; while such a model would take considerable effort to generate, it would be worth investigating this in future iterations of this product.

The use of a single platform featuring bnAb production from genetically modified HIV-specific T cells (TbnAbs), thus recruiting ADCC activity from endogenous NK cells, allows for spatial and temporal coordination between the innate and adaptive components of an immune attack. Coupling monoclonal antibody (mAb) production to T cells will also serve to target mAb production to lymphoid tissues. On their own, high-molecular-weight molecules like antibodies do not penetrate the lymph node cortex,[44] which represent critical anatomical sites of the disease.[22,45,46] Recruitment of these compartments of immunity toward the goal of eradicating HIV has not been simultaneously tested in murine models of latency; this approach allows for synergy between immune arms, broadens the target range of the immune therapy, and provides further insight into what defines an effective anti-HIV reservoir immune response.

Conclusions

Here we have provided rationale to move this HIV therapeutic strategy from our pre-clinical studies into murine models of HIV/AIDS, ultimately bringing us closer to a definitive cure for HIV/AIDS. Demonstration of the efficacy of this approach also provides a new immune therapeutic model not just for HIV/AIDS but also for other diseases that may benefit from cell therapy with antibody-producing T cells, such as cancer or autoimmunity.

MATERIALS AND METHODS

10-1074 Antibody Construct Design

The 10-1074 antibody construct included codon-optimized heavy and light chains of the 10-1074 separated by 2A cleavage sequences (with the constant region of the heavy IgG1 chain substituted with the constant region of IgG3), 2A and furin cleavage site, and truncated CD19 (to quantify transduction efficiency). 10-1074 is a broadly neutralizing antibody isolated from an African individual chronically infected with a subtype A virus (as described in Mouquet et al.[47]).

Production of Retroviral Vector

Plasmid constructs were synthesized by Genscript, subcloned into an Moloney murine leukemia virus (M-MLV) retroviral backbone, and expanded using the QIAGEN Endofree Plasmid Maxi Kits (QIAGEN, Hilden, Germany). Plasmids were transfected into Phoenix Eco cells using Lipofectamine 3000 kits (ThermoFisher, Waltham, MA, USA), as

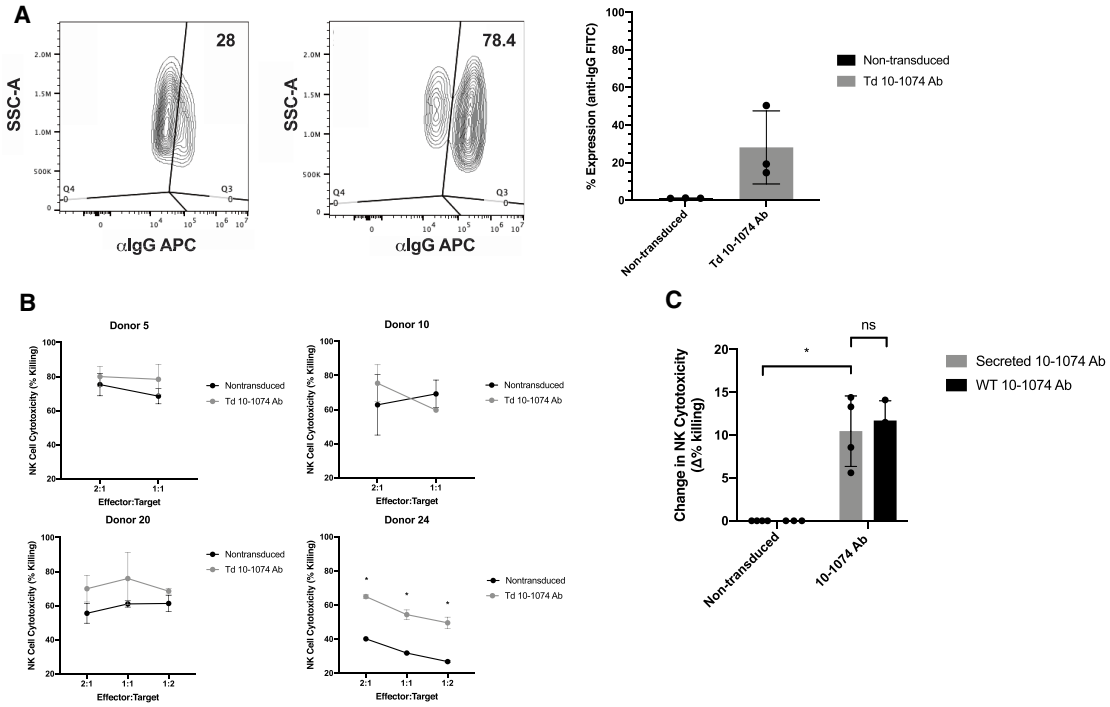

Figure 5. T Cell-Secreted Antibodies from HIV-Specific T Cells Elicit ADCC

(A) Sample flow cytometry figure shown. HIV gp120-coated CEM.NKr.CCR5 cells were used as target cells. Binding to HIV envelope of secreted 10-1074 antibody was measured by expression of secondary anti-human IgG (H+L) on the cell surface by flow cytometry; n = 3. Error bars denote standard deviation. (B) Cytotoxicity assays from four donors show higher killing (over allo-based killing) of HIV envelope-expressing cells that were co-cultured with NK cells in the presence of nontransduced (black lines) or transduced supernatant (gray lines) from the donors indicated. Error bars denote standard deviation. (C) ADCC as measured by the change in NK cell killing of HIV envelope expressing target cells (HeLa) upon addition of antibody containing transduced HIV-specific T cell supernatant. Each graph represents one donor. Circles represent mean of 3 technical replicates. Error bars depict standard deviation. Student's t tests were used for statistical analysis, where ns denotes $p > 0.05$, *$p < 0.005$. (C) Change in NK cell cytotoxicity upon addition of antibody containing transduced HIV-specific T cell supernatant (gray bar) at 2:1 E:T ratio was compared with change in cytotoxicity upon addition of WT 10-1074 antibody (black bar, 250 ng/mL). Circles depict individual donors; n = 4. Error bars depict the standard deviation. Student's t tests were used for statistical analysis, where ns denotes $p > 0.05$, *$p = 0.015$.

per manufacturer's protocol. Supernatant was then collected and used to transduce PG13 producer cell lines (ATCC, Manassas, VA, USA). Polybrene (Santa Cruz Biotechnology, Dallas, TX, USA) was added at a concentration of 8 μg/mL. PG13 cells transduced with the plasmid constructs were single-cell sorted by flow cytometry (using CD19 as marker of transduced cells), and clones were expanded and cryopreserved.

Peripheral Blood Samples

Peripheral blood samples were obtained from deidentified, healthy donor buffy coats from the NIH, through the Department of Transfusion Medicine, or commercially from AllCells. Peripheral blood samples were processed within 24 h of receipt (< 3 days post collection), using Ficoll-Paque Plus Density Gradient Media (GE Life Science, Pittsburgh, PA, USA) to obtain PBMCs. PBMCs were either frozen for future use or immediately used for monocyte isolation.

Manufacture of HIV-Specific T Cells

Monocyte-derived dendritic cells were used as antigen-presenting cells and were generated by separating adherent cells from PBMCs, as previously described.[38] After 2 h adherence on plates, non-adherent cells were collected and cryopreserved (these cells were subsequently used as the T cell fraction). Adherent cells were cultured with GM-CSF (R&D, Minneapolis, MN, USA) and IL-4 (R&D, Minneapolis, MN, USA) and incubated for 72 h at 37°C. One day prior to stimulation, adherent cell fraction (refered to as dendritic cells, DCs) were pulsed with overlapping peptide library spanning Gag, Nef, and Pol (JPT Peptide Technologies, Berlin, Germany) and matured with GM-CSF, TNF-α, IL-1b, IL-4, IL-6, prostaglandin E2 (PGE-2), and lipopolysaccharides (LPS) or GM-CSF, INFγ, IL-4, and LPS (R&D, Minneapolis, MN, USA). Within 16 h following maturation, DCs were irradiated at 30 Gy and cocultured with the non-adherent fraction of PBMCs at a ratio of 1:10 (DC: T cell). Subsequent stimulations used autologous phytohemagglutinin (PHA) blasts as antigen-presenting cells, made from phytohemagglutinin- and IL-2 stimulated autologous PBMCs, and gene-modified K562 feeder cells, as previously described.[38] PHA blasts and K562s were irradiated at 30 Gy and 200 Gy, respectively, prior to stimulation. T cells were stimulated at a ratio of 1:4 PHA blast to T cell for the second stimulation and

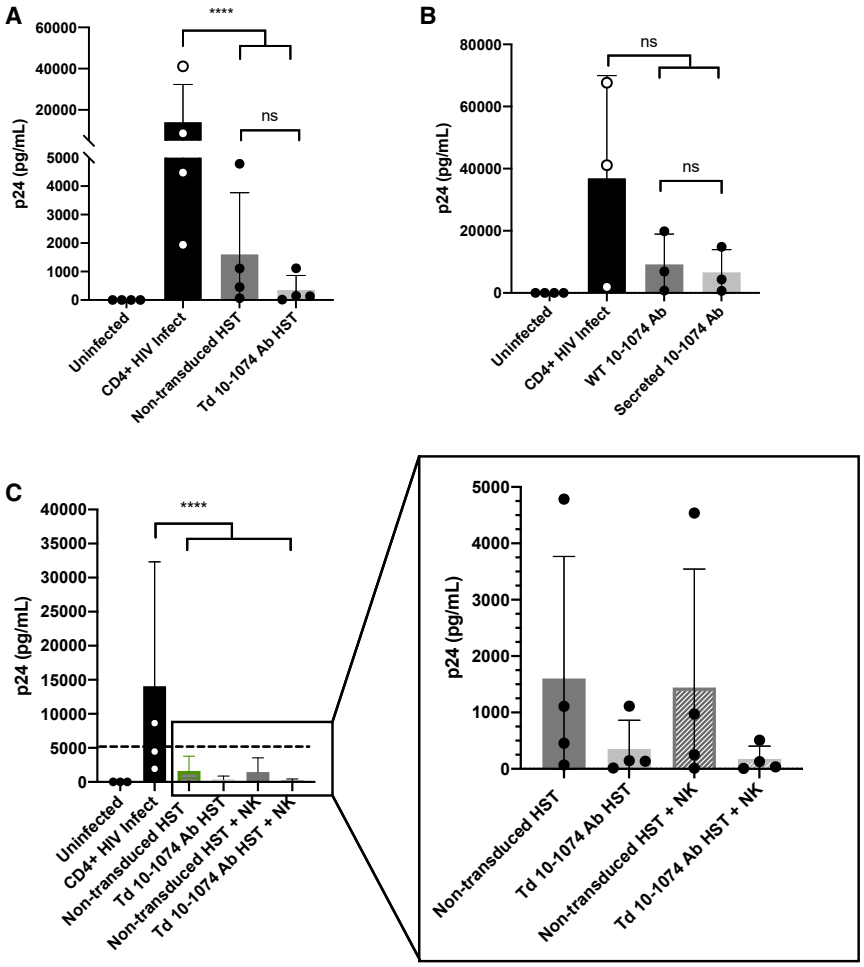

Figure 6. Genetic Modification of HIV-Specific T Cells to Secrete 10-1074 Antibody Increases Anti-Viral Efficacy against HIV-Infected Targets

(A) HIV p24 concentration in cell supernatant was measured by p24 ELISA and shown in pg/mL. Mean p24 levels are shown from SF162 HIV-infected autologous CD4+ T cells (black bar, no effectors), nontransduced HIV-specific T cells (dark gray bar), and transduced HIV-specific T cells (light gray bar) plated at 10:1 E:T ratios; n = 4. Error bars denote standard deviation. (B) Mean p24 levels are shown from SF162 HIV-infected autologous CD4+ T cells (black bar, no effectors), WT 10-1074 antibody (dark gray bar, 250 ng/mL), and HIV-specific T cell-secreted antibody (light gray bar); n = 4. Error bars denote standard deviation. (C) Mean p24 levels are shown from SF162 HIV-infected autologous CD4+ T cells (black bar, no effectors), nontransduced HIV-specific T cell (dark gray bar), transduced HIV-specific T cell (light gray bar), nontransduced HIV-specific T cell + autologous NK cells (dark gray bar, stripes), transduced HIV-specific T cell + autologous NK cells (light gray bar, stripes); n = 4. Error bars denote standard deviation. (A–C) One-way ANOVA was used to test differences in control (CD4+ HIV infect) versus treatment groups, followed by a Dunnett's multiple comparison test, where ****$p < 0.0001$. Paired t tests were used for statistical analysis between treatment groups, where ns denotes $p > 0.05$.

1:1:4 of T cells:PHA blasts:gene-modified K562 for the third stimulation.[48] Gene-modified K562 cells, expressing co-stimulatory molecules 4-1BB Ligand (4-1BBL), CD80, CD83, and CD86, were shared by Dr. Cliona Rooney (Baylor College of Medicine).

Transduction of HIV-Specific T Cells

Viral transduction of antigen-specific T cells was performed as previously described,[28] with some modifications. Three days after the second stimulation, T cells were transduced with concentrated retroviral supernatant (concentrated 1:3 with RetroX concentrator; Takara, Kusatsu, Japan). Non-tissue culture plates were treated with 50 μg/mL Retronectin (Takara, Kusatsu, Japan) overnight at 4°C. Retroviral supernatant was added to each well and centrifuged at 2,000 × *g* for 2 h at 30°C. Following viral centrifugation, cells were plated at 5×10^5 cells/well with the addition of 50 U/mL IL-2 (R&D, Minneapolis, MN, USA). Supernatants were collected two to three days following transduction and frozen for functional assays.

Five to seven days following the third stimulation, cells were collected for functional assays and cryopreserved in freezing media containing 50% fetal bovine serum (FBS), 40% RPMI, and 10% dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA).

Flow Cytometry

Cell phenotype and transduction efficiency were determined by flow cytometry, using the following cell surface markers: CD3 (clone SK7), CD8a (clone RPA-T8), CD4 (clone OKT4), CD16 (clone 3G8), CD56 (clone HCD56), and CD19 (clone REA675). Stained cells were run on a Beckman Coulter Cytoflex. Data were analyzed using the FlowJo software.

INF-γ ELISpot

Specificity to HIV peptides Gag, Nef, and Pol ("GNP"; JPT, Berlin, Germany) was determined by IFN-γ ELISpot assays. Medium (no peptide) and an irrelevant peptide (actin) were used as negative controls, and Staphylococcus enterotoxin B (SEB) was used as a positive control. Positive results were defined as at least double the number of IFN-γ spot-forming units (SFU) in the negative control, with at least 10 SFU/1 × 10^5 cells plated. ELISpot plates were scanned and analyzed by Zellnet.

Cytokine Secretion

Cytokine secretion of virus-naive donor-derived HIV-specific T cells secreting 10-1074 antibody was determined using Bio-plex Pro Human Cytokine 17-plex Assay (BioRad, Hercules, CA, USA). Cellular supernatants were collected 6 days following stimulation and cryopreserved until the assay was performed. T cell

secretion of GM-CSF, TNF-α, monocyte chemoattractant protein 1 (MCP-1), IL-4, IL-5, IL-13, and IL-17 were measured.

10-1074 Antibody ELISA

Secretion of antibody by HIV-specific T cells was tested by ELISA. HIV Env gp120 recombinant human protein (MyBioSource, San Diego, CA, USA) was used to coat high-binding microplates (Sigma-Aldrich, St. Louis, MO, USA). Supernatants collected from both HIV-specific T cells and non-specific T cells were used as primary analytes as the 10-1074 antibody variable region would bind the gp120 protein-coated plate. Horseradish peroxidase (HRP)-labeled goat anti-Human IgG (H+L) cross-absorbed secondary antibody (ThermoFisher, Waltham, MA, USA) was used to detect plate-bound primary antibody by binding to the Fc portion of the construct. Levels of 10-1074 antibody were quantified using a standard curve generated using purified WT 10-1074 antibody (obtained through the NIH AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases [NIAID], NIH: 10-1074 mAb from Dr. Michel C. Nussenzweig).[49]

HIV Binding

The following reagents were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: 69T1RevEnv Cells from Dr. Joseph Dougherty[32] and CEM.NKR CCR5+ cells from Dr. Alexandra Trkola.[50–52] HIV envelope expression on HeLa cells was measured 7 days and 2 passages after removing from tetracycline. CEM.NKr.CCR5 cells were incubated with 1.5 μg of HIV-1 YU2 gp120 recombinant protein (MyBioSource.com, San Diego, CA, USA) per 1 million CEM cells for 75 min. HIV envelope-expressing HeLa cells or CEM.NKr.CCR5 cells coated with HIV-1 gp120 were fixed with 4.2% paraformaldehyde (BD Biosciences, San Jose, CA, USA) for 20 min at 4°C. Cells were washed in chilled fluorescence-activated cell sorting (FACS) buffer (PBS + 2% FBS) and incubated in supernatant containing secreted antibody from both nontransduced and transduced cell supernatant for 1 h at room temperature. Cells were washed an additional two times in FACS buffer and incubated with goat anti-human IgG (H+L) fluorescein isothiocyanate (FITC) (Life Technologies, Carlsbad, CA, USA) for 30 min at room temperature before analysis by flow cytometry.

ADCC

HIV envelope-expressing HeLa cells described above (AIDS Reagent Program; 69T1 RevEnv Cells, 3336) were used as target cells. Target cells were labeled with 10-1074 antibody secreted from HIV-specific T cells, and europium cytotoxicity assays (Perkin Elmer, Waltham MA, USA) were performed. Media alone and nontransduced supernatant were used as negative controls and equivalent concentration of WT 10-1074 antibody (250 ng/mL) was used as a positive control. NK cells were expanded from PBMCs as previously described[53,54] and used as effector cells.

Viral Inhibition Assay (p24)

The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 SF162 Virus from Dr. Jay Levy.[55] CD4+ selected PBMCs (target cells) were activated with IL-2 and PHA for 3-4 days before infection with a laboratory strain of HIV (SF162, described above). Infected target cells were co-cultured with genetically modified HIV-specific T cells at a ratio of 10:1 effector to target cells. A single dose of WT 10-1074 antibody was plated at 250 ng/mL. Supernatant was collected and measured for HIV p24 levels on days 3, 5, and 8 post infection and coculture. P24 levels were quantified by p24 ELISA (ABL, Rockville, MD, USA).

Statistics

Data presented are summarized as mean ± standard deviation. We tested for normality between nontransduced and transduced conditions using the Shapiro-Wilk test. For data passing the normality test, we used paired t tests; for those that do not, we used Wilcoxon tests. For virus inhibition assay results, we tested for significant differences between mean control (CD4+ HIV Infect) and treatment groups (varied) using a one-way ANOVA with Dunnett's multiple comparison test. Statistical differences between treatment groups were tested using paired t tests. p values less than 0.05 were used to determine significance. Analysis was performed using Graphpad PRISM.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omtm.2020.08.015.

AUTHOR CONTRIBUTIONS

A.B.P. performed experiments, analyzed data, and wrote the manuscript. A.B.P., R.B.J., and C.R.Y.C. designed experiments and wrote and edited the manuscript. Y.R., M.K., and D.S. performed experiments for the manuscript. H.G., D.F.N., C.M.B., and R.M.L. helped with experimental design and troubleshooting, and edited the manuscript. P.J.H. helped edit the manuscript.

CONFLICTS OF INTEREST

C.R.Y.C., P.J.H., and C.M.B. are co-founders of a biotechnology company, Mana Therapeutics, developing T cell therapies for cancer. A.B.P., C.R.Y.C., R.B.J., D.F.N., and C.M.B. have filed a patent describing the methods and products described in this paper.

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following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: Anti-HIV-1 p24 Monoclonal (KC57)-PE from NIAID, Division of AIDS (catalog # 13449). The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 SF162 gp140 trimer, from Dr. Leo Stamatatos. Graphical abstract was created with BioRender.com.

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