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Reprint of: Virus-Specific T Cells: Broadening Applicability

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Abstract

Virus infection remains an appreciable cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). Although pharmacotherapy and/or antibody therapy may help prevent or treat viral disease, these drugs are expensive, toxic, and often ineffective due to primary or secondary resistance. Further, effective treatments are limited for many infections (eg, adenovirus, BK virus), which are increasingly detected after alternative donor transplants. These deficiencies in conventional therapeutics have increased interest in an immunotherapeutic approach to viral disorders, leading to adoptive transfer of virus-specific cytotoxic T lymphocytes (VSTs), which can rapidly reconstitute antiviral immunity post-transplantation without causing graft-versus-host disease. This review will explore how the VST field has improved outcomes for many patients with life-threatening viral infections after HSCT, and how to broaden applicability beyond the "patient-specific" products, as well as extending to other viral diseases even outside the context of HSCT.

Keywords

Adoptive T cell therapy; Cytomegalovirus; BK virus; HIV

INTRODUCTION

It is more than 26 years since the first proof-of-principle studies conducted by Riddell et al. demonstrated that virus-specific T cell clones from a healthy donor could be generated ex vivo from autologous cytomegalovirus (CMV)-infected fibroblasts. When adoptively transferred into an allogeneic hematopoietic stem cell transplantation (HSCT) recipient, these virus-specific T cells (VSTs) could prevent CMV infection without causing graft-

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versus-host disease (GVHD) [1]. Since then, numerous trials of adoptive immunotherapy with VSTs derived from transplant donors have established their safety and potency for both the prevention and treatment of CMV disease. Application of VST has subsequently expanded, first to the generation of Epstein-Barr virus (EBV)-specific T cells [2–4] and then to the generation of multivirus-specific T cells targeting common post-transplantation viral pathogens, including adenovirus, BK virus, and human herpesvirus type 6 [2,5]. These studies indicated that the techniques used to elicit VSTs could successfully be applied to numerous viruses.

Central to the development and application of VSTs has been technology-based progress in the generation of VSTs. Historically, the process required a lengthy, 8- to 10-week culture period. In the process of optimization, which notably includes the use of gas-permeable culture flasks for rapid T cell expansion, the technique has become simpler and cheaper. Today, it is possible to generate VSTs from autologous antigen-presenting cells pulsed with viral peptide libraries in less than 14 days. More recently, groups have used T cells isolated directly from donor leukocytes on the basis of their binding viral peptide/HLA tetramers or dissociable streptamers, or on expression of activation markers or cytokines after short-term in vitro sensitization [2]. Despite these efforts to speed VST production, the acute nature of viral illness in immunosuppressed individuals often demands immediate availability of the T cell product. Furthermore, viruses complicating organ transplantation, such as EBV, present a particular problem for adoptive T cell transfer therapies. Although immunosuppressed, organ transplant recipients, in contrast to HSCT recipients, are not tolerant of adoptively transferred T cells, even if the cell donor is HLA-matched. These contingencies have spurred the development of banked off-the- shelf VST products. Select closely HLA-matched cell products can be shipped for same-day use. Despite the challenges, however, such third-party donor VST banks are being developed, as we discuss in this review.

Finally, successful management of post-HSCT viral complications has stimulated research into the treatment of viral diseases outside the context of HSCT. VSTs are now being explored in a broad range of inherited and acquired immunodeficient states. Here we review the latest approaches for generating VSTs for these new indications and the results of clinical trials in transplantation- and non-transplantation-related polyomavirus and human immunodeficiency virus (HIV) infections.

THIRD-PARTY VSTS

The principal constraints to the broader application of adoptive therapy with transplant donor-derived T cells are logistic in nature. The relatively low incidence of refractory infectious complications in the post-transplantation period makes it impractical to generate viral-specific populations for all HSCT recipients at risk. At the same time, the aggressive nature of these infections requires rapid treatment of patients who do not respond to first line antiviral therapy. Thus, an 8- to 10-week wait is too long once a patient has been identified as needing treatment. Therefore, the use of longer manufacturing approaches means that T cells need to be generated before the patient develops an infection. Although recent approaches bypass this constraint, rapid selection of low-frequency populations of T cells might not be possible. Some of the limitations in the generation and application of donor-

derived viral-specific adoptive cell therapy can be overcome by using banked, off-the shelf, or so-called third-party T cells.

Limitations of Viral Capture Strategies

Rapid selection by tetramer or streptamer depends on identifying an HLA allele and the viral epitope presented by that HLA allele. Variants of prevalent HLA alleles differ in the capacity to present specific viral epitopes, making it challenging to use this capture method of selection for patients not bearing common HLA alleles. Other logistic constraints include the fact that some donors may be unwilling or unable to provide the secondary donations needed to generate VSTs. In addition, although possible, it is difficult to generate viral-specific populations from donors who have not been previously sensitized to the virus in question.

HLA Restriction

More critical to the efficacy of VSTs generated from the HCT donor is the issue of HLA restriction. In the HLA-nonidentical transplant setting, viral-specific cytotoxic T lymphocytes (CTLs) may be restricted in cytotoxicity through an HLA allele not shared by the HCT recipient and thus ineffective in treating host infected targets. This issue is especially problematic if the VST line is not assessed for HLA restriction before infusion. With the increased use of haploidentical HCT donors, the issue of ensuring appropriate HLA restriction of VSTs will become paramount. These limitations, and an attempt to provide access to VSTs for a growing number of centers, have led groups at Baylor College of Medicine, Children's National Medical Center, University of Edinburgh, Memorial Sloan Kettering Cancer Center (MSKCC), and University of Tubingen to explore the use of banked partially HLA-matched viral-specific CTLs derived from third-party donors (eg, healthy individuals other than the HCT donor or the patient). This approach has now gained traction and is being attempted by a growing number of centers [2].

Treatment of Post-Transplantation Lymphoproliferative Disease

As pioneered by Dorothy Crawford and reported by Haque et al. [3], the group at the University of Edinburgh used partially HLA-matched EBV-specific T cells derived from a bank of 70 lines generated from healthy EBV-seropositive volunteer blood donors to treat 33 solid organ transplant patients with EBV post-transplantation lymphoproliferative disease. In this study, 52% of patients achieved a complete response or partial response that was sustained for ≥6 months. Since that time, other groups have expanded this experience to treat an expanding number of viral infections primarily in HCT recipients. These centers include, but are not limited to, Children's National Medical Center, the University of Aberdeen, Baylor College of Medicine, The Karolinska Institute, The University of Tubingen and MSKCC. Reports on fewer than 200 HSCT recipients treated with third-party VSTs confirm the potential efficacy and limited risk of toxicities, including GVHD [4].

Clinical Experience with VST Cell Banks

Banks of appropriate diversity have been generated and in addition to their immediate accessibility, these banks of third-party donor-derived VSTs provide unique advantages for recipients of HLA nonidentical HSCT. Because banked T cells are characterized by their

HLA restriction, T cells restricted by an HLA allele expressed by the virus-infected cells in the patient can be selected. Indeed, in a survey of consecutive transplant recipients at MSKCC, from a bank of 132 GMP grade CMVpp65-specific T cell lines, we could identify appropriately restricted lines for 93% of HLA-nonidentical HSCT recipients and 98% of cord blood transplant recipients. In contrast, examination of the HLA restrictions of CMVpp65-specific T cells generated from the donors of HLA-nonidentical HSCT grafts showed that they were restricted by an HLA shared by the transplant recipient in only 60% to 70% of cases [4]. Working with a more limited bank, the group at Baylor demonstrated that a bank of just 32 tri-VST lines was sufficient to provide suitable HLA-restricted T cells for 90% of the patients referred for treatment in their multicenter trial [2]. More recently, they treated 38 patients with multiple viral infections from a bank of 59 lines generated with specificity for adenovirus, BK virus, CMV, EBV, and human herpesvirus 6 [5]. A third advantage is that certain patients may fail to respond to VSTs specific for epitopes presented by one HLA allele and may respond to treatment with T cells from a different third-party donor specific for a different epitope presented by a different shared HLA allele.

Adoptively transferred third-party T cells have a demonstrated role in the initial responses observed. However, the durability of responses is both surprising and unexplained. Transplant donor-derived VSTs persist long-term. Indeed, as reported by Heslop et al. [6], the group at Baylor detected genetically marked donor-derived EBV-specific T cells as long as 10 years after adoptive transfer. In contrast, third-party T cells, although detected for as long as 90 days after infusion into immunodeficient HSCT recipients, do not achieve durable engraftment [4]. Nevertheless, the responses induced are usually sustained even in patients who are still markedly lymphopenic.

The mechanisms contributing to the sustained responses observed are unknown. It is possible that the initial transient expansion of VSTs is sufficient to control asymptomatic latent infections. Small numbers of the third-party T cells may persist long enough at sites of infection to sustain control until reconstitution of donor-derived viral immunity is established. Alternatively, the allogeneic third-party T cells may facilitate cross-presentation of viral antigens to donor-derived effectors. Indeed, the groups at Baylor and Children’s National Medical Center have reported on the identification of non-EBV tumor antigen-directed T cells in individuals treated with autologous EBV-CTLs, suggesting that antigen spread can occur even in the absence of an allogenic stimulus. Ongoing research examining interactions between third-party T cells and the transplant recipient may elucidate the mechanisms contributing to the reconstitution of viral immunity. In addition, questions remain regarding the ideal method for generating VSTs, as well as the characteristics of the cell therapy products and the recipient that are most likely to translate into treatment success.

Future Directions for Third-Party VSTs

Although the experience with third-party VSTs is still limited, the results reported to date are quite promising. Trials directly comparing donor and third-party T cells have not yet been performed; however, in the larger single-arm trials from MSKCC and Baylor College of Medicine, results of treatment with partially HLA-matched third-party donor-derived T

cells have been comparable to those achieved with transplant donor-derived T cells in the treatment of EBV lymphomas and only slightly inferior in the treatment of drug-refractory CMV infections or persistent CMV viremia. However, it is important to note that there are no completed prospective randomized clinical trials comparing third-party T cells and standard therapy; therefore, it is important to acknowledge that making banked VSTs available to more patients requires building on the multicenter trials that have been and are currently being performed. Several multicenter trials are currently open and reporting promising results in terms of both feasibility and efficacy.

ADOPTIVE T CELL THERAPY FOR POLYOMAVIRUS DISEASES

First reported in 1953, polyomaviruses (PyVs) are small, double-stranded DNA viruses widely distributed throughout the vertebrate phylum that are notable for their propensity to cause malignancy (hence the name “polyoma”). The first reported human PyVs, both discovered in 1971, were BK virus (BKV) in a patient (“BK”) who developed hemorrhagic cystitis (HC) and JC virus (JCV) in a patient (“JC”) with progressive multifocal leukoencephalopathy (PML). Since then, many PyVs have been isolated in humans, but the only new PyV contributing definitively to human disease is the Merkel cell virus (MCV), described in 2006. Found in 80% of patients with Merkel cell cancer (MCC), MCV is significant for the being the only oncogenic human PyV identified so far. Like many DNA viruses, PyVs are acquired early in life through asymptomatic infection. In immunocompetent individuals, the cellular and humoral responses suppress, but do not eliminate, the virus, which then acquires lifelong latency. Diseases associated with PyV occur only when the virus reactivates because of compromised cellular immunity. PyV disease can occur in inherited or acquired immune deficiency diseases or following immunosuppressive therapy, particularly in the context of stem cell and organ transplantation. PyV diseases can have fatal outcomes, and, unfortunately, there are no antiviral drugs effective against PyV. However, the close association between disease and immunodeficiency is a clear rationale for the development of cellular immunotherapeutic approaches to control the virus [7].

Immunity to PyV and the Development of Adoptive T Cell Therapy

PyVs have several well-conserved and well-defined antigens: the small and large T antigens (sT and LT), and the VP1 capsid protein responsible for cellular tropism of specific viruses to the central nervous system (JCV), the urothelium (BKV), and the skin (MCV). Whereas there is close homology between T antigens in human PyVs, VP1 is more virus-specific. Current technologies developed for the ex vivo expansion of VSTs for the treatment of CMV, adenovirus, and EBV infections using PepMixes of immunodominant viral proteins are readily applied to the generation of BKV-, JCV-, and MCV-specific T cells [2,8,9]. Indeed, the very close (>80%) homology between JCV and BKV proteins means that a single cell product targeting LT and VP1 antigens recognizes both viruses. MCV is more distinct from the others, and only a truncated LT is expressed in tumors, necessitating specific MCV antigen-directed approaches. In addition to ex vivo expanded T cell products, the isolation of PyV-specific T cells from healthy seropositive donors for adoptive transfer is under investigation [10].

BKV

BKV reactivation occurs in the majority of individuals after HSCT, but causes HC in <10% of HSCT recipients. A BKV-associated nephropathy can also occur, but this syndrome is more common after renal transplantation [11].

Several investigators have included BKV peptide libraries in the production of multivirus-specific T cells (MVSTs) to treat or prevent viral reactivation after allogeneic HSCT. In a study at Baylor College of Medicine, approximately 60% of the multivirus-specific products exhibited activity against BKV. In 7 patients with BKV HC who received HLA-matched VST products, 5 exhibited complete response and 1 showed a partial response [8]. Our group generated MVSTs from PepMixes of CMV, EBV, adenovirus, and BKV to prevent viral reactivation after HSCT. In a phase I study at the National Heart, Lung and Blood Institute (NCT02108522), 0 of 9 patients receiving escalating doses of MVSTs infused at day +14 post-HSCT developed BKV-HC. At the M.D. Anderson Cancer Center, a third-party bank of BKV-specific T cells generated from a wide array of BKV peptides was used to successfully treat 10 patients with BKV HC after HSCT. Five patients had complete resolution of symptoms and gross hematuria, 4 had a partial response, marked by a decrease in HC from grade 4 (red cell transfusion requirement/renal impairment) to grade 3 (urinary blood clots), or from grade 3 to grade 2 (macroscopic hematuria). One patient relapsed after an initial partial response [12]. These promising results suggest that BKV-specific T cells may be effective in the prophylaxis and treatment of HC, but confirmation in larger studies is needed. BKV-specific T cells have yet to be reported in BKV-associated nephropathy after solid organ transplantation.

JCV

PML is a rare but disastrous complication of JCV reactivation. The disease is typically rapidly progressive, with death occurring in weeks from progressive encephalopathy. Individuals at elevated risk for PML include HSCT and organ transplant recipients, persons developing AIDS, patients with multiple sclerosis receiving natalizumab (which blocks T cell entry into the central nervous system), patients with lymphoma receiving multiple chemotherapies, and patients with various immunodeficiency diseases [13,14]. PML can respond to a reduction in immunosuppression when possible. The successful use of adoptively transferred JCV-specific T cells was first reported in 2011. A patient with post-transplantation PML receiving donor VP1- and LT-specific T cells cleared the virus and regained cognitive function At the National Institutes of Health, JCV-specific T cells are generated by PepMix stimulation of closely matched healthy related donors. Patients receive a dose of 1×10^6 JCV-specific T cells/kg. Early results suggest efficacy in recipients who receive good-quality products. This study is ongoing (NCT02694783).

MCV

MCC is a highly malignant, chemotherapy-resistant skin cancer with a propensity to metastasize comparable to that of melanoma [15]. It occurs most commonly in older adults and in immunocompromised individuals. Despite surgical re-section and chemotherapy, the 5-year survival rate for metastatic MCC is <25%. However, checkpoint inhibitors such as pembrolizumab can be effective in patients with advanced disease, indicating a potential for

T cell-based therapies [16,17]. In a single case report, a patient with metastatic MCC (MCV $^{+}$) received autologous CD8^{+} cells specific for a single LT epitope after lesion-targeted radiation to up-regulate MHC class I antigen presentation by the tumor. Two of 3 metastases regressed permanently after treatment [18]. This study has encouraged our group to explore strategies to treat MCC with MCV-specific T cells generated from PepMixes from an array of MCV proteins to generate both MHC class I and class II epitopes.

Generating effective MCV-specific T cells faces several challenges. First, autologous T cells may lack MCV immunity, necessitating the generation of MCV T cells from immunocompetent closely matched related donors. Second, in immunocompetent patients with MCC, the tumor cells might have already developed strategies to evade T cell cytotoxic attack.

In summary, in many ways PyV diseases represent ideal targets for treatment with adoptively transferred T cells. PyVST-specific T cell therapy is at an early stage of development, but these preliminary results strongly suggest that the approach will be effective in treating BKV disease. Adoptive cell therapy for other PyV diseases is confronted by different challenges, however. In PML, the rapidity of disease progression mandates rapid intervention, necessitating the availability “off-the-shelf” third-party VST products. For better efficacy, for treating MCC, MCV-specific T cells will need to be combined with other treatments, such as lymphodepletion, to address unfavorable alterations to the immune milieu and strategies to circumvent tumor evasion.

BROADENING APPLICABILITY TO HIV

Worldwide, almost 37 million people are infected with HIV [19]. HIV-1 specifically attacks the CD4^{+} T cells of the immune system. Without intervention, HIV infection can progress to life-threatening acquired immunodeficiency syndrome (AIDS). Although antiretroviral therapy (ART) prevents progression to AIDS and prolongs life expectancy, it is not curative.

Despite the poor prognosis of HIV^{+} individuals who are not on ART, a subset, termed elite controllers, can naturally control viremia. Elite controllers have dominant Gag-specific CTL responses, suggesting that T cell immunity can control viremia [20–22]. The possibility of T cells eradicating HIV is illustrated by the spectacular cure of the “Berlin patient,” an HIV^{+} male who developed leukemia [23]. He received a stem cell transplant from a donor with a mutation in the HIV coreceptor gene *CCR5-Δ32*, which confers resistance to HIV infection. Currently, his HIV is undetectable despite discontinuation of ART. Initial T cell therapies for HIV were unsuccessful owing to single CD8 epitope specificity and exclusion of CD4 cells, resulting in limited persistence of cells in vivo [24–26]. Addressing these limitations, groups have sought to develop better T cell therapies with the goal of curing HIV.

T Cell Therapies for HIV

Requirements for effective and long-lasting immunity against HIV include the infusion of HIV-specific T cell clones or polyclonal CTLs, and the genetic modification of T cells with artificial T cell receptors (TCRs) and chimeric antigen receptors (CARs).

HIV-Specific T Cell Clones

Clinical trials have focused on isolating $CD8^+$ T cells that show strong IFN-γ and cytotoxicity responses to HIV. Examples include T cells specific for HLA-A2-restricted epitopes in gp120, p17, p24, and Nef [26]. When infused into 6 patients, a trend toward increased (albeit short-lived) $CD4^+$ T cell counts and decreased plasma and cell-associated viral levels was seen. In another study using $CD8^+$ Gag-specific T cells, 3 HIV^+ individuals who received 5 $CD8^+$ T cell infusions showed decreases in infected $CD4^+$ T cells, but no decreases in viral load compared with preinfusion levels [27]. In another study, 2 autologous CTL clones expanded against HIV Gag and Pol were infused into an HIV^+ individual with an increasing viral load despite ART, with no significant changes in CD8 or CD4 lymphocyte levels or viral load [28]. The lack of success using HIV-specific $CD8^+$ T cell clones may have been be due to antigen escape [25], low in vivo levels of HIV epitopes recognized by the T cell clones [99], or lack of $CD4^+$ T cell help [29].

Multi-HIV Antigen-Specific T Cells

To circumvent the problems of single epitope-specific T cells, polyclonal HIV-specific T cells expanded against multiple HIV antigens have been developed. Multiepitopespecific CTLs recognizing Gag, Nef, and Pol can suppress in vitro HIV replication. These polyclonal HIV-specific T cells include $CD4^+$ T cells, which can improve in vivo persistence and can be expanded against HIV peptides irrespective of the patient's HLA type, thereby broadening their therapeutic applicability. Autologous multiepitope-specific T cells are now in an ongoing clinical trial (NCT02208167). Another study demonstrated that multiepitope-specific T cells can be generated from HIV-naïve donors, opening up the possibility of antiviral T cell therapy in HIV^+ individuals with hematologic malignancies necessitating allogeneic HSCT [30].

Artificial TCRs

Engineering artificial TCRs is an attractive T cell therapy strategy for cancer and HIV. Such TCRs can be designed to target sequences that contribute significantly to viral fitness, such as the HLA-A*02-restricted P17 epitope SLYNTVATL (A2-SL9), associated with lower HIV levels in chronic infection. Although a promising approach, the artificial TCR strategy for treating HIV is currently on hold based on reports of lethal cardiac toxicity from an affinity-enhanced TCR trial in cancer patients.

CARs

CAR cells work in an MHC-unrestricted manner, directly binding surface antigens on the target cell and activating the T cell. Much has been learned from the first use of CARs in patients with $CD19^+$ malignancies. Although highly effective, they cause significant toxicity from cytokine release [31–33], and their use may necessitate previous lymphodepleting chemotherapy [34]. In the HIV setting, CAR cells must have low toxicity, have a low potential for viral escape, and be minimally immunogenic to permit durable viral suppression. In a Phase II trial (NCT01013415), 24 HIV^+ participants received a single infusion of T cells transduced with a CD4zeta CAR containing the extracellular domain of human CD4, which binds to HIV Env glycoprotein with or without postinfusion IL-2 [35].

IL-2 did not enhance the survival of infused T cells, but did traffic to rectal tissues, with decreases in rectal tissue-associated HIV. In another Phase II study, 40 HIV^+ participants on highly active antiretroviral therapy (HAART) received either CD4zeta-modified T cells or unmodified T cells [36]. Infusion of CAR-modified T cells decreased the HIV burden from baseline compared with the infusion of unmodified T cells. CD4zeta-CAR-modified T cells were detected up to 11 years postinfusion. Infused CAR T cells had stable levels of engraftment and persisted for more than 11 years, with a predicted half-life of more than 16 years [37]. A Phase I trial in HIV^+ individuals on HAART (NTC01013415) of autologous CD4zeta-CAR-modified T cells, with or without IL-2, is currently ongoing.

Zinc Finger Nuclease HIV Coreceptor Disruption

Several clinical studies are currently underway to evaluate the safety and efficacy of infusing $CD4^+$ T cells that have HIV coreceptor deletions of CCR5 via zinc finger nuclease (ZFN) knockdown. A group from University of Pennsylvania studied 12 HAART recipients enrolled in an open-label, nonrandomized, uncontrolled study in which each patient received a single dose of 10^9 ZFN-modified (CCR5-targeting) autologous $CD4^+$ T cells (NCT00842634) [38]. These modified VSTs had a half-life of 48 weeks and were detected in all participants up to 42 months. Blood levels of HIV DNA were decreased in most participants. The need for homozygous *CCR5-Δ32* knockdown was demonstrated in a heterozygous individual who received CCR5 ZFN-modified autologous $CD4^+$T cells and exhibited a marked reduction in viral load [38]. Other clinical studies (NCT01252641 and NTC01044654) are ongoing. A continuing concern is that other sites in the genome where CCR5 ZFNs show cross-reactivity could cause off-target side effects [39,40].

In summary, adoptive T cell therapy for HIV is rapidly developing. Perhaps the most important question is whether HIV VSTs can not only decrease the dependency on ART, but also target latent reservoirs to achieve a durable HIV cure. Strategies targeting HIV using VSTs in conjunction with latency reversing agents to reactivate dormant, HIV-infected cells and render them susceptible to both drug and cell-based attacks are being explored [41–45].

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REFERENCES

1. Riddell SR, Watanabe KS, Goodrich JM, Li CR, Agha ME, Greenberg PD. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. Science. 1992;257:238–241. [PubMed: 1352912]
2. Bollard CM, Heslop HE. T cells for viral infections after allogeneic hematopoietic stem cell transplant. Blood. 2016;127:3331–3340. [PubMed: 27207801]
3. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood. 2007;110:1123–1131. [PubMed: 17468341]

4. O'Reilly RJ, Prockop S, Hasan AN, Koehne G, Doubrovina E. Virus-specific T-cell banks for 'off the shelf' adoptive therapy of refractory infections. Bone Marrow Transplant. 2016;51:1163–1172. [PubMed: 27042851]
5. Tzannou I, Papadopoulou A, Naik S, et al. Off-the-shelf virus-specific T cells to treat BK virus, human herpesvirus 6, cytomegalovirus, Epstein-Barr virus, and adenovirus infections after allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2017;35:3547–3557. [PubMed: 28783452]
6. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010;115:925–935. [PubMed: 19880495]
7. Muranski P, Davies S. Adoptive immunotherapy for diseases associated with human polyomaviruses BK, JC and Merkel cell carcinoma virus. Cytotherapy. 2017;19, in press.
8. Papadopoulou A, Gerdmann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. Sci Transl Med. 2014;6:242ra83.
9. Blyth E, Clancy L, Simms R, et al. BK virus-specific T cells for use in cellular therapy show specificity to multiple antigens and polyfunctional cytokine responses. Transplantation. 2011;92:1077–1084. [PubMed: 22002347]
10. Mani J, Jin N, Schmitt M. Cellular immunotherapy for patients with reactivation of JC and BK polyomaviruses after transplantation. Cytotherapy. 2014;16:1325–1335. [PubMed: 24934303]
11. Arthur RR, Shah KV, Baust SJ, Santos GW, Saral R. Association of BK viruria with hemorrhagic cystitis in recipients of bone-marrow transplants. N Engl J Med. 1986;315:230–234. [PubMed: 3014334]
12. Olson AL, Muftuoglu M, Kaur I, et al. Efficacy of third party BK virus (BKV) specific cytotoxic T-lymphocytes generated by ex vivo expansion for the treatment of BKV infection in stem cell transplant recipients, a phase 2 trial. Blood. 2016;128, 504. [PubMed: 27235138]
13. Pavlovic D, Patera AC, Nyberg F, Gerber M, Liu M. Progressive multifocal leukoencephalopathy: current treatment options and future perspectives. Ther Adv Neurol Disord. 2015;8:255–273. [PubMed: 26600871]
14. Bloomgren G, Richman S, Hotermans C, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med. 2012;366:1870–1880. [PubMed: 22591293]
15. Balduzzi A, Luccini G, Hirschm HH, et al. Polyomavirus JC-targeted T-cell therapy for progressive multiple leukoencephalopathy in a hematopoietic cell transplantation recipient. Bone Marrow Transplant. 2011;46:987–992. [PubMed: 20921942]
16. Kaufman HL, Russell J, Hamid O, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. Lancet Oncol. 2016;17:1374–1385. [PubMed: 27592805]
17. Nghiem PT, Bhatia S, Lipson EJ, et al. PD-1 blockade with pembrolizumab in advanced Merkel-cell carcinoma. N Engl J Med. 2016;374:2542–2552. [PubMed: 27093365]
18. Chapuis AG, Afanasiev OK, Iyer JG, et al. Regression of metastatic Merkel cell carcinoma following transfer of polyomavirus-specific T cells and therapies capable of re-inducing HLA class-I. Cancer Immunol Res. 2014;2:27–36. [PubMed: 24432305]
19. Centers for Disease Control and Prevention. HIV/AIDS basic statistics. 2016 Available at:, https://www.cdc.gov/hiv/basics/statistics.html, Accessed November 6, 2017.
20. Chen H, Li C, Huang J, et al. CD4+ T cells from elite controllers resist HIV-1 infection by selective upregulation of p21. J Clin Invest. 2011;121:1549–1560. [PubMed: 21403397]
21. Ndhlovu ZM, Proudfoot J, Cesa K, et al. Elite controllers with low to absent effector CD8+ T cell responses maintain highly functional, broadly directed central memory responses. J Virol. 2012;86:6959–6969. [PubMed: 22514340]
22. Ndhlovu ZM, Stampouloglou E, Cesa K, et al. The breadth of expandable memory CD8+ T cells inversely correlates with residual viral loads in HIV elite controllers. J Virol. 2015;89:10735–10747. [PubMed: 26269189]
23. Hütter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med. 2009;360:692–698. [PubMed: 19213682]

24. Chapuis AG, Casper C, Kuntz S, et al. HIV-specific CD8+ T cells from HIV+ individuals receiving HAART can be expanded ex vivo to augment systemic and mucosal immunity in vivo. Blood. 2011;117: 5391–5402. [PubMed: 21422474]
25. Koenig S, Conley AJ, Brewah YA, et al. Transfer of HIV-1-specific cytotoxic T lymphocytes to an AIDS patient leads to selection for mutant HIV variants and subsequent disease progression. Nat Med. 1995;1:330–336. [PubMed: 7585062]
26. Lieberman J, Skolnik PR, Parkerson GR 3rd, et al. Safety of autologous, ex vivo-expanded human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte infusion in HIV-infected patients. Blood. 1997;90:2196–2206. [PubMed: 9310470]
27. Brodie SJ, Lewinsohn DA, Patterson BK, et al. In vivo migration and function of transferred HIV-1-specific cytotoxic T cells. Nat Med. 1999;5:34–41. [PubMed: 9883837]
28. Tan R, Xu X, Ogg GS, et al. Rapid death of adoptively transferred T cells in acquired immunodeficiency syndrome. Blood. 1999;93:1506–1510. [PubMed: 10029578]
29. Lam S, Sung J, Cruz C, et al. Broadly-specific cytotoxic T cells targeting multiple HIV antigens are expanded from HIV+ patients: implications for immunotherapy. Mol Ther. 2015;23:387–395. [PubMed: 25366030]
30. Patel S, Lam S, Cruz CR, et al. Functionally active HIV-specific T cells that target gag and nef can be expanded from virus-naïve donors and target a range of viral epitopes: implications for a cure strategy after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2016;22:536–541. [PubMed: 26721209]
31. Maus MV, Grupp SA, Porter DL, June CH. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. Blood. 2014;123:2625–2635. [PubMed: 24578504]
32. Ramos CA, Savoldo B, Dotti G. CD19-CAR trials. Cancer J. 2014;20:112–118. [PubMed: 24667955]
33. Davila ML, Brentjens R, Wang X, Rivière I, Sadelain M. How do CARs work?: early insights from recent clinical studies targeting CD19. Oncoimmunology. 2012;1:1577–1583. [PubMed: 23264903]
34. Zhang T, Cao L, Xie J, et al. Efficiency of CD19 chimeric antigen receptor-modified T cells for treatment of B cell malignancies in phase I clinical trials: a meta-analysis. Oncotarget. 2015;6:33961–33971. [PubMed: 26376680]
35. Masiero S, Del Vecchio C, Gavioli R, et al. T-cell engineering by a chimeric T-cell receptor with antibody-type specificity for the HIV-1 gp120. Gene Ther. 2005;12:299–310. [PubMed: 15496956]
36. Mitsuyasu RT, Anton PA, Deeks SG, et al. Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. Blood. 2000;96:785–793. [PubMed: 10910888]
37. Deeks SG, Wagner B, Anton PA, et al. A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. Mol Ther. 2002;5:788–797. [PubMed: 12027564]
38. Scholler J, Brady TL, Binder-Scholl G, et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. Sci Transl Med. 2012;4:132ra53.
39. Tebas P, Stein D, Tang WW, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N Engl J Med. 2014;370:901–910. [PubMed: 24597865]
40. van Lunzen J, Glaunsinger T, Stahmer I, et al. Transfer of autologous gene-modified T cells in HIV-infected patients with advanced immunodeficiency and drug-resistant virus. Mol Ther. 2007;15:1024–1033.
41. Gabriel R, Lombardo A, Arens A, et al. An unbiased genome-wide analysis of zinc-finger nuclease specificity. Nat Biotechnol. 2011;29:816–823. [PubMed: 21822255]
42. Pattanayak V, Ramirez CL, Joung JK, Liu DR. Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection. Nat Methods. 2011;8:765–770. [PubMed: 21822273]
43. Deeks SG. HIV: shock and kill. Nature. 2012;487:439–440. [PubMed: 22836995]
44. Lopez M A multi-step pace towards a cure for HIV: kick, kill, and contain. AIDS Rev. 2013;15:190–191. [PubMed: 24002204]

45. Schiedel M [HIV-1 eradication with the “shock and kill” strategy]. Pharm Unserer Zeit. 2010;39:171–173. [PubMed: 20425765]