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Immunotherapy for transplantation-associated viral infections

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Review Series

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections following allogeneic hematopoietic stem cell transplantation (HSCT) are a major cause of morbidity and mortality. Early clinical trials demonstrate that adoptive transfer of donor-derived virus-specific T cells to restore virus-specific immunity is an effective strategy to control CMV and EBV infection after HSCT, conferring protection in 70%–90% of patients. The field has evolved rapidly to develop solutions to some of the manufacturing challenges identified in early clinical studies, such as prolonged in vitro culture, optimization of the purity of the virus-specific T cell product, the potential limitations of targeting a single viral antigen, and how to manage the patient with a virus-naive donor. This Review both discusses the seminal early studies and explores cutting-edge novel technologies that broaden the feasibility of and the scope for delivering virus-specific T cells to patients after HSCT.

Immunotherapy for transplantation-associated viral infections

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Introduction

Viral infections cause morbidity and mortality in immunosuppressed patients following allogeneic hematopoietic stem cell transplantation (HSCT) (1). This principally reflects the inability of the depressed host immune system to limit viral replication and dissemination, and loss of T cell function is central to this effect (2). Despite advances in prophylactic and preemptive pharmacotherapy, antiviral therapeutics are limited by toxicity and to some extent by lack of efficacy in breakthrough infections (3). Immunotherapeutic strategies to accelerate reconstitution of virus-specific immunity and to hasten T cell recovery after HSCT remain a compelling alternative to drug treatments. This Review will discuss cytomegalovirus (CMV) and Epstein-Barr virus (EBV) in the post-HSCT setting, with a focus on CMV- and EBV-directed virus-specific T cells (VSTs). Published data additionally support the use of VSTs in the settings of solid organ transplantation and profound non-HSCT immunosuppression (4–6), but detailed discussion is beyond the scope of the Review. Emerging evidence supports the use of VSTs to treat a broader range of viral targets, including varicella-zoster virus, adenovirus, and BK virus. Cutting-edge translation of this multi-VST technology will be reviewed (7–9).

CMV: biology and pathogenicity in the post-HSCT setting. CMV infects 50%–80% of the population and maintains a latent reservoir in mononuclear leukocytes. Containment of CMV in its latent state engages a large proportion of the host immune repertoire: in young adults, 1%–2% of CD4 and CD8 T cells are CMV-reactive, rising to up to 30%–40% in the elderly (10–13). For the majority of CMV-infected individuals, asymptomatic reactivation is countered effectively by innate and adaptive immunity. In the immunocompromised HSCT patient, unconstrained viral replication and dissemination can lead to end-organ damage, CMV disease, and increased mortality (14, 15). The efficacy of conventional antiviral therapies such as ganciclovir and foscarnet to treat CMV end-organ disease is limited (16).

Sixty to eighty-five percent of CMV-seropositive patients will experience CMV dissemination after HSCT, particularly in the context of T cell–depleted or matched unrelated donor (MUD) grafts. In CMV-seronegative patients, primary infection is prevented through selection of CMV-seronegative grafts (17), but where matched CMV-seronegative donors are unavailable, 20%–40% of CMV-seronegative patients who receive CMV-seropositive grafts will develop primary CMV infection. Untreated, 50% of HSCT patients with CMV reactivation will develop CMV disease (14, 15).

Current clinical practice uses surveillance programs to monitor CMV DNA burden by quantitative PCR (16, 18). Preemptive antiviral pharmacotherapy (asymptomatic patients with rising CMV DNA titers) and prophylactic therapy (pharmacotherapy prior to detection of CMV DNA) can reduce the incidence of CMV disease after HSCT, but have not yet definitively correlated with improved overall survival (18–20). An additional consideration of prophylactic/preemptive pharmacotherapy for CMV is that drug toxicities (including neutropenia with consequent bacterial infection, and renal impairment) and to a lesser extent drug resistance remain problematic. Novel antiviral pharmacotherapies are under investigation (e.g., maribavir, letermovir, brincidofovir) but have not yet clearly demonstrated superiority/lesser toxicity compared with conventional agents (21, 22).

Immunotherapeutic strategies to hasten T cell recovery after HSCT remain a compelling alternative/adjunct to drug treatments. European Blood and Marrow Transplant Registry data report a reduction in transplant-related and overall mortality when CMV-seropositive patients receive CMV-seropositive grafts. In contrast, CMV-seropositive patients in receipt of T cell–depleted CMV-seronegative donor or cord blood grafts are at highest risk from CMV-associated morbidity and mortality

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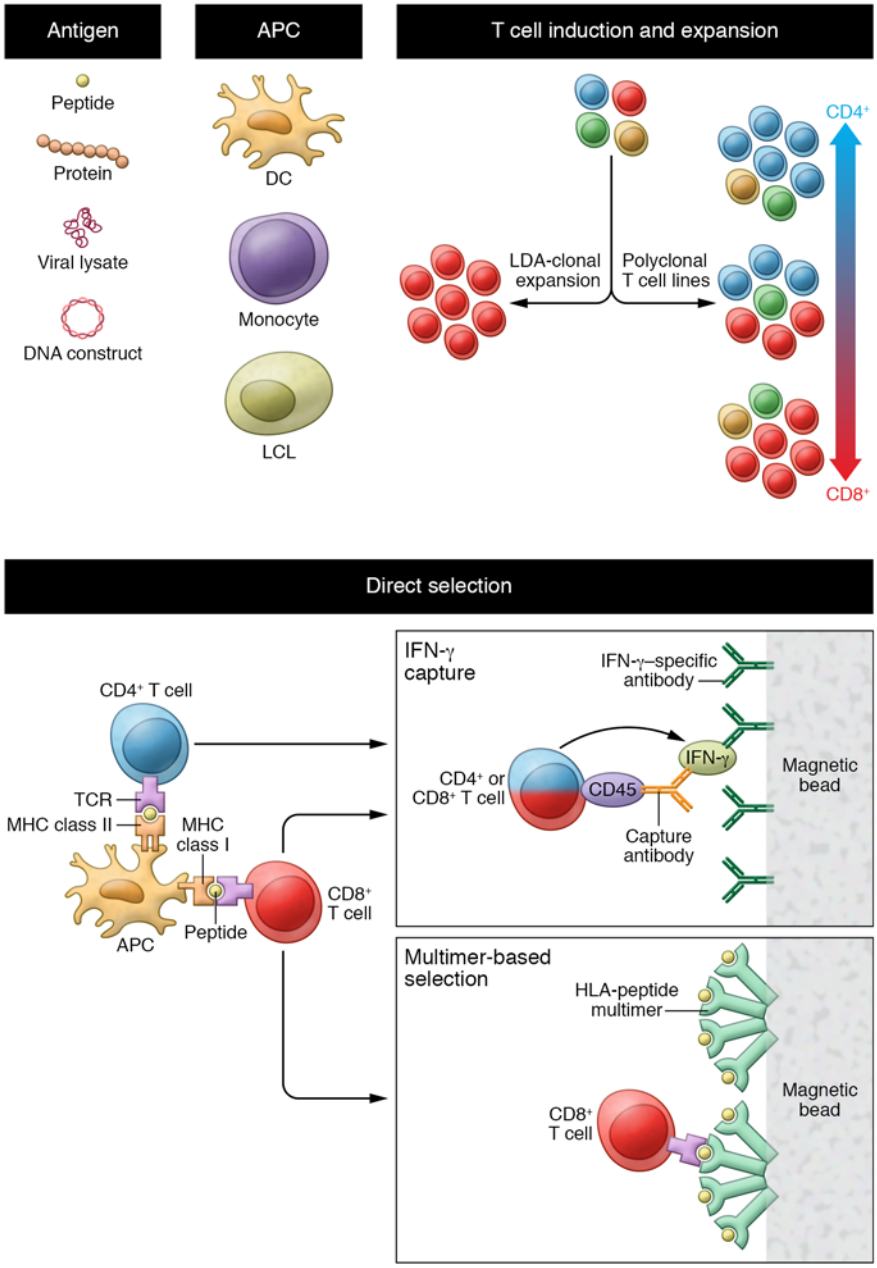

Figure 1. VST manufacture and direct selection methodology. VSTs can be generated by coculture of virus-derived peptides, proteins, or viral lysates with antigen-presenting cells (APCs) and T cells. However, these techniques are time-consuming and difficult to reproduce to good manufacturing practice (GMP) standards. Recently developed coculture methods include the use of gene-modified APCs that are engineered to present immunogenic viral peptides to T cells. This may allow for the generation of virus-reactive T cells from virus-naive donors. Direct selection techniques can permit rapid generation of VSTs to GMP standards and are now being used in clinical trials. Selection is delivered through IFN-γ capture or through multimer-based selection. LCL, lymphoblastoid cell lines; MoDC, monocyte-derived dendritic cell; LDA, limiting dilution assay.

(23, 24). Patients with severe graft-versus-host disease (GVHD) and drug-induced T cell dysfunction are also at high risk of CMV-related morbidity.

We can conclude that pharmacotherapy for CMV has limitations, that transfer/reconstitution of CMV immunity can limit reactivation/dissemination of CMV, and that CMV-seropositive HSCT patients stand to benefit significantly from VSTs.

CMV: immune responses in health and disease. To determine optimal approaches to T cell therapies for CMV, it is pertinent to review the immune response to primary infection/viral reactivation. The CMV virion comprises a 230-kb double-stranded linear DNA genome encapsulated by a protein-rich tegument with abundant pp150 (U32) and pp65 (UL83) proteins. During the infective phase, three subgroups of viral proteins are synthesized: immediate-early (IE), early (E), and late (L) (25). The breadth of T cell responses to CMV in healthy subjects is heterogeneous. In one study of 33 volunteers exposed to overlapping 15-mer peptides derived from 213 CMV ORFs, immune reactivity to 151 of the 213 ORFs was demonstrated. CMV-seropositive subjects recognize a median of 12 (CD4) and 8 (CD8) CMV ORFs, which amounts to a circulating CMV memory repertoire of greater than 20% in at least one-third of older subjects (26). This broadens the list of CMV-specific target antigens beyond the current gold standards pp65 and IE-1.

In healthy subjects, CMV replication is effectively contained by the innate and adaptive immune systems. Neutralizing antibodies to envelop glycoprotein B (27) can prevent blood-borne spread, but in general, humoral responses to CMV confer limited protection (25). In contrast, preclinical studies confirm that CMV-specific T cells are critical to recovery from CMV infection: CD8 T cells can prevent CMV recurrence, and CD4 T cells can deliver antiviral functions such as cytotoxicity and cytokine production (25, 28).

In CMV-seropositive HSCT patients, the risk of viremia inversely correlates with reconstitution of CMV-specific T cell immunity. The frequency of CMV-specific T cells identified within a graft correlates inversely with reported CMV infection after HSCT (29, 30), and reconstitution of CMV-reactive CD8 T cells is associated with protection against CMV (31). In one study of T cell–replete sibling allograft recipients where recipient and donor were CMV seropositive, CMV reactivation was followed by rapid CMV-reactive CD8 T cell reconstitution, and the presence of more than 10 CMV-reactive CD8 T cells/μl blood was associated with protective immunity. This was not observed in a matched cohort of CMV-seropositive recipients treated with CMV-seronegative grafts (29). Recovery of CMV-specific CD4 responses is also critical to effective antiviral responses, and several groups have advocated for restoration of both antigen-specific CD4 and CD8 T cell populations to deliver long-term control of CMV (32).

Table 1. Clinical studies of donor-derived CMV-specific T cells

Cell therapy	No. of patients	Date of study	Activation	Acute GVHD	Dose	CMV-related outcome	Ref.[A]
CD8 T cell clones	14	1995	Autologous fibroblasts pulsed with CMV virion proteins	3 Grade 1–2 GVHD	Intrapatient dose escalation; range 33 × 10^6/m^2 to 1 × 10^9/m^2	Reconstitution CMV immunity in 14/14	38
Polyclonal T cell lines	8	2002	CMV lysate	None	1 × 10^7 T cells/m^2	5/8 cleared after dose 1; 1/8 cleared after dose 2; 1/8 did not clear; 1/8 non-evaluable	36
Polyclonal T cell lines	16	2003	CMV Ag-pulsed DCs	3 Grade 1 skin GVHD	1 × 10^5/kg	8/16 cases did not require antiviral drugs; further reactivation in 2/16 only	34
CD8 T cells (multimer selected)	9	2005	NLV-HLA-A02 pentamers	2 Grade 1–2 GVHD	1.23 × 10^3/kg to 3.3 × 10^4/kg	8/9 cleared viremia	51
CD4 T cell clones	25	2005	CMV antigen	1 Grade 2 GVHD	1 × 10^5/kg to 1 × 10^6/kg	7/25 reactivated CMV; 5/25 CMV disease (2 died)	39
Polyclonal T cell lines	9	2007	NLVPMVATV (HLA-A2 restricted) pulsed DCs	3 Grade 3 GVHD (fatal in 1)	Target dose 2 × 10^7/m^2	Transient detection CMV-specific T cells by NLV-tetramer staining; 2/9 reactivated CMV without requirement antiviral drugs	37
Polyclonal T cell lines	12	2008	DCs transduced with Ad5f35pp65 adenoviral vector encoding CMV-pp65	2 Grade 3; 2 grade 2 GVHD	2 × 10^7/m^2	Reconstitution CMV immunity in 12/12; no requirement for antiviral drugs	42
Polyclonal CD4 and CD8 T cells (gamma catch)	18	2010	CMV-pp65 protein	1 Possible GVHD	Mean dose 21 × 10^3/kg pp65-specific T cells	Partial or complete viral clearance in 15/18	55
Polyclonal CD4 and CD8 T cells (gamma catch)	18 (11 preemptive; 7 prophylactic)	2011	Recombinant pp65 or overlapping pp65 peptide pool	5 Grade 1; 2 grade 2; 1 grade 3 GVHD	Target dose 1 × 10^4 CD3 T cells/kg	CMV-reactive T cells in 11/11 preemptively treated patients; 7/7 patients treated prophylactically did not reactivate CMV	56
CD8 T cells (multimer selected)	2	2011	Peptides derived from pp65	None	0.37 × 10^5 to 2.2 × 10^5 CMV-pp65-CTL/kg	2/2 complete responses	41
Polyclonal T cell lines	7	2012	Peptides derived from IE-1 and pp65	None	2.5 × 10^5 to 5 × 10^5 CD3+CMV CTL/kg	5/7 developed CMV-specific CTL activity in the blood; 2/7 no response	81
Polyclonal CD4 and CD8 T cells (gamma catch)	6	2012	Two CMV-pp65 peptides	None	0.6 × 10^6 to 17 × 10^6 T cells (comprising 54%–96% CMV-pp65–specific CD8 T cells)	6/6 cleared viremia	82
CD8 T cells (multimer selected)	2	2012	NLV-containing HLA-A02 pentamers	None	0.8 × 10^4 to 10.8 × 10^4 cells/kg	2/2 complete responses	83
Polyclonal T cell lines	16	2015	15-mer peptides spanning pp65	None	5 × 10^5/kg (× 1 dose) to 1 × 10^6/kg (× 3 weekly doses)	14/16 cleared viremia (including 2 with CMV disease)	84

[A]Some cited studies incorporate results of other published studies. Ag, antigen.

Cellular therapies for CMV

A number of considerations help guide appropriate use of VSTs in post-HSCT patients. Prophylactic therapy poses several challenges: it will expose some patients to unnecessary therapy with the attendant risks of T cell alloreactivity and GVHD, it may not be feasible from a health economics perspective because of a costly cell manufacture process, and it is not yet clear whether the absence of antigenic stimulation from replicating virus may compromise engraftment/expansion of VSTs in vivo.

Preemptive therapy based on PCR-directed surveillance will reduce overtreatment. Concerns mainly relate to deliverability and, in particular, whether good manufacturing practice–compliant (GMP-compliant) technology can generate VSTs at the tempo required for effective clinical application (25). The majority of published clinical studies describe prophylactic or preemptive therapy. Outcomes have been encouraging, but comparisons between studies are difficult because of both the heterogeneous composition of the cell products (namely CD4/CD8 ratio, total cell dose, and range of CMV-target specificities) and the highly variable study design (timing of infusion, preemptive versus prophylactic administration, patient CMV risk group) (33–37). See Table 1 for an overview of clinical studies of CMV-specific T cells after HSCT and Figure 1 for a schematic illustration of different methods of VST manufacture, including direct selection methodology.

CMV-specific T cell clones. Initial clinical studies used CMV-specific T cell clones to reduce the potential risk of GVHD. In one study, large numbers of donor-derived CD8 T cell clones expanded for over 8 weeks in vitro by repeated restimulation were infused at day 30–40 after HSCT into low-risk patients. Encouragingly, CD8 T cell expansion was observed, but was not maintained in the absence

Table 2. Clinical studies using donor-derived multi-VSTs

Cell therapy	No. of patients	Date of study	Activation	Acute GVHD	Dose	CMV-related outcome	Ref.
EBV, ADV	14	2009	Monocytes/LCLs transduced with ADV vector	3 Skin rashes	5×10^6 to 1.35×10^8 cells/m^2	11 Patients treated prophylactically remain negative; 2/3 ADV infection cleared virus	85
CMV, EBV	3	2010	DCs pulsed with EBV-LMP2, CMV-pp65, CMV-IE peptides	1 Grade 1 GVHD	Median cell dosage 0.6×10^6/kg/infusion	2/2 patients cleared virus; 1/1 patient did not reactivate virus (patients treated prophylactically)	86
CMV-specific or multi-VSTs (CMV, EBV, ADV)	40	2013	pp65-pulsed MoDCs or MoDCs transfected with Ad5f35pp65 adenoviral vector encoding CMV-pp65	No increase in acute or chronic GVHD related to viral-specific CTLs	Dose 2×10^7 cells/m^2	CTL recipients had CMV immune reconstitution and less frequent reactivation with only 1 case requiring pharmacotherapy	87[A]
Multi-VSTs (CMV, EBV, ADV)	10	2013	DC nucleofection with DNA plasmids encoding viral antigen	1 Grade 1 GVHD	Dose range 0.5–2 × 10^7 cells/m^2	Complete viral eradication in 8/10	88
Multi-VSTs (CMV, EBV, ADV, HHV-6, BK virus)	11	2014	Immunodominant antigen pepmixes	1 Stage II skin GVHD	Dose range 0.5×10^7 to 2×10^7 cells/m^2	94% Virological and clinical response (sustained)	7
Multi-VSTs (CMV, EBV, ADV, VZV)	10	2015	Ad5f35 encoding CMV-pp65 (+ selected EBNA-1, LMP EBV epitopes; commercial VZV vaccine)	3 Grade 2–4 GVHD	Dose 2×10^7/m^2 VSTs	Reconstitution CMV immunity in 10/10; 6/10 reactivated CMV and 1/10 required antiviral drugs	8
Multi-VSTs (CMV, EBV, ADV)	26	2015	EBV-LCLs transduced with Ad5f35pp65 adenoviral vector encoding CMV-pp65	2 Skin rashes	5×10^6/m^2 to 1×10^8/m^2	10/11 cleared CMV; 5/6 cleared ADV; 6/6 cleared EBV; 1 patient progressed	43
Multi-VSTs (CMV, EBV, ADV)	3	2015	Monocytes/LCLs transduced with Ad5f35pp65 adenoviral vector encoding CMV-pp65	None reported	5×10^6/m^2 to 1×10^7/m^2	Treatment: 1/1 cleared virus; prophylaxis: 2/2 no reactivation	62
Multi-VSTs (CMV, EBV, ADV)	6	2016	Immunodominant antigen pepmixes	1× grade 1 and 1× grade 2 GVHD	5×10^6/m^2 to 4.6×10^7/m^2	Complete response in 2/2 with EBV and 2/5 with CMV	89

[A]Incorporates results of other published studies. ADV, adenovirus; VZV, varicella-zoster virus.

of CD4 help, and the logistics of this prolonged manufacture were costly and nonscalable (38). A similar study of CMV-reactive CD4 T cell clones in the haploidentical HSCT setting resulted in detectable CD4 and CD8 CMV-specific responses in treated patients (39). This early work confirmed the potential of adoptive therapy for CMV after HSCT without apparent complication by GVHD, but highlights some of the challenges of manufacture.

CMV-specific T cell lines. An alternative to CMV-specific T cell clones is the use of CMV-specific T cell lines. Oligo- or polyclonal CMV-reactive T cell cultures are enriched for CMV-reactivity by coculture with CMV lysate, proteins, or peptides pulsed onto antigen-presenting cells (APCs), either peripheral blood mononuclear cells (PBMCs) (36) or monocyte-derived dendritic cells (MoDCs) (35). MoDCs have some distinct advantages in this role by virtue of their ability to cross-present via MHC class I to CD8 T cells, though their inclusion adds a further manufacturing step.

One clinical study of a single infusion of mainly CD4 CMV-specific T cells (1×10^7/m^2) in patients with persistent CMV DNAemia at 4 weeks after pharmacotherapy showed plasma CMV clearance in 63% of patients. Critics of this study argue that viral loads were modest in half of the patients, that most patients were responding to antiviral pharmacotherapy, and that endogenous reconstitution was likely to contribute to the responses observed (36). In a separate study of preemptive intervention following detection of CMV DNAemia by PCR, T cells cocultured over 3 weeks with MoDCs pulsed with CMV lysate were infused into patients after HSCT. Outcomes were promising: no cases of CMV disease or GVHD were reported, 50% of all treated patients cleared the virus without antiviral pharmacotherapy, and despite very low cell doses (<1×10^3 CMV T cells/kg), massive in vivo expansion of transferred cells was observed several days after infusion (34).

The HLA-A2–restricted pp65 peptide NLVPMVATV (NLV) can be used in lieu of CMV lysate, and in T cell/MoDC coculture NLV yields a product highly enriched for CMV-reactive CD8 T cells (62% NLV-specific T cells) compared with CMV lysate/MoDCs (0.2%–6.5% CMV-specific CD8 T cells) (37). A potential disadvantage of this manufacturing process is that the final cell product largely comprises terminally differentiated CD8 T cells, and a clinical study of this product demonstrated relatively poor expansion and a short duration of persistence in vivo (40). Sensitization of allogeneic T cells in vitro to a pool of 15-mer peptides spanning pp65 was demonstrated to generate oligoclonal CMV-specific T cells to three different peptides. Seventeen patients were infused, and in 15 cases CMV viremia was cleared, with oligoclonal CMV-specific T cells persisting for up to 2 years in some cases

(41). A major disadvantage of the HLA-A2–restricted NLV peptide approach is the restriction of benefit to HLA-A2$^+$ patients only.

This work broadly confirms that generation of CMV-specific cell lines is feasible and that infusion is not associated with a significant risk of GVHD. There are compelling data to suggest that virus-specific transferred T cells can engraft and expand and have the potential to mediate clinical responses.

Multispecific T cell lines. Multi-VST lines represent an interesting option to target multiple viral infections using one adoptive cell product. Such lines can be manufactured either with APC systems similar to those described in the preceding section (using overlapping peptide pools from multiple viruses), or with gene transfer approaches. The latter are exemplified by the use of an adenoviral vector encoding the CMV-associated pp65 antigen to transduce APCs (MoDCs and EBV-transformed lymphoblastoid cell lines [LCLs]) before coculture with PBMCs or naive cord blood. This method delivers both MHC class I–dependent processing and expansion of CMV-reactive CD8 T cells, and MHC class II–dependent processing and presentation of adenovirus/EBV/CMV–associated peptides to drive expansion of virus-specific CD4 T cells.

The adenoviral transfer vector promotes antiadenoviral T cell specificity (bispecific cytotoxic T lymphocytes [CTLs]) (42), and if EBV-transformed B cells are used in lieu of MoDCs, then additional EBV-specificity is generated (trispecific CTLs) (43). The relative benefits of trispecific antiviral T cells produced in this way must be weighed against the significantly longer in vitro manufacturing process required to generate them (44). A clinical study of trispecific CTLs administered prophylactically after HSCT in the absence of immunosuppression demonstrated CMV-specific reactivity in 70% of patients, and 40% of patients had detectable circulating T cells to pp65-specific HLA pentamers with no increase in the incidence of GVHD. In this study, CMV- and EBV-specific T cell numbers rose in the absence of viral reactivation, but adenoviral-CTL expansion was only observed in the context of adenoviral infection (43). Bispecific CTLs generated by coculture with adenovirus-transduced MoDCs that were infused 28 days after HSCT delivered similar outcomes to those described above. All treated patients showed CMV-specific immunity, and 5 of 12 had T cells detectable by pp65-specific HLA pentamer analysis (42).

More recent studies have used nucleofection to introduce DNA plasmids encoding multiple immunogenic antigens from CMV, EBV, and adenovirus into APCs (45), or have used viral antigen–derived 15-mer peptide libraries (pepmix) with APCs to deliver a product with a broader CMV-reactive T cell repertoire (46). Extending the scope of antiviral adoptive cell transfer to simultaneously target three viruses has been shown to deliver sustainable responses in patients, and the main reason for therapeutic failure is donor seronegativity (7). Table 2 summarizes some of the clinical trials of multi-VSTs in the post-HSCT setting.

Direct selection techniques. In generating novel immunotherapies for viral infection after HSCT, the two greatest clinical priorities are the prevention of toxicity and the augmentation of clinical responses. For these reasons, direct selection of CMV-specific T cells from donor samples is an attractive option when they are present at sufficiently high frequencies. Given the high frequencies of circulating CMV-reactive T cells in seropositive individuals, direct selection is likely to yield sufficient starting material for ex vivo manufacture. Direct selection is a rapid process resulting in an enriched starting material. This helps to avoid or reduce ex vivo culture, which may translate into a more cost-effective and GMP-compatible manufacturing process.

Direct CMV-reactive T cell selection can be performed in one of two ways: binding of class I HLA–peptide multimers (47, 48), or selection according to induction of cytokine secretion (often IFN-γ) in response to viral proteins/peptides (49). Alternative approaches target surface antigens upregulated following presentation of viral antigen on some form of APC. The resulting cellular composition will differ between methods such that class I HLA multimers will select only CD8 T cells, whereas IFN-γ catch (also known as gamma catch) technology will deliver a CD4 T cell–skewed product.

Multimer-based selection. Multimer-based T cell selection is currently limited to donors who have one of a restricted number of HLA-restricting alleles and detectable responses to specific immunodominant epitopes. As the technology grows, multimers using an increasing number of alleles and epitopes will become available. Their mode of action is to display peptide-loaded recombinant HLA complexes to T cells, selecting out those with T cell receptors (TCRs) of the correct specificity. There are a number of cell selection reagents available, including tetramers, pentamers, and streptamers. Similar frequencies of multimer-positive T cells can be detected following selection using all three techniques, but tetramers possess the lowest background signal and can potentially deliver the purest product (50).

The first study to demonstrate the feasibility of tetramer-based selection of highly purified CMV peptide–specific CD8 T cells from the blood of CMV-seropositive donors delivered a therapeutic product to nine HSCT patients within 4 hours of cell selection. A median dose of 8.6×10^3 CMV-specific CD8 T cells/kg at a median purity of 95.6% was infused, and transferred cells remained detectable in all patients at 10 days. Four of seven patients treated preemptively did not require concomitant antiviral pharmacotherapy. Notably, one patient with antiviral drug–resistant CMV achieved disease control within 8 days of infusion (51).

Streptamers possess one major advantage over other forms of selection: they bind target reversibly. Reversible target binding is achieved through monomeric Strep-tagging of MHC molecules loaded with a selected target peptide to which reactive CD8 T cells bind with low affinity. Streptamer multimerization at the T cell surface through binding of the Strep-Tactin backbone increases binding avidity to T cell targets before selection. The subsequent addition of biotin, which competitively binds Strep-Tactin with higher affinity, displaces Strep-Tactin multimers from the Strep-tagged MHC molecules bound to CMV-reactive CD8 T cells. In this monomeric format, Strep-tagged MHC spontaneously dissociates from the selected CD8 T cells as a result of the low-affinity singular bond (48, 52). Two prospective randomized studies using these products have been reported in abstract form: CMV-ACE/ASPECT (NCT01220895, ClinicalTrials.gov) and CMV-IMPACT (NCT01077908) (53, 54). Initial results suggest that they may enhance the tempo of immune reconstitution compared with an untreated control group, and that they may reduce subsequent CMV reactivation rates.

IFN-γ catch. Direct selection of antigen-specific cells can be performed by IFN-γ catch, whereby short-term antigen stimu-

lation ex vivo induces T cell recall responses. IFN-γ–secreting T cells then undergo magnetic selection following labeling with an IFN-γ–targeted mAb conjugated to a leukocyte-specific (CD45) mAb. The resulting cellular product comprises CD4 and CD8 T cells recognizing multiple viral epitopes selected independently of HLA-specificity (unlike multimer-based selections). One clinical study evaluated 18 patients with drug-refractory CMV viremia after HSCT who had no prior history of GVHD. The mean T cell dose achieved in this study was 21.3×10^3/kg (albeit the dose did not correlate with in vivo expansion), and the CD4/CD8 ratio was 2:1. GVHD occurred in only 1 patient, and 15 of 18 patients had at least 1 log reduction in CMV DNA. In 12 evaluable patients, CMV-reactive T cells expanded in vivo, and in a subgroup, cells were still detectable at 6 months. In all 12 cases, there was either clearance or a significant reduction in CMV viremia (55).

A phase I/II study of IFN-γ catch technology generated pp65-specific T cells for 25 patients after HSCT. Sibling donor PBMCs were coincubated with recombinant pp65 or overlapping pp65 peptides and were selected by immunomagnetic IFN-γ catch technology before administration in the prophylactic/preemptive setting. Products were generated for 25 patients, but were not infused in seven patients because of the presence of grade 1 or greater GVHD prior to infusion. In the prophylactic cohort, patients did not require antiviral pharmacotherapy over 6 months of follow-up. Of 11 patients treated preemptively, two required no antiviral pharmacotherapy and seven required only a single course of treatment. GVHD (greater than grade 1) arose de novo in 3 of 18 patients, but this was not associated with product purity. Predictably, the predominant circulating CMV-reactive T cells at 4 weeks after infusion were effector memory and terminally differentiated effector cells (56).

Both multimer-based and IFN-γ catch–based selection processes offer rapid GMP-compatible manufacture and scalability. Enrichment for virus-specificity may also limit the potential risks of alloimmunity. Potential limitations include the need for additional donor leukapheresis to collect sufficient starting material, which can be burdensome, particularly in the unrelated donor setting. Additionally, this method has a requirement for circulating VSTs, and is therefore not available to virus-naive donors or CMV-seropositive donors who do not respond to pp65 or IE-1 stimulation (57).

Third-party VSTs. In cases in which the donor graft is CMV-naive, manufacture of VSTs is more challenging. In this setting, third-party VSTs collected from virus-immune subjects with common HLA types represent a potential therapeutic option. Biobanks of cryopreserved VSTs can be swiftly accessed, and published data suggest that despite the degree of mismatch inherent in this strategy, it is feasible, is associated with significant clinical responses, and does not precipitate high GVHD rates (58–61). In one clinical study, patients failing 7 days of viral pharmacotherapy for CMV reactivation after HSCT were eligible to receive an initial dose of up to 2×10^7 viral-specific T cells/m^2, followed by 2 weekly infusions thereafter in the event of a response. Seventeen of 19 evaluable patients achieved a response (9 complete, 8 partial), 10 patients demonstrated expansion of CMV-reactive T cells in the blood, and de novo GVHD occurred in only 2 patients (60). This approach clearly represents an important development — firstly for patients who lack donor-derived VST options, and secondly for those who require urgent therapy and for whom the delays inherent in GMP manufacture of an individualized product are clinically inappropriate. The trade-off comes by virtue of the degree of mismatch and the consequent possibility of shorter persistence and/or risks of alloimmunity, although the latter is not borne out by early studies.

New directions in CMV cellular therapies

T cells derived from virus-naive donors or cord blood are now being used as starting material to generate CMV-specific T cells in vitro with antigen presentation delivered through donor-derived pp65-transduced PBMCs or LCLs, or APCs incubated with pepmixes. Interestingly, epitope mapping reveals that CMV-reactive T cells generated in this way recognize different epitopes from those recognized by seropositive adult donors, but they still appear to be able to elicit a clinical response and confer protection (62, 63).

Gene modification of T cells by retroviral transduction with a synthetic CMV-specific TCR could offer an alternative option for patients with uncontrolled CMV infection and a CMV-seronegative donor (64). CMV-specific TCR-transduced T cells offer the potential advantages of a targeted therapy with a relatively rapid manufacture process using naive starting material, but the disadvantages include high costs, feasibility issues, increased regulatory complexity, and the risk of viral immune escape by virtue of targeting of a single epitope.

It is often the case that viral reactivation after HSCT arises in patients with GVHD receiving treatment with systemic immunosuppression. The efficacy and persistence of CMV-specific T cells have been shown to be compromised by corticosteroids (29), and for this reason, strategies to facilitate CMV-specific T cell resistance to commonly used immunosuppressive agents, and in particular corticosteroids, may be of critical importance to the field (65, 66).

EBV: biology and pathogenicity in the post-HSCT setting

EBV is a human herpesvirus with a seroprevalence of 95% in adults. Primary infection manifests as infectious mononucleosis, but following clinical recovery, EBV persists in latent form, predominantly in B cells. In the post-HSCT setting, gross impairment of T cell function liberates EBV-infected B cells (usually donor-derived) to outgrow, causing post-transplant lymphoproliferative disorder (PTLD). PTLD risk is particularly high in patients receiving enhanced immune suppression for the treatment of GVHD, and in those receiving anti-thymocyte globulin (57, 67).

The advent of PCR-based EBV monitoring, preemptive therapy, and prompt administration of rituximab has made a huge impact on the mortality associated with PTLD after HSCT, although part of this reduction is attributable to ascertainment bias, and intervention in many who may have spontaneously cleared the virus. Despite these measures, up to one-third of patients with clinically apparent PTLD will succumb to uncontrolled disease, such that cellular therapeutic options are of great clinical relevance (68).

In considering potential immunotherapeutic approaches for PTLD, an understanding of the replicative cycle of EBV and latency-associated antigen expression is critical. Most healthy EBV-seropositive subjects have a broad repertoire of EBV-reactive T cells that mainly target the early-cycle lytic antigens. In the immunocompromised patient after HSCT, highly immunogenic type

Table 3. Clinical studies using donor-derived EBV-specific T cells

Cell therapy	No. of patients	Date of study	Activation	Acute GVHD	EBV-related outcome	Ref.
Polyclonal EBV-specific T cells	1	1997	Coculture with EBV-LCL	None reported	No response	90
Polyclonal EBV-specific T cells	1	1998	Coculture with EBV-LCL	Reactivation of acute skin GVHD	CR	91
Polyclonal EBV-specific T cells	6	2000	Coculture with EBV-LCL	None reported	EBV DNA levels reduced in 5/6; 1 death from PTLD	92
Polyclonal EBV-specific T cells	4	2007	Coculture with EBV-LCL	None reported	CR in 3 of 4 patients with PTLD recurrence post-rituximab; reduced EBV DNA in 1 patient without PTLD	77
Polyclonal EBV-specific T cells	113	2010	Coculture with EBV-LCL	8 recipients had recurrence of GVHD, but no de novo cases arose after CTL treatment	11/13 receiving treatment for PTLD achieved CR; prophylaxis prevented PTLD in 101/101	72[A]
Polyclonal EBV-specific T cells (gamma catch)	6	2010	Rapid expansion with peptides from 11 EBV antigens	None reported	3/6 complete responses	93
Polyclonal EBV-specific T cells	14	2012	Coculture with EBV-LCL	None reported	Established PTLD: 68% achieved durable CR	76
Polyclonal EBNA-1 protein–specific T cells (gamma catch)	10	2013	Rapid expansion using EBNA-1	1 Grade 2 GVHD	Established EBV viremia ± PTLD: clinical and virological responses in 7/10 cases	74

[A]Incorporates results of several other published studies (79, 80, 84). CTL, cytotoxic T lymphocyte; CR, complete response.

3 latency viral antigens such as Epstein-Barr nuclear antigen-2 (EBNA-2), EBNA-3a, EBNA-3b, EBNA-3c, and EBNA-LP are seen in addition to type 1 latency antigens (EBNA-1) and type 2 latency antigens (latent membrane proteins 1 and 2 [LMP1 and LMP2]).

Immunotherapy and manufacture of EBV-reactive T cells

The first clinical studies of immunotherapy for PTLD used unmanipulated donor lymphocyte infusions. Clinical responses were observed, but at the cost of significant toxicity from GVHD (69). The generation of a more virus-specific, less alloreactive therapy, EBV-specific T cells (EBVSTs), has followed similar strategies to those outlined for CMV. Initial studies using the B95-8 laboratory strain of EBV to develop LCLs demonstrated that these potent APCs can successfully expand EBVSTs ex vivo; administration of these cells induced clinical responses in patients. However, the use of live virus to generate patient products brings with it regulatory challenges (57, 70, 71). One further disadvantage of this method, particularly where there is urgent clinical need, is the long in vitro process (up to 3 months). Interestingly, the long culture period does not adversely affect EBVST persistence in vivo (72) and is also purported to reduce alloreactivity in vitro. In fact, any residual alloreactivity identified by in vitro assessment has not been shown to confer an increased risk of GVHD (73). Alternative methods to generate EBVSTs include use of whole viral proteins such as EBNA-1 to expand EBNA-1–specific VSTs, and this has been the subject of a phase I trial of PTLD after HSCT (74). To address the issues of prolonged in vitro processing, novel methods using direct cell selection via multimer binding and IFN-γ capture (EBNA-1–based stimulation) are also being explored (74).

Clinical responses to EBVSTs. There have been multiple clinical studies of EBVSTs generated by coculture with EBV-transformed LCLs for prophylaxis or treatment of PTLD. Across two major centers (St. Jude Children's Research Hospital and Baylor College of Medicine), 114 patients received EBVSTs. The outcomes were impressive: of 101 treated patients receiving prophylaxis with EBVSTs, 0% developed PTLD compared with 11.5% in the control group. In a cohort with established PTLD, 11 of 13 treated patients achieved sustained complete response to treatment. Importantly, there were few short-term and no long-term toxicities associated with treatment: only 8 of 114 total treated patients experienced a recurrence of GVHD after EBVST treatment (70–72, 75). Similar response rates were observed in a cohort of patients with PTLD at the Memorial Sloan Kettering Cancer Center (MSKCC), where complete response was achieved in 10 of 14 treated patients (76). Notably, the Pavia group reported responses even in patients who had failed rituximab (77).

There is a minority of patients who fail to respond or lose their responses to EBVSTs. In some cases, EBV variants possess deletions in immunodominant epitopes, such that EBVSTs generated against the WT epitope are unable to recognize and bind the variant epitope. The MSKCC group reported 3 patients who did not respond to EBVSTs: the transferred donor T cells recognized the B95-8–transformed LCLs, but not the EBV strain expressed on patient tumors. For one PTLD patient whose tumor derived from recipient (not donor) B cells, the fact that the synthesized donor EBVSTs skewed toward a donor-specific HLA antigen (A11011) that was not present on the recipient cells resulted in immune evasion. This patient subsequently responded to a third-party cell line (76). This case demonstrates the value of determining the origin of the tumor. See Table 3 for clinical trials of donor-derived EBVSTs and Table 2 for a summary of clinical studies of multi-VSTs.

EBV: off-the-shelf third-party cells. PTLD is an aggressive fast-paced tumor that requires urgent treatment, and this must be central to decision making about the source and manufacture methods used to generate EBVSTs. Banks of HLA-matched EBVSTs derived from healthy EBV-seropositive subjects are available where cells

are needed urgently. However, shorter persistence of these cells due to mismatching at one or more loci and alloreactivity are both risks. Published data suggest that the concept is feasible, that clinical responses are possible or likely, and that GVHD does not occur at higher rates than in the control population (57, 59, 76).

An early study of third-party EBVSTs for PTLD after solid organ transplantation or HSCT screened EBVST donations before administration to confirm specific killing of donor LCLs as a surrogate readout for efficacy (78). Impressive clinical responses were reported: there was a reduction in tumor burden in 64% of treated patients at 5 weeks, which was maintained in 52% at 6 months of follow-up. There was no reported increase in the incidence of GVHD (59). Further, using the same technology at another center, MSKCC observed clinical responses in 4 of 5 PTLD patients after HSCT (76, 79).

Overall response rates using third-party EBVSTs for PTLD (50%–70% responses) are slightly lower than that observed with donor-derived EBVSTs, but they nonetheless remain a therapeutic option for a subgroup of PTLD patients. Multiple infusions or repeat dosing may be required because of the shorter persistence of these cells in vivo.

EBV: new directions. The high seroprevalence of EBV (greater than 95%) reminds us that strategies to manufacture EBVSTs from EBV-naive donors are less critical than strategies for CMV, whose population prevalence is as low as 50%. However, it is important to be able to manipulate cord blood for this purpose, and adenoviral transfer vector–based transduction of APCs (including LCLs) can be used to generate de novo EBVSTs from cord blood grafts.

Other potential developments in EBV immunotherapy include gene modification of T cells by retroviral transduction with a synthetic EBV-specific TCR. This could potentially offer an alternative option for patients with PTLD and an EBV-seronegative donor (80).

Conclusions and future directions

CMV and EBV primary infection and reactivation after HSCT can be formidable clinical problems for which effective pharmacotherapeutics have substantial limitations. Accelerated reconstitution of antiviral immunity through adoptive cellular immunotherapy offers a more physiological solution.

In the setting of EBV-associated PTLD, clinical responses to EBVSTs are reported in 70%–90% of patients with minimal toxicity, and third-party cell banks now permit rapid administration in urgent cases. There is an expanding literature on CMV-specific T cell therapies that suggests their safety and efficacy, but without the robust confirmation of a phase III clinical study (as yet) to support this.

Strategies to select virus-specific cells to high purity have major implications for scalability and deliverability to a wider range of patients than can presently be treated using these methods. Novel developments that provide antiviral adoptive cell transfer options for HSCT patients with seronegative grafts also address an area of significant unmet clinical need.

It is clear that VSTs can potentially deliver clinical benefit and possess an acceptable safety profile. In order to move this technology into the mainstream, optimized GMP-grade delivery and infrastructure must be supported and developed. Work to transition antiviral T cell therapies from academic centers to commercial entities will help to facilitate broader implementation and improve accessibility of these therapies to patients and clinicians. This will also facilitate the larger randomized studies that are required to confirm efficacy, and to establish how these cellular therapies integrate with the newer antiviral pharmacotherapies. In this respect, studies of both HLA multimer–selected and third-party cells have been executed or initiated, the results of which will be crucial to establishing a more robust evidence base, particularly with regard to appropriate control groups. A greater focus on health care economics and reimbursement pathways will also be critical to the final positioning of these therapies in the overall treatment strategies for viral infections. Further analysis, characterization, and comparison of the phenotype and function of VSTs generated by different methods are also key, particularly given paradigm shifts in other T cell manufacturing processes to promote expansion, function, and persistence.

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