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Pathogen-Specific T Cells Beyond CMV, EBV and Adenovirus

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Abstract

Purpose of Review Infectious diseases contribute significantly to morbidity and mortality in recipients of allogeneic haematopoietic stem cell transplantation (aHSCT), particularly in the era of highly immunosuppressive transplant regimens and alternate donor transplants. Delayed cellular immune recovery is a major mechanism for the increased risk in these patients. Adoptive cell therapy with ex vivo manipulated pathogen-specific T cells (PSTs) is increasingly taking its place as a treatment strategy using donor-derived or third party–banked cells.

Recent Findings The majority of clinical trial data in the form of early-phase studies has been in the prophylaxis or treatment of cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenovirus (AdV). Advancements in methods to select and enrich PSTs offer the opportunity to target the less common viral pathogens as well as fungi with this technology. Early clinical studies of PSTs targeting polyomaviruses (BK virus and JC virus), human herpesvirus 6 (HHV6), varicella zoster virus (VZV) and *Aspergillus* spp. have shown promising results in small numbers of patients. Other potential targets include herpes simplex virus (HSV), respiratory viruses and other invasive fungal species.

Summary In this review, we describe the burden of disease of this wider spectrum of pathogens, the progress in the development of manufacturing capability, early clinical results and the opportunities and challenges for implementation in the clinic.

Keywords Haemopoietic stem cell transplantation · Adoptive immunotherapy · Virus-specific T cells · Fungus-specific T cells · Pathogen-specific T cells · Immune reconstitution

Introduction

Allogeneic haematopoietic stem cell transplantation (aHSCT) remains the only curative treatment for a number of haematological malignancies and bone marrow failure syndromes despite the many promising advances in targeted therapies. Immunosuppression through pre-transplant conditioning regimens and in the post-transplant setting results in

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significant morbidity and mortality from opportunistic infections [1]. Post-transplant immune reconstitution is complex and modulated by donor, recipient and iatrogenic factors. Whilst innate immunity takes between 1 and 3 months to recover, adaptive immunity can take 1 year or more [2]. The resulting B and T cell lymphopenia renders the transplant recipient vulnerable to opportunistic infections, viral and fungal infections in particular. Infection is a leading cause of transplant-related mortality [3, 4].

The use of prolonged immunosuppressive medications remains a mainstay of treatment to prevent graft failure and control graft versus host disease (GvHD). In recent years, there have been increasing numbers of less well-matched transplants, such as haploidentical, human leukocyte antigen (HLA)–mismatched and umbilical cord blood (UCB) transplants [5]. These have necessitated longer periods of more intense immunosuppression, as well as an increase in the use of T cell depletion agents such as anti-thymocyte globulin (ATG), in order to reduce morbidity associated with the increased incidence and severity of GvHD in these patients. The direct relationship between T cell depletion and increased incidence of opportunistic infections has been well demonstrated [6–8].

Conventional antiviral and antifungal pharmacotherapies have many limitations including drug toxicity and the emergence of resistant organisms. Ganciclovir and its oral PEGylated form valganciclovir have been shown to cause pancytopenia in 31% and neutropenia in 20% of bone marrow transplant patients [9, 10]. Foscarnet and cidofovir both cause significant nephrotoxicity. Antifungal agents such as amphotericin B, azoles and echinocandins have adverse effects on renal and hepatic function. They have interactions with other commonly used medications in this patient group and are usually required to be given for longer periods of time than antiviral therapies. The incidence of antiviral and antifungal resistance is rising, and chronic infection has been linked to T cell exhaustion [11, 12]. Additionally, a number of opportunistic viral and fungal infections do not have effective pharmacological treatments.

Adoptive cell therapy through the transfer of T cells that are specific to viral and fungal antigens from donor to recipient has been explored as a treatment strategy to promote rapid cellular immune recovery following aHSCT, primarily in the treatment of cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenovirus. In this review, we explore the development and use of pathogen-specific T cells in a wider spectrum of pathogen targets, including human herpesvirus 6 (HHV6), herpes simplex virus (HSV), varicella zoster virus (VZV), polyomaviruses (BK and JC virus), respiratory viruses and fungi (*Aspergillus*, *Candida* and *Rhizopus* spp.). We also address evolving methods of cell manufacture, and areas for future research.

Immune Reconstitution Post Transplantation

The profound immunosuppression experienced by patients following aHSCT is a direct effect of chemotherapy given to attain remission prior to transplant, and pre-transplant conditioning regimens. There is heterogeneity in these regimens, as well as in a large number of clinical variables including patient factors (age, prior treatment, comorbidities, indication for transplant) [13], stem cell source [14], donor type [15], the use of T cell depletion strategies and post-transplant immunosuppression [16–18]. These factors are important determinants of the pace of immune recovery following transplant. Despite this heterogeneity, there is a consistent pattern of cellular immune reconstitution observed in the majority of patients [2]. Understanding this pattern allows for correlation with infections that might occur in higher frequencies during certain post-transplant periods and for the design of specific targeted therapies.

T cell–mediated immunity shows the most prolonged post-transplant recovery course and occurs by two main mechanisms. In contrast to B cells, mature T cells are transferred in the graft and persist through the conditioning process [19]. Early T cell reconstitution occurs via the peripheral expansion of these cells, mediated by elevated levels of cytokines IL-7 and IL-15 and a relatively decreased level of T regulatory (Treg) cells [20, 21]. The memory T cells generated in this manner have a limited T cell receptor (TCR) repertoire dependent on previous donor antigen exposure. The second mechanism of T cell reconstitution involves de novo differentiation of naïve T cells from donor stem cells and subsequent thymic maturation. The development of a broad TCR repertoire, maturation of a $CD4^+$ T cell compartment including functional Treg cells and removal of alloreactive T cells can take 4–6 years [22, 23]. This is delayed by involution of the thymus in older individuals and myeloablative conditioning regimens which markedly reduce thymic activity in the first 6 months post transplantation [24], resulting in an increased risk of infections and an increased incidence of GvHD.

Disease Burden of Opportunistic Pathogens in the Post-transplant Period

The kinetics of immune recovery correlates with the range and timing of infectious complications following transplant. The first month following aHSCT is associated with an increased risk of infections by bacteria and *Candida* spp. due to defects in innate immunity and loss of mucosal barriers. Following this, viral and fungal infections predominate, with persistent T cell defects the major contributor to both early (< 100 days) and late (> 100 days) infectious complications [2]. Infections account for 15–30% of deaths in the early post-transplant period and 10–40% of deaths in the late post-transplant period

[25]. Although less common than viral or bacterial infections, invasive fungal infections are responsible for the majority of deaths related to infection (50–80%) [25]. In addition to transplant-related mortality directly attributable to infection, it is estimated that infections contribute to up to 70–80% of other causes of non-relapse mortality [4] and significant morbidity in the post-transplant period.

Varicella Zoster Virus

VZV is a human-specific α-herpesvirus with ubiquitous seropositivity in the community. Primary varicella infection (chicken pox) usually occurs in childhood and results in lifelong latent infection. Viral replication occurs in a variety of cells including dendritic cells, keratinocytes and neurons to elicit a predominantly Th1 cell–mediated cellular immune response. Following the period of primary infection, the virus establishes dormancy of the neurons of the dorsal root ganglia [26]. Reactivation results in herpes zoster (shingles) and is associated with a painful dermatomal rash, often complicated by chronic post-herpetic neuralgia. A more severe primary infection or disseminated reactivation of VZV can frequently affect immunocompromised patients with significant morbidity and mortality. Herpes zoster occurs in up to 30% of patients following aHSCT, with prophylaxis with an antiviral such as acyclovir considered standard of care for at least 12 months, reducing this risk to 5–10% [27]. Despite this, a risk of reactivation in the second year post aHSCT remained 8.8% in one study [28] and was 6.1% even in those who continued prophylaxis into the second year until immunosuppression had ceased. An inactivated recombinant subunit–adjuvanted VZV vaccine has recently been developed, demonstrating high immunogenicity in kidney transplant recipients, but it has yet to be evaluated in recipients of aHSCT [29].

Human Herpesvirus 6

HHV6 is a β-herpesvirus that exists as two species, HHV6A and HHV6B, with 75–95% of common nucleotide sequences [30]. Primary infection occurs in > 90% of individuals before the age of 2 years and is associated with an acute febrile illness and viral exanthem. Whilst immune control is thought to be predominantly Th2 cell–mediated, a low-level humoral response in the feto-maternal transfer of passive HHV6-specific IgG is thought to contribute to lower levels of primary infection in children 3–9 months of age [31]. Latency is established in a variety of tissues including monocytes, T cells and salivary glands [32]. HHV6 reactivation occurs in > 50% of aHSCT patients post transplantation, with clinically significant manifestations including encephalitis, myocarditis, pneumonitis and bone marrow suppression [33, 34]. Viral reactivation has been shown to be associated with myeloablative regimens [35], and a higher risk of clinically severe, life-threatening disease is associated with UCB and haploidentical transplants, T cell depletion and active GvHD [36]. Pharmacotherapy with antiviral agents such as cidofovir, ganciclovir and foscarnet has shown control against viraemia, but the effect of these agents on mortality is unknown [37].

Herpes Simplex Virus

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2, respectively) are highly prevalent α-herpesviruses that primarily affect the oral and genital mucosa, respectively, prior to establishing latency in the cell bodies of local innervating neurons [38]. Prior to the routine use of prophylactic acyclovir, viral reactivation and reinfection occurred in up to 70% of patients post aHSCT [39]. HSV-1 has a significant risk for systemic involvement in the immunocompromised host, with a diverse range of clinical manifestations, including meningoencephalitis, pneumonia, hepatitis, oesophagitis and bone marrow suppression [40]. Immune control and viral latency are reliant on a cellular immune response, with HSV-specific $CD8^+$ T cells secreting IFN-γ activated early after the onset of infection [41]. Additionally, the cytolytic effects of $CD8^+$ T cells are postulated to be important in clearing local infection [42]. Therefore, patients with impaired cellular immunity are at a significantly increased risk of developing disseminated infection. Despite routine HSV prophylaxis with acyclovir, an estimated 15–20% of aHSCT recipients will demonstrate detectable HSV infection [43, 44]. Furthermore, the emergence and increasing prevalence of drug-resistant HSV in 28–45% of isolates has a significant impact on aHSCT mortality rates [45, 46]. Alternative antiviral pharmacotherapies carry the same problems with adverse effects as previously outlined. Several vaccines against primarily HSV-2 have shown promising virus-specific immune responses in animals, but this has not translated into human trials, leading to their early termination [38]. Although there is future scope for an effective vaccine solution for the general population, vaccines are less likely to induce adequate immune responses in the immunosuppressed.

Polyomaviruses

BK and JC polyomaviruses (BKV and JCV, respectively) are small non-enveloped dsDNA viruses, representing two of the 13 known human polyomavirus species. Like the herpesviruses, BKV and JCV infection is common but these viruses rarely cause clinical manifestations in immunocompetent hosts. In the immunosuppressed host, BKV is associated with haemorrhagic cystitis in the aHSCT setting and nephropathy post renal transplantation. JCV causes a progressive multifocal leukoencephalopathy (PML) post transplantation which

has a > 80% mortality rate and few treatment options [47]. BK virus–related haemorrhagic cystitis occurs in up to 25% of paediatric and 54% of adult aHSCT recipients [48], whereby a triad of dysuria with urothelial damage, high-level BK viruria and haemorrhagic inflammation occurs following stem cell engraftment [49]. Myeloablative conditioning, unrelated donor grafts and acute GvHD are risk factors for developing BK virus–related haemorrhagic cystitis [50, 51]. There are currently no effective prophylactic therapies to prevent BK haemorrhagic cystitis with a recent randomised study showing no preventative effect in using fluoroquinolones [52]. Treatment of BK haemorrhagic cystitis often involves prolonged IV administration of cidofovir, a nucleotide analogue with a long half-life and significant adverse effects of nephrotoxicity [53]. Together with high nursing requirements, urological interventions and transfusion support to control bleeding, the management of BK haemorrhagic cystitis leads to significant patient morbidity [51].

Respiratory Viruses: Influenza and Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is a medium-sized enveloped RNA virus of the *Paramyxoviridae* family whilst influenza viruses are single-stranded RNA viruses of the *Orthomyxoviridae* family. Influenza A and B are responsible for the majority of human infections with antigenicity based on the haemagglutinin and neuraminidase membrane glycoproteins. These viruses are commonly seasonally acquired, affecting up to 20% of aHSCT recipients and resulting in significant morbidity and poor survival [54–56]. During the pandemic H1N1 influenza outbreak, 30% of all hospitalised influenza patients with haematological malignancy required ICU admission with high inpatient mortality [57]. Rapid progression from upper to lower respiratory tract infection is often complicated by secondary bacterial infection, mechanical ventilation, admission to intensive care unit and increased mortality [54, 55, 57]. Lymphopenia, increased age, GvHD and mismatched unrelated donors are known risk factors for progression to lower respiratory tract infection [58, 59]. The current main pharmacotherapy for influenza is oseltamivir, a neuraminidase inhibitor which can prevent the progression to pneumonia and reduce viral shedding. It requires early administration in the infective period and can induce drug resistance [57, 59]. For RSV infection, ribavirin with or without an immunomodulator can reduce the rate of progression to pneumonia [55]. Overall, the most important aspect in the care of immunocompromised patients with RSV or influenza is early recognition and supportive care. Seasonal influenza vaccinations are recommended annually, but vaccine responses can be inadequate in the early post-aHSCT period [60, 61].

Invasive Fungal Infections: *Aspergillus*, *Candida* and *Rhizopus*

Infection with invasive moulds (*Aspergillus* spp. and *Rhizopus* spp.) and yeasts (*Candida* spp.) occurs in up to 10% of aHSCT recipients with associated high mortality rates [62, 63]. *Aspergillus* species are the most common cause of invasive fungal infection (IFI) in aHSCT, whereby inhalational exposure to air-borne conidia in the setting of neutropenia and innate immune deficiency leads to invasive pulmonary aspergillosis. *Rhizopus* species cause rhino-orbital-cerebral mucormycosis, and invasive *Candida* yeasts are frequently observed with a concerning emergence of drug-resistant non-*Candida albicans* spp. seen [64, 65]. Recipients of aHSCT are a well-recognised high-risk group for IFIs with the most vulnerable risk period being pre-engraftment or during post-engraftment GvHD [62]. In addition to mortality rates exceeding 50%, the development of IFI leads to prolonged hospital admissions, intensive care unit stays and increased health care costs [62, 66]. Antifungal prophylaxis with a mould-active agent such as posaconazole or voriconazole is recommended in high-risk groups; however, breakthrough IFIs often occur. Emergence of rare drug-resistant non-*Aspergillus* moulds has been observed, and an overall mortality benefit for the use of antifungal prophylaxis has not been shown [67, 68]. The diagnostic work-up for IFIs is currently challenging and imprecise, resulting in the extended use of empirical antifungal agents which are expensive as well as toxic [66, 69]. Given these considerations, alternative therapies for the management of IFIs in the immunodeficient host are clearly needed.

Multipathogen Infections

Infection with multiple pathogens (viral, bacterial or fungal), either concurrently or sequentially, contributes significantly to the cumulative infectious burden following aHSCT [70]. The detection of four or more double-stranded viruses at any time within the early period following aHSCT is associated with poor patient survival [70]. CMV co-infection is frequently observed following aHSCT and a recognised risk factor for pulmonary *Aspergillus* infection, BK haemorrhagic cystitis and RSV respiratory tract infection, although the additional impact of CMV to the infection is unknown [63, 71]. Lower respiratory tract viral infections also predispose patients to secondary pulmonary aspergillosis, and viral co-infection is diagnosed in up to 40% of pulmonary IFIs [72]. Managing multiple infectious pathogens in hosts with multiple immune deficits requires a more innovative approach than current single-pathogen targeted pharmacotherapies.

Adoptive T Cell Therapy for Immune Reconstitution

Adoptive T cell therapy is a mechanism to induce rapid reconstitution of pathogen-specific, T cell–mediated immunity and has been shown to be safe and effective in the treatment of CMV, EBV and adenovirus (AdV) infections in early-phase studies [73–77]. In one study limited to patients who had failed conventional antiviral therapies, the majority of patients (93%) achieved complete virological control with pathogen-specific T cells (PSTs) [74].

Whilst a large number of small studies have been performed on the clinical use of adoptively transferred PSTs in EBV, CMV and AdV infections, there are very few of a wider array of pathogens (Table 1). In the 25 years since the first reports of the use of antigen-specific T cells for immune reconstitution [85–90], there have been major advances in manufacturing methodologies, facilitating the broadening of antigen targets to less common opportunistic pathogens. These are summarised in Tables 2 and 3.

Manufacturing methods of the ACT product utilise either ex vivo expansion or ex vivo isolation or a combination of both. The former relies on ex vivo tissue culture to produce cell numbers in the range for therapeutic use, whilst the latter assumes in vivo expansion of much smaller numbers of isolated cells in the presence of antigen in the recipient. The majority of methods rely on ex vivo stimulation, activation and expansion of antigen-specific T cells. This requires an antigen source with or without a specialised antigen-presenting cell. Live or attenuated virus, virus-infected cells or lysates, vectors, plasmids or peptide mixes have been used as the stimulating antigen by various groups. Some of these require knowledge of immunogenic epitopes and HLA specificity of the immune response. For the extended pathogen spectrum, this information is largely unavailable so antigen sources have included therapeutic vaccines (seasonal influenza vaccine [93] or live attenuated varicella vaccine [92]), overlapping peptide mixes (RSV [96•], BKV [91, 94] and HHV6 [80••]) or individual epitopes (HSV [95]) that expose T cells to an array of epitopes. Gene modification methods have also been used, such as transfection of antigen-presenting cells with viral vectors containing target virus DNA [81, 103], or nucleofection of plasmids containing viral DNA [76].

The search for appropriate epitopes for fungal infections is more challenging due to the complexity of fungal pathogens and the large number of potentially immunogenic proteins. This has been addressed by the use of fungal lysates. There is significant cross-reactivity of the cellular immune response in some pathogens [104]. A combination of fungal lysates from *Aspergillus*, *Candida* and *Rhizopus* species has been shown to cross-react with *Fusarium* and *Lomentospora* (*Scedosporium*) species [101]. This raises the possibility that fungus-specific T cells could be protective against or treat multiple fungal infections. Despite promising pre-clinical results, there has been only one published clinical study on the safety and efficacy of ACT in humans [78]. This study showed that *Aspergillus fumigatus*-specific T cell therapy was safe to use without significant toxicity or GvHD post aHSCT and contributed to a higher rate of clinical resolution of invasive *Aspergillus* infection when used in combination with conventional pharmacotherapies, compared to those therapies alone. Other good manufacturing practice (GMP)–compliant methods for generating *Aspergillus*-specific T cells have since been described [97, 100, 102], and a GMP-compliant commercial *A. fumigatus* water-soluble lysate has become available. Clinical trials using these methods are underway (ACTRN12618001540202, EudraCT no. 2013-002914-11).

Antigen stimulation–independent ex vivo direct isolation methods such as major histocompatibility complex (MHC) multimer/streptamer selection have not been extensively utilised outside of EBV, CMV and AdV due to the limited knowledge of epitopes in the extended pathogen spectrum. Moreover, these methods are limited largely to MHC class I/$CD8^+$ T cell responses, a disadvantage in pathogens whose immune response is largely mediated through MHC class II/$CD4^+$ T cells (fungi and some viruses, including BKV and JCV). As more is understood of the immune response and MHC class II multimers become more widely available, this approach may become more feasible.

Cytokine capture is an alternative method of isolating PSTs using magnetic beads ex vivo to select cytokine-producing T cells following in vivo antigen stimulation. The benefit of this strategy is that it selects both $CD4^+$ and $CD8^+$ PSTs if whole antigen sources (such as overlapping libraries of peptides) are used [105]. Beyond CMV, EBV and AdV, this method has been used in the treatment of BKV [83].

Recipient-Specific Manufacture Versus Cell Banking

The first studies of adoptive T cell therapy used donor-derived cells which were autologous to the engrafted haemopoietic system [86, 89, 90] and were seen to persist in the recipient for many years [106]. In some cases, transferred clones were able to respond to antigen with expansion of transferred cells seen in the peripheral blood at the time of viral reactivation [107]. Time-of-need or advance manufacture of donor-derived PST products is costly and labour-intensive, requiring a unique product for each donor-recipient pair. Donors may be required to undergo multiple cell donations and generally need to be seropositive for the pathogen target. Advances in processes in the last 25 years have significantly shortened manufacturing times to approximately 2 weeks [76]. Despite this, for a number of pathogens in the extended spectrum (polyomaviruses, invasive fungal infections), effective therapy needs to be commenced as soon as possible to minimise

Table 1 Existing studies of pathogen-specific T cells targeted beyond CMV, EBV and AdV in the post-aHSCT setting

Study	Specificity	No. of patients	Cell source	Manufacturing	Cell dose	Safety/AEs	Clinical response
Perruccio et al. (2005) [78]	*A. fumigatus*	10 treated 13 control	Donor-derived	Ex vivo expansion	1×10^5–3×10^6/kg (dose escalation in protocol)	aGvHD 1 (cell dose 3×10^6/kg) No AEs for patients given 1×10^6/kg or less	Treated group: 9/10 cleared invasive aspergillosis, 1/10 died of *Aspergillus* pneumonia Control group: 7/13 cleared, 6/13 died
Balduzzi et al. (2011) [79]	JCV (PML)	1	Donor-derived	Ex vivo expansion	0.5–1.0×10^6/kg	Nil	CR
Papadopoulou et al. (2014) [80••]	BKV (HC) HHV6 (+ CMV/AdV/EBV)	7 2	Donor-derived	Ex vivo expansion	0.5–$2 \times 10^7/m^2$	BKV: aGvHD 1 HHV6: nil	BKV: CR 5, PR 1, NR 1 HHV6: CR 2
Ma et al. (2015) [81]	VZV prophylaxis (multipathogen product with CMV/AdV/VZV/EBV specificity) Co-administered with antivirals	10	Donor-derived	Ex vivo expansion	$2 \times 10^7/m^2$	aGvHD DN 2 cGvHD 7	8/10: higher immune reconstitution at day 100 versus day 0 10/10: no detectable VZV at 12 months; some patients ceased antiviral therapy from 4 months post transplantation
Olson et al. (2016) [82]	BKV (HC)	10 to date	Third-party donor bank	Ex vivo expansion	0.48–1×10^3/kg	aGvHD DN 1 aGvHD Ra 1	CR 8 PR 1 NE 1 (relapse)
Pello et al. (2017) [83]	BKV (HC)	1	Donor-derived	Direct isolation/ cytokine capture	0.34×10^4/kg	Nil	CR
Tzannou et al. (2017) [84]	BKV (HC, aN) HHV6 (+ CMV/AdV/EBV)	20 4	Third-party donor bank	Ex vivo expansion	$2 \times 10^7/m^2$	BKV: aGvHD 2, cGvHD 3 HHV6: nil	BKV: CR 6, PR 15 HHV6 – PR 3, NE 1

AEs adverse events, *aGvHD* acute graft versus host disease, *aN* acute nephritis, *BKV* BK virus, *cGvHD* chronic graft versus host disease, *CR* complete response, *DN* de novo, *HC* haemorrhagic cystitis, *HHV6* human herpesvirus 6, *JCV* JC virus, *NE* not evaluable, *NR* no response, *PML* posterior multifocal leukoencephalopathy, *PR* partial response, *Ra* reactivated

Table 2 Clinical and pre-clinical manufacturing protocols of virus-specific T cells beyond CMV, EBV and AdV

Study	Target(s)	Manufacturing method	In vitro specificity	Cell dose	Mean CD4/CD8 %
Blyth et al. (2011) [91]	BKV	MoDCs pulsed with overlapping peptide mixes spanning VP1/VP2/VP3/large T/small T BKV antigens	10/11 had cytokine response to at least 1 BKV protein (11/15 cultures tested for antigen specificity)	NR	12.5/15.2
Blyth et al. (2012) [92]	VZV	MoDCs pulsed with VZV vaccine	16/18 cytokine-responsive to VZV antigen 1/18 dominated by NK cells, no response 1/18 not tested	NR	54.2/28.7
Gaundar et al. (2012) [93]	Influenza	MoDCs pulsed with influenza vaccine (Fluvax), then tested against overlapping 15mer pepmixes of influenza antigens	7/7	NR	78.1/14.9
Papadopoulou et al. (2014) [80••]	HHV6 BKV (CMV) (EBV) (AdV)	PBMCs stimulated by overlapping 15mer pepmix covering targets pp65/IE1 (CMV), LMP/BZLF1/EBNA1 (EBV), penton/hexon (AdV), VP1/large T (BKV), U11/U14/U90 (HHV6)	14/48 pentaspecific 9/48 quadrispecific 12/48 trispecific 11/48 bispecific 1/48 monospecific 1/48 no response	$0.5–2 \times 10^7/m^2$	57/35
Lamarche et al. (2017) [94]	BKV	MoDCs derived from 5 healthy individuals and 8 kidney transplant patients infected with clinically significant BKV nephropathy pulsed with overlapping peptide libraries from VP1/large T antigens	5/5 cultures from healthy individuals 8/8 cultures from BKV-infected kidney transplant patients Absolute cell numbers significantly lower for transplant patients	NR	> 90% of total $CD4^+$ and $CD8^+$ cells, mean values of each not reported
Ma et al. (2017) [95]	HSV-1	MoDCs stimulated by 3 HLA-A1 and 4 HLA-A2 HSV-1 peptides generated from HSV-seropositive donors	9/9	NR	60/35
Vasileiou et al. (2019) [96•]	RSV Influenza PIV-3 hMPV	PBMCs stimulated with 15mer overlapping peptides of 12 different respiratory viral antigens; NP1/MP1 (influenza A), F/N (RSV), F/HN/M/N (PIV-3), F/M/M2-1/N (hMPV)	12/12 quadrispecific	NR	74.4/18.1

BKV BK virus, *HHV6* human herpesvirus 6, *hMPV* human metapneumovirus, *HSV-1* herpes simplex virus 1, *MoDC* monocyte-derived dendritic cell, *PBMC* peripheral blood mononuclear cell, *NR*– not reported, *PIV-3* parainfluenza virus 3, *RSV* respiratory syncytial virus, *VZV* varicella zoster virus

Table 3 Pre-clinical manufacturing study protocols of fungus-specific T cells

Study	Target(s)	Manufacturing method	In vitro specificity
Tramsen et al. (2009) [97]	*A. fumigatus*	MoDCs incubated with *A. fumigatus* water-soluble antigen extract	3/3
Khanna et al. (2011) [73]	*C. albicans* *A. fumigatus* (CMV) (EBV) (AdV)	MoDCs pulsed with multiple antigens: hexon (AdV), pp65 (CMV), LMP2 (EBV), Crf1 p41 (*A. fumigatus*) and MP65 (*C. Albicans*) Single-pathogen-specific and multipathogen-specific products made	7 donors made 4 specific and 1 multipathogen product each 7/7 AdV specific 7/7 EBV specific 7/7 *C. albicans* specific 6/7 *A. fumigatus* specific 6/7 multipathogen products quadrispecific 1/7 multipathogen products trispecific Fewer absolute numbers of each specific T cell population in multipathogen versus single pathogen products
Gaundar et al. (2012) [98]	*A. fumigatus*	MoDCs pulsed with *A. fumigatus* lysate	8/8
Tramsen et al. (2013) [99]	*A. fumigatus* *C. albicans* *R. oryzae*	MoDCs pulsed with water-soluble cellular extracts of *A. fumigatus*, *C. albicans* and *R. oryzae*	3/3 multispecific, showing cross-specificity when tested with other species in same fungal genus
Bacher et al. (2015) [100]	*A. fumigatus*	PBMCs stimulated by *A. fumigatus* lysate in RPMI, CD8 and Treg cells depleted, $CD137^+$ population immunomagnetically enriched	13/13
Deo et al. (2016) [101]	*A. terreus* *C. krusei* *R. oryzae*	MoDCs pulsed with pure strains of isolated fungal lysates of *A. terreus*, *C. krusei* and *R. oryzae*	11/11 multispecific for the 3 fungal antigens, as well as cross-reactivity for *C. albicans*, *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus flavus* and *Lomentospora prolificans*
Papadopoulou et al. (2019) [102]	*A. fumigatus*	PBMCs pulsed with *A. fumigatus* lysate or peptide mixes	Peptide-pulsed PBMC showed more consistent enrichment than lysate-pulsed PBMC Lysate-pulsed PBMCs showed higher T cell repertoire diversity than peptide-pulsed PBMCs

MoDC monocyte-derived dendritic cell, *PBMC* peripheral blood mononuclear cell

mortality and significant end-organ damage, rendering this method of manufacture less feasible in the clinical setting.

Third-party donor-derived cell banks have been developed as a more readily available source of partially HLA-matched PSTs in trials mainly targeting CMV, EBV and adenovirus [75, 80••, 84]. Whilst these appear safe and effective in the setting of salvage for established viral infections, their long-term persistence is not well understood. Nonetheless, it appears that they result in control of viral infection in recipients over a follow-up period of at least 12 months [74]. For the extended pathogen spectrum, third party–banked cells hold several potential advantages, including ready availability in the infrequent event of clinical need, the ability to manufacture and characterise products for ideal product choice when needed and the ability to target donors of appropriate serostatus or HLA type in advance. A recent phase II study has utilised banked PSTs specific to a number of viral infections including BKV and HHV6 [84], and a phase II study using banked BKV-specific PSTs reported promising preliminary results in 10 patients and is ongoing [82]. Outside the scope of haematopoietic stem cell transplantation, third party–banked cells have potential applicability against pathogen targets following solid organ transplants, where donor-derived cells are not available or unsuitable due to HLA mismatch [108–110].

The limitations of third-party cell banks include a higher theoretical risk of GvHD which has, not to date, been demonstrated in practice; the logistical planning and resource cost of generation of the bank; and the need for a representative population of donors that cover common HLA subtypes, with the possibility of a suitable product not being available for a particular patient. A recent study has shown that it is possible to locally generate a third-party cell bank to provide a suitable partially matched HLA product against CMV, EBV and AdV to > 90% of aHSCT patients in that same population using only 30 donors [111•]. A multicentre study [75] used a similar number of banked cell lines to effectively treat severe CMV, EBV and AdV post aHSCT.

In the wider spectrum of pathogens addressed by this review, the establishment of third-party cell banks would benefit from an improved understanding of immunodominant HLA types for these pathogens. Selection of third-party PST products is based on the level of HLA match with the patient whilst also taking into consideration if the pathogen-specific activity is mediated through shared HLA antigens [111•]. T cell responses against CMV and EBV are highly predictable with individuals typically displaying strong responses to a limited number of epitopes of known HLA restriction [112, 113]. This simplifies the characterisation of T cell products which can be screened with commercially available MHC tetramers or assessed for activity against individual peptides using assays to detect cytotoxic activity or cytokine production.

Mapping responses to specific HLAs can be attempted by assessing activity against antigen preparations using HLA blocking antibodies or by comparing responses to antigens presented by HLA-matched and HLA-mismatched target cells. However, confirmation of HLA restriction can be complicated by a lack of suitable blocking antibodies, alloreactivity to mismatched targets and promiscuity of $CD4^+$ T cells to multiple MHC class II HLA loci. To simplify HLA mapping, tools such as single HLA-expressing cell lines may prove to be a valuable resource [114]. Another approach for antigens with a large number of potential epitopes is to use prediction algorithms to identify candidate peptides with a high probability of binding to specific HLA types [115].

Conclusions and Future Directions

Infectious disease morbidity remains high in recipients of aHSCT, and adoptive cell therapy with PSTs is increasingly recognised as a modality for both treatment of the infection and repair of the underlying immune deficit. Investigations of a wider spectrum of viral and fungal pathogens as targets for adoptive cell therapies are underway. The last 25 years have seen advancement and innovation in the manufacture and enrichment of PSTs. However, their application is still limited by logistics and time-consuming, labour-intensive enrichment procedures. Off-the-shelf third-party cells are promising and address a number of these challenges, but their clinical efficacy has yet to be shown in randomised studies. The persistence of infused third-party cells and their role in the generation of adaptive immunity post transplantation remains uncharacterised and is an area for future study. There is scope for expansion of knowledge in understanding of antigen targets in the broader array of opportunistic pathogens both in deepening the understanding of the ways T cells target infections and in the potential development of multimer/streptamer direct isolation methods for pathogens in the wider spectrum. The development of infrastructure for manufacture and distribution of cellular therapies in the wider context offers the opportunity for centralised manufacture for PSTs for a number of pathogens that could be distributed at the time of clinical need.

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Compliance with Ethical Standards

Conflict of Interest Wei Jiang, Barbara Withers, Gaurav Sutrave, Leighton E. Clancy and Michelle I. Yong declare that they have no conflict of interest.

Emily Blyth has two patents: patent AU2015902675 issued and patent AU2018/050630 pending.

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References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. Bone Marrow Transplant. 2005;36:757. https://doi.org/10.1038/sj.bmt.1705140.
2. Mehta RS, Rezvani K. Immune reconstitution post allogeneic transplant and the impact of immune recovery on the risk of infection. Virulence. 2016;7(8):901–16. https://doi.org/10.1080/21505594.2016.1208866.
3. Tanaka Y, Kurosawa S, Tajima K, Tanaka T, Ito R, Inoue Y, et al. Analysis of non-relapse mortality and causes of death over 15 years following allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2016;51(4):553–9. https://doi.org/10.1038/bmt.2015.330.
4. Young J-AH, Logan BR, Wu J, Wingard JR, Weisdorf DJ, Mudrick C, et al. Infections after transplantation of bone marrow or peripheral blood stem cells from unrelated donors. Biol Blood Marrow Transplant. 2016;22(2):359–70. https://doi.org/10.1016/j.bbmt.2015.09.013.
5. D'Souza A, Fretham C. Center for International Blood and Marrow Transplant Research summary slides—HCT trends and survival data. 2018. https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/pages/index.aspx. Accessed 22/4/19 2019.
6. Yamasaki S, Aoki J, Mori J, Mizuno S, Uchida N, Ohashi K, et al. Better disease control before allogeneic stem cell transplantation is crucial to improve the outcomes of transplantation for acute myeloid leukemia patients with extramedullary disease. Bone Marrow Transplant. 2019. https://doi.org/10.1038/s41409-019-0527-z.
7. Bacigalupo A, Lamparelli T, Bruzzi P, Guidi S, Alessandrino PE, di Bartolomeo P, et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). Blood. 2001;98(10):2942–7. https://doi.org/10.1182/blood.V98.10.2942.
8. Kawamura K. Effect of antithymocyte globulin on HLA-mismatched unrelated transplantation. Int J Hematol. 2019. https://doi.org/10.1007/s12185-019-02597-y.
9. Goodrich JM, Mori M, Gleaves CA, Du Mond C, Cays M, Ebeling DF, et al. Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation. N Engl J Med. 1991;325(23):1601–7. https://doi.org/10.1056/nejm199112053252303.
10. Schmidt GM, Horak DA, Niland JC, Duncan SR, Forman SJ, Zaia JA. A randomized, controlled trial of prophylactic ganciclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplants; the City of Hope-Stanford-Syntex CMV Study Group. N Engl J Med. 1991;324(15):1005–11. https://doi.org/10.1056/nejm199104113241501.
11. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol. 2009;10(1):29–37. https://doi.org/10.1038/ni.1679.
12. Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J Virol. 2003;77(8):4911–27.
13. Kollman C, Howe CWS, Anasetti C, Antin JH, Davies SM, Filipovich AH, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. Blood. 2001;98(7):2043–51. https://doi.org/10.1182/blood.V98.7.2043.
14. Heining C, Spyridonidis A, Bernhardt E, Schulte-Mönting J, Behringer D, Grüllich C, et al. Lymphocyte reconstitution following allogeneic hematopoietic stem cell transplantation: a retrospective study including 148 patients. Bone Marrow Transplant. 2007;39:613. https://doi.org/10.1038/sj.bmt.1705648.
15. Baron F, Storer B, Maris MB, Storek J, Piette F, Metcalf M, et al. Unrelated donor status and high donor age independently affect immunologic recovery after nonmyeloablative conditioning. Biol Blood Marrow Transplant. 2006;12(11):1176–87. https://doi.org/10.1016/j.bbmt.2006.07.004.
16. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood. 2014;124(3):344–53. https://doi.org/10.1182/blood-2014-02-514778.
17. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238. https://doi.org/10.1016/j.bbmt.2009.06.019.
18. Vadakekolathu J, Rutella S. T-cell manipulation strategies to prevent graft-versus-host disease in haploidentical stem cell transplantation. Biomedicines. 2017;5(2):33.
19. Fry TJ, Mackall CL. Immune reconstitution following hematopoietic progenitor cell transplantation: challenges for the future. Bone Marrow Transplant. 2005;35:S53. https://doi.org/10.1038/sj.bmt.1704848.
20. Bolotin E, Annett G, Parkman R, Weinberg K. Serum levels of IL-7 in bone marrow transplant recipients: relationship to clinical characteristics and lymphocyte count. Bone Marrow Transplant. 1999;23:783. https://doi.org/10.1038/sj.bmt.1701655.
21. Williams KM, Gress RE. Immune reconstitution and implications for immunotherapy following haematopoietic stem cell transplantation. Best Pract Res Clin Haematol. 2008;21(3):579–96. https://doi.org/10.1016/j.beha.2008.06.003.
22. Sutrave G, Blyth E, Gottlieb DJ. Cellular therapy for multiple pathogen infections after hematopoietic stem cell transplant. Cytotherapy. 2017;19(11):1284–301. https://doi.org/10.1016/j.jcyt.2017.07.012.
23. Auletta JJ, Lazarus HM. Immune restoration following hematopoietic stem cell transplantation: an evolving target. Bone Marrow Transplant. 2005;35(9):835–57.
24. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood. 2010;115(19):3861–8. https://doi.org/10.1182/blood-2009-12-234096.
25. Parody R, Martino R, Rovira M, Vazquez L, Vázquez MJ, de la Cámara R, et al. Severe infections after unrelated donor allogeneic hematopoietic stem cell transplantation in adults: comparison of cord blood transplantation with peripheral blood and bone marrow transplantation. Biol Blood Marrow Transplant. 2006;12(7):734–48. https://doi.org/10.1016/j.bbmt.2006.03.007.
26. Sartori AM. A review of the varicella vaccine in immunocompromised individuals. Int J Infect Dis. 2004;8(5):259–70. https://doi.org/10.1016/j.ijid.2003.09.006.

27. Boeckh M, Kim HW, Flowers MED, Meyers JD, Bowden RA. Long-term acyclovir for prevention of varicella zoster virus disease after allogeneic hematopoietic cell transplantation—a randomized double-blind placebo-controlled study. Blood. 2006;107(5):1800–5. https://doi.org/10.1182/blood-2005-09-3624.
28. Erard V, Guthrie KA, Varley C, Heugel J, Wald A, Flowers MED, et al. One-year acyclovir prophylaxis for preventing varicella-zoster virus disease after hematopoietic cell transplantation: no evidence of rebound varicella-zoster virus disease after drug discontinuation. Blood. 2007;110(8):3071–7. https://doi.org/10.1182/blood-2007-03-077644.
29. Vink P, Ramon Torrell JM, Sanchez Fructuoso A, Kim SJ, Kim SI, Zaltzman J, et al. Immunogenicity and safety of the adjuvanted recombinant zoster vaccine in chronically immunosuppressed adults following renal transplant: a phase III, randomized clinical trial. Clin Infect Dis. 2019. https://doi.org/10.1093/cid/ciz177.
30. Dominguez G, Dambaugh TR, Stamey FR, Dewhurst S, Inoue N, Pellett PE. Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A. J Virol. 1999;73(10):8040–52.
31. Becerra A, Gibson L, Stern LJ, Calvo-Calle JM. Immune response to HHV-6 and implications for immunotherapy. Curr Opin Virol. 2014;9:154–61. https://doi.org/10.1016/j.coviro.2014.10.001.
32. De Bolle L, Naesens L, De Clercq E. Update on human herpesvirus 6 biology, clinical features, and therapy. Clin Microbiol Rev. 2005;18(1):217–45. https://doi.org/10.1128/CMR.18.1.217-245.2005.
33. Boutolleau D, Fernandez C, André E, Imbert-Marcille B-M, Milpied N, Agut H, et al. Human herpesvirus (HHV)-6 and HHV-7: two closely related viruses with different infection profiles in stem cell transplantation recipients. J Infect Dis. 2003;187(2):179–86. https://doi.org/10.1086/367677.
34. Brennan Y, Gottlieb DJ, Baewer D, Blyth E. A fatal case of acute HHV-6 myocarditis following allogeneic haemopoietic stem cell transplantation. J Clin Virol. 2015;72:82–4. https://doi.org/10.1016/j.jcv.2015.09.013.
35. de Pagter PJ, Schuurman R, Keukens L, Schutten M, Cornelissen JJ, van Baarle D, et al. Human herpes virus 6 reactivation: important predictor for poor outcome after myeloablative, but not non-myeloablative allo-SCT. Bone Marrow Transplant. 2013;48(11):1460–4. https://doi.org/10.1038/bmt.2013.78.
36. de Pagter PJ, Schuurman R, Meijer E, van Baarle D, Sanders EA, Boelens JJ. Human herpesvirus type 6 reactivation after haematopoietic stem cell transplantation. J Clin Virol. 2008;43(4):361–6. https://doi.org/10.1016/j.jcv.2008.08.008.
37. Lin R, Liu Q. Diagnosis and treatment of viral diseases in recipients of allogeneic hematopoietic stem cell transplantation. J Hematol Oncol. 2013;6(1):94. https://doi.org/10.1186/1756-8722-6-94.
38. Whitley R, Baines J. Clinical management of herpes simplex virus infections: past, present, and future [version 1; peer review: 2 approved]. F1000Res. 2018;7(1726). https://doi.org/10.12688/f1000research.16157.1.
39. Saral R, Burns WH, Laskin OL, Santos GW, Lietman PS. Acyclovir prophylaxis of herpes-simplex-virus infections. N Engl J Med. 1981;305(2):63–7. https://doi.org/10.1056/nejm198107093050202.
40. Bustamante CI, Wade JC. Herpes simplex virus infection in the immunocompromised cancer patient. J Clin Oncol. 1991;9(10):1903–15. https://doi.org/10.1200/jco.1991.9.10.1903.
41. Mueller SN, Jones CM, Smith CM, Heath WR, Carbone FR. Rapid cytotoxic T lymphocyte activation occurs in the draining lymph nodes after cutaneous herpes simplex virus infection as a result of early antigen presentation and not the presence of virus. J Exp Med. 2002;195(5):651–6. https://doi.org/10.1084/jem.20012023.
42. Ghiasi H, Cai S, Perng G-C, Nesburn AB, Wechsler SL. Perforin pathway is essential for protection of mice against lethal ocular HSV-1 challenge but not corneal scarring. Virus Res. 1999;65(2):97–101. https://doi.org/10.1016/S0168-1702(99)00107-0.
43. Chakrabarti S, Milligan DW, Collingham KE, Ratcliffe D, Pillay D, Cane PA. Resistance to antiviral drugs in herpes simplex virus infections among allogeneic stem cell transplant recipients: risk factors and prognostic significance. J Infect Dis. 2000;181(6):2055–8. https://doi.org/10.1086/315524.
44. Stránská R, Schuurman R, Nienhuis E, Goedegebuure IW, Polman M, Weel JF, et al. Survey of acyclovir-resistant herpes simplex virus in the Netherlands: prevalence and characterization. J Clin Virol. 2005;32(1):7–18. https://doi.org/10.1016/j.jcv.2004.04.002.
45. Frobert E, Burrel S, Ducastelle-Lepretre S, Billaud G, Ader F, Casalegno JS, et al. Resistance of herpes simplex viruses to acyclovir: an update from a ten-year survey in France. Antivir Res. 2014;111:36–41. https://doi.org/10.1016/j.antiviral.2014.08.013.
46. Kakiuchi S, Tsuji M, Nishimura H, Yoshikawa T, Wang L, Takayama-Ito M, et al. Association of the emergence of acyclovir-resistant herpes simplex virus type 1 with prognosis in hematopoietic stem cell transplantation patients. J Infect Dis. 2017;215(6):865–73. https://doi.org/10.1093/infdis/jix042.
47. Mateen FJ, Muralidharan R, Carone M, van de Beek D, Harrison DM, Aksamit AJ, et al. Progressive multifocal leukoencephalopathy in transplant recipients. Ann Neurol. 2011;70(2):305–22. https://doi.org/10.1002/ana.22408.
48. Ruggeri A, Roth-Guepin G, Battipaglia G, Mamez AC, Malard F, Gomez A, et al. Incidence and risk factors for hemorrhagic cystitis in unmanipulated haploidentical transplant recipients. Transpl Infect Dis. 2015;17(6):822–30. https://doi.org/10.1111/tid.12455.
49. Cesaro S, Dalianis T, Hanssen Rinaldo C, Koskenvuo M, Pegoraro A, Einsele H, et al. ECIL guidelines for the prevention, diagnosis and treatment of BK polyomavirus-associated haemorrhagic cystitis in haematopoietic stem cell transplant recipients. J Antimicrob Chemother. 2018;73(1):12–21. https://doi.org/10.1093/jac/dkx324.
50. Giraud G, Priftakis P, Bogdanovic G, Remberger M, Dubrulle M, Hau A, et al. BK-viruria and haemorrhagic cystitis are more frequent in allogeneic haematopoietic stem cell transplant patients receiving full conditioning and unrelated-HLA-mismatched grafts. Bone Marrow Transplant. 2008;41(8):737–42. https://doi.org/10.1038/sj.bmt.1705962.
51. Gilis L, Morisset S, Billaud G, Ducastelle-Lepretre S, Labussiere-Wallet H, Nicolini FE, et al. High burden of BK virus-associated hemorrhagic cystitis in patients undergoing allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2014;49(5):664–70. https://doi.org/10.1038/bmt.2013.235.
52. Knoll GA, Humar A, Fergusson D, Johnston O, House AA, Kim SJ, et al. Levofloxacin for BK virus prophylaxis following kidney transplantation: a randomized clinical trial. JAMA. 2014;312(20):2106–14. https://doi.org/10.1001/jama.2014.14721.
53. Ljungman P, Deliliers GL, Platzbecker U, Matthes-Martin S, Bacigalupo A, Einsele H, et al. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. The infectious diseases working party of the European Group for Blood and Marrow Transplantation. Blood. 2001;97(2):388–92.
54. Sim SA, Leung VKY, Ritchie D, Slavin MA, Sullivan SG, Teh BW. Viral respiratory tract infections in allogeneic hematopoietic stem cell transplantation recipients in the era of molecular testing. Biol Blood Marrow Transplant. 2018;24(7):1490–6. https://doi.org/10.1016/j.bbmt.2018.03.004.
55. Shah JN, Chemaly RF. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. Blood.

2011;117(10):2755–63. https://doi.org/10.1182/blood-2010-08-263400.
56. Hutspardol S, Essa M, Richardson S, Schechter T, Ali M, Krueger J, et al. Significant transplantation-related mortality from respiratory virus infections within the first one hundred days in children after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2015;21(10):1802–7. https://doi.org/10.1016/j.bbmt.2015.06.015.
57. Tramontana AR, George B, Hurt AC, Doyle JS, Langan K, Reid AB, et al. Oseltamivir resistance in adult oncology and hematology patients infected with pandemic (H1N1) 2009 virus, Australia. Emerg Infect Dis. 2010;16(7):1068–75. https://doi.org/10.3201/eid1607.091691.
58. Martino R, Porras RP, Rabella N, Williams JV, Ramila E, Margall N, et al. Prospective study of the incidence, clinical features, and outcome of symptomatic upper and lower respiratory tract infections by respiratory viruses in adult recipients of hematopoietic stem cell transplants for hematologic malignancies. Biol Blood Marrow Transplant. 2005;11(10):781–96. https://doi.org/10.1016/j.bbmt.2005.07.007.
59. Nichols WG, Guthrie KA, Corey L, Boeckh M. Influenza infections after hematopoietic stem cell transplantation: risk factors, mortality, and the effect of antiviral therapy. Clin Infect Dis. 2004;39(9):1300–6. https://doi.org/10.1086/425004.
60. Ljungman P, Avetisyan G. Influenza vaccination in hematopoietic SCT recipients. Bone Marrow Transplant. 2008;42(10):637–41. https://doi.org/10.1038/bmt.2008.264.
61. Ambati A, Einarsdottir S, Magalhaes I, Poiret T, Bodenstein R, LeBlanc K, et al. Immunogenicity of virosomal adjuvanted trivalent influenza vaccination in allogeneic stem cell transplant recipients. Transpl Infect Dis. 2015;17(3):371–9. https://doi.org/10.1111/tid.12382.
62. Girmenia C, Raiola AM, Piciocchi A, Algarotti A, Stanzani M, Cudillo L, et al. Incidence and outcome of invasive fungal diseases after allogeneic stem cell transplantation: a prospective study of the Gruppo Italiano Trapianto Midollo Osseo (GITMO). Biol Blood Marrow Transplant. 2014;20(6):872–80. https://doi.org/10.1016/j.bbmt.2014.03.004.
63. Yong MK, Ananda-Rajah M, Cameron PU, Morrissey CO, Spencer A, Ritchie D, et al. Cytomegalovirus reactivation is associated with increased risk of late-onset invasive fungal disease after allogeneic hematopoietic stem cell transplantation: a multicenter study in the current era of viral load monitoring. Biol Blood Marrow Transplant. 2017;23(11):1961–7. https://doi.org/10.1016/j.bbmt.2017.07.025.
64. Chapman B, Slavin M, Marriott D, Halliday C, Kidd S, Arthur I, et al. Changing epidemiology of candidaemia in Australia. J Antimicrob Chemother. 2017;72(4):1103–8. https://doi.org/10.1093/jac/dkw422.
65. Kennedy KJ, Daveson K, Slavin MA, van Hal SJ, Sorrell TC, Lee A, et al. Mucormycosis in Australia: contemporary epidemiology and outcomes. Clin Microbiol Infect. 2016;22(9):775–81. https://doi.org/10.1016/j.cmi.2016.01.005.
66. Ananda-Rajah MR, Cheng A, Morrissey CO, Spelman T, Dooley M, Neville AM, et al. Attributable hospital cost and antifungal treatment of invasive fungal diseases in high-risk hematology patients: an economic modeling approach. Antimicrob Agents Chemother. 2011;55(5):1953–60. https://doi.org/10.1128/AAC.01423-10.
67. Lamoth F, Chung SJ, Damonti L, Alexander BD. Changing epidemiology of invasive mold infections in patients receiving azole prophylaxis. Clin Infect Dis. 2017;64(11):1619–21. https://doi.org/10.1093/cid/cix130.
68. Ethier MC, Science M, Beyene J, Briel M, Lehrnbecher T, Sung L. Mould-active compared with fluconazole prophylaxis to prevent invasive fungal diseases in cancer patients receiving chemotherapy or haematopoietic stem-cell transplantation: a systematic review and meta-analysis of randomised controlled trials. Br J Cancer. 2012;106(10):1626–37. https://doi.org/10.1038/bjc.2012.147.
69. Heng SC, Morrissey O, Chen SC, Thursky K, Manser RL, Nation RL, et al. Utility of bronchoalveolar lavage fluid galactomannan alone or in combination with PCR for the diagnosis of invasive aspergillosis in adult hematology patients: a systematic review and meta-analysis. Crit Rev Microbiol. 2015;41(1):124–34. https://doi.org/10.3109/1040841X.2013.804033.
70. Hill JA, Mayer BT, Xie H, Leisenring WM, Huang M-L, Stevens-Ayers T, et al. The cumulative burden of double-stranded DNA virus detection after allogeneic HCT is associated with increased mortality. Blood. 2017;129(16):2316–25. https://doi.org/10.1182/blood-2016-10-748426.
71. Yong MK, Slavin MA, Kontoyiannis DP. Invasive fungal disease and cytomegalovirus infection: is there an association? Curr Opin Infect Dis. 2018;31(6):481–9. https://doi.org/10.1097/QCO.0000000000000502.
72. Goncalves SM, Lagrou K, Rodrigues CS, Campos CF, Bernal-Martinez L, Rodrigues F, et al. Evaluation of bronchoalveolar lavage fluid cytokines as biomarkers for invasive pulmonary aspergillosis in at-risk patients. Front Microbiol. 2017;8:2362. https://doi.org/10.3389/fmicb.2017.02362.
73. Khanna N, Stuehler C, Conrad B, Lurati S, Krappmann S, Einsele H, et al. Generation of a multipathogen-specific T-cell product for adoptive immunotherapy based on activation-dependent expression of CD154. Blood. 2011;118(4):1121–31. https://doi.org/10.1182/blood-2010-12-322610.
74. Withers B, Blyth E, Clancy LE, Yong A, Fraser C, Burgess J, et al. Long-term control of recurrent or refractory viral infections after allogeneic HSCT with third-party virus-specific T cells. Blood Adv. 2017;1(24):2193–205. https://doi.org/10.1182/bloodadvances.2017010223.
75. Leen AM, Bollard CM, Mendizabal AM, Shpall EJ, Szabolcs P, Antin JH, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. Blood. 2013;121(26):5113–23. https://doi.org/10.1182/blood-2013-02-486324.
76. Gerdemann U, Katari UL, Papadopoulou A, Keirnan JM, Craddock JA, Liu H, et al. Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for adenovirus, EBV, and CMV infections after allogeneic hematopoietic stem cell transplant. Mol Ther. 2013;21(11):2113–21. https://doi.org/10.1038/mt.2013.151.
77. Feucht J, Opherk K, Lang P, Kayser S, Hartl L, Bethge W, et al. Adoptive T-cell therapy with hexon-specific Th1 cells as a treatment of refractory adenovirus infection after HSCT. Blood. 2015;125(12):1986–94. https://doi.org/10.1182/blood-2014-06-573725.
78. Perruccio K, Tosti A, Burchielli E, Topini F, Ruggeri L, Carotti A, et al. Transferring functional immune responses to pathogens after haploidentical hematopoietic transplantation. Blood. 2005;106(13):4397–406. https://doi.org/10.1182/blood-2005-05-1775.
79. Balduzzi A, Lucchini G, Hirsch HH, Basso S, Cioni M, Rovelli A, et al. Polyomavirus JC-targeted T-cell therapy for progressive multiple leukoencephalopathy in a hematopoietic cell transplantation recipient. Bone Marrow Transplant. 2011;46(7):987–92. https://doi.org/10.1038/bmt.2010.221.
80.•• Papadopoulou A, Gerdemann U, Katari UL, Tzannou I, Liu H, Martinez C, 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(242):242ra83-ra83. https://doi.org/10.1126/scitranslmed.3008825 **This study showed the feasibility of rapid manufacture of multipathogen specificity (CMV, EBV,**

adenovirus, HHV6 and BKV) from stem cell donors and the clinical effect in viral control in patients with viral infection and virus-related disease post transplantation.

81. Ma CK, Blyth E, Clancy L, Simms R, Burgess J, Brown R, et al. Addition of varicella zoster virus-specific T cells to cytomegalovirus, Epstein-Barr virus and adenovirus tri-specific T cells as adoptive immunotherapy in patients undergoing allogeneic hematopoietic stem cell transplantation. Cytotherapy. 2015;17(10):1406–20. https://doi.org/10.1016/j.jcyt.2015.07.005.
82. Olson AL, Muftuoglu M, Kaur I, Li L, Abueg G, Chemaly R, 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. Am Soc Hematol. 2016.
83. Pello OM, Innes AJ, Bradshaw A, Finn SA, Uddin S, Bray E, et al. BKV-specific T cells in the treatment of severe refractory haemorrhagic cystitis after HLA-haploidentical haematopoietic cell transplantation. Eur J Haematol. 2017;98(6):632–4. https://doi.org/10.1111/ejh.12848.
84. Tzannou I, Papadopoulou A, Naik S, Leung K, Martinez CA, Ramos CA, 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(31):3547–57. https://doi.org/10.1200/jco.2017.73.0655.
85. Riddell SR, Greenberg PD. The use of anti-CD3 and anti-CD28 monoclonal antibodies to clone and expand human antigen-specific T cells. J Immunol Methods. 1990;128(2):189–201.
86. 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 (New York, NY). 1992;257(5067):238–41.
87. Rooney CM, Smith CA, Ng CY, Loftin S, Li C, Krance RA, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet (London, England). 1995;345(8941):9–13.
88. Smith CA, Ng CY, Heslop HE, Holladay MS, Richardson S, Turner EV, et al. Production of genetically modified Epstein-Barr virus-specific cytotoxic T cells for adoptive transfer to patients at high risk of EBV-associated lymphoproliferative disease. J Hematother. 1995;4(2):73–9. https://doi.org/10.1089/scd.1.1995.4.73.
89. Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med. 1995;333(16):1038–44. https://doi.org/10.1056/nejm199510193331603.
90. Rooney CM, Smith CA, Ng CY, Loftin SK, Sixbey JW, Gan Y, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998;92(5):1549–55.
91. Blyth E, Clancy L, Simms R, Gaundar S, O'Connell P, Micklethwaite K, et al. BK virus-specific T cells for use in cellular therapy show specificity to multiple antigens and polyfunctional cytokine responses. Transplantation. 2011;92(10):1077–84. https://doi.org/10.1097/TP.0b013e31823328c0.
92. Blyth E, Gaundar SS, Clancy L, Simms RM, Bilmon I, Micklethwaite KP, et al. Clinical-grade varicella zoster virus-specific T cells produced for adoptive immunotherapy in hemopoietic stem cell transplant recipients. Cytotherapy. 2012;14(6):724–32. https://doi.org/10.3109/14653249.2012.663486.
93. Gaundar SS, Blyth E, Clancy L, Simms RM, Ma CKK, Gottlieb DJ. In vitro generation of influenza-specific polyfunctional CD4+ T cells suitable for adoptive immunotherapy. Cytotherapy. 2012;14(2):182–93. https://doi.org/10.3109/14653249.2011.613932.
94. Lamarche C, Orio J, Georges-Tobar V, Pincez T, Goupil M, Dahmani A, et al. Clinical-scale rapid autologous BK virus-specific T cell line generation from kidney transplant recipients with active viremia for adoptive immunotherapy. Transplantation. 2017;101(11):2713–21. https://doi.org/10.1097/tp.0000000000001698.
95. Ma CK, Clancy L, Deo S, Blyth E, Micklethwaite KP, Gottlieb DJ. Herpes simplex virus type 1 (HSV-1) specific T-cell generation from HLA-A1- and HLA-A2-positive donors for adoptive immunotherapy. Cytotherapy. 2017;19(1):107–18. https://doi.org/10.1016/j.jcyt.2016.09.013.
96.• Vasileiou S, Turney AM, Kuvalekar M, Mukhi SS, Watanabe A, Lulla P, et al. Rapid generation of multivirus-specific T lymphocytes for the prevention and treatment of respiratory viral infections. Haematologica. 2019. https://doi.org/10.3324/haematol.2018.206896 **In this study, multivirus-specific PSTs were generated to 12 immunodominant respiratory viral antigens derived from respiratory syncitial virus, influenza, parainfluenza and human metapneumonvirus via a GMP-compliant manufacturing methodology. The expanded cells showed multipathogen specificity without activity against non-infected autologous or allogeneic targets *ex vivo*. *In vivo* viral clearance was demonstrated in 4 out of 5 post-aHSCT patients with respiratory viral infections who were administered the cell product. This represents a potential new treatment for such infections, given the lack of conventional therapeutic options.**
97. Tramsen L, Koehl U, Tonn T, Latge JP, Schuster FR, Borkhardt A, et al. Clinical-scale generation of human anti-Aspergillus T cells for adoptive immunotherapy. Bone Marrow Transplant. 2009;43(1):13–9. https://doi.org/10.1038/bmt.2008.271.
98. Gaundar SS, Clancy L, Blyth E, Meyer W, Gottlieb DJ. Robust polyfunctional T-helper 1 responses to multiple fungal antigens from a cell population generated using an environmental strain of Aspergillus fumigatus. Cytotherapy. 2012;14(9):1119–30. https://doi.org/10.3109/14653249.2012.704013.
99. Tramsen L, Schmidt S, Boenig H, Latge JP, Lass-Florl C, Roeger F, et al. Clinical-scale generation of multi-specific anti-fungal T cells targeting Candida, Aspergillus and mucormycetes. Cytotherapy. 2013;15(3):344–51. https://doi.org/10.1016/j.jcyt.2012.11.014.
100. Bacher P, Jochheim-Richter A, Mockel-Tenbrink N, Kniemeyer O, Wingenfeld E, Alex R, et al. Clinical-scale isolation of the total Aspergillus fumigatus-reactive T-helper cell repertoire for adoptive transfer. Cytotherapy. 2015;17(10):1396–405. https://doi.org/10.1016/j.jcyt.2015.05.011.
101. Deo SS, Virassamy B, Halliday C, Clancy L, Chen S, Meyer W, et al. Stimulation with lysates of Aspergillus terreus, Candida krusei and Rhizopus oryzae maximizes cross-reactivity of anti-fungal T cells. Cytotherapy. 2016;18(1):65–79. https://doi.org/10.1016/j.jcyt.2015.09.013.
102. Papadopoulou A, Alvanou M, Koukoulias K, Athanasiou E, Lazaridou A, Savvopoulos N, et al. Clinical-scale production of Aspergillus-specific T cells for the treatment of invasive aspergillosis in the immunocompromised host. Bone Marrow Transplant. 2019. https://doi.org/10.1038/s41409-019-0501-9.
103. Blyth E, Clancy L, Simms R, Ma CKK, Burgess J, Deo S, et al. Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. Blood. 2013;121(18):3745.
104. Castellano-Gonzalez G, Clancy LE, Gottlieb D. Prospects for adoptive T-cell therapy for invasive fungal disease. Curr Opin Infect Dis. 2017;30(6):518–27. https://doi.org/10.1097/qco.0000000000000403.
105. Baugh KA, Tzannou I, Leen AM. Infusion of cytotoxic T lymphocytes for the treatment of viral infections in hematopoetic stem

cell transplant patients. Curr Opin Infect Dis. 2018;31(4):292–300. https://doi.org/10.1097/qco.0000000000000456.

106. Heslop HE, Slobod KS, Pule MA, Hale GA, Rousseau A, Smith CA, 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(5):925–35. https://doi.org/10.1182/blood-2009-08-239186.
107. Heslop HE, Ng CY, Li C, Smith CA, Loftin SK, Krance RA, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. Nat Med. 1996;2(5):551–5.
108. Barrett AJ, Prockop S, Bollard CM. Reprint of: virus-specific T cells: broadening applicability. Biol Blood Marrow Transplant. 2018;24(3s):S1–s6. https://doi.org/10.1016/j.bbmt.2017.12.787.
109. Chinen J, Buckley RH. Transplantation immunology: solid organ and bone marrow. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S324–S35. https://doi.org/10.1016/j.jaci.2009.11.014.
110. Prockop SE, Vatsayan A. Epstein-Barr virus lymphoproliferative disease after solid organ transplantation. Cytotherapy. 2017;19(11):1270–83. https://doi.org/10.1016/j.jcyt.2017.08.010.
111.• Withers B, Clancy L, Burgess J, Simms R, Brown R, Micklethwaite K, et al. Establishment and operation of a third-party virus-specific T cell bank within an allogeneic stem cell transplant program. Biol Blood Marrow Transplant. 2018;24(12):2433–42. https://doi.org/10.1016/j.bbmt.2018.08.024 **Findings from this study showed that only a small number of carefully selected third-party donors are required to generate a PST bank covering the majority of common HLA restrictions. Whilst this study was limited to CMV, EBV and AdV, other studies have used third party–banked cells for BKV and HHV6 [103].**
112. Sukdolak C, Tischer S, Dieks D, Figueiredo C, Goudeva L, Heuft HG, et al. CMV-, EBV- and ADV-specific T cell immunity: screening and monitoring of potential third-party donors to improve post-transplantation outcome. Biol Blood Marrow Transplant. 2013;19(10):1480–92. https://doi.org/10.1016/j.bbmt.2013.07.015.
113. Bieling M, Tischer S, Kalinke U, Blasczyk R, Buus S, Maecker-Kolhoff B, et al. Personalized adoptive immunotherapy for patients with EBV-associated tumors and complications: evaluation of novel naturally processed and presented EBV-derived T-cell epitopes. Oncotarget. 2018;9(4):4737–57. https://doi.org/10.18632/oncotarget.23531.
114. D'Orsogna LJ, van der Meer-Prins EMW, Zoet YM, Roelen DL, Doxiadis IIN, Claas FHJ. Detection of allo-HLA cross-reactivity by virus-specific memory T-cell clones using single HLA-transfected K562 cells. In: Christiansen FT, Tait BD, editors. Immunogenetics: methods and applications in clinical practice. Totowa: Humana; 2012. p. 339–49.
115. Paul S, Lindestam Arlehamn CS, Scriba TJ, Dillon MB, Oseroff C, Hinz D, et al. Development and validation of a broad scheme for prediction of HLA class II restricted T cell epitopes. J Immunol Methods. 2015;422:28–34. https://doi.org/10.1016/j.jim.2015.03.022.