Appendix

Chief investigators at participating sites

- Prof Peter J. Shaw

  Children's Hospital Westmead, Westmead, New South Wales, Australia

- Dr Matthew Greenwood

  Royal North Shore Hospital, St Leonards, New South Wales, Australia

- Associate Professor Tracey O'Brien

  Sydney Children's Hospital, Randwick, New South Wales, Australia

- Dr Chris Fraser

  Lady Cilento Children's Hospital, Brisbane, Queensland, Australia

- Dr Agnes Yong

  Royal Adelaide Hospital, Adelaide, South Australia, Australia

- Dr Lochie Teague

  Starship Children's Hospital, Auckland, New Zealand

- Prof David Gottlieb

  Westmead Hospital, Westmead, New South Wales, Australia

Methods

Detailed participant inclusion and exclusion criteria

1. Inclusion criteria
   - Recipients of myeloablative or non-myeloablative allogeneic transplantation for any indication.
   - Viral reactivation or infection with cytomegalovirus (CMV), adenovirus (AdV) or Epstein-Barre virus (EBV) *must* be present at the time of infusion as determined by:

- For CMV
    - CMV detectable by antigen detection, polymerase chain reaction (PCR) or culture in peripheral blood or tissue biopsy or by immunohistochemical staining on tissue biopsy specimen
- For AdV
    - Presence of AdV as detected by PCR, antigen detection or culture in body fluids including blood, stool, urine or nasopharyngeal secretions
- For EBV
    - Elevated EBV detectable in peripheral blood by PCR *or*
    - Presence of documented EBV related post-transplant lymphoproliferative disorder (PTLD) diagnosed by tissue biopsy *or*
    - Elevated EBV detectable in the blood by PCR *and* clinical or imaging findings consistent with EBV lymphoma

- Failure of standard therapy as defined by:
    - For CMV
        - The continued presence of detectable CMV virus or antigen after at least 14 days of antiviral therapy with intravenous Ganciclovir or Foscarnet
        - Recurrence of detectable CMV virus or antigen after at least 2 weeks of prior antiviral therapy
    - For AdV

- A less than 50% reduction in viral load in blood or any site of disease as measured by PCR or any quantitative assay despite use of therapy as determined by the treating physician;
- Standard therapy may include intravenous Cidofovir within the limits of renal function

  - For EBV
    - A less than 50% decrease in the size of EBV lymphoma or
    - A less than 50% decrease in the EBV viral load in peripheral blood despite use of appropriate therapy as determined by the treating physician which may include:
      - Reduction in immunosuppression
      - Rituximab 375mg/m^2 up to 4 infusions
      - Cytotoxic chemotherapy

- Adequate hepatic and renal function (< 3 x upper limit of normal for AST (SGOT), ALT (SGPT), < 2 x upper limit of normal for total bilirubin, serum creatinine)
- ECOG status 0 to 3 or Lansky score 30-100
- Patient (or legal representative) has given informed consent.

2. Exclusion criteria

- Use of anti-lymphocyte globulin (ALG, ATG, anti-CD52 or other broad spectrum lymphocyte antibody) given in the 4 weeks immediately prior to infusion or planned within 4 weeks after infusion.
- Grade II or greater graft versus host disease within 1 week prior to infusion.

- Prednisone or methylprednisolone at a dose of > 1 mg/kg (or equivalent in other steroid preparations) administered within 72 hours prior to cell infusion.
- ECOG status 4 or Lansky score <30
- Privately insured in or outpatients in New South Wales participating centers

3. Patients on corticosteroid and immunosuppressive medication must also adhere to the following rule:
    - Patients receiving corticosteroids at a dose of less than 1 mg/kg body weight should have received corticosteroids for 7 days without dose reduction prior to infusion of virus-specific T-cells.

Virus specific T-cell (VST) product release criteria and shipping information

1. Prior to release of VSTs for infusion the following quality control criteria will all be satisfied:
    - Post-thaw viability > 50% by trypan blue exclusion
    - Bacterial and fungal sterility on culture of pre-freeze product
    - PCR negativity for Mycoplasma antigens on pre-freeze product
    - Less than 2% $CD19^+$, less than 2% $CD14^+$
2. Cryopreserved VST products were transported to peripheral centers in a validated dry shipper with a temperature data logger and product storage and transport form, in accordance with local guidelines.

Tables

Supplementary Table S1. Acute GVHD - staging for organ involvement

Stage	Skin	Liver	Gut
1	maculopapular rash <25% of body	bilirubin 25-40 umol/L	diarrhea 500 - 1000 ml/day nausea/vomiting + biopsy
2	25-50% of body	40-74 umol/L	1000-1500 ml/day
3	generalized erythroderma	75-200 umol/L	1500-2500 ml/day
4	bullae/desquamation	>300 umol/L	>2500 ml/day or severe abdominal pain or ileus

Supplementary Table S2. Acute GVHD - overall clinical grading based on individual organ staging

Grade	Skin	Liver	Gut	Clinical Performance
I	1 to 2	0	0	Normal
II	1 to 3	1 (&/or) 1		Mild decrease
III	2 to 3	2 to 3 (&/or) 2 to 3		Marked decrease
IV	2 to 4	2 to 4 (&/or) 2 to 4		Incapacitated

Supplementary Table S3. Chronic GVHD – staging for organ involved

	Mild (Score 1)	Moderate (Score 2)	Severe (Score 3)
Skin	<18% BSA with disease signs but NO sclerotic features	19-50% BSA OR involvement with superficial sclerotic features "not hidebound" (able to pinch)	>50% BSA OR deep sclerotic features "hidebound" (unable to pinch) OR impaired mobility, ulceration or severe pruritus
Mouth	Mild symptoms with disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs with partial limitation of oral intake	Severe symptoms with disease signs on examination with major limitation of oral intake
Eyes	Mild dry eye symptoms not affecting ADL (requiring eyedrops <3x per day) OR asymptomatic signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL (requiring drops >3x per day or punctal plugs), WITHOUT vision impairment	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) OR unable to work because of ocular symptoms OR loss of vision caused by keratoconjunctivitis sicca
GI tract	Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	Symptoms associated with mild to moderate weight loss (5- 15%)	Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs OR esophageal dilation

Liver	Elevated Bilirubin, AP*, AST or ALT <2 x ULN	Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	Bilirubin or enzymes > 5 x ULN
Lungs†	Mild symptoms (shortness of breath after climbing one flight of steps) FEV1 60-79% OR LFS 3-5	Moderate symptoms (shortness of breath after walking on flat ground) FEV1 40-59% OR LFS 6-9	Severe symptoms (shortness of breath at rest; requiring 0_2) FEV1 <39% OR LFS 10-12
	LFS score calculated by adding FEV1 and DLCO score as follows: ≥80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6.		
Joints and fascia	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) AND not affecting ADL	Tightness of arms or legs OR joint contractures, erythema thought due to fasciitis, moderate decrease ROM AND mild to moderate limitation of ADL	Contractures WITH significant decrease of ROM *AND* significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
Genital tract	Symptomatic with mild signs on exam AND no effect on coitus and minimal discomfort with gynecologic exam	Symptomatic with moderate signs on exam AND with mild dyspareunia or discomfort with gynecologic exam	Symptomatic WITH advanced signs (stricture, labial agglutination or severe ulceration) AND severe pain with coitus or inability to insert vaginal speculum

This table is adapted from Figure 1 Organ scoring of chronic GVHD in Filipovich *et al*.[1]
*AP may be elevated in growing children, and not reflective of liver dysfunction.
†Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS) is preferred, but if DLCO (adjusted for hematocrit but not alveolar volume) is not available, grading using FEV1 should be used. GVHD indicates graft versus host disease; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal

Supplementary Table S4. CMV assay by participating center

Participating Center	CMV PCR quantitation assay
Lady Cilento Children's Hospital, Brisbane	Qiagen Artus® PCR kit
Royal Adelaide Hospital, Adelaide	'In-house' PCR assay
Royal North Shore Hospital, Sydney	Roche COBAS®AmpliPrep/COBAS® TaqMan® CMV test
Sydney Children's Hospital, Sydney	Argene CMV R-gene®
The Children's Hospital at Westmead, Sydney	Argene CMV R-gene®
Westmead Hospital, Sydney	Roche COBAS®AmpliPrep/COBAS® TaqMan® CMV test

Abbreviations: CMV, cytomegalovirus; PCR, polymerase chain reaction.

Supplementary Table S5. Detailed CMV drug resistance mutations

Patient	UL54	UL97
1	Q578H, T700A, E756D, A834P	M460V
5	I726T	ND
6	ND	ND
10	ND	ND
11	L802M*	ND
12	I726T	ND
13	I726T	ND
14	ND	ND
15	ND	A594V
16	ND	ND
25	ND†	ND
27	ND	ND

Abbreviations: ND- no mutation detected.
*CMV drug mutation 7 days after 1st VST.
†No sequence results available for UL54 primer 1-4 due to technical issues.

Supplementary Table S6. Non-VST targeted infections

Patient	Viral
1*, 10, 15, 18	Rhinovirus RTI
2, 3, 6, 11, 22	EBV reactivation (quantitative) not requiring therapy
2,4	Herpes zoster
4	Hepatitis C-related liver cirrhosis (progression)
5, 17*	Human metapneumovirus RTI
5*, 24*	Respiratory syncytial virus RTI
5*, 6*, 16*	Herpes simplex virus 1 ulceration (oral)
5, 11, 13	BK virus reactivation (urine)
9*	Rhinovirus + Influenza A(H3) RTI
10	Rhinovirus + Parainfluenza RTI
11*†	Disseminated adenovirus (blood, GIT, stool, urine)
13*	Polyoma virus (non-JC, non-BK) in CSF
13*	EBV PCR+ in brain, CSF and BMAT without viral cytopathic tissue change
13*	Polyoma virus in BMAT without viral cytopathic change
19*	Rotavirus diarrhea
24*	Human herpesvirus 6 (blood, marrow, CSF)
24*†	Cerebral EBV PTLD
26	Adenovirus reactivation (blood) not requiring therapy
27	Parainfluenza RTI

Patient	Bacterial
1	Aeromonas in stool culture
1*	Escherichia coli UTI
6*	Streptococcus pneumoniae pneumonia/sepsis
9*†	Pseudomonas putida pneumonia/sepsis + Enterococcus faecium pneumonia/sepsis
10	Pseudomonas aeruginosa (sputum)
14*	Serratia marcescens bacteremia (line-related)
16*	Enterococcus faecium UTI/sepsis
17*†	Enterococcus faecium pneumonia/empyema
18*	Pseudomonas aeruginosa RTI
18*, 28	Clostridium difficile (stool)
23*	Enterococcus faecalis and Enterobacter cloacae UTI
24*	Corynebacterium species (BAL)
24*	Pseudomonas aeruginosa bacteremia
Patient	**Fungal**
6	Candida albicans (sputum)
8	Candida glabrata (vaginal thrush)
9*	Saccharomyces cerevisiae (BAL)
9*, 12*	Treatment for presumed respiratory invasive fungal infection
15*†	Presumed Pneumocystis jirovecii pneumonia
24*	Candida species (BAL)
Patient	**Presumed infection - no organism identified**
7*	RTI
20*	RLL pneumonia
24*	Orbital cellulitis
26*	Febrile (not neutropenic)
Patient	**Parasitic**
13*†	Disseminated toxoplasma gondii (cerebral, CSF, blood and BMAT)

Abbreviations: VST, virus-specific T cells; RTI, respiratory tract infection; EBV, Epstein-Barr virus; GIT, gastrointestinal; CSF, cerebral spinal fluid; BM, bone marrow; PTLD, post-transplant lymphoproliferative disease; UTI, urinary tract infection; BAL, bronchoalveolar lavage; RLL, right lower lobe.

*Associated with hospitalization or prolongation of hospitalization.

†Contributed to death.

Reference

1. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant.* 2005;11(12):945-956.