Autologous adoptive T-cell therapy for recurrent or drug-resistant cytomegalovirus complications in solid organ transplant patients: A single-arm open-label phase I clinical trial

Corey Smith[1], Leone Beagley[1], Sweera Rehan[1], Michelle A. Neller[1], Pauline Crooks[1], Matthew Solomon[1], Chien-Li Holmes-Liew[2,3], Mark Holmes[2,3], Scott McKenzie[4,5], Peter Hopkins[4,5], Scott Campbell[3,5], Ross Francis[3,5], Daniel Chambers[4,5] and Rajiv Khanna[1, 5]

[1]QIMR Centre for Immunotherapy and Vaccine Development, QIMR Berghofer Medical Research Institute, Brisbane, Australia; [2]South Australian Lung Transplant Unit, Department of Thoracic Medicine, Royal Adelaide Hospital, Adelaide, Australia and Faculty of Medicine, University of Adelaide, South Australia; [3]Department of Nephrology, Princess Alexandra Hospital, Brisbane, Australia; [4]Northside Heart and Lung and Queensland Lung Transplant Service, The Prince Charles Hospital, Brisbane, Australia; [5]School of Medicine, University of Queensland, Brisbane, Australia

Address for correspondence: Prof. Rajiv Khanna, QIMR, Berghofer, Medical Research Institute, 300 Herston Rd, Brisbane, Australia 4029

Tel: 61-7-3362 0385; Fax: 61-7-3845 3510; E mail: rajiv.khanna@qimrberghofer.edu.au

Main summary of the manuscript

Autologous T-cell therapy in SOT recipients with recurrent or drug-resistant CMV-associated complications is safe and may provide clinical benefit, especially when standard therapies are not effective or are contraindicated.

Abstract

Background

Opportunistic infections including cytomegalovirus (CMV) are a major cause of morbidity and mortality in solid organ transplant (SOT) recipients. The recurrent and protracted use of anti-viral drugs with eventual emergence of drug resistance represents a significant constraint to therapy. While adoptive T-cell therapy has been successfully used in haematopoietic stem cell transplant recipients, its extension to the SOT setting poses a considerable challenge because of the inhibitory effects of immunosuppressive drugs on the virus-specific T-cell response *in vivo*, and the perceived risk of graft rejection.

Methods

In this prospective study, 22 SOT recipients (13 renal, 8 lung and 1 heart) with recurrent or ganciclovir-resistant CMV infection were recruited and of these, 13 patients were treated with *in vitro*-expanded autologous CMV-specific T cells. These patients were monitored for safety, clinical symptoms and immune reconstitution.

Results

Autologous CMV-specific T-cell manufacture was attempted for 21 patients, and was successful in 20 cases. The use of this adoptive immunotherapy was associated with no therapy-related serious adverse events. Eleven (84%) of the thirteen treated patients showed improvement in symptoms, including complete resolution or reduction in DNAemia, CMV-associated end organ disease and/or

the cessation or reduced use of anti-viral drugs. Furthermore, many of these patients showed co-incident increased frequency of CMV-specific T cells in peripheral blood following completion of T-cell therapy.

Conclusions

The data presented here demonstrate for the first time the clinical safety of CMV-specific adoptive T-cell therapy and its potential therapeutic benefit for SOT patients with recurrent and/or drug-resistant CMV infection or disease.

Key words: immunotherapy, T cells, virus infection, transplant, cytomegalovirus

Introduction

Clinical management of cytomegalovirus (CMV) infection in solid organ transplant (SOT) recipients remains a major challenge. The incidence of early CMV-associated complications in SOT recipients has significantly reduced since the advent of virostatic therapy based on ganciclovir [1]. The inhibition of viral reactivation by either the prophylactic or pre-emptive administration of ganciclovir has therefore become critical in the prevention of CMV-associated disease. However, late CMV reactivation can be more problematic to manage, especially in patients who are unable to reconstitute anti-viral T cell immunity [2]. Furthermore, the emergence of ganciclovir-resistant CMV reactivation or disease poses major difficulties in clinical management, with significant morbidity and mortality due to drug-associated toxicity, immunomodulatory impact and allograft loss [3]. Alternative safe and effective therapeutic options for ganciclovir-resistant CMV are lacking. Additional anti-viral management strategies, using foscarnet or cidofovir, are associated with nephrotoxicity, and require intravenous administration and hospitalisation. Genes conferring resistance to ganciclovir are also associated with resistance to foscarnet and cidofovir. Newer antiviral therapeutics such as maribavir and letermovir may offer alternatives for the treatment of CMV, with the potential for reduced side-effects [4, 5]. Reduction in immunosuppression can be

used to improve viral control, but increases the risk of graft rejection. Over the last decade, a number of immune-based diagnostic tests have been developed which can help to identify patients who are at increased risk of developing early or late CMV-associated complications [6]. These studies have clearly shown that the reconstitution of anti-viral T-cell immunity is absolutely critical in protecting SOT recipients from CMV infection or reactivation [7-9].

The reconstitution of CMV immunity through the administration of CMV-specific T cells offers an attractive option to enhance the control of CMV in SOT recipients. This approach has been used very effectively to treat CMV-associated complications in haematopoietic stem cell transplant (HSCT) recipients. However, this is typically dependent upon the use of HSCT donor-derived material to generate CMV-specific cell therapy, whereas in the context of SOT recipients, autologous immune cells from heavily immunosuppressed individuals are required to generate an effective T-cell therapy. Whilst showing some promising results with an autologous CMV-specific T-cell therapy in an SOT patient, a previous case study also raised potential safety concerns [10]. As a consequence, the development of this approach has been limited due to the perceived difficulties in generating T cells from highly immunosuppressed SOT recipients, and the potential risks associated with graft rejection following T-cell administration. Despite these concerns, we recently demonstrated in case reports the use of autologous T-cell therapies for CMV disease in two transplant recipients with no evident side effects [11, 12] As a consequence of these promising results, we initiated a formal phase I study to assess the safety of autologous therapy in a larger cohort of lung, heart and kidney transplant recipients with CMV-associated complications. We demonstrate here the capacity to generate CMV-specific T cells from the majority of recruited SOT recipients despite these underlying complications. Following adoptive immunotherapy, we detected only grade 1 and 2 adverse events potentially associated with T-cell infusion, and saw no evidence for any impact on graft function or survival. More importantly, the majority of the patients showed resolution of clinical symptoms, which was coincident with anti-viral T-cell reconstitution in a proportion of the treated patients.

Materials and Methods

Patient recruitment and study design

This single-arm open-label phase I study was performed according to the principles of the Declaration of Helsinki and approved by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee, The Prince Charles Hospital Human Research Ethics Committee and the Royal Adelaide Hospital Research Ethics Committee. This study was registered under the Australian New Zealand Clinical Trial Registry (ACTRN12613000981729). Patients were deemed eligible for the study if they met at least one of the four following criteria: (A) CMV reactivation or disease (as defined by histology) following successful initial therapy; (B) Persistent CMV disease, i.e. no response to 2 weeks of salvage foscarnet or other second-line anti-viral agent; (C) Persistent CMV replication (more than 6 weeks by PCR) despite appropriate anti-viral therapy; or (D) Any CMV reactivation or disease where anti-viral therapy is contraindicated on the basis of intolerance or end organ limitation (e.g. renal impairment, marrow dysfunction). CMV disease refers to organ dysfunction due to CMV infection and/or evidence of tissue invasive viral infection. Anti-viral drug therapy was administered as per the institutional guidelines. Standard prophylaxis for lung transplant recipients was valganciclovir until 9 months post-transplant. CMV IgG and lifelong valganciclovir was administered to D+R- lung transplant patients. Standard prophylaxis for renal transplant recipients included 3 months of valganciclovir for D+R+ and D-R+ patients, 6 months of valganciclovir for D+R- patients, and no treatment for D-R- patients. Standard prophylaxis for heart transplant recipients was valganciclovir until 6 months post-transplant for D+R+ patients, valganciclovir until 12 months for D+R- patients, and valacyclovir for D-R- patients. Patients received up to six doses of *in vitro*-expanded T cells at 1–2 × 10^7 cells/m^2 at fortnightly intervals. Each participant was monitored for safety, clinical symptoms, viral load and immune reconstitution for 28 weeks after the completion of adoptive T-cell therapy. Viral load monitoring was undertaken using COBAS TaqMan PCR assay

(Roche Molecular Diagnostics, Pleasanton, CA) as described previously [13]. The lower limit of detection in this assay is 150 copies/mL.

Manufacture and adoptive transfer of CMV-specific T cells

To manufacture the CMV-specific T-cell therapy for each patient, peripheral blood mononuclear cells (PBMC) were stimulated with a clinical-grade custom peptide pool (JPT Technologies, Berlin, Germany) which includes pre-defined HLA class I and class II-restricted peptide epitopes from pp65, pp50, IE-1, gH and gB (Supplemental Table 1) [14-16]. These cells were cultured in Grex-10 culture flasks (Wilson Wolf Corporation, Saint Paul, MN) at a starting cell density of 2–5 × 10^6 cells/cm^2. These cultures were supplemented with recombinant IL-21 (40 ng/mL) on Day 0, and recombinant IL-2 (120 IU/mL) on Day 2 and every three days thereafter. On Day 14, expanded T cells were harvested and frozen in 1 mL single-dose aliquots in Albumex 4 (CSL Behring, Broadmeadows, Australia) containing 10% dimethyl sulfoxide (WAK-Chemie Medical GmbH, Steinbach, Germany). A dedicated good manufacturing practice-accredited cell therapy manufacturing facility (Q-Gen Cell Therapeutics) was used for T-cell therapy manufacturing and storage. At the completion of *in vitro* culture, T cells were phenotypically and functionally characterised using Multitest 6-Colour TBNK Reagent (BD Biosciences, San Jose, CA) and intracellular cytokine staining (detailed below). Microbiological testing was performed as recommended under British Pharmacopoeia 2015 (Appendices XVIE and XVIA) on all T-cell products, for the presence/absence of microbiological contamination (fungal and bacterial, including mycoplasma) and endotoxin (<3 EU/mL). For adoptive transfer, T cells were thawed into 19 mL clinical grade normal saline and infused intravenously over a period of 5–10 min.

Intracellular cytokine analysis of CMV-specific T cells

To characterise the T-cell therapy and PBMC isolated from follow-up blood samples, cells were stimulated with CMV peptide epitopes and assessed for the expression of IFN-γ, TNF and IL-2, and mobilisation of CD107 using intracellular cytokine assay as described previously [17]. Cells were

acquired using a BD LSR Fortessa with FACSDiva software (BD Biosciences). Post-acquisition and Boolean analyses were performed using FlowJo software (FlowJo LLC, Ashland, OR).

Results

Patient characteristics and T-cell therapy manufacturing

The primary objective of this study was to assess the safety of autologous T-cell therapy in SOT recipients with CMV-associated complications. The clinical characteristics of the participants included in this study are provided in Table 1. CMV reactivation was managed in patients 1553PAH06, 1553PAH08 and 1553PAH09 by a combination of antiviral therapy and reduction in immunosuppression prior to enrolment in the clinical trial. In total, 21 SOT recipients (13 renal, 8 lung, 1 heart) were included in the study. Two of the lung transplant patients included in the follow-up analyses were previously treated under the Special Access Scheme of the Therapeutic Goods Administration [11, 12].. Of the 21 patients analysed, 13 SOT recipients were allocated to intervention and received a maximum of six doses of adoptive T-cell therapy, whilst one patient discontinued therapy after a single dose and no immune monitoring was performed. Of the remaining eight patients, seven did not receive adoptive T-cell therapy due to improvement in their clinical status, and we failed to manufacture therapy for one patient.

CMV-specific T cells were successfully expanded from 20 of the 21 patients, and their antigen specificity was assessed by intracellular IFN-γ analysis (Table 2). The CMV peptide pool-expanded cells were predominantly $CD3^+$ $CD8^+$ T cells (Fig 1A), with a median specificity of 51.2% (Fig 1B). The frequency of IFN-γ-producing $CD8^+$ T cells did not differ significantly between kidney and lung/heart transplant recipients (Fig. 1C) or pre-transplant CMV seropositive and CMV seronegative individuals (Fig. 1D). A marked improvement in the polyfunctionality of the CMV-specific T cells was observed following *in vitro* expansion, with an increase in the proportion of cells capable of producing IFN-γ, TNF and CD107a (Fig 1E). T cells generated from the majority of the patients showed reactivity against multiple peptide epitopes encoded by multiple CMV antigens (Table 2).

Clinical outcomes following adoptive immunotherapy

None of the patients who received adoptive CMV-specific T-cell therapy showed treatment-related grade 3, 4 or 5 adverse events (Table 3 and Supp. Table 2). All adverse events that were deemed at least possibly attributable to T-cell infusion were grade 1 and 2, and included fatigue and malaise. Importantly, no adverse events associated with a change in the graft status were detected. Clinical follow-up of patients allocated to T-cell therapy intervention indicated that 11 of the 13 patients showed objective improvement in their symptoms. These included reduction or resolution of CMV reactivation and/or disease and improved response to anti-viral drug therapy. The median peak viral load prior to adoptive T-cell therapy in the 11 patients who showed a clinical response was 3.2×10^4 CMV copies/mL of blood (range 1.4×10^3 – 3.44×10^5 copies). Following adoptive immunotherapy, the median viral load dropped to 1.2×10^3 CMV copies/mL of blood (range 0–7.9×10^3 copies; Table 4). Furthermore, many of these patients showed resolution of CMV disease symptoms (Table 4). More importantly, following the completion of adoptive T-cell therapy, the use of anti-viral drug therapy was either completely stopped (5/11) or significantly reduced (6/11; Table 4).

Virological and immunological monitoring following T-cell therapy

To assess the impact of adoptive T-cell therapy on CMV-specific T cell immune reconstitution, we conducted a longitudinal intracellular cytokine analysis following immunotherapy, and overlaid this analysis with virological monitoring in each patient. Representative data from eight patients, including seven who showed an objective response to adoptive immunotherapy, are shown in Fig. 2. The shaded box represents the analysis period pre-treatment and the arrows represent each infusion of autologous *in vitro*-expanded CMV-specific T cells. This analysis revealed evidence of immunological reconstitution post-therapy in association with control of viraemia. This is best exemplified in patient 1553PAH08, whose proportion of IFN-γ-producing CMV-specific T cells

increased from 0.03% prior to the first infusion to 9.3% at the completion of the follow-up period, with a concordant reduction in viral load and cessation of anti-viral drug therapy (Fig. 2A). A similar improvement in peripheral T-cell immunity following the commencement of T-cell infusions was also evident in other patients, including 1553PAH09, 1553PCH02 and 1553PCH04 (Fig. 2A). It is important to note that immune reconstitution in these patients was observed in spite of the continuation of immunosuppressive therapies prescribed prior to adoptive T-cell therapy (Table 1). Coincident with immune reconstitution, we also observed improvement in the functional quality of CMV-specific T-cell responses, characterised by an increased proportion of T cells co-expressing IFN-γ, TNF and CD107 (Fig. 2B). In contrast, patient 1553RAH01, who did not respond clinically to therapy, showed no evidence of immunological reconstitution post-therapy. Patient 1553PCH01 experienced a decline in CMV-specific T cell immunity, coincident with contracting a secondary infection that caused bilateral bronchopneumonia. Follow-up immunological analysis was not possible for patient 1553PCH03, who died early after the commencement of therapy due to complications related to CMV infection. Interestingly, although patients 1553PAH06 and 1553PCH05 showed clinical improvement, there was no change in the frequency of CMV-specific T cells in their peripheral blood following adoptive T-cell therapy.

Discussion

In this study we describe the first formal evaluation of autologous CMV-specific T-cell therapy in SOT recipients. In a cohort of patients recruited due to evidence of drug resistance or intolerance, or persistent viral reactivation or disease, we demonstrate no evidence of severe adverse events or any negative impact on the graft following T-cell administration. While this study was not designed to access efficacy, we saw evidence of viral control following T-cell therapy in the majority of treated patients. This provides a platform to evaluate the potential efficacy of T-cell therapy in a larger cohort of SOT recipients, with the inclusion of a control arm.

The therapeutic efficacy of donor-derived CMV-specific T-cell therapy in HSCT recipients has provided clear evidence of the potential therapeutic benefits of adoptive cell therapy in both preventing and treating viral disease in transplant patients [18, 19]. In contrast with CMV-specific T cells generated from healthy CMV-seropositive individuals for administration in HSCT recipients, which has now been administered to over 100 patients [20, 21], autologous CMV-specific immunotherapy in SOT recipients is dependent upon the capacity to generate CMV-specific T cells from immunosuppressed individuals. However, case reports in both SOT and HSCT recipients have shown its potential efficacy [10-13]. Consistent with these previous observations, we were able to generate CMV-specific T cells from 20 of the 21 patients for whom T-cell manufacture was attempted in the current study. Our observations indicate that despite the heavy immunosuppressive regimes used to prevent graft rejection, the majority of these individuals were able to prime a CMV-specific T-cell response and, in some cases, patients had a high precursor frequency in their PBMC prior to T-cell expansion. We did note functional defects in the CMV-specific T cells in the peripheral blood of SOT recipients as recently reported [22], characterised by a reduced capacity to express TNF and IFN-γ, which could potentially mediate increased sensitivity to viral reactivation. Importantly, and likely due to the removal of these cells from the

immunosuppressive environment, this phenotype could be reversed following *in vitro* stimulation, with the majority of expanded CMV-specific T cells co-expressing CD107a, TNF and IFN-γ.

Both virological and immunological monitoring provided evidence of the potential benefit that immunological reconstitution following adoptive immunotherapy can have upon viral control in SOT patients. Although the study was not designed to assess efficacy, and in some patients the changes were transient, there was evidence in multiple patients that immune reconstitution coincided with reduction in or resolution of viral reactivation. This is particularly important for the SOT recipients who had developed drug resistance or had either ongoing or a previous history of CMV-associated end-organ disease. Furthermore, we also showed that adoptive T-cell therapy can be safely used, concurrently with immunosuppressive therapies, for preventing CMV-associated complications in patients who are unable to tolerate standard anti-viral drug therapy.

In summary, whilst efficacy needs to be confirmed in controlled trials, this prospective, multi-centre, open-label study has confirmed the feasibility and safety of CMV-targeted autologous adoptive T-cell therapy in SOT recipients. It therefore provides an important platform for subsequent controlled studies and for the future application of adoptive T-cell therapy in SOT recipients, especially in a setting where no appropriate treatment options are available.

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Conflict of interest

Potential conflicts of interest. RK and CS receive research and consultancy funding from Atara Biotherapeutics Inc. RK is also appointed as an advisor on the Atara Biotherapeutics Scientific Advisory Board. RK and CS hold international patents or patent applications which cover CMV epitope sequences and their use in adoptive immunotherapy. RF has received non-financial support from Amgen Pharmaceuticals and Novartis Pharmaceuticals, outside the submitted work. SC has received non-financial support from Astellas, Merck Sharp & Dohme, Shire and Novartis, as well as personal fees from Novartis, outside the submitted work. SM has received non-financial support and personal fees from Astellas, Bayer, Boehringer Ingelheim, Bristol-Meyers Squibb, Novartis, Pfizer, and Servier all outside the submitted work. Other authors declare no conflict of interest.

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Figure Legends

Figure 1: **Phenotypic and functional characteristics of CMV-specific T cells expanded for adoptive immunotherapy**. (A) The phenotypic characteristics of CMV peptide pool-expanded T cells were assessed using standard TBNK analysis, measuring the surface expression of CD3 (T cells), CD8 ($CD8^+$ T cells), CD4 ($CD4^+$ T cells), CD16 and CD56 (NK cells) and CD19 (B cells). (B) PBMC (*ex vivo*) or cultured T cells (Day 14) were assessed for the intracellular production of IFN-γ following recall with the CMV peptide pool or with individual HLA-matched peptides. The data represent the proportion of $CD8^+$ T cells producing IFN-γ. (C) Comparison of CMV-specific T cell responses generated from either kidney or heart/lung transplant patients (D) Comparison of CMV-specific T-cell responses generated from either CMV-seronegative recipients (R-) or CMV-seropositive recipients (R+). (E) CMV peptide pool-stimulated T cells were assessed for intracellular cytokine production (IFN-γ, TNF, IL-2) and degranulation (CD107a) following recall with the CMV peptide pool. The data represent the proportion of the total antigen-specific T cells producing each combination of effector functions.

Figure 2: **Immunological and virological monitoring following adoptive cellular therapy.** (A) PBMC samples from patients before and after T-cell therapy were assessed for IFN-γ-producing CMV-specific T cells following stimulation with the CMV peptide pool. The data represent an overlay of the number of IFN-γ-producing $CD8^+$ T cells and the CMV load in copies/mL from eight patients. The shaded area indicates the time period prior to adoptive T-cell therapy and the arrows represent T-cell infusions. (B) Polyfunctional cytokine (IFN-γ, TNF, IL-2) and degranulation (CD107a) analysis was performed on PBMC samples following stimulation with the CMV peptide pool. Heat-maps represent the

proportion of total antigen-specific T cells producing each combination of effector functions. For 1553RAH01, polyfunctional analysis could not be performed due to the low level of CMV-specific T cell immunity.

Table 1: Clinical profile of SOT recipients enrolled in the study

Patient Code	Age/ Sex	Organ	Criteria for Recruitment[a]	Immuno-suppression	Anti-Viral Treatment	Drug Resistance	CMV Disease History	Donor/ Recipient CMV Status
1553PAH01	61M	Kidney	B,C	TAC; MMF; MePRD	GCV; FOS; IVIG	GCV[b]	Pan-enteritis[c]	+/-
1553PAH02	45F	Kidney	A	TAC; MMF; PRD	VGCV	Nil[d]	Colitis[c]	+/+
1553PAH03	57M	Kidney	A	CSA; PRD	VGCV; GCV	Nil	None	Unk/+
1553PAH04	64F	Kidney	A	TAC; MMF; PRD	VGCV	Nil	Colitis[e]	+/+
1553PAH05	23M	Kidney	C	TAC; MMF; PRD	VGCV; GCV; FOS; LEF	GCV[b]	Colitis[c] Pneumonitis[e]	+/+
1553PAH06	57M	Kidney	A	TAC; MMF; PRD	VGCV; GCV	GCV	Colitis[e]	-/-
1553PAH07	26F	Kidney	A	TAC; MMF; PRD	VGCV; GCV	Nil	Colitis[e]	+/+
1553PAH08	26M	Kidney	B,C	TAC; MMF; PRD	VGCV; GCV; FOS	Nil	None	+/-
1553PAH09	44M	Kidney	C	TAC; MMF; PRD; MePRD	VGCV; GCV	Nil	None	+/-
1553PAH10	53F	Kidney	A	TAC; MMF; PRD	VGCV; GCV	Nil	None	+/+
1553PAH11	45M	Kidney	C	TAC; MMF; PRD	VGCV; GCV	Nil	None	+/-
1553PAH12	43F	Kidney	C	TAC; MMF; PRD	VGCV; GCV	Nil	Colitis[c]	+/-
1553PAH13	53M	Kidney	A	TAC; MMF; PRD	VGCV; GCV	Nil	None	+/+
1553PCH01	62M	Lung	B	EVR, PRD	GCV; FOS	GCV	Oesophagitis[c]	-/-
1553PCH02	55M	Lung	A	TAC; MMF; EVR; AZA; PRD	VGCV; GCV; FOS; IVIG	GCV	Colitis[c]	+/+

1553PCH03	62F	Lung	C	TAC; MMF; EVR; AZA; MYF	VGCV; GCV; FOS; IVIG	GCV, CDV	Pneumonitis[c]	+/-
1553PCH04	29F	Lung	A	CSA; TAC; MMF; EVR	VGCV: GCV; FOS; IVIG; LEF	GCV	Pneumonitis[c] Colitis[c]	+/-
1553PCH05	66M	Lung	A	CSA; TAC; MMF; AZA	VGCV; GCV	Nil	Colitis[c] Mouth ulcer[c]	+/-
1553RAH01	64M	Lung	D	TAC; PRD	VGCV; GCV; IVIG	Nil	Pneumonitis[c]	+/-
SASRAH01	41F	Lung	A,B	TAC; PRD; AZA; EVR; LEF; MePRD	VGCV; GCV; FOS	GCV; UL97; L595S	Hepatitis[c] Pneumonitis[c]	+/-
SASSVH01	56M	Lung	A,B	NA	VGCV: GCV; FOS; CDV	GCV; L595S; FOS; UL54; L415N; S734P; I840T	None	+/-
1553PCH06	61M	Heart	D	CSA; MMF	VGCV	Nil	None	+/+

Abbreviations: AZA, azathioprine; CDV, cidofovir; CSA, cyclosporin; EVR, everolimus; FOS, foscarnet; GCV, ganciclovir; LEF, leflunomide; MePRD, methylprednisolone; MMF, mycophenolate; NA, not available; PRD, prednisolone; TAC, tacrolimus; VGCV, valganciclovir.

[a] A: CMV reactivation or disease (as defined by histology) following successful initial therapy; B: Persistent CMV disease, i.e. no response to 2 weeks of salvage foscarnet or other second line anti-viral agent; C: Persistent CMV replication (more than 6 weeks by PCR) despite appropriate anti-viral therapy; D: Any CMV reactivation or disease where anti-viral therapy is contraindicated on the basis of intolerance or end organ limitation (e.g. renal impairment, marrow dysfunction).

[b] Clinical evidence of resistance, standard mutations not detected.

[c] Histologically confirmed CMV disease.

[d] Nil: No evidence of resistance.

[e] Clinical diagnosis of CMV disease, not confirmed by histology.

Table 2: CMV-specific reactivity of *in vitro*-expanded T cells from SOT recipients

Patient Code	Recipient HLA Type	Donor HLA Type	CMV-Specific T cell Response[a]		CMV Epitopes Targeted
			Ex vivo	Day 14	
1553PAH01	A1 A11 B8 B60	A31 A33 B51 B58	0.24	0.0	NA
1553PAH02	A2 A34 B44 B75	A1 A2 B44 B44	5.15	79.9	NLV (pp65, A2); VLE/YIL (IE-1, A2) DEL (IE-1, B44)
1553PAH04	A2 A25 B7 B35	A2 A24 B7 B62	0.43	47.6	RPH (pp65, B7); TPR (pp65, B7)
1553PAH05	A24 A34 B56 Cw1 Cw7	A3 A31 B51 B7	0.05	24.3	QYD (pp65, A24)
1553PAH06	A2 A32 B7 B27	A2 A11 B13 B46	17.67	77.2	NLV (pp65, A2); RPH (pp65, B7); TPR (pp65, B7)
1553PAH07	A2 A2 B44 B51	A2 A2 B7 B44	0	36.5	NLV (pp65, A2)
1553PAH08	A1 A29 B8 B52	A1 A2 B44 B57	0	22.9	VTE (pp50, A1); ELR/K (IE-1, B8);
1553PAH09	A3 A29 B44 B45 Cw6 Cw16	A2 A3 B7 B51	0.09	48.4	TRA (pp65, Cw6)
1553PAH10	A11 A24 B7 B55 Cw7 Cw7	A2 A31 B62 B60	3.14	66.0	RPH (pp65, B7); TPR (pp65, B7); QYD (pp65, A24); AYA (IE-1, A24)
1553PAH11	A3 A24 B35 B60	A2 A23 B44 B62	3.21	59.1	IPS (pp65, B35); AYA (IE-1, A24)
1553PAH12	A25 A68 B8 B35	A1 A11 B8 B35	0.44	61.6	IPS (pp65, B35); ELR/K (IE-1, B8)
1553PAH13	A2 A11 B35 B35 Cw4 Cw4	A11 A32 B58 B62 Cw4 Cw7	3.21	60.2	NLV (pp65, A2); IPS (pp65, B35)
1553PCH01[b]	A3 A31 B38 B65 Cw8	A2 A3 B7 B65	0.00	56.9	KAR (IE-1; A31)
1553PCH02	A1 A3 B42 B57 Cw17	A2 A3 B7 B62	0.87	57.3	TRA (pp65, Cw6); VTE (pp50, A1)
1553PCH03	A1 A3 B7 B8 Cw7 Cw7	A1 A2 B51 B57	8.74	48.0	RPH (pp65, B7); TPR (pp65, B7); YSE (pp65, A1); VTE (pp50; A1); QIK (IE-1; B8); CRV (IE-1; Cw7)
1553PCH04	A2 A11 B44 B50 Cw5 Cw6	A32 A62 B44 B53	6.35	63.6	TRA (pp65, Cw6)
1553PCH05	A2 A3 B27 B49 Cw1 Cw7	A3 A29 B50 B51	1.32	26.9	NLV (pp65, A2)

1553RAH01	A2 A23 B44 B44	NA	0.00	31.9	NA
SASRAH01[c]	A1 A11 B7 B35 Cw4 Cw7	NA	0.73	11.68	RPH (pp65, B7); TPR (pp65, B7); YSE (pp65, A1); VTE (pp50, A1); IPS (pp65, B35);
SASSVH01[c]	A1 A3 B7 B8 Cw7 Cw7	NA	14.22	43.94	RPH (pp65, B7); TPR (pp65, B7); VTE (pp50; A1); ELR (IE-1; B8); QIK (IE-1; B8);
1553PCH06	A2 A24 B44 B56 Cw1 Cw5	A1 A3 B7 B8	17.13	71.4	NLV (pp65, A2); VLE/YIL (IE-1, A2)

Abbreviations: NA, not available

[a] CMV responses were determined as the proportion of $CD8^+$ T cells producing IFN-γ

[b] The KAR peptide was added to the CMV peptide pool for stimulation

[c] HLA-specific peptide pools were generated to manufacture T cells for these patients

Table 3: Safety assessment after T-cell therapy

Adverse events[a]	Number of incidents	Frequency of occurrence[b]
Grade 1 – Mild		
Nausea	2	1 (8%)
Malaise	2	1 (8%)
Fatigue	2	1 (8%)
Altered taste sensation	2	1 (8%)
Grade 2 – Moderate		
Fatigue	1	1 (8%)
Halitosis	1	1 (8%)
Microangiopathic haemolytic anaemia	1	1 (8%)

[a] Events possibly or probably related to the T-cell therapy. No adverse events were deemed to be definitely related to the T-cell therapy.

[b] Number of patients who received T cell therapy and experienced the adverse event.

Table 4: Clinical responses following adoptive T-cell therapy

Patient Code	Organ	Timing of First Infusion Post-Transplant (days)	No. of Infusions	Total T-cell Dose ($\times 10^6$)	Peak CMV Load Pre-Infusion ($\times 10^3$)	CMV Load at First Infusion ($\times 10^3$)	Peak CMV Load Post-Infusion ($\times 10^3$)	Anti-Viral Therapy Pre-Infusion	Anti-Viral Therapy Post-Infusion	Clinical Symptoms/ Management Post-Infusion
1553PAH05	Kidney	411	1	45.25	1.4	0.32	0.32	VGCV; GCV; FOS; LEF	FOS; LEF[a]	DNAemia and CMV disease symptoms resolved
1553PAH06	Kidney	262	6	245	12	0.13	0.78	VGCV; GCV	Nil	DNAemia and CMV disease symptoms resolved
1553PAH08	Kidney	187	5	226	54	2.8	7.9	VGCV; GCV; FOS	VGCV; IVIG	Reduction in DNAemia and resolution of CMV disease symptoms
1553PAH09	Kidney	237	5	180	10	0	1.4	VGCV; GCV	VGCV	Diarrhoea resolved; immunosuppression reduced
1553PCH01	Lung	3403	6	210	8	0	0.12	GCV; FOS	Nil	FOS stopped without viral reactivation
1553PCH02	Lung	724	3	108	48	0	2.3	VGCV; GCV; FOS; IVIG	Nil	Reduction in DNAemia
1553PCH03	Lung	1480	2	42	12	24	45	VGCV; GCV; FOS; IVIG	GCV	Died of multi-organ failure
1553PCH04	Lung	979	6	168	17	4.7	2.9	VGCV; GCV; FOS; IVIG; LEF	IVIG; LEF	Reduction in DNAemia

1553PCH05	Lung	1075	6	241	47	0	0	VGCV; GCV	VGCV	Reduction in DNAemia
1553RAH01	Lung	861	3	104	18.9	1.3	17.6	VGCV; GCV	GCV; FOS; IVIG	Ongoing elevated CMV PCR, however no end-organ disease
SASRAH01	Lung	266	4	120	344	0	1	VGCV; GCV; FOS	Nil	Drug-independent resolution of DNAemia
SASSVH01[b]	Lung	790	2 1	38.7 (cycle 1) 22.2 (cycle 2)	95.4	1.8	2.5	VGCV; GCV; FOS; CDV	CDV	Reduction in DNAemia
1553PCH06	Heart	637	6	204	1.5	0	0	VGCV	Nil	VGCV ceased after T cell therapy

Abbreviations: CDV, cidofovir; FOS, foscarnet; GCV, ganciclovir; IVIG, intravenous CMV immunoglobulin; LEF, leflunomide; VGCV, valganciclovir.

[a] Used as part of CMV treatment due to its antiviral activity.

[b] This patient received two cycles of T-cell therapy.

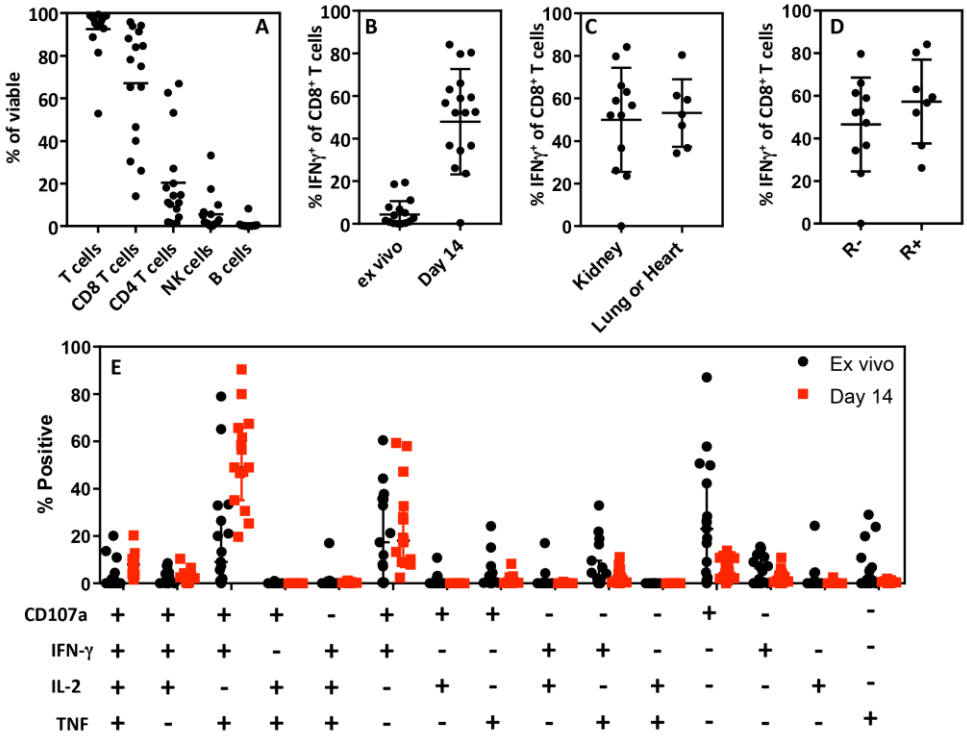

Figure 1

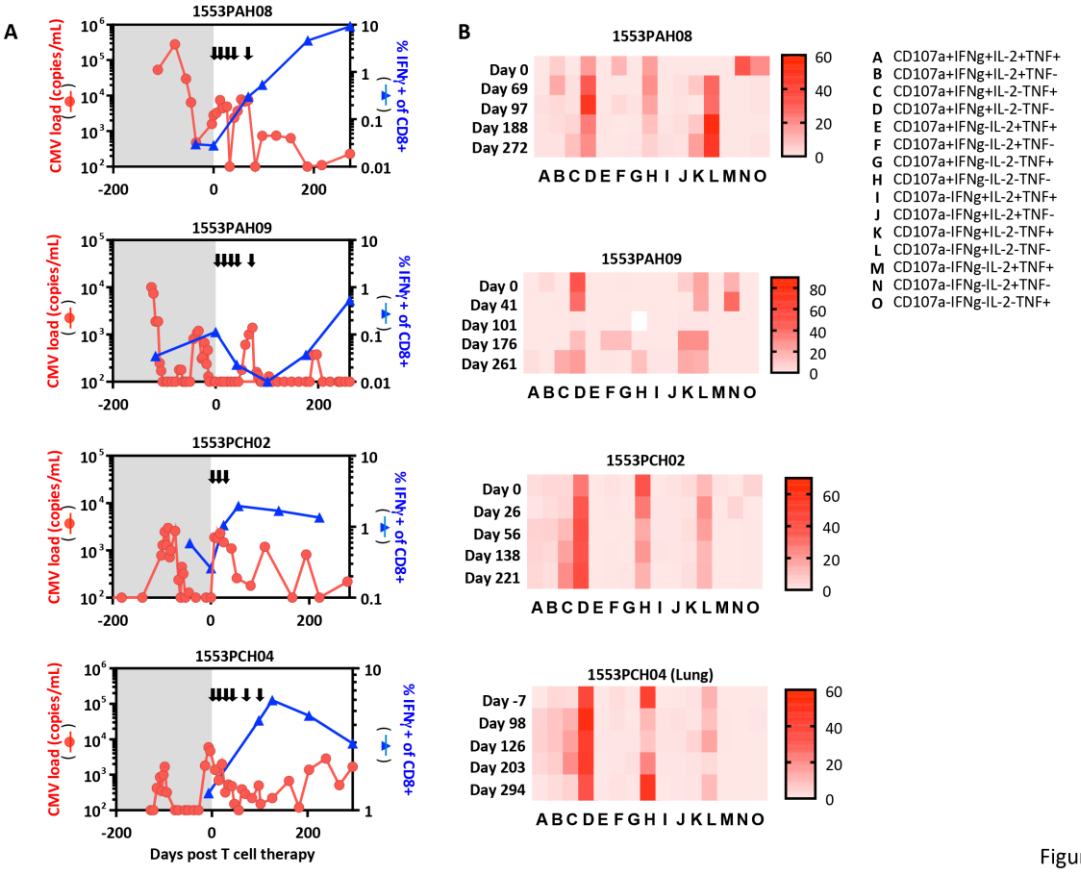

Figure 2

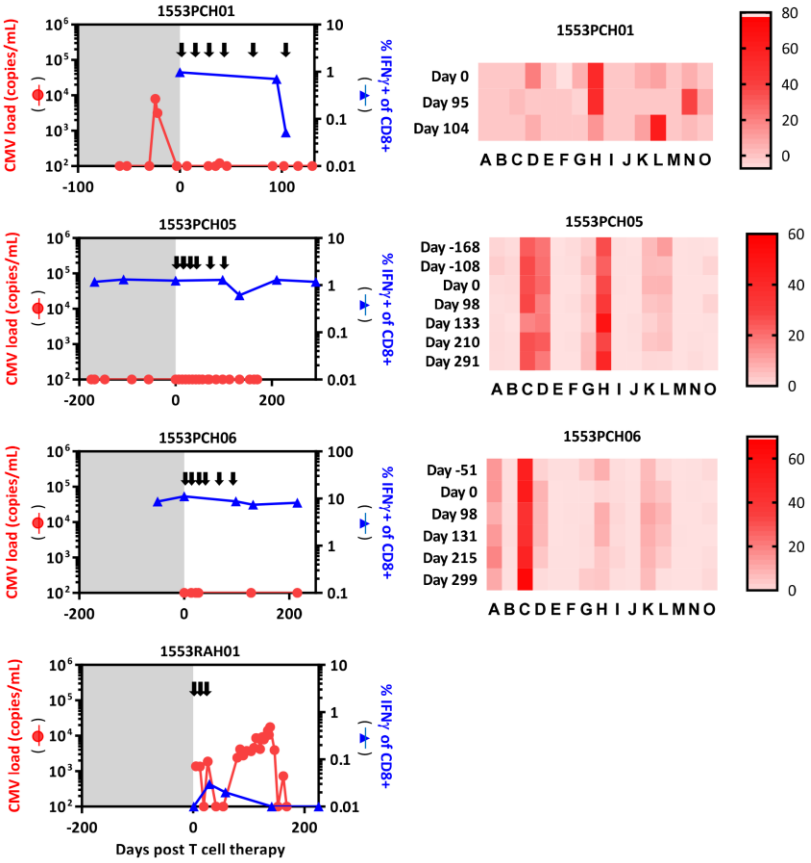

Figure 2