Taylor & Francis
Taylor & Francis Group

Acta Clinica Belgica
International Journal of Clinical and Laboratory Medicine

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/yacb20

Treatment of a patient with severe cytomegalovirus (CMV) infection after haploidentical stem cell transplantation with donor derived CMV specific T cells

Joline Ingels, Saskia De Smet, Kelly Heyns, Nele Lootens, Jonas Segaert, Tom Taghon, Georges Leclercq, Karim Vermaelen, Evelyne Willems, Etienne Baudoux, Tessa Kerre, Frédéric Baron & Bart Vandekerckhove

To cite this article: Joline Ingels, Saskia De Smet, Kelly Heyns, Nele Lootens, Jonas Segaert, Tom Taghon, Georges Leclercq, Karim Vermaelen, Evelyne Willems, Etienne Baudoux, Tessa Kerre, Frédéric Baron & Bart Vandekerckhove (2021) Treatment of a patient with severe cytomegalovirus (CMV) infection after haploidentical stem cell transplantation with donor derived CMV specific T cells, Acta Clinica Belgica, 76:6, 482-486, DOI: 10.1080/17843286.2020.1752446

To link to this article: https://doi.org/10.1080/17843286.2020.1752446

Published online: 14 Apr 2020.

ACTA CLINICA BELGICA
2021, VOL. 76, NO. 6, 482–486
https://doi.org/10.1080/17843286.2020.1752446

Taylor & Francis
Taylor & Francis Group

CASE REPORT

Treatment of a patient with severe cytomegalovirus (CMV) infection after haploidentical stem cell transplantation with donor-derived CMV-specific T cells

Joline Ingels[a,b], Saskia De Smet[b], Kelly Heyns[c], Nele Lootens[b], Jonas Segaert[d], Tom Taghon[a], Georges Leclercq[a], Karim Vermaelen[c], Evelyne Willems[e], Etienne Baudoux[e], Tessa Kerre[a,d], Frédéric Baron[e] and Bart Vandekerckhove[a,b]

[a]Department of Diagnostic Sciences, Ghent University, Ghent, Belgium; [b]Cell Therapy Unit, Department of Regenerative Medicine, Ghent University Hospital, Ghent, Belgium; [c]Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium; [d]Department of Internal Medicine, Ghent University Hospital, Ghent, Belgium; [e]Department of Medicine, Division of Hematology, University of Liège, Liège, Belgium

ABSTRACT

Objectives: Cytomegalovirus (CMV) infection is one of the most common complications in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients. The classic antiviral treatments have shown clinical efficacy but are often associated with drug resistance. Reconstitution of CMV-specific cellular immunity is essential in controlling CMV infection; therefore, adoptive transfer of CMV-specific T cells is a promising treatment option. We treated a patient with a multidrug resistant CMV infection after haploidentical HSCT with CMV-specific T cells.

Methods: The T cells were derived from the HSCT donor who was CMV seropositive. We generated the T cells by a short-term Good Manufacturing Practice (GMP) grade protocol in which a leukapheresis product of the HSCT donor was stimulated with the immunodominant antigen pp65 and interferon-γ secreting cells were isolated. A total of 5×10^5 T cells were administered to the patient within 30 hours after leukapheresis.

Results: The patient was closely monitored for reconstitution of antiviral T cell immunity and viral replication after adoptive T cell transfer. We observed an in vivo expansion of both $CD4^+$ and $CD8^+$ CMV-specific T cells associated with a significant decrease in viral burden and clinical improvement.

Conclusion: This case report further supports the feasibility and effectiveness of adoptive donor T cell transfer for the treatment of drug resistant CMV infections after allo-HSCT.

KEYWORDS
CMV infection; adoptive T cell transfer; stem cell transplantation

Case report

Cytomegalovirus (CMV) infection has remained an important cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). This is especially the case in recipients of HLA-mismatched or HLA-haploidentical HSCT, as well as in those given T cell depleted grafts [1–3]. Standard antiviral agents such as ganciclovir have significantly reduced the incidence of early-onset CMV disease. However, prolonged administration is associated with significant hematopoietic toxicity, drug resistance, delayed CMV-specific T cell reconstitution, and development of late-onset CMV infection [4]. Previous reports have demonstrated that T-cell immunity is essential in controlling CMV infections [5]. Therefore, a promising approach to treat refractory CMV infection after allo-HSCT in patients who lack anti-CMV immunity has been the adoptive transfer of CMV-specific T cells from the original stem cell donor [6,7].

We here report the adoptive transfer of CMV-specific T cells in a 9-year-old girl for the treatment of CMV infection after allo-HSCT. The girl received CD34-selected peripheral blood stem cells from her HLA-haploidentical mother as treatment for a refractory neuroblastoma. Conditioning regimen consisted of melphalan 140 mg/m^2, Thiotepa 10 mg/m^2, fludarabine 200 mg/m^2 and thymoglobuline 10 mg/kg. The infused graft contained 16.6×10^6 $CD34^+$ cells/kg recipient with residual T cells of 0.02×10^6 $CD3^+$ cells/kg recipient. She experienced CMV infection 24 days after transplantation (Figure 1.). Ganciclovir, foscavir and cidofovir all failed to eradicate CMV infection and CMV sequencing demonstrated a L773V substitution in the CMV polymerase domain (a mutation known to cause resistance to ganciclovir, foscavir and cidofovir) [8].

Because of the rapid increase in viral load (Figure 1) and the demonstration of the mutation in the CMV polymerase domain, it was decided the treat the patient with CMV virus-specific T cells of the mother HSC donor on day 104 after transplantation. At the same time, the patient was started on letermovir in addition to cymevene + foscavir combination. The CMV serostatus of the mother was repeated and

CONTACT Bart Vandekerckhove bart.vandekerckhove@ugent.be Department of Diagnostic Sciences, Ghent University, Ghent, Belgium

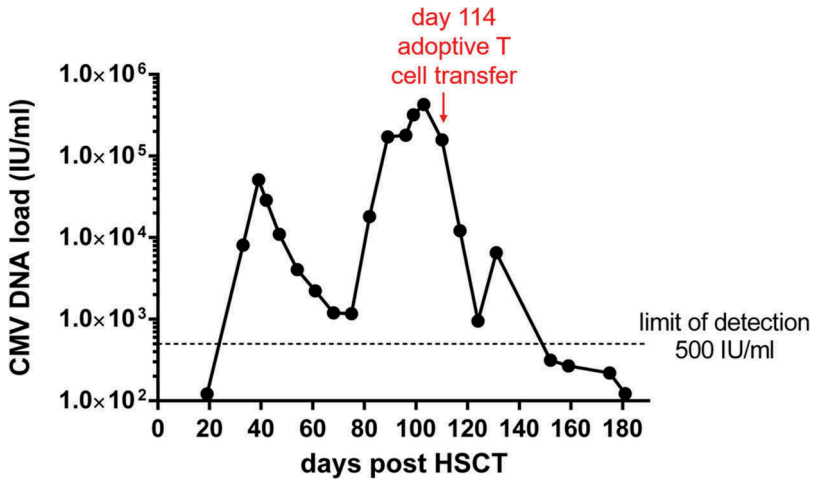

Figure 1. Time course of viral load. CMV-DNA load was assessed in peripheral blood by PCR at different time points after HSCT. Time point of infusion of CMV-specific T cells is indicated in the graph.

confirmed to be seropositive: CMV IgM-, IgG$^+$. Next, we assessed the specific cellular immunity by quantifying the peripheral blood T cells reactive to the immunodominant antigen of CMV phosphoprotein-65 (pp65). Isolated PBMC were incubated with Peptivator CMVpp65, a 15-mer peptide pool covering the complete primary structure of the pp65 antigen and subsequently IFNγ production was visualized by intracellular staining and flowcytometric analysis. We detected a robust CMVpp65-specific CD4$^+$ T cell population (Figure 2(a)), while virtually no specific CD8$^+$ T cells were observed. The frequency of specific CD4$^+$ T cells was clearly above background (criterium: at least twice background levels) and was considered adequate to obtain a sufficient number of CMV-specific T cells for adoptive transfer. An apheresis of the mother was scheduled 4 days later.

As our patient was <18 years, she failed the inclusion criteria of our clinical trial protocol (Eudra CT 2013-004953-26). We therefore decided to perform the

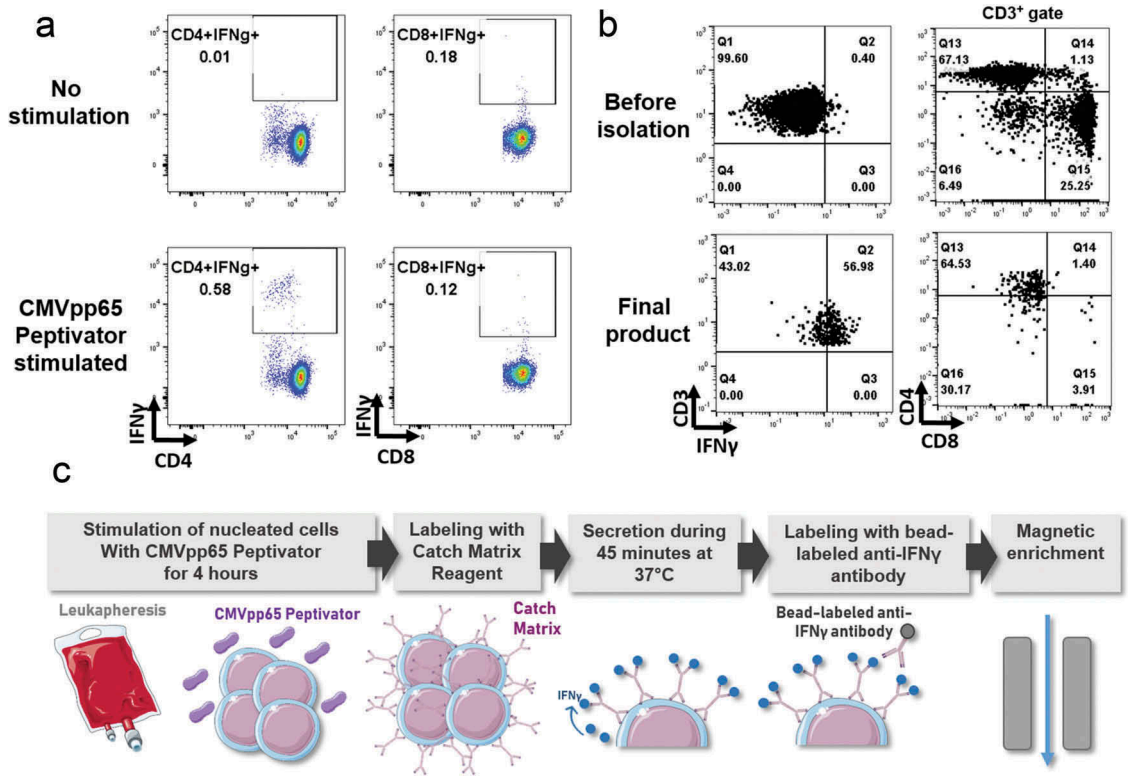

Figure 2. Detection of CMVpp65-specific T cells in PBMC and isolation from a leukapheresis of the family HSCT donor. (a) PBMC of the HSCT donor were stimulated for 6 hours with CMVpp65 Peptivator and as a control without, intracellularly stained for IFNγ and analyzed by flowcytometry. Dot plots represent IFNγ expression in the CD3$^+$CD4$^+$ or CD3$^+$CD8$^+$ population. (b) IFNγ expression in CD3$^+$ T cells and CD4$^+$ and CD8$^+$ T cell distribution before isolation and in the isolated fraction (final product). (c) Schematic overview of the production process for the isolation of CMVpp65-specific T cells by the IFNγ Capture technology.

procedure conforming to the hospital exemption procedure legislation and notified the federal agency for medicines and health products (FAMHP) thereof. This procedure is meant to enable the production of a particular medicinal product specifically designed for a particular patient for which no similar medicine is available on the market. This allowed us to produce the product in a timely fashion.

The leukapheresis product was collected from 7.7 liters of blood volume of the mother donor and contained 5.7×10^9 nucleated cells. The leukapherate was processed the following day using the CliniMACS cytokine capture technology in a licensed Good Manufacturing Practice (GMP) facility (Figure 2(c)). Following a standardized production protocol, a total of 1×10^9 nuclear cells were stimulated with Peptivator CMVpp65 to induce IFNγ production. After 4 hours, cells were labelled with a bispecific antibody (Catchmatrix Reagent) that binds to the cells using its CD45 specificity and is able to catch secreted IFNγ on the cell membrane using its IFNγ specificity. After a wash step and appropriate dilution, the coated cells were incubated for exactly 45 minutes at 37°C to allow for secretion of IFNγ and capture on the surface of the cells by the Catchmatrix Reagent. Next, cells were labelled with a second IFNγ-specific antibody coupled to a magnetic bead resulting in bead covered CMVpp65-specific T cells. The cell mixture was then passed through a magnetic field to separate the bead-coated CMVpp65-specific cells from the uncoated cells. We recovered 5.4×10^5 viable $CD3^+$ T cells, of which 2.7×10^5 were $CD4^+IFN\gamma^+$ and 0.1×10^5 $CD8^+IFN\gamma^+$ T cells (Figure 2(b)). The cells were washed and resuspended in Plasmalyte with 4% human serum albumin. Total T cell dose was 24.4×10^3 T cells/kg patient. We performed extensive quality testing on process intermediates as well as on the final product. Mycoplasma testing was performed using a PCR method according to the European Pharmacopoeia immediately after culture and proved negative. Endotoxin levels were <4.4 IU/kg patient. Sterility testing was performed on the apheresis product, after Peptivator stimulation and on the final product. However, as the definitive results were not available at the time of release, a gram-stain was performed on the final product and was negative. The whole production process from stimulation to release was completed within 12 hours.

The CMV-specific T cells were infused intravenously as a single dose on the same day of isolation, 114 days after HSCT. There were no infusion-related acute toxicities. Although donor and recipient are HLA-haploidentical, no GvHD related to the T cell product was induced up to 14 weeks after infusion.

Survival and in vivo expansion of CMVpp65-specific T cells was assessed at 2, 4 and 8 weeks following adoptive T cell transfer (Figure 3). CMV-specific $CD4^+$ T cells were readily detectable in the peripheral blood of the patient 2 weeks after infusion, and expanded thereafter reaching a similar frequency as found in CMV seropositive healthy individuals. This response remained stable during the follow-up period of 8 weeks. Interestingly, although a low number of $CD8^+$ T cells were present in the T cell product (Figure 2(b)), CMVpp65-specific $CD8^+$ T cells strongly expanded over time and even exceeded the frequencies found in healthy CMV seropositive individuals at 8 weeks post-infusion. The expansion of CMVpp65-specific T cells was accompanied by a significant reduction of CMV DNA copies in peripheral blood to <500 IU/ml blood which is the lower limit of detection of this assay (Figure 1).

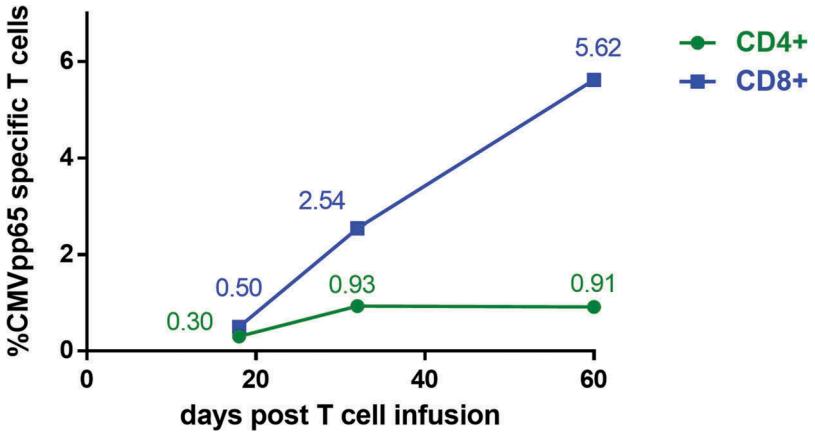

Figure 3. Expansion of CMVpp65-specific T cells after adoptive T cell transfer. CMVpp65-specific T cell response was assessed in peripheral blood of the recipient at 2, 4 and 8 weeks after adoptive T cell transfer. PBMC were stimulated with CMVpp65 Peptivator for 6 hours and intracellularly stained for IFNγ. The percentage of $IFN\gamma^+$ T cells obtained after stimulation with CMVpp65 Peptivator was corrected for background staining by subtracting the percentage of $IFN\gamma^+$ cells measured in the absence of stimulation.

Discussion

In this case report, we describe the successful adoptive transfer of CMV-specific T cells derived from a haploidentical HSCT family donor in a patient with a refractory CMV infection after HSCT.

The successful production and transfer of CMV-specific T cells requires a suitable donor, a validated production protocol and coordinated logistics. As our patient presented with a life-threatening CMV infection, the cell therapy product had to be administered as rapidly as possible. Therefore, the throughput time from donor screening to collection of apheresis and infusion of the T cells had to be minimized. From the moment the intent to treat was decided, it took 2 days to screen the donor for CMV-specific T cells and another 2 days for the apheresis and isolation of the cells. The final product was transported immediately after production to the transplant centre and administered within 4 hours after production. This very short throughput time is only possible when fully validated procedures and trained dedicated production and QC personnel is available at all time. In addition, for the isolation of the T cells, we applied the GMP-compatible IFNγ Capture technology in which the CMV-specific T cells are directly isolated from a leukapheresis without the need for prolonged cultures. This technology allowed us to isolate the T cells and release the product within one day after the apheresis.

We administered a low dose of 24.4×10^3 T cells/kg body weight and observed an expansion of CMV-specific T cell immunity, associated with a strong reduction in CMV copies. Clinical effects with low T cell doses have also been observed in a study by Feuchtinger et al., in which haploidentical T cells administered at a median dose of 7.1×10^3 T cells/kg were associated with strong reductions in CMV viremia [6]. As in our case, these low doses did not result in GvHD, which is a potential life-threatening toxicity when administering T cells in a haploidentical setting.

CMV-specific T cell immunity has been demonstrated to be crucial in the effective control of CMV infection in posttransplant patients. After T cell infusion, we observed an expansion of both $CD4^+$ and $CD8^+$ T cells. Cytotoxic $CD8^+$ T cells destroy virus-infected cells, while helper $CD4^+$ T cells regulate the induction of effector $CD8^+$ T cells and maintenance of $CD8^+$ T memory cells [9,10]. Multiple studies suggest that functionality of both T cell subsets is required to achieve an effective control of CMV infection [11,12]. The strong reduction of CMV copies we observed is thus most likely attributable to the combined reconstitution of $CD4^+$ and $CD8^+$ T cell immunity. Of note, the viral load in blood was already decreasing at the time of T cell infusion following the introduction of letermovir. Eighteen days before infusion the patient had profound T-lymphopenia (4.9 $CD3^+$/μl with no detected naive $CD4^+$ T cells) and T cell counts normalized concurrent with the clearance of the CMV infection, to 452 $CD3^+$/μl 3 days after infusion and 2077 $CD3^+$/μl 1 month after T cell infusion. We believe that the increase in peripheral T cell levels is a consequence of the viral clearance and of peripheral expansion of infused T cells in this context of profound lymphopenia. Further, it is unlikely that the posttransplant-generated naïve T cells would contain such a high percentage of CMV reactive T cells at the measured time points. It is therefore implausible that endogenous reconstitution of CMV reactive T cells contributed to the decrease in viral load.

Acknowledgments

We thank prof. dr. Jerina Boelens for sterility testing and Gram stain.

Disclosure statement

The authors report no declarations of interest.

Funding

This work was supported by the Kinderkankerfonds.

References

[1] Green ML, Leisenring W, Xie H, et al. Cytomegalovirus viral load and mortality after haemopoietic stem cell transplantation in the era of pre-emptive therapy: a retrospective cohort study. Lancet Haematol. 2016;3:e119–e127.

[2] Chang Y-J, Zhao X-Y, Huo M-R, et al. Immune reconstitution following unmanipulated HLA-mismatched/haploidentical transplantation compared with HLA-identical sibling transplantation. J Clin Immunol. 2012;32:268–280.

[3] Liu J, Kong J, Chang YJ, et al. Patients with refractory cytomegalovirus (CMV) infection following allogeneic haematopoietic stem cell transplantation are at high risk for CMV disease and non-relapse mortality. Clin Microbiol Infect. 2015;21:1121.e9-1121.e15.

[4] Ahmed A. Antiviral treatment of cytomegalovirus infection. Infect Disord - Drug Targets. 2011;11:475–503.

[5] Cwynarski K, Ainsworth J, Cobbold M, et al. Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. Blood. 2001;97:1232–1240.

[6] Feuchtinger T, Opherk K, Bethge WA, et al. Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. Blood. 2010;116:4360–4367.

[7] Einsele H, Roosnek E, Rufer N, et al. Infusion of cytomegalovirus (CMV)–specific T cells for the treatment of

CMV infection not responding to antiviral chemotherapy. Blood. 2002;99:3916–3922.
[8] Chou S, Ercolani RJ, Sahoo MK, et al. Improved detection of emerging drug-resistant mutant cytomegalovirus subpopulations by deep sequencing. Antimicrob Agents Chemother. 2014;58:4697–4702.
[9] Laidlaw BJ, Craft JE, Kaech SM. The multifaceted role of CD4+ T cells in CD8+ T cell memory. Nat Rev Immunol. 2016;16:102–111.
[10] Janeway's Immunobiology. Ninth Edition. By Kenneth Murphy and Casey Weaver; with contributions by Allan Mowat, Leslie Berg, and David Chaplin; with acknowledgment to: charles A. Janeway, Jr., Paul Travers, and Mark Walport. New York: Garland Science. Tay Q Rev Biol. 2018;93:59.
[11] Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. Clin Microbiol Rev. 2009;22:76–98.
[12] Walter EA, Greenberg PD, Gilbert MJ, 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;333:1038–1044.