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

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1 INTRODUCTION

- **Cytomegalovirus (CMV) infection** has remained an important cause of **morbidity and mortality** after allogeneic hematopoietic stem cell transplantation (**allo-HSCT**) [1].
- Previous reports have demonstrated that **T-cell immunity** is essential in controlling CMV infections [2].
- 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 [3].

We here report the **treatment** of a patient with **multidrug resistant CMV infection** after haploidentical HSCT **with CMV specific T cells** of the **HSCT donor**.

2 CASE DESCRIPTION

A 9 years old girl received HSC from her HLA-haploidentical mother. She experienced a multidrug resistant CMV infection 24 days after transplantation **(fig 1.)**.

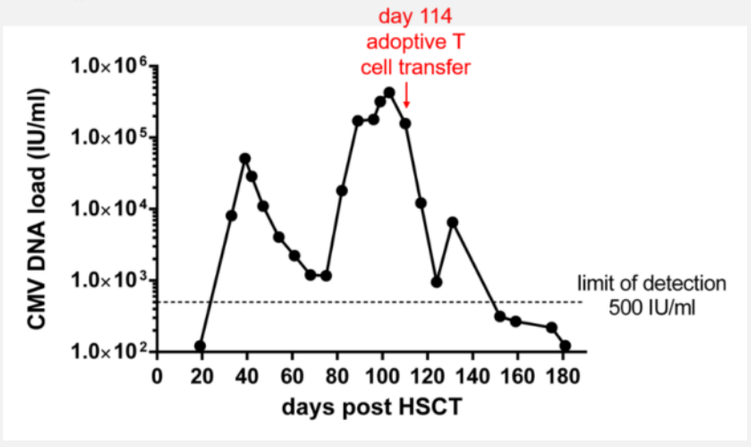

Fig. 1, Time course of viral load. CMV-DNA load was assessed in peripheral blood by PCR at different time points after HSCT. Time point of infusion of CMV specific T cells is indicated in the graph.

The mother donor was CMV seropositive and screened for the presence of CMV specific cellular immunity by stimulating peripheral blood mononuclear cells (PBMC) with a library peptide pool covering the CMVpp65 protein and evaluating the production of IFNγ by the T cells. We observed a robust population of CMV specific $CD4^+$ T cells, while virtually no CMV specific $CD8^+$ T cells were detected **(fig. 2.)**

The frequence of CMVpp65 specific T cells was considered adequate to obtain a sufficient number of CMV specific T cells for adoptive transfer.

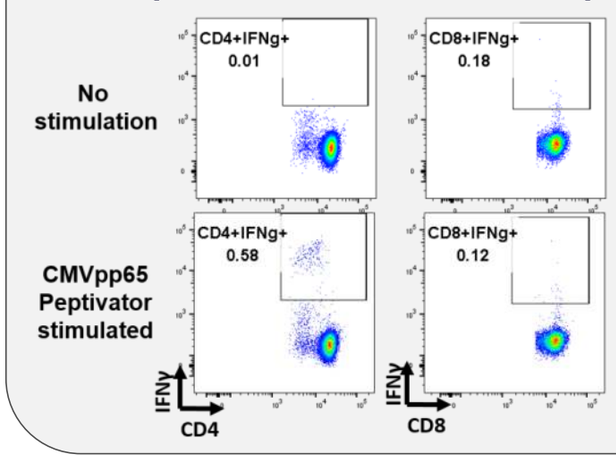

Fig. 2. Detection of CMVpp65 specific T cells in PBMC. PBMC of the HSCT donor were stimulated for 6 hours with CMVpp65 Peptivator and as a control without, intracellularly stained for IFNγ and analyzed by flowcytometry. Dot plots represent IFNγ expression in the $CD3^+CD4^+$ or $CD3^+CD8^+$ population.

[1] Green, M.L. 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] Cwynarski, K. et al. Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. Blood 2001, 97, 1232–1240.
[3] Feuchtinger, T. 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.

3 RESULTS

We collected a leukapheresis product of the mother donor. A total of $1x10^9$ nuclear cells of the leukapheresis product were stimulated with CMVpp65 peptide pool and IFNγ secreting cells were isolated by the IFNγ Capture technology in a licensed GMP facility (**fig. 3**),

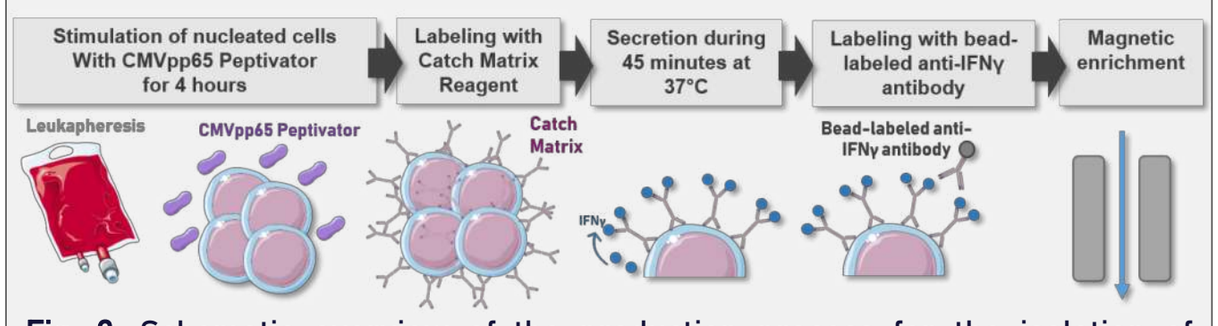

Fig. 3. Schematic overview of the production process for the isolation of CMVpp65 specific T cells by the IFNγ Capture technology.

We recovered $5.4x10^5$ viable $CD3^+$ T cells, of which $2.7x10^5$ were $CD4^+IFN\gamma^+$ and $0.1x10^5$ $CD8^+IFN\gamma^+$ T cells (**fig. 4**). Total T cell dose was $24.4x10^3$ T cells/kg patient. The T cell product was released and administered to the patient one day after apheresis.

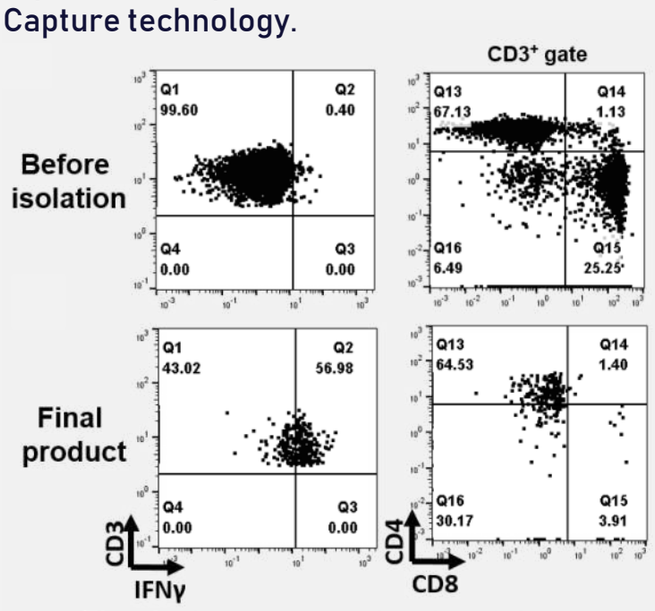

Fig. 4. IFNγ expression in CD3+ T cells and CD4+ and CD8+ T cell distribution before isolation and in the isolated fraction (final product).

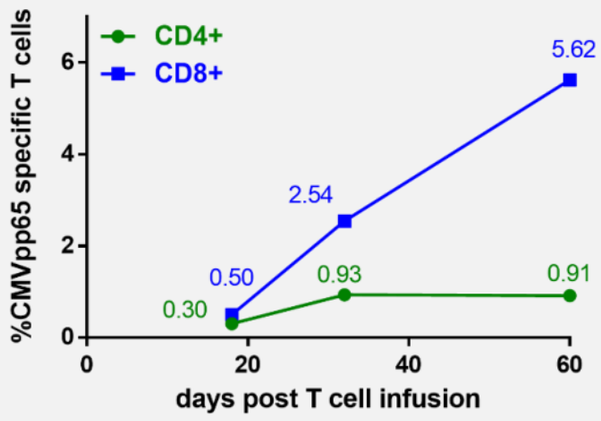

Fig. 5. PBMC of the patient collected 2, 4 and 6 weeks after adoptive transfer, were stimulated for 6 hours with CMVpp65 Peptivator and as a control without, intracellularly stained for IFNγ and analyzed by flowcytometry. The percentage of IFNγ+ T cells obtained after stimulation with CMVpp65 Peptivator were corrected for background staining by subtracting the percentage of IFNγ+ cells measured in the absence of stimulation.

Survival and in vivo expansion of CMVpp65 specific T cells was assessed at 2, 4 and 8 weeks following adoptive T cell transfer. We observed an expansion of CMVpp65 specific CD4+ T cells and surprisingly of CD8+ T cells. The expansion was accompanied by a significant reduction of CMV DNA copies in peripheral blood to <500 IU/ml blood **(fig. 1.)**

4 CONCLUSION

We here described the successful adoptive transfer of CMV specific T cells derived from a haploidentical HSCT family donor in a patient with a refractory CMV infection. Our data further support the feasibility and effectiveness of this treatment option.

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