Peptide-stimulated expansion of virus-specific T cells for preventative treatment after allogeneic stem cell transplantation

R. Gary¹, M. Aigner¹, A. Moosmann², and A. Gerbitz¹

¹Department of Internal Medicine 5, University Hospital Erlangen, Erlangen, Germany
²DZIF Research Group, Helmholtz Zentrum München, Munich, Germany

A phase I/IIa clinical trial was initiated to prevent or pre-emptively treat reactivation of CMV and EBV in patients after allogeneic hematopoietic stem cell transplantation (HSCT). For donor T-cell expansion, HLA class I and II matched peptides were chosen that cover antigens from all phases of CMV and EBV infection.

Materials & Methods

Selected CMV- and EBV-derived GxP Peptides (9- to 15mers) were synthesized and pooled by JPT Peptide Technologies. For each virus, pools of 17 peptides (10 restricted through HLA class I, 7 restricted through HLA class II) were generated. The peptides have different HLA restrictions, such that approximately 80% of the European population is covered by at least one matched peptide from each virus. To ensure efficacy, HLA class I only peptides, that were shown to elicit responses in a majority of donors with the relevant HLA, were included. Lyophilized pools are ready-to-use in the GMP-conform manufacturing process. For peptide stimulation, T-cells are obtained as PBMC as a small fraction of the G-CSF-mobilized stem cell graft. After initial cryopreservation, PBMC are stimulated with the two peptide pools in two separate fractions for two hours and washed. Pooled PBMC are then transferred into a closed bag system and expanded over 9 days. On day 9 after peptide stimulation, T cells are harvested and aliquoted.

To test functionality of expanded T cells, further analyses comprise flow cytometry of HLA-multimer-binding cells and intracellular IFN-γ detection upon re-stimulation with peptide pools. In addition, the TCR repertoire of the T-cell product will be analyzed by TCR-β chain next-generation sequencing to identify long living T cells within the product and the host after adoptive transfer.

Results

Stimulation of PBMC from G-CSF-mobilized allogeneic donors using two separate peptide pools for CMV and EBV results in a strong expansion of CMV- and EBV-specific T cells after 9 days of cultivation. As shown for HLA A*02:01-positive donors in Figure 1, the expansion of HLA-multimer binding T cells ranges from 7.5 to more than 100 fold.

**Figure 1:** Expansion of CMV (top panel) and EBV (bottom panel) specific T cells from G-CSF mobilized stem cells using HLA matched peptides. Stimulation was performed on day 0 followed by a 9 day expansion period without any further stimulation. A total of 33,28% specific T cells was achieved (16,79% CMV, 16,49% EBV) using HLA multimer staining.
PBMC from G-CSF-mobilized or non-mobilized donors can be used as starting material for T-cell expansion. By peptide stimulation, naïve T cells are efficaciously depleted, while effector and effector memory cells are expanded. Thus, the risk of GvHD after adoptive transfer is minimized, since alloreactive cells are largely derived from the naïve T-cell compartment. Analysis of the peptide stimulated T-cell product using next generation sequencing of the TCR V-beta repertoire (4) demonstrated that only a limited number of T-cell receptors with specificity for each peptide was found within the product, which further increases the safety profile.

Discussion & Conclusions
With the current manufacturing protocol, CMV/EBV specific T-cells can be expanded in a GMP compliant fashion. This is mandatory for clinical trials within the EU legislation. The protocol allows for stringent quality control of the T-cell product. Our exclusive use of defined GxP peptides with known HLA class I and II restrictions will make it possible to use HLA class I multimers (and potentially class II multimers) for a comprehensive quantitative analysis of the specific T-cells in the product, and to track them in patients especially on a molecular level by next generation TCR V-beta sequencing. Since we avoid the use of hundreds to thousands of potentially irrelevant peptides, as for example provided by using overlapping peptides for stimulation, we decrease the probability that T-cells with unwanted specificities that accidentally cross-react with some of these peptides. Activation of T cells by peptide stimulation seems to be an important factor for the efficacy of the product as T cells will be adoptively transferred at an early stage after allogeneic HSCT and therefore will have to survive in a host treated with immunosuppressive agents such as Cyclosporine A. Moreover, it is very likely that our specificities of choice will be more efficaciously activated if the number of potentially competing peptides in the loading reaction is limited. Still, it is important to include peptides from a range of antigens from different phases of viral replication.

The manufacturing process has so far included a limited number of HLA allotypes, but can be extended to other specificities for which defined peptides become available. Thus, this protocol is a versatile platform for manufacturing T-cells with specificity for foreign antigens in the setting of allogeneic stem cell transplantation.

References
1. "Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for Adenovirus, EBV and CMV after allogeneic hematopoietic stem cell transplantation" Gerdemann et al., Molecular Therapy (2013)