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## Control of cytomegalovirus viremia after T cell depleted allogeneic stem cell transplantation

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# Chapter 8

Summary

Following allogeneic stem cell transplantation (alloSCT), most patients experience a period of profound and prolonged T cell deficiency due to the immune suppression and/or T cell depletion (TCD). In this period the patients are at risk for developing infectious complications by reactivation of endogenous herpes like viruses cytomegalovirus (CMV), Epstein-Barr virus (EBV) and varicella zoster virus (VZV). Reactivation of endogenous CMV is the most frequently occurring herpes virus reactivation following alloSCT. Approximately 60% of alloSCT recipients are seropositive for CMV and are therefore at risk for endogenous reactivation of latent CMV. CMV reactivation can lead to potentially fatal CMV disease, comprising CMV pneumonitis, CMV colitis or CMV encephalitis. The aim of this thesis was to evaluate factors that influence the incidence of CMV disease after TCD alloSCT. These factors include the conditioning regimen, serostatus of the donor, pharmacological intervention following alloSCT and adoptive T cell transfer for treatment of refractory CMV reactivation or CMV disease.

CMV disease can be prevented by pre-emptively treating CMV reactivation using ganciclovir intravenously. In a pre-emptive treatment strategy, antiviral therapy is initiated when the viral load is above a predetermined PCR threshold. An effective oral treatment for pre-emptive CMV therapy would enable prevention and treatment of CMV in an outpatient setting and would lead to reduced patient burden and health-care costs. In **chapter 2** we demonstrate that pre-emptive treatment with oral valganciclovir is equally effective in reducing CMV DNA load in allogeneic stem cell recipients compared to intravenous ganciclovir. Severe adverse effects were not observed and CMV disease did not occur. The percentage of patients receiving erythrocyte transfusions was higher in the group of patients receiving ganciclovir, which is possibly the result of co-morbidity in the admitted patients treated with ganciclovir intravenously. Pre-emptive treatment of CMV viremia episodes in allogeneic stem cell recipients with either valganciclovir or ganciclovir led to a similar median CMV DNA load reduction in plasma of approximately  $0.1 \log_{10}$  copies/ml/day. We concluded that oral valganciclovir (900 mg, twice daily) is equally effective and safe as intravenous ganciclovir (5 mg/kg, twice daily) in the treatment of CMV reactivation aiming to prevent CMV disease following alloSCT. The vast majority of alloSCT recipients, without any clinical signs and symptoms of CMV disease when the first laboratory signs of CMV infection are detected, can benefit from treatment with an oral drug, without the need for hospitalization. For patients with suspected symptomatic CMV infections intravenously administered ganciclovir remains the first choice drug, as the course of CMV disease can be rapidly progressive and ultimately fatal.

It has been established that alloSCT with reduced intensity conditioning (RIC) can be successfully performed in individuals with a wide variety of different diseases and results in reduced risk of transplant-related mortality. Durable donor engraftment and favorable response of the disease with no graft versus host disease (GVHD) was reported for the in vitro TCD alloSCT protocol using RIC with fludarabine, anti-thymocyte globulin (ATG), busulphan and Campath-in-the-bag. It can be hypothesized that following RIC more residual

recipient T cells survive the conditioning regimen and can confer protective immunity following alloSCT. In **chapter 3** we demonstrate that there was no significant difference in incidence and severity of CMV infections within 100 days following alloSCT preceded by RIC compared to a conventional MAC. The onset of CMV DNA detection in plasma following alloSCT, the duration of a CMV infection, the peak load, the area under the DNAemia curve, the number and duration of pre-emptive CMV treatment episodes, as well as the number of recurrent infections within 100 days following alloSCT were comparable after RIC and MAC. This comparable severity after RIC and MAC may be explained by TCD as both patient groups received TCD grafts. By itself, TCD of the graft is associated with an increased risk of CMV infections, which seems to be reflected by the high overall incidence of CMV infections (51%) within 100 days following alloSCT in this study. As RIC relatively spares recipient hematopoietic cells, recipients who depend on recipient CMV-specific T cells were expected to benefit most from RIC in control of CMV reactivation. CMV seropositive recipients ( $R^+$ ) transplanted with a CMV seronegative donor ( $R^+D^-$ ) depend on residual recipient CMV-specific T cells, as the graft of the donor does not contain memory CMV-specific T cells. In this study no statistical difference in frequency and severity of CMV reactivation was present  $R^+D^-$  patients compared to CMV seropositive recipients transplanted with a CMV seropositive donor ( $R^+D^+$ ). However, a non-significant increase of frequency and severity of CMV reactivation was observed in  $R^+D^-$  patients compared to  $R^+D^+$  and as expected this difference was more pronounced in MAC compared to RIC. This difference did not reach statistical significance presumably due to small numbers of patients and a short follow-up of 100 days following alloSCT.

In **chapter 4** we specifically investigated the effect of donor CMV serostatus on the incidence of CMV disease after TCD alloSCT in a larger cohort of CMV seropositive patients. CMV-specific T cells may be transferred with the graft from CMV seropositive donors and provide protection against CMV disease. However, profound T cell depletion may eradicate these CMV-specific T cells. To determine the effect of donor CMV serostatus, we analyzed the incidence of CMV disease after TCD alloSCT in 157 CMV seropositive recipients, comprising 51  $R^+D^-$  patients and 106  $R^+D^+$  patients. Furthermore, we determined the origin of CMV-specific T cells in a selection of 25  $R^+D^-$  patients to determine whether primary donor-derived CMV-specific T cell responses could be demonstrated. The duration of CMV reactivations and the incidence of CMV disease were higher in  $R^+D^-$  patients compared to  $R^+D^+$  patients. In  $R^+D^-$  patients, CMV-specific  $CD4^+$  and  $CD8^+$  T cells were mainly of recipient origin. However, in 53% of  $R^+D^-$  patients donor-derived CMV-specific T cells were detected within the first year, even as early as 3 months following TCD alloSCT. We conclude that donor CMV serostatus significantly influenced the clinical severity of CMV reactivations indicating the role of CMV-specific memory T cells transferred with the graft, despite the ultimate formation of primary donor-derived CMV-specific T cell responses in  $R^+D^-$  patients. Considering the pivotal role of CMV-specific T cells in preventing CMV disease, improving CMV-specific T cell reconstitution in patients by adoptive transfer of CMV-specific T cells

can be an attractive treatment modality. However, the use of adoptive T cell transfer is not commonplace, as questions regarding safety and efficacy still need answering. In **chapter 5** we analyzed the safety and efficacy of adoptive transfer of CMV pp65-specific CD8<sup>+</sup> T cell lines to restore CMV-specific T cell immunity by performing a phase I/II clinical study on adoptive transfer of in vitro-generated donor-derived or patient-derived CMV pp65-specific CD8<sup>+</sup> T cell lines. Peripheral blood mononuclear cells from CMV seropositive donors or patients were stimulated with HLA-A\*0201-restricted and/ or HLA-B\*0702-restricted CMV pp65 peptides (NLV/TPR) and 1 day after stimulation interferon-gamma producing T cells were enriched using the CliniMACS Cytokine Capture System, and cultured with autologous feeders and low-dose interleukin-2. After 7–14 days of culture, quality controls were performed and the CMV-specific T cell lines were administered or cryopreserved. The T cell lines generated contained 0.6–17 x 10<sup>6</sup> cells, comprising 54%–96% CMV pp65-specific CD8<sup>+</sup> T cells, and showed CMV-specific lysis of target cells. Fifteen CMV-specific T cell lines were generated, of which 8 were administered to patients with refractory CMV reactivation. Seven cell lines were generated but not administered because patients had cleared the CMV reactivation by the time the cell line was generated (n=4), due to a relapse of the malignant disease (n=1), or patients died due to the progressive CMV disease before infusion of the CMV-specific T cells (n=2). After administration, no acute adverse events and no graft versus host disease were observed and CMV loads disappeared. In several patients, a direct relation between administration of the T cell line and the in vivo appearance of CMV pp65-specific T cells could be documented. In conclusion, administration of CMV pp65-specific CD8<sup>+</sup> T cell lines was found to be feasible and safe.

In contrast to CMV, little is known about VZV-specific CD8<sup>+</sup> T cell immunity because validated VZV-derived immunodominant peptides for Human Leucocyte Antigen (HLA) class I are lacking. Because of this lack of validated VZV-derived immunodominant peptides for HLA class I, the analysis of VZV-specific CD8<sup>+</sup> T cell responses is hampered. To be able to analyze the role of CD8<sup>+</sup> T cells in VZV reactivation, we set out to identify epitopes for VZV by a new pentamer-based epitope discovery method. In **chapter 6** we describe our search for immunogenic antigens for VZV to develop VZV-specific pentamers and the development of multimeric HLA complexes to identify VZV-specific CD8<sup>+</sup> T cells. Potential HLA-A2 binding peptides from the putative immediate-early (IE) 62 protein of VZV were tested for binding, and peptides with sufficient binding capacity were used to generate pentamers. Patients with VZV reactivation following TCD alloSCT were screened with these pentamers, leading to the identification of the first validated HLA class I-restricted epitope of VZV. In 42% of HLA-A2 positive patients following VZV reactivation, these IE62-ALW-HLA-A2-specific T cells could be detected ex vivo. We demonstrated that despite the low frequencies, it is possible to detect VZV-specific CD8<sup>+</sup> T cells, allowing ex vivo analysis of immune responses to VZV infection, reactivation, and possibly VZV vaccination.

Despite pre-emptive antiviral treatment the incidence of CMV disease in CMV seropositive alloSCT patients is still 10% at 1 year following alloSCT. This illustrates the necessity for

adequate CMV-specific T cell immunity for long-term control of CMV and prevention of CMV disease. In **chapter 7** we provide an overview of factors relevant for prevention of CMV disease after alloSCT. GVHD and the use of an unrelated or HLA mismatched donor was found to be associated with an increased risk of developing CMV disease despite pre-emptive antiviral treatment, either due to systemic immune suppression needed to prevent or treat GVHD or due to eradication of recipient derived CMV-specific memory T cells by the alloreactive donor T cell response mediating GVHD. T cell depletion was found to be associated with an increased risk for CMV reactivation but not with an increased risk for CMV disease. It can be hypothesized that because immune suppression is in general not needed after TCD alloSCT, CMV-specific T cells are not hampered by immune suppression to provide protective immunity in case of CMV reactivation. Donor CMV serostatus significantly appears to influence CMV-specific T cell reconstitution and the risk of developing CMV disease. The incidence of CMV related complications and mortality is lower in R<sup>+</sup>D<sup>+</sup> patients compared to R<sup>+</sup>D<sup>-</sup> patients. In R<sup>+</sup>D<sup>-</sup> patients memory CMV-specific T cells are not present in the donor graft and recipient-derived virus-specific T cells can be (partially) eradicated by the conditioning regimen and/or by an alloreactive T cell response mounted by donor T cells. Finally, we reviewed the available studies on the safety and efficacy of adoptive transfer of donor CMV-specific T cells for the prevention and treatment of CMV disease following alloSCT. All trials published thus far are phase-1/2 trials, demonstrating safety, proof of concept and association between adoptive T cell therapy (ACT) and viral clearance. A major limitation for ACT following alloSCT is that treatment of active GVHD with systemic immune suppression was an exclusion criterion for administration of CMV-specific ACT in all trials. Another major limitation of clinical applicability of CMV-specific ACT is that isolation of CMV-specific memory T cells from the donor is restricted to CMV seropositive donors. Adoptive transfer of T cells isolated from healthy third-party donors may be a solution for R<sup>+</sup>D<sup>-</sup> patients as donor CMV-specific T cells are not readily available for these patients. Trials demonstrate that ACT with third-party donor derived virus-specific T cells is feasible, safe and may be effective in treating persistent CMV reactivation and CMV disease. However long-term persistence of these T cells is uncertain.

In conclusion, the aim of this thesis was to evaluate the factors that influence the incidence of CMV disease after TCD alloSCT. We determined that prevention of CMV disease is safe and feasible using pre-emptive treatment with oral valganciclovir. We did not demonstrate a reduced risk for CMV reactivation or disease in patients treated with a RIC regimen and TCD alloSCT. GVHD and the use of immune suppression following alloSCT were found to be important risk factors for the development of CMV disease. T cell reconstitution was found to be improved in CMV seropositive patients transplanted with a CMV seropositive donor, which leads to a decreased risk of developing CMV disease. Donor derived CMV-specific primary T cell responses were detected in the majority of R<sup>+</sup>D<sup>-</sup> patients within one year after transplantation.