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

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Chapter 9

General discussion

Developments in CMV-specific antiviral medication

We investigated the safety and efficacy of pre-emptive therapy using (val)ganciclovir following TCD alloSCT and demonstrated that valganciclovir was equally effective to ganciclovir in preventing CMV disease[1]. In our study of 107 patients following TCD alloSCT, CMV disease did not occur following pre-emptive therapy with (val)ganciclovir. Based on results obtained by us and others[2-4], pre-emptive strategies using valganciclovir are now the golden standard for prevention of CMV disease following alloSCT despite considerable side effects such as myelotoxicity and nephrotoxicity[5]. Although in our study on the safety and efficacy of pre-emptive therapy using (val)ganciclovir no CMV disease was observed, 13 patients had no or only moderate response to (val)ganciclovir. Larger studies demonstrate that despite a pre-emptive therapy strategy, the incidence of CMV disease is still 10% at 1 year following alloSCT[6, 7]. Our study was not designed to identify factors associated with an increased risk of treatment failure. In the review of literature that we performed, it is demonstrated that GVHD and the use of an unrelated or HLA-mismatched donor are important risk factors for developing CMV disease despite pre-emptive therapy[8]. In these patients prolonged use or prophylactic use of antiviral medication may be beneficial to allow more time for CMV-specific T cell reconstitution to develop while suppressing CMV reactivation and preventing CMV disease. However, due to the side effects, prolonged treatment with ganciclovir or valganciclovir as prophylaxis is not feasible[9-12]. Foscarnet and cidofovir are alternatives to (val)ganciclovir, but are also not suitable for prophylaxis due to considerable side effects.

Maribavir, brincidofovir and letermovir have been described as promising new anti-CMV drugs[13], possibly suitable for prophylaxis. Maribavir was not beneficial over placebo in a phase II trial[14] and is therefore not recommended for CMV prophylaxis after alloSCT. Phase III trials with maribavir are ongoing (NCT02927067 and NCT02931539) but for pre-emptive treatment of CMV reactivation rather than prophylaxis. Brincidofovir (also known as CMX001) was effective in decreasing the incidence of CMV reactivation in a phase II study as prophylaxis following alloSCT. However, efficacy to prevent CMV reactivation was only achieved in a dosage which was associated with increased gastrointestinal symptoms[15]. A phase III trial was performed (SUPPRESS, NCT01769170) and although the results of this trial have not yet been published, a manufacturer statement reported that prophylaxis with brincidofovir did not prevent CMV reactivation. In contrast to maribavir and brincidofovir, prophylaxis with Letermovir was demonstrated to be safe and effective to prevent CMV disease in a double blinded randomized control trial in CMV seropositive patients following alloSCT[16]. However, after cessation of the prophylaxis (predetermined at 100 days after alloSCT), the incidence of clinically significant CMV reactivation increased. The incidence of CMV disease was low in the letermovir group and in the placebo group, 1.5% and 1.8% at 24 weeks after alloSCT. The incidence and frequency of side effects was comparable to placebo with, most notably no increase in myelotoxic or nephrotoxic evens in the letermovir group. With the FDA approval based on this phase III trial, it can be concluded that prophylaxis for

CMV reactivation after alloSCT is possible using letermovir as an alternative for pre-emptive treatment with (val)ganciclovir.

Although Letermovir for CMV prophylaxis appears promising, trials directly comparing letermovir prophylaxes to pre-emptive therapy with (val)ganciclovir to prevent CMV disease following alloSCT have not been performed. Thus far no clinical superiority in efficacy of letermovir has been demonstrated compared to pre-emptive (val)ganciclovir. Besides efficacy, additional questions remain to be answered before CMV prophylaxis with Letermovir can be recommended over pre-emptive therapy with (val)ganciclovir. First, in order to prevent CMV disease, letermovir should provide protection during the period in which CMV-specific T cell immunity reconstitutes. It may take longer than the 100 days used in the trial to bridge this period especially in high-risk populations such as CMV seropositive recipients transplanted with CMV seronegative donors or during GHVD and treatment with immune suppression. However, prolonged use (i.e. longer than 14 weeks) of letermovir may eventually induce letermovir resistance. Second, it is not known whether CMV-specific T cell reconstitution is effective during letermovir prophylaxis, as Letermovir suppresses CMV viremia completely[17, 18]. By completely suppressing CMV reactivation, CMV-specific antigen presentation may also be suppressed, possibly hampering CMV-specific T cell reconstitution. Future studies should focus on development of Letermovir resistance and on the influence of letermovir use of T cell reconstitution before the place of letermovir prophylaxis for prevention of CMV disease following alloSCT is determined.

CMV-specific vaccination after alloSCT

Since CMV-specific T cells are essential for long-term control of CMV reactivation, interventions to accelerate CMV-specific T cell reconstitution may significantly contribute to the prevention of CMV disease. Traditionally, T cell immunity can be boosted by vaccination, an intervention in which antigen is presented in conjunction with a stimulatory adjuvant. A beneficial effect of CMV vaccination on CMV-specific T cell reconstitution may overcome the aforementioned possibly impaired CMV-specific antigen presentation when Letermovir prophylaxis is applied. It is not clear what the nature of the CMV-specific T cell reconstitution would be following vaccination, whether recipient or donor memory CMV-specific T cells are expanding on a recall response or whether naïve donor CMV-specific T cells develop into effector T cells by a primary immune response. We have demonstrated that a primary CMV-specific T cell response is possible shortly after alloSCT. However, the majority of patients depend on recipient CMV-specific T cells during the first year after alloSCT[19]. It can therefore be anticipated that CMV-specific vaccination early after alloSCT will primarily lead to a boosting of CMV-specific memory T cells. In CMV seropositive patients transplanted with a CMV seronegative donor (R^+D^-), no CMV-specific memory T cells of donor origin are present and CMV vaccination early after transplantation will boost memory CMV-specific T cell response from recipient origin. However, recipient CMV-specific memory T cells are at risk of being eradicated when an alloreactive immune response eradicates the recipient

hematopoietic cells. When this alloreactive immune response eradicates the boosted recipient CMV-specific memory T cells, the effect of vaccination will be abrogated. In that case, vaccination can be only effective when it induces a primary immune response from donor origin.

A commercially available CMV-specific vaccine has not yet been developed. Several phase I/II trials demonstrated that CMV-specific vaccination can boost pre-existing memory T cells[20-23]. Two randomized controlled trials have been performed to determine the clinical benefit of CMV-specific vaccination after alloSCT. The first trial was a randomized, double blind, placebo-controlled trial that investigated safety and efficacy of a CMV DNA vaccine (TransVax) in 108 patients following alloSCT[24]. Although the frequency of CMV reactivation did not differ between the vaccine recipients and the controls, the combined endpoint of clinically significant CMV viremia and initiation of antiviral therapy was significantly reduced in vaccine recipients. Despite this significant effect on the combined endpoint, no difference in occurrence of CMV disease and CMV-specific T cell reconstitution could be demonstrated. The second randomized trial investigated the safety and efficacy of a chimeric peptide vaccine containing a CMV pp65 derived $CD8^+$ T cell epitope combined with a tetanus T helper epitope (CMVPepVax) in 36 patients following alloSCT[25]. This study demonstrated a significant effect after CMVPepVax vaccination with a significant rise in pp65-specific $CD8^+$ T cells, reduced incidence of CMV reactivation and usage of antiviral treatment, and increased relapse free survival compared to patients with observation only. This trial provides proof of principle that vaccination can improve CMV-specific T cell reconstitution after alloSCT. However, in this trial only HLA-A2 positive patients could be vaccinated due to the HLA restriction of the peptide in the CMVPepVax vaccine. Vaccination of non-HLA-A2 patients would require multiple vaccines with different CMV peptides or a single vaccine with multiple CMV peptides. Currently, a clinical trial (#NCT02506933) is being performed to determine the efficacy to prevent CMV disease after alloSCT with an attenuated poxvirus Modified Vaccine Ankara (MVA) containing 3 immunodominant CMV antigens (pp65, IE1 and IE2, Triplex). This vaccine was demonstrated to be safe and effective in inducing CMV-specific T cell responses in CMV seronegative and CMV seropositive healthy adults[26]. The results of this phase 2 trial must be awaited.

It has thus far not been demonstrated that CMV-specific vaccination can induce primary immune responses from donor origin. The efficacy of vaccination after alloSCT to induce a primary immune response depends on the immune status of the alloSCT recipient. This immune status is influenced by T cell reconstitution, occurrence of GVHD and use of immune suppression[27]. The optimal timing of vaccination after alloSCT to induce primary T cell responses is not clear. In our study on the origin of CMV-specific T cells early after alloSCT, the majority of the analyzed R^+D^- patients developed a CMV-specific primary immune response within the first year after TCD alloSCT, some patients even as early as 3 months after TCD alloSCT[19]. In a T cell depended pneumococcal vaccination it was demonstrated that the primary immune response rate increased from 54% at 3 months to 94% at 9 months after

alloSCT, demonstrating a time dependent effect probably due to T cell reconstitution[28]. To induce a primary immune response, presentation of the antigen by antigen presenting cells is mandatory. Dendritic cells (DC) are professional antigen presenting cells, which can be pulsed with antigen and used for vaccination after alloSCT (DC vaccination). In DC vaccination, donor derived DC are pulsed with pathogen specific peptides and transferred to the patient [29]. This approach, although time consuming and laborious, may be more effective to induce a primary CMV-specific T cell response and prevent CMV disease compared to peptide only vaccination in R^+D^- patients[20, 30]. Analysis of efficacy of CMV-specific vaccination to induce a primary immune response should include chimerism analysis to determine the origin of the induced CMV-specific immunity to exclude the effect of boosting residual recipient CMV-specific T cells after vaccination.

Due to the increased risk for CMV disease in R^+D^- patients, accelerating CMV-specific T cell reconstitution is especially important for these patients to prevent CMV disease. Because vaccination after transplantation does not yet reliably induce primary CMV-specific immune responses, vaccinating the CMV seronegative donor prior to harvesting the stem cell graft may be an effective approach to avoid the R^+D^- serostatus combination. Thus far, CMV vaccination of the donor prior to transplantation was attempted in one trial, but was not feasible because the time between donor identification and transplantation was not enough to perform adequate vaccination[24]. CMV vaccination of CMV seronegative donor prior to alloSCT to avoid the R^+D^- combination has never been studied.

VZV-specific T cell reconstitution after alloSCT

Cellular immunity is essential for preventing reactivation of VZV leading to the clinical syndrome of herpes zoster. Ex vivo analysis of VZV-specific T cell reconstitution after TCD is hampered by the lack of clinically validated immunodominant peptides needed for artificial HLA class I constructs (tetramers or pentamers). Therefore, we developed and validated the first VZV-specific pentamer (IE62-ALW-A2) by determining immunogenic antigens for VZV using a pentamer-based epitope discovery method[31].

Using this VZV-specific pentamer it was possible to detect VZV-specific $CD8^+$ T cells upon VZV reactivation after TCD alloSCT in 63% of HLA-A2 patients after TCD alloSCT. Compared to CMV-specific T cells, the frequency of IE62-ALW-$CD8^+$ T cells in ex vivo analysis was low (mean 0.04%, range 0.01%-0.11%). This lower frequency may be explained by differences in viral tropism and replication between CMV and VZV. Whereas CMV resides and reactivates regularly in monocytes and vascular endothelial cells, VZV resides in neurons, which are immune privileged sites, and reactivates only sporadically. We also demonstrated that antigenic stimulation by VZV reactivation following alloSCT leads to an increase in IE62-ALW-A2-specific T cells. It is demonstrated that introduction of VZV antigens to T cells in this situation leads to a boost in VZV-specific memory T cells, providing protection when VZV reactivates[32]. VZV-specific vaccination provides antigenic stimulation to boost T cell immunity to prevent VZV reactivation. As discussed earlier, the efficacy of vaccination after alloSCT is

determined by the immune competence of the patient after alloSCT, which is influenced by factors like the occurrence of GVHD and/or treatment with immune suppression affecting T cell reconstitution. The efficacy of vaccination in general is defined by a predefined rise in antibody titer with at least partially recovered B and T cell immunity. However, a recent study in VZV vaccination in alloSCT recipients demonstrated VZV vaccination induced T cell responses in the absence of a B cell antibody response[33]. Therefore, to determine vaccination efficacy after alloSCT additional immunological assays are necessary[34]. The IE62-ALW-A2 pentamer can potentially be used for ex vivo analysis of efficacy of VZV vaccination of HLA A2 positive patients after TCD alloSCT. Future directions on vaccination after alloSCT should focus on determining the individual immune competence to allow for optimal vaccination and protection from preventable diseases such as herpes zoster.

Origin of CMV-specific T cells

Understanding the mechanisms leading to successful CMV-specific T cell reconstitution is important for future attempts to improve CMV-specific T cell reconstitution and prevent CMV disease after alloSCT. We demonstrated that in CMV positive recipients (R^+) transplanted with a CMV seronegative donor (R^+D^-) CMV-specific T cells are mainly of recipient origin and that in time primary CMV-specific T cell responses can develop from donor origin[19]. Selecting a CMV negative donor will exclude the possibility of donor derived CMV-specific memory T cells to provide protection in the first months following alloSCT. Protection in that period depends solely on residual recipient CMV-specific T cells until the development of a primary CMV-specific T cell response of donor origin.

As discussed earlier, residual recipient CMV-specific T cells may be the target of an alloreactive response and may therefore be eradicated. It can therefore be hypothesized that prevention of an alloreactive T cell response can help to preserve recipient CMV-specific T cells. T cell depletion is used to prevent GVHD after alloSCT by preventing alloreactive T cell responses. T cell *re*pletion by adding back small numbers of T cells to the graft is performed with the intent to induce an alloreactive T cell response to eradicate the residual malignant cells (GVL) with lower risk of inducing GVHD. In a study by Chalandon the origin of CMV-specific T cells was compared after T cell *replete* and T cell *depleted* alloSCT[35]. CMV-specific T cells could be demonstrated in only 1/6 R^+D^- patients following T cell replete alloSCT compared to 2/2 R^+D^- patients following T cell depleted alloSCT. In the R^+D^- patients following T cell depleted alloSCT, the CMV-specific T cells were of recipient origin. This study demonstrates that lowering the chance to induce an alloreactive T cell response by TCD may allow persistence of residual CMV-specific T cells providing long-term control of CMV reactivation.

Donor Lymphocyte Infusion is used to induce an alloreactive immune response targeting minimal residual disease (MRD) and residual hematopoietic cells, aiming at conversion to full donor chimerism. It could be hypothesized that the application of DLI poses a potential risk of developing CMV disease in R^+D^- patients because of the eradication of recipient CMV-specific T cells by the induced alloreactive immune response. However, apart from developing CMV

disease in the setting of acute GVHD and subsequent treatment with immune suppression following DLI, it is unclear whether the incidence of CMV disease is increased after DLI in R^+D^- patients. It has been demonstrated that following DLI in R^+D^- patients, the recipient T cells were indeed eradicated. However, these cells were directly replaced by donor derived CMV-specific T cells, indicating a donor derived primary T cell response[36].

Whether or not recipient CMV-specific T cells are present in the patient following TCD alloSCT presumably also depends on the conditioning strategy used prior to alloSCT. In the study by Grimaldi and Sellars, demonstrating persistence of recipient CMV-specific T cells, patients received reduced intensity conditioning (RIC). In RIC a less toxic condition regimen is used with relative sparing of recipient hematopoietic cells including recipient CMV-specific T cells. In our study, the incidence and severity of CMV reactivation following alloSCT was comparable after RIC and myeloablative condition (MAC)[37]. This may be explained by the additional Anti Thymocyte Globulin (ATG) used in RIC conditioning in our study. This additional T cell depletion, used to avoid graft rejection by recipient T cells may not only eradicate alloreactive T cells from recipient origin, but also residual CMV-specific T cells. Also in our study the observation period was short, only 100 days following alloSCT.

Monitoring MRD by measuring total leucocyte chimerism (TLC) is important to predict relapse of the malignant disease for which alloSCT was indicated. Upon a rise of recipient TLC, interventions such as DLI are performed. However, It has been shown that following CMV reactivation the TLC demonstrated more recipient origin, especially in R^+D^- patients[36, 38]. A recent trial of 45 recipient of TCD alloSCT for severe aplastic anemia confirmed this positive correlation between recipient chimerism and CMV reactivation[39]. CMV reactivation caused a massive expansion of CMV-specific T cells from recipient, thereby influencing the TLC. Unlike following TCD alloSCT for hematologic malignancies, full donor chimerism was not promoted in these patients and mixed chimerism was not treated with DLI. This state of mixed T cell chimerism persisted for years even after stopping immune suppression, indicating a state of mutual tolerance of donor and recipient T cells. In this case TLC may not be a marker of minimal residual disease and DLI should not be performed to achieve full donor chimerism.

Adoptive cell transfer for prevention and treatment of CMV disease following alloSCT

Although it may take a period of several months to even years, eventually most R^+D^- patients develop CMV-specific immunity. If time is allowed for successful immune reconstitution and primary CMV-specific T cell responses develop, CMV reactivation will be controlled and CMV disease will be prevented. This paves the way for strategies to bridge the period of impaired immunity via adoptive T cell transfer (ACT) to prevent CMV disease. Although the rationale for ACT is clear, thus far no evidence for efficacy has been demonstrated in formal phase 3 trials. As discussed in our review on prevention of CMV disease following alloSCT, all trials published thus far are phase-1/2 trials with relatively small numbers of patients[8]. These trials suggest safety, proof of concept, and an association between ACT and viral clearance. Although restoration of anti-viral immunity after CMV-specific ACT was demonstrated, it

remains unclear whether all immune responses seen following ACT were causally related to the ACT, or that CMV-specific T cell responses developed irrespective of the ACT. This should be the focus of a formal randomized controlled clinical trial.

The purpose of ACT can be prophylactic (e.g. early administration to prevent CMV related complications) or therapeutic when administered in case of persistent CMV reactivation or overt CMV disease. The purpose of ACT may affect the choice of the techniques used for generating the T cell. In general, 2 different approaches are used to produce CMV-specific T cell products for adoptive transfer. Techniques without expansion or only minimal expansion generate less differentiated CMV-specific T cells. Stem cell characteristics such as multi-potency and self-renewal capacity have been demonstrated within these less differentiated T cells[40]. Adoptive transfer of these cells may lead to more effective CMV-specific T cell reconstitution and persistence than transfer of in vitro expanded effector T cells. If time for in vivo proliferation is granted, i.e. in the absence of persistent CMV reactivation or CMV disease as in prophylactic ACT and in the absence of immunosuppression or GVHD, the use of non-expanded CMV-specific T cell lines may be best suited for reconstitution of CMV-specific T cell immunity. Techniques using expansion by repeated stimulation generate large numbers of more differentiated CMV-specific effector T cells. However, repeated stimulation may lead to exhaustion and reduced persistence following ACT[41, 42]. If the goal of ACT is to temporarily overcome persistent CMV reactivation or CMV disease, transfusing large numbers of CMV-specific effector T cells may be sufficient to bridge and allow CMV-specific T cell reconstitution to develop. Future research to demonstrate efficacy of ACT should tailor the technique for isolating CMV-specific T cells and generating the T cell lines to the purpose of ACT.

Two factors hamper proper assessment of the clinical relevance of ACT following alloSCT, the exclusion of patients with active GVHD treated with systemic immune suppression and the exclusion of R^+D^- patients. GVHD and treatment with systemic immune suppression are major risk factors for CMV disease and these patients may benefit the most from CMV-specific ACT. Considering the body of evidence that ACT with in-vitro selected CMV-specific T cells is safe with minimal risk of inducing concurrent GVHD, future trials may consider including patients with active GVHD, especially when using cell products with high purity. In case of ongoing immune suppressive therapy the numbers of CMV-specific T cells used for ACT may need to be higher to overcome the immune suppression. R^+D^- patients are at greatest risk of developing CMV disease, due to delayed reconstitution of virus-specific immunity and may benefit greatly from CMV-specific ACT. However, most trials thus far isolate CMV-specific T cells from CMV seropositive donors. In theory, CMV-specific T cells from the CMV seropositive recipient harvested prior to the alloSCT procedure (autologous CMV-specific T cells) could be used for ACT in R^+D^- patients. Autologous CMV-specific ACT has not been extensively studied in a clinical trial. Successful treatment of CMV disease in one R^+D^- patient with ACT using autologous CMV-specific T cells is described in one case report[43]. In our study on CMV-specific ACT, autologous CMV-specific T cell lines were generated for 3 R^+D^- patients,

but these cell lines were never administered[44]. One patient died due to CMV disease during the cell production period and two patients cleared the CMV reactivation before the CMV-specific T cell product could be administered. In theory, transfusing autologous CMV-specific T cells could pose a risk for inducing graft rejection. In addition, the infused recipient CMV-specific T cells could be eradicated by alloreactive donor T cells in case of an alloreactive response from donor T cells, either as part of the desired GVL effect or as part of GVHD. Despite these considerations, the use of autologous CMV-specific ACT should be studied, as the treatment options for R^+D^- patients with CMV disease are limited.

A different solution for R^+D^- patients may be the use of CMV-specific T cells isolated from CMV seropositive third-party donors (TPD). Using TPD CMV-specific T cells allows the formation of a bank of stored T cell lines from CMV seropositive donors. In such a bank CMV-specific T cell lines from donors partially HLA matched with the ACT recipient can be stored, for example an HLA–A2 restricted CMV-specific T cell line for HLA-A2 positive alloSCT recipients. TPD CMV-specific T cell lines can be used "off the shelf" for the treatment of persistent CMV viremia or CMV disease which eliminates delays caused by obtaining fresh cells from the donor, T cell isolation, processing and quality control. It has been demonstrated that all T cells have the potential to cross-react to allo-HLA molecules, thereby inducing GVHD[45]. Therefore, potential toxicity risks include the risk for graft rejection by an alloreactive response to donor hematopoietic cells or induction of GVHD by an alloreactive response to recipient tissue antigens by the adoptively transferred TPD T cells. Trials indicate that ACT with TPD virus-specific T cells is feasible, probably safe and may be effective in treating persistent CMV reactivation and CMV disease[46, 47]. However, long-term persistence of these T cells is unlikely. The level of HLA matching between the TPD and the respective patient and stem cell donor impacts on the persistence of the adoptively transferred virus-specific T cells. Despite the concerns regarding the persistence of TPD T cells, a short-term effect as demonstrated in the recent clinical trials may be sufficient for bridging a period of severe CMV-specific T cell deficiency, thereby preventing or treating CMV disease and allowing for the development of subsequent CMV-specific immunity from the stem cell donor T cell repertoire for long-term control of CMV viremia.

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