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

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Chapter 7

Control of cytomegalovirus viremia after allogeneic stem cell transplantation; a review on CMV-specific T cell reconstitution

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Abstract

Recipients of allogeneic stem cell transplantation (alloSCT) are at risk for reactivation of endogenous herpesviruses due to profound and prolonged T cell deficiency following conditions such as GVHD, immunosuppression and/or T cell depletion. Reactivation of endogenous CMV is the most frequently occurring herpesvirus reactivation following alloSCT. Antiviral medication is often used in pre-emptive treatment strategies initiated when increases in CMV viral loads are detected as a result of active reactivation of the virus. 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 this review, we analyzed the available studies on the influence of donor CMV status on CMV-specific T cell reconstitution and CMV disease. Furthermore, 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, including studies on adoptive transfer of third-party CMV-specific T cells as a possible alternative when donor T cells are not available.

Introduction

Allogeneic stem cell transplantation (alloSCT) is a potentially curative treatment for various hematological diseases[1]. Following alloSCT, patients experience a period of profound and prolonged T cell deficiency in which they are at risk for developing infectious complications, including reactivations of endogenous herpesviruses like cytomegalovirus (CMV)[2], Epstein-Barr virus (EBV)[3] and varicella zoster virus (VZV)[4]. Infections with herpesviruses usually occur during childhood, and are controlled via the development of virus-specific T cell responses and ultimate formation of immunological memory. Despite virus-specific T cells control, herpesviruses are not completely cleared resulting in latent infections with equilibrium between the viruses and the virus-specific T cells. The frequencies of circulating memory T cells directed against these latent viruses are relatively high in immune competent, CMV or EBV infected hosts and can comprise up to 40% of the complete T cell repertoire[5, 6]. These high frequencies are presumably the result of repeated stimulation by frequent reactivations of CMV or EBV during life. Reactivation of endogenous CMV is the most frequently occurring herpes virus reactivation following alloSCT. In Western Europe and the United states 45-60% of alloSCT recipients is seropositive for CMV and therefore at risk for endogenous reactivation of latent CMV infection[7, 8].

CMV-specific T cells are essential for long-term control of CMV reactivation following alloSCT[9-11]. Failing CMV-specific T cell immunity, either quantitatively due to eradication by the conditioning regimen or qualitatively due to immune suppression or exhaustion by chronic antigen stimulation[12, 13] leads to impaired control of CMV reactivation, and may result in CMV disease, such as CMV pneumonitis, CMV colitis or CMV encephalitis[14]. Antiviral medication used in a pre-emptive treatment strategy can prevent CMV disease during this period of impaired CMV-specific T cell immunity. In a pre-emptive treatment strategy, viral load is routinely monitored using quantitative PCR and antiviral therapy is initiated when the viral load is above a predetermined PCR threshold. Multiple trials have demonstrated the safety and efficacy of pre-emptive strategies using oral valganciclovir[15]. However, despite the use of a pre-emptive strategy, the incidence of CMV disease is still 10% at 1 year following alloSCT in CMV seropositive patients[16, 17], illustrating the need for effective CMV-specific T cell immunity.

Several circumstances can increase the risk for CMV disease despite pre-emptive antiviral treatment (**table 1**). Graft versus Host Disease (GVHD) is associated with an increased risk of CMV disease despite pre-emptive antiviral treatment[18-22]. The use of an unrelated or HLA mismatched donor also implies an increased risk of developing CMV disease[20-22]. This may be caused by a higher risk of GVHD due to HLA mismatches requiring prolonged systemic immune suppression for prevention or treatment. Donor derived alloreactive T cells cause GVHD by targeting non-hematopoietic cells in the tissues and organs of the patient. Treatment of GVHD with systemic immune suppression not only suppresses alloreactive T cells responsible for the GVHD but also CMV-specific T cells. The increased risk of developing CMV disease in the presence of GVHD can also be caused by eradication

of recipient derived CMV-specific memory T cells by the profound alloreactive donor T cell response mediating the GVHD[23, 24]. If adequate reconstitution of donor-derived CMV-specific T cells is not yet sufficiently in place, these patients suffer from impaired anti-viral immune control.

GVHD can be prevented by immunosuppression after alloSCT or by T cell depleted (TCD) alloSCT[25-27]. In TCD alloSCT strategies, mature donor T cells are depleted from the stem cell graft. Various methods are used to deplete T cells from the graft resulting in different levels of TCD including $CD34^+$ selection resulting in almost complete T cell depletion or lymphocyte-depleting antibodies such as anti-thymocyte globulin (ATG)[28] or alemtuzumab[29, 30]. Although TCD strategies are effective in preventing GVHD and long-term post-transplant immune suppression is rarely needed, TCD also contributes to T cell impairment. Several studies demonstrate a higher incidence of CMV *reactivation* following TCD alloSCT. However, with the exception of $CD34^+$ selection, the incidence of CMV *disease* is not increased compared to non-TCD alloSCT[18, 19, 21, 31, 32]. The avoidance of immunosuppression after TCD alloSCT strategies, may allow even small numbers of CMV-specific T cells to reconstitute effective CMV-specific T cell immunity controlling CMV reactivation. This is confirmed by the finding that the high incidences of CMV disease after $CD34^+$ selected alloSCT can be reduced by adding back small numbers of T cells to the $CD34^+$ selected stem cell graft[33, 34]. This positive effect of even minimal numbers of donor T cells in the graft on the prevention of CMV disease leads to a preference of selecting a CMV seropositive donor for a CMV seropositive recipient.

Table 1. Overview of evidence for factors potentially associated with increased risk for CMV disease after allogeneic stem cell transplantation

Factor	Number of patients	Outcome on CMV Disease	Reference
GVHD	117 R^+ and R^-	Increased incidence CMV disease at day 100 in grade II–IV acute GVHD vs grade 0–I acute GVHD (17.1% vs 1.3%)	18
	162 R^+or D^+	Increased risk for CMV disease in acute GVHD (OR 9.7)	19
	1571 R^+	Increased risk for CMV disease in acute or chronic GVHD (Adjusted HR 4.1)	20
	186 R^+or D^+	Increased risk for CMV disease in steroid therapy for moderate- to-severe GVHD (HR 4.7)	21
	488 recipients with CMV reactivation after alloSCT	Increased risk for refractory CMV reactivation in acute GVHD (HR 1.9)	22
Unrelated/HLA mismatched donor	1571 R^+	Increased risk for CMV disease with unrelated or HLA mismatched donor (Adjusted HR 2.1)	20
	186 R^+or D^+	Increased risk for CMV disease with non-HLA-identical donors (HR 2.7)	21
	488 recipients with CMV reactivation after alloSCT	Increased risk for refractory CMV reactivation with HLA mismatched family donors (HR 2.0)	22
TCD	117 R^+ and R^- (15 (12.8%) received ATG for TCD)	No difference in CMV disease in conventional and ATG based TCD	18
	162 R^+or D^+	No difference in CMV disease in patients treated with ATG and not treated with ATG	19
	186 R^+ and R^- (23 alemtuzumab/ATG for T cell depletion)	No difference in CMV disease following RIC in conventional and alemtuzumab/ ATG based TCD	21
	73 R^+ and R^-	No CMV disease following Alemtuzumab based TCD; all matched related donor	31
	107 R^+ and R^-	No CMV disease within 100 days following Alemtuzumab based TCD, additional ATG in transplantation unrelated donor	32

Abbreviations: CMV = cytomegalovirus; R^+ = recipient CMV seropositive; R^- = recipient CMV seronegative; D^+ = Donor CMV seropositive; GVHD = graft vs host disease; OR = odds ratio; HR = hazard ratio; HLA = human leucocyte antigen; ATG = antithymocyte globulin; TCD = T cell depletion

Influence of donor CMV serostatus on CMV specific T cell reconstitution and CMV disease

Donor CMV serostatus and the associated presence or absence of donor CMV-specific memory T cells in the graft impact on the incidence of CMV related complications especially in CMV seropositive recipients undergoing alloSCT. In different studies the incidence of CMV related complications in CMV seropositive recipients transplanted with a CMV seropositive donor (R^+D^+) versus CMV seropositive recipients transplanted with a CMV seronegative donor (R^+D^-) has been investigated for TCD and non-TCD alloSCT programs (**table 2**). These studies demonstrate better CMV-specific T cell reconstitution[35-37] and less CMV related complications and deaths in R^+D^+ patients compared to R^+D^- patients[19, 36-41].

In R^+D^- patients CMV-specific memory T cells are not present in the graft and as a result the short term anti-viral immunity depends on residual recipient-derived CMV-specific memory T cells[42] and/or on the ultimate formation of a donor-derived primary CMV-specific T cell response. Recipient-derived T cells may be affected by the conditioning regimen prior to the alloSCT. Furthermore, all residual recipient derived lymphopoietic cells, including the T cells, may be attacked after the alloSCT by an alloreactive T cell response mounted by donor T cells. This is demonstrated by a study in which absence of CMV-specific T cells and CMV disease was seen in R^+D^- patients following T cell replete and not in T cell depleted alloSCT, indicating the eradication of residual CMV-specific T cells due to an alloreactive T cell response[43]. Consequently, adequate development of a donor derived primary T cell response is warranted for long-term anti-viral immune protection. Donor derived primary T cells can originate post transplant from donor stem cells via thymic development or from mature naive T cells present in the graft. Because the function of the thymus is anticipated to be greatly impaired in mature recipients following alloSCT, a primary T cell response derived from donor stem cells via thymic development cannot be expected shortly after alloSCT[43, 44]. High numbers of naive mature donor T cells present in the graft as seen in umbilical cord blood transplantation (UCBSCT) could hypothetically prevent CMV disease via the development of a primary CMV-specific immune response. However, despite the high number of naive T cells in the graft, delayed immune reconstitution and a high incidence of CMV disease is observed following UCBSCT[45-47].

Although it may take a period of 6 months to even several 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 is controlled and CMV disease is prevented. This paves the way for strategies to bridge the period of impaired immunity via adoptive T cell transfer (ACT) to prevent CMV disease.

Table 2. Effect of donor CMV serology on survival, clinical endpoints and CMV-specific T cell reconstitution in CMV positive recipients

Endpoint	No of CMV^+ recipients (R^+D^+ vs R^+D^-)	Outcome	Reference
Survival	298; TCD in all patients (177 vs 121)	Lower mortality in R^+D^+ (42% versus 56%)	36
	531; TCD in all patients (331 vs 200)	Lower mortality in R^+D^+; even in R^+D^+ unrelated donor vs R^+D^- sibling donor	38
	29349 (19385 vs 9964)	Superior overall survival R^+D^+ in MAC, not in RIC	39
	10638 (7008 vs 3630)	Superior overall survival R^+D^+ in MAC and RIC	40
CMV disease or treatment	298 (177 vs 121)	Lower incidence of CMV disease (3% versus 13%) in R^+D^+	36
	178; T cell repletion (128 vs 50)	More recurrent need for antiviral therapy in R^+D^- (16% vs 0.8)	37
	147 (78 vs 69)	Increased failure of pre-emptive treatment in R^+D^-	41
CMV-specific T cell reconstitution	69 (55 vs 14)	More CMV-specific T cell responses detectable at day 100 in R^+D^+ compared to R^+D^- (82% vs 42%)	35
	298; TCD in all patients (177 vs 121)	CMV-specific $CD4^+$ and $CD8^+$ T cell counts higher in R^+D^+	36
	178; T cell repletion (128 vs 50)	CMV-specific $CD8^+$ T cell counts higher in R^+D^+ compared to R^+D^-	37

Abbreviations: CMV = cytomegalovirus; R^+ = recipient CMV seropositive; D^+ = donor CMV seropositive; D^- = donor CMV seronegative; TCD = T cell depletion; MAC = myeloablative conditioning; RIC = reduced intensity conditioning

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

Several trials have been performed using ACT of purified populations of CMV-specific T cells isolated via different in-vitro strategies (**table 3**)[48-59]. ACT in these trials was either prophylactic or pre-emptive or intended for treatment of persistent CMV reactivation or CMV disease. All trials published thus far are phase-1/2 trials with relatively small numbers of patients. These studies suggest safety, proof of concept, and an association between ACT and viral clearance, but no formal phase-3 efficacy trials have been performed yet. Restoration of anti-viral immunity after CMV-specific ACT was demonstrated, however it remained 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.

In general, 2 different approaches are used to produce CMV-specific T cell products for adoptive transfer. CMV-specific T cell products can consist of in vitro selected and expanded CMV-specific T cell lines or non-expanded CMV-specific T cells. T cell lines consisting of expanded CMV-specific T cells can be produced by repeated stimulation of peripheral blood derived mononuclear cells (PBMCs) with CMV derived antigens. After repeated stimulation in the course of several weeks, T cells specific for the antigens used for stimulation will be the main component of the cultures due to preferential outgrowth. The advantage of this technique is than combined $CD4^+$ and $CD8^+$ T cell lines are usually generated, depending on the antigens used for stimulation. The antigens used for stimulation also determine the broadness of specificity of the T cells lines, ranging from specificity to a single peptide to protein spanning peptide pools or viral proteins. Although expansion techniques will lead to large numbers of CMV-specific T cells, the down side is that repeated stimulation may lead to exhaustion of the expanded T cells leading to reduced persistence following ACT[54, 60]. However, 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.

CMV-specific T cell lines can also be generated from isolated CMV-specific T cells without or after only minimal expansion, aiming to prevent T cell exhaustion. In vivo, naive T cells differentiate to effector T cells upon first activation by antigen encounter and into memory T cells, which can mount another response after re-exposure to the antigen. Memory T cells can either be less differentiated central memory T cells or more differentiated effector memory T cells[61]. Stem cell characteristics such as multi-potency and self-renewal capacity have been demonstrated within the less differentiated central memory T cell compartment [62]. Experimental studies demonstrated the capacity of a single naive $CD8^+$ T cells to repopulate and develop into various memory and effector subsets and transferring even very low numbers of less differentiated T cells may be effective for successful T cell reconstitution[63-65]. Therefore, adoptively transferring less differentiated CMV-specific T cells may lead to more effective CMV-specific T cell reconstitution and persistence than transferring in vitro expanded effector T cells. CMV-specific T cell products generated with no or very limited expansion can be made by selection of T cells producing interferon gamma (IFN γ) upon in-vitro stimulation with

CMV derived antigens using the cytokine capture assay and a magnetic bead isolation system (MACS)[66, 67]. Using stimulation with CMV antigens as basis for isolation allows isolation of both $CD4^+$ and $CD8^+$ CMV-specific T cells. Another method is the highly specific isolation based on interaction of the T cell receptor with CMV peptide-HLA-multimer complexes (e.g. tetramers and streptamers) loaded on isolation beads[68, 69]. In this technique however, isolation is only possibly for $CD8^+$ T cells, specific for the peptide used and with known HLA restriction pattern. In non-expansion techniques the number of CMV-specific T cells generated is lower compared to techniques based on T cell expansion, however with lower risk of culture-induced T cell exhaustion and better proliferative capacity and persistence. If time for in vivo proliferation is granted, i.e. in the absence of persistent CMV viremia or CMV disease as in prophylactic ACT and in the absence of immunosuppression or GVHD, using non-expanded CMV-specific T cell lines may be best suited for reconstituting CMV-specific T cell immunity.

A major limitation for interpreting the clinical relevance of ACT following alloSCT has been the exclusion of active GVHD treated with systemic immune suppression in all trials. However, 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 ACT 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, e.g. derived from an expansion-based approach to generate sufficient number of CMV-specific T cells. A potential future approach for patients with uncontrolled CMV viremia and active GVHD may be treatment with CMV-specific T cells rendered resistant to corticosteroids by gene editing techniques[70]. In this approach the glucocorticoid receptor gene of CMV-specific T cells is disrupted leading to corticosteroid resistance. Adoptively transferring these cells for ACT may be effective in treating CMV disease during corticosteroid treatment for GVHD. This technique is still in a preclinical stage, and safety and efficacy have to be determined in clinical trials.

A 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. However, especially CMV seropositive patients transplanted with a graft from a CMV seronegative donor (R^+D^-) are at greatest risk of developing CMV disease, due to delayed reconstitution of virus-specific immunity. 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 post-transplant for prevention or treatment of CMV disease in R^+D^- patients. However, transfusing autologous CMV-specific T cells poses a risk of inducing graft rejection and would be eradicated by alloreactive donor T cells in case of GVHD. Autologous CMV-specific ACT has not yet been studied in a clinical trial. A different solution for R^+D^- patients may be the use of CMV-specific T cells isolated from CMV seropositive third-party donors (TPD).

Table 3. Overview of trials using Adoptive Cell Transfer to prevent or treat CMV disease

Trial phase	Purpose	Method of generating T cell line	Number of patients	Clinical outcome	Reference
2	Pre-emptive treatment of CMV viraemia	Stimulation with donor DCs pulsed with HLA-A2 restricted CMV pp65 peptide NLV or transduced with adenoviral vector encoding CMV protein pp65 ; cultured for 21 days	50; D^+ recipients compared to contempory controls	No increase in GVHD Reduction of patients who required CMV directed antiviral therapy (17% vs 36%)	48
1-2	Treatment of refractory CMV reactivation or CMV disease	Isolation of IFN γ -producing cells after stimulation with pp65protein; infused without further in vitro expansion	18; 16 D^+ recipients, 2 D^- recipients received third-party T cells	1 case of GVHD 15/18 cases CMV infection cleared or viral burden reduced	49
1-2	Pre-emptive treatment of CMV, EBV or AdV reactivation	Stimulation with donor DCs transduced with immunodominant viral antigens from EBV, CMV and AdV; cultured for 2–3-weeks	10 D^+ recipients	Complete virological responses in 80% Decrease in viral load correlated with an increase in the frequency of virus-specific T cells	50
1	Pre-emptive treatment of CMV viraemia or treatment of CMV disease	Stimulated with 15-mer peptide pool spanning CMV derived protein pp65; cultured for 28 days	17; 16 D^+ recipients , 1 D^- recipient received third-party T cells	No de novo GHVD 15/17 cases cleared CMV viraemia, including 3/5 cases with CMV disease	51
1-2	Prophylactic treatment CMV, EBV, AdV and VZV related complications	Stimulated with donor DCs transduced with adenoviral vector encoding CMV protein pp65 and EBV epitopes or pulsed with a VZV vaccine; cultured for 21 days	10 D^+ recipients	3/10 grade II-IV de novo GVHD No CMV disease (7 CMV reactivation, 1 required antiviral therapy)	52

1-2	Treatment of refractory CMV reactivation	Stimulation with HLA-A2 and/ or HLA-B7 CMV pp65 peptides (NLV/TPR), enrichment IFN γ -producing T cells after 1 day; cultured for 7–14 days	6 HLA-A2$^+$ or B7$^+$ D$^+$ recipients	No GVHD Efficacy of administered CMV pp65-specific CD8$^+$ T cell lines demonstrated in several patients	53
1	Prophylactic treatment	Stimulation with donor DCs pulsed with CMV derived pp65 NLV peptide; cultured for 21 days	9 HLA-A2$^+$ D$^+$ recipients with a HLA-A2$^+$ donor	3/9 cases Grade 3 acute GVHD 1 patient died due to gastrointestinal GVHD	54
1-2	Pre-emptive treatment EBV, AdV, CMV, BK, and HHV-6 reactivation	Stimulation with overlapping peptide libraries of EBV, CMV, AdV, BK virus and HHV; cultured for 9-11 days	11 patients No generation of CMV-specific T cell lines possible in R$^+$D$^-$ patients	1/11 grade II de novo GVHD 94% virological and clinical response rate	55
1-2	Prophylactic and pre-emptive treatment	Stimulated with recombinant pp65 or pool of overlapping peptides from CMVpp65 and after 1 day isolation and storage IFN γ -producing T cells	25 D$^+$ recipients	8/25 cases GVHD (5 grade I) Expansions of CD4$^+$ and CD8$^+$ CMV-specific T cells shortly after adoptive transfer	56
1-2	Prophylactic and pre-emptive treatment	Stimulation with donor DCs and CMV viral lysate; cultured for 2 weeks	30 D$^+$ recipients	4/30 developed Grade II-III GvHD Massive expansions of CMV -specific T cells	57
1-2	pre-emptive treatment	Stimulation with donor DCs and CMV antigen and cultured for 14-21 days	16 D$^+$ recipients	3/16 cases Grade I acute GVHD Massive expansions of CMV -specific T cells after adoptive transfer	58
1-2	Prophylactic treatment	Stimulation with autologous CMV infected fibroblasts; cultured for 7-14 days. CMV-specific CD8$^+$ T cells cloned from cultures by limiting-dilution method	14 D$^+$ recipients	No toxic effects Increased in vivo cytotoxic activity to CMV after infusions	59

Abbreviations: DC = dendritic cell; D$^+$ = donor CMV seropositive; GVHD = graft versus host disease; IFN = interferon; HLA = human leucocyte antigens; UCB = umbilical cord blood transplantation; EBV = Epstein Barr virus; AdV = adenovirus

Adoptive cell transfer using third-party CMV specific T cells

Using CMV-specific T cells from third-party donors (TPD) allows the formation of a bank of stored T cell lines from CMV seropositive donors with different HLA types. TPD CMV-specific T cell lines can be used "off the shelf" for treating persistent CMV viremia or CMV disease which eliminates delays caused by obtaining fresh cells from the donor, T cell isolation, processing and quality control. The potential efficacy as well as the potential toxicity of this approach is likely to be associated with the level of HLA matching between the third party donor and the respective patient and stem cell donor. 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. It has been demonstrated that T cells, including CMV-specific T cells, harbor the capacity to cross-react to one or more allo-HLA molecules, thereby potentially inducing GVHD[71]. Vice versa, rejection of the adoptively transferred TPD T cells by anti-HLA/alloreactive donor derived T cells may also occur, consequently hampering efficacy.

In one recent multicenter trial 50 patients with severe refractory CMV, EBV or adenovirus infections were treated with banked TPD virus-specific T cells[72]. The cell lines used for adoptive transfer in this trial were generated by expansion, generating large numbers of CMV-specific T cells. The cumulative rate of complete responses (decrease of viral load below limit of detection and resolution of symptoms) or partial responses (decrease of viral load of at least 50% and alleviation of symptoms) was 74%. Development of de novo GVHD was seen in only 2/50 patients. Another recent study described ACT with TPD CMV-specific T cells in 8 R^+D^- patients with persistent CMV reactivation and no visible frequencies of circulating CMV-specific T cells[73]. The cell products used in this trial were generated without expansion, resulting in low numbers of CMV-specific T cells. In all but one patient survival/persistence of TPD CMV-specific T cells could not be demonstrated in peripheral blood of the patients after infusion. In one patient TPD T cells were found back at detectable frequencies after the adoptive transfer. In contrast to the other patients in this study, in this single case there was a complete HLA match between the patient, the stem cell donor and the TPD T cells.

These 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. 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 most likely impacts on the rejection of the adoptively transferred virus-specific T cells. However, the induction of an alloreactive response to reject the TPD takes time to develop and will depend on a functional T cell compartment in the patient. Therefore, the use of a third party T cell product with confers immediate protection is probably preferred over a product in which the T cells depend on profound in vivo proliferation for protection because by the time the third party T cells may have sufficiently proliferated, these cells may be eradicated by a developing alloreactive response by patient T cells. Thus, for immediate short-term protection in R^+D^- patients

with refractory CMV viremia or CMV disease, large numbers of TPD CMV-specific T cells produced by expansion techniques may be more effective than non-expanded 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.

Although the clinical results so far suggest that ACT with TPD CMV-specific T cells is safe, the induction of GVHD is still a major concern when using partially HLA matched TPD T cells and may correlate with in vivo persistence of the TPD T cells. The low incidence of GVHD after TPD ACT observed in the clinical studies may at least in part be explained by the rapid rejection and/or limited persistence of TPD T cells. If TPD were to persist and proliferate, GVHD may manifest as collateral damage.

A potential future alternative to TPD T cells is CMV-specific T cell Receptor (TCR) transfer to T cells from the CMV negative donor[74]. If stem cell donor derived T cells will be used, they will be likely to persist and expand after infusion. However, a potential danger is still the induction of GVHD due to a co-expressed endogenous alloreactive TCR. A clinical trial is currently undertaken to determine to the incidence of GVHD and the efficacy to generate CMV-specific T cells responses by infusing CMV TCR transduced T cells (ClinicalTrials.gov NCT02988258). Another potential future approach for R^+D^- patients is adoptively transferring CMV-specific T cells developed from the naive repertoire of the CMV seronegative donor. Although the procedure is experimental and the procedure is time-consuming, two studies demonstrated proof of principle that generation of CMV-specific T cells from a naive T cell repertoire is possible[75, 76].

Conclusion

Despite the use of pre-emptive strategies to control CMV viremia, CMV disease is not prevented in all patients. Patients with impaired CMV-specific T cell immunity due to GVHD, systemic immune suppression or absence of CMV-specific memory T cells in the graft have the greatest risk of developing CMV disease. The selection of a CMV seropositive donor for a CMV seropositive patient will potentiate efficient CMV-specific T cell reconstitution and significantly reduces the incidence of CMV disease. Multiple trials demonstrated that ACT with CMV-specific donor T cells is feasible and safe. However, the ultimate proof of efficacy of these strategies must come from future placebo controlled phase-3 clinical trials with significant patient numbers. In patients with GVHD treated with immune suppression, larger numbers of T cells may be required for ACT to overcome the effect of the immune suppression. ACT using third-party off the shelf donor derived CMV-specific T cell lines may be applied to provide short-term protection and temporary control of persistent CMV reactivation and disease in alloSCT patients. However, no long-term survival/persistence of partially HLA matched third-party donor derived CMV-specific T cells is likely to occur. The use of larger numbers of third-party CMV-specific T cells may be effective in bridging the period

of impaired immunity, despite the eradication by an alloreactive response. The execution of a large trial evaluating the effect of third party T cell products on CMV reactivation and -disease, the relevance of HLA-matching between third party donor and recipient, and the persistence of third party donor T cells in relation to the occurrence of GVHD would greatly enhance our knowledge to prevent and treat CMV disease in immunocompromised patients.

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