Monday, March 28th

09:00-10:30

Oral 9: Cellular therapies

Nervous system regulates thymic regeneration after immune injuries

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Abstract

Paradoxical to its importance as the main organ responsible for generating T cells throughout life, the thymus is extremely sensitive to negative insults including cytoreductive chemo- or radiation therapy, infections and GVHD. Insufficient recovery of thymic function has been directly correlated with increased risk of opportunistic infection and poor clinical outcome in transplanted recipients. Therefore, strategies to regenerate thymic function and immune reconstitution represent a significant unmet clinical need. While multiple paracrine and endocrine pathways have been shown to regulate thymic function, the regulatory role of the peripheral nervous system remains largely unknown. Given its previously described role in modulating bone marrow hematopoiesis after damage, we sought to investigate if the sympathetic nervous system (SNS) mediated similar effects on thymic regeneration after immune insults. We observed that thymic concentration of epinephrine and norepinephrine was increased in mice after sub-lethal total body irradiation (SLTBI), reaching a maximum concentration at day 4 and returning to its baseline levels at day 14 post-SLTBI. Daily administration of epinephrine or norepinephrine directly resulted in a significant decrease in thymic regeneration post-SLTBI. We identified that SNS-related negative regulation of thymic recovery occurs specifically through an α1A/D dependent mechanism, given that administration of tamsulosin, an α1A/D specific inhibitor was able to enhance thymic cellularity post-SLTBI, while no effect was observed after the administration of pan-α and pan-β adrenergic antagonists. Besides the autonomic nervous system, sensory nociceptive C-fibers have been shown to pose a strong peripheral input to the immune system, mainly via the transient receptor cation channels TRPV1 and TRPA1, which among others, serve as receptors for multiple endogenous and exogenous reactive ligands. We observed that within the thymus TRPA1 was highly expressed in the medulla and sparsely in the cortex, mainly representing intraparenchymal small nerve fibers. Nerve fibers were also found to adjacent to vessels, while interestingly thymic endothelial cells expressed TRPA1 as well. On the other hand, TRPV1 expression was weak and primarily localized at the subcortical area. While thymic cellularity was unimpaired in a TRPV1 KO setting, TRPA1 KO mice exhibited significantly lower thymic cellularity at baseline and after SLTBI, suggesting that TRPA1 represents a critical factor for organ regeneration after insults. Consistently, administration of cinnamaldehyde and allyl-isothiocyanate, two potent, exogenous TRPA1 agonists, significantly enhanced thymic regeneration post-SLTBI. Our study attributes for the first time functional roles to distinct peripheral nervous system compartments in the context of endogenous thymic regeneration. Most importantly, the pharmacological modulation of negative SNS-imposed or positive TRPA1/nociceptive-imposed signals represents a novel therapeutic approach to enhance thymic regeneration and immune recovery in immunocompromised patients.

Disclosure of conflict of interest

None
Prospective multicenter pilot phase II Study of sequential infusion of donor lymphocyte infusion (DLI) and Cytokine Induced Killer (CIK) cells for patients with relapse after allogeneic transplantation.

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Abstract

Introduction: Disease relapse is a major cause of mortality following allogeneic hematopoietic stem cell transplantation (alloHSCT) for hematologic malignancies. When allogeneic transplant fails, donor lymphocyte infusion (DLI) is one of the main clinical option, but this treatment is complicated by a high risk of acute graft-versus-host disease (GvHD). Donor’s Cytokine Induced Killer (CIK) cells have shown Graft versus Leukemia (GvL) activity with little GvHD and, therefore, may represent an ideal alternative candidate to treat post-transplant relapse. We report the final results of a phase II multicenter pilot study, whose objective was to evaluate the safety and efficacy of sequential administration of donor derived unmanipulated DLI and CIK cells in patients with recurrent hematologic cancers after alloHSCT. Methods Seventy-four patients relapsed after matched related (N=42) or unrelated donor (n=32) alloHSCT, were enrolled in the study. This phase II multicenter study was authorized by Istituto Superiore di Sanità, as for Advanced Therapeutic Medicinal Product (ATMP) regulations, and approved by the Agenzia Italiana del Farmaco (AIFA). The trial was registered as EUDRACT number 2008-003185-26; Clinical Trial.gov: NCT01186809. Results We evaluated 74 patients (including 16 children and 58 adults) treated with sequential administration of unmanipulated donor lymphocytes infusions (DLI) followed by three infusions of donor derived CIK cells. Two patients died before starting therapy due to disease progression, 9 patients died during the DLI administrations due to disease progression, 1 patient developed aGvHD and was not further treated with CIK cells and 1 patient was withdrawn from the protocol. Therefore, 61 patients received at least one CIK administration and 43 completed study protocol. The first 12 patients were treated with increasing numbers of CIK cells, in groups of three patients per dose level. Since dose limiting toxicity (DLT) was never observed (acute GvHD of grade IV), the highest dose planned (5 x 10^6/kg, 5 x 10^7/kg and 10 x 10^6/kg) was then administered to all patients. As per protocol, clinical response was determined 100 days after the last CIK administration and the study was analyzed on an intent to treat basis. Acute GvHD was observed in a total of 12 patients (16%): grade 1-2 (n=7) and 3-4 (n=5). During follow up, chronic GvHD was observed in 11 patients (15%) (4 mild, 5 moderate and 2 severe). An early death occurred in 2 (3%) patients, progression of disease was observed in 41 (55%) patients, a stable disease in 8 patient (11%), a complete remission in 20 (27%) and a partial remission in 3 (4%), for an overall response rate of 31%. Progression free survival (PFS) and overall survival (OS) were significantly associated (p<0.0001) with the type of relapse since at 3 years it was 13% and 25% vs. 57% and 64 % for patients enrolled due to a hematologic vs a molecular/cytogenetic relapses, respectively (Figure 1 A-B). By multivariate analysis, the type of relapse and a short time from alloHSCT to relapse (<6 months) were the significant predictors of survival (HR 3.37, 95%CI 1.59-7.16 and 2.1, 95%CI 1.10-3.90). Conclusion Our study shows that administration of CIK cells is feasible in patients with recurrent hematologic cancer after alloHSCT with a relatively low toxicity in terms of GvHD. Particularly in the setting of the molecularly relapsed patients, long-term survival can be achieved.
**Figure 1A. Progression Free Survival by relapse type**

![Graph showing progression free survival by relapse type.](image)

- **Molecular+Cytogenetic**: 57%
- **Hematologic**: 13%

**N at risk (events)**
- **Hematologic**: 44 (38) 5 (0) 4 (0) 3 (0) 3 (0) 3 (0) 3 (0) 0
- **Molec+Cytog**: 30 (12) 14 (0) 8 (0) 5 (0) 5 (0) 3 (0) 2

**Figure 1B. Overall Survival by relapse type**

![Graph showing overall survival by relapse type.](image)

- **Molecular+Cytogenetic**: 64%
- **Hematologic**: 25%

**N at risk (events)**
- **Hematologic**: 44 (28) 15 (3) 9 (1) 6 (0) 6 (1) 5 (1) 5 (1) 0
- **Molec+Cytog**: 30 (8) 19 (0) 12 (1) 6 (0) 5 (0) 3 (0) 2

*P<0.0001

*P=0.0004*
Disclosure of conflict of interest

None of authors have any conflict of interest to declare with respect to this academic non-sponsored trial.
Rapid Process for the Generation of Functional Chimeric Antigen Receptors against Novel Targets

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Abstract

Introduction: Chimeric antigen receptor (CAR) T cells bind to surface antigens via a single-chain variable fragment (scFv) and elicit potent anti-tumor activity. To our knowledge, no CAR development platform exists that allows the rapid generation, screening, biochemical characterization, and identification of functional CAR constructs against novel targets integrating multiple state-of-the-art high-throughput technologies. Experimental procedures: We have established a rapid process for the development of functional CARs against novel targets using fully human antibody phage display, compatible vector systems for bacterial and mammalian expression, as well as high-throughput functional and biochemical characterization assays. The individual steps of our process were validated using different tumor antigens and we here describe the complete workflow for one model antigen expressed on multiple myeloma cells. Using two rounds of panning selections from a large naïve fully-human antibody phage display library we generated a pool of scFvs that bind to the model antigen. After confirming the reactivity of the polyclonal phage population by time-resolved fluorescence assay we generated multiple monoclonal binders in scFv, scFv-Fc and CAR formats. In addition to determining antigen binding of the soluble antibody formats we established a high-throughput flow cytometry assay simultaneously determining CAR surface expression and antigen binding. Furthermore, we adapted protocols for small-scale lentiviral transduction and expansion of primary CAR T cells followed by a sensitive, high-throughput luciferase-based cytotoxicity assay using multiple CAR candidates. Finally, in collaboration with Wasatch Microfluidics we performed high-throughput surface plasmon resonance measurements and epitope binning of candidate binding domains. Results: After two rounds of antibody phage selections we obtained 1,323 scFv binders. The subsequent bacterial expression of 163 soluble scFv clones yielded 23 unique monoclonal binders. Surprisingly, a comparison of these 23 binders in different formats showed that none of the traditional screening formats, including soluble scFv and scFv-Fc fusion constructs, correlated with CAR binding. CARs showing high combined expression and antigen binding were found to have affinities in the low nanomolar range (18.4-22.9nM). They also displayed significant cytotoxicity of myeloma cell lines spontaneously expressing the antigen (53-79% killing at an effector:target-ratio of 10:1), but healthy cells expressing lower levels of the antigen were relatively spared. Importantly, in a murine xenograft model of multiple myeloma we found that our newly generated CAR T cells specifically killed tumor cells without overt toxicities. Conclusion: We show that the process that we have developed allows the rapid generation of multiple candidate scFvs, screening, as well as functional and biochemical characterization of CARs targeting novel antigens within 2 months. Determining key properties of binding domains, which have been shown to shape CAR T cell function and phenotype, during initial screening enables the rational selection of CAR candidates prior to low-throughput downstream analyses. In addition, our data suggest that the common strategy of reformattting existing monoclonal immunoglobulins into CAR binding domains, while fast and usually reliable, may not produce ideal CAR constructs.

Disclosure of conflict of interest

Adam Miles is an employee of Wasatch Microfluidics
The Infusion of Multi-Antigen Specific T-Cell Products for the Prevention of Viral Infections after T-Cell Depleted Allogeneic Stem Cell Transplantation

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Abstract

Viral infections and disease relapse are major complications in the interval between T cell depleted allogeneic stem cell transplantation (TCD alloSCT) and donor lymphocyte infusion (DLI). The infusion of selected donor T cells can be an effective method to restore anti-viral and anti-tumor immunity early after TCD alloSCT. In this phase I/II study (EU FP7 T control) the feasibility and safety of the generation and preemptive administration of donor T cells targeting CMV, EBV and AdV to restore viral immunity together with T cells targeting tumor associated antigens (TAA) and minor histocompatibility antigens (MiHA) to boost the graft versus leukemia (GVL) response is evaluated. Efficacy is assessed by in-vivo appearance/expansions of target Ag-specific T cells in the peripheral blood detected with direct tetramer staining, and the effect on viral reactivation and disease relapse until DLI was given 20 weeks later. HLA-A*02+ patients treated for a hematological malignancy with an HLA-matched TCD alloSCT from a CMV+ and/or EBV+ donor were included. 6-8 weeks after alloSCT, T cells directed against HLA-A*02-restricted peptides of CMV, EBV and AdV, and the TAA NY-ESO-1, WT-1, RHAMM, PRAME and proteinase 3 were isolated under GMP conditions in 1 day, using the reversible streptamer-nanobead technology by cliniMACS selection out of the naive and memory T cell compartment from 2*10^9 donor PBMC. Depending on the patient/donor HLA-typing, additional streptamers targeting viral peptides in HLA-A*01, A*24, B*07, or B*08 were added to the procedure as well as the HLA-A*02/HA-1h streptamer in case of MiHA disparity in the GVL direction. At the moment of the interim-analysis of this trial, 21 multi Ag-specific T cell products have been generated that consisted of 0.4-26*10^6 cells with purities of 46-99% target Ag-specific T cells within the T cell compartment. 19 products were administered without infusion related complications or GVHD; 2 patients experienced GVHD at the day of infusion and did, therefore, not receive their product. 14 patients were analyzed at this stage. All 14 donors were EBV+ and AdV+ and 5/14 donors were CMV+. All products consisted for 99% of virus-specific memory T cells, while the remaining 1% included TAA, MiHA and naïve virus-specific T cells. No product-related adverse events were reported. 13 patients completed follow-up; 1 patient died during follow-up. 2 patients showed disease relapse before DLI without obvious coinciding expansion of TAA or MiHA-specific T-cells. More sensitive techniques may be needed to visualize these cells. None of the patients experienced AdV reactivations, although in 1 patient AdV-specific T cells appeared after infusion of our T cell product. In 6 patients CMV reactivations were observed. 2 patients received the product from a CMV+ donor and 4 patients from a CMV- donor. In all 6, CMV-specific T cells were detected and CMV was cleared. 3 patients experienced an EBV reactivation. In 2 patients, the virus was cleared without obvious expansion of EBV-specific T cells. 1 patient required treatment for an EBV-PTLD; ultimately EBV-specific T cells appeared and the virus was cleared. In conclusion, we have shown that the streptamer-nanobead based generation and adoptive transfer of donor-derived multi Ag-specific T cell products is feasible and safe and can be used as a strategy to prevent viral infections between TCD alloSCT and DLI.

Disclosure of conflict of interest

None
CD19 targeted CAR-T therapy versus chemotherapy in re-induction treatment of refractory/relapsed acute lymphoblastic leukemia: results of a case-controlled study

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Abstract

Introduction CD19 targeted CAR-T cells (CART19s) have potent anti-leukemia activities in patients with refractory/relapsed acute lymphoblastic leukemia (R/R ALL). This case-controlled single center study was designed to determine the safety and efficacy of CART19 therapy for R/R ALL in contrast to chemotherapy. Patients and Methods A matched case-controlled study, in which each patient treated with CART19s (CART19 group, 22 patients) was paired with 3 control subjects selected among R/R ALL patients treated with conventional or salvage chemotherapy (chemotherapy group), was performed. Patients relapsed after allogeneic hematopoietic stem cell transplantation (allo-HSCT) received donor lymphocyte infusion after chemotherapy if donor lymphocytes were available. Results Patient characteristics were shown in Figure 1A. The complete remission rate was significantly higher in CART19 group than in chemotherapy group (90.5% [95% confidence interval (CI), 76.2 to 99.9] VS 38.1% [95% CI, 32.0 to 44.2], P<0.001). For patients relapsed after allo-HSCT and chemotherapy, the CR rates in the 2 groups were 100% VS 48.0% (P=0.0099) and 84.6% VS 30.8% (P=0.0432), respectively. Among patients who had complete remission, a higher percentage in CART19 group had results below the threshold for minimal residual disease (0.01% marrow blasts) (100% vs 25.0%, P<0.001). In the survival analysis, the overall survival rate at 12 months was significantly higher in CART19 group than in chemotherapy group (51.5% vs 12.7% ; hazard ratio, 0.576 [95 % CI, 0.09839 to 0.5865]; P=0.048) (Figure 1B). 22.2% and 46.7% post-transplant patients in CART19 group and chemotherapy group complicated with graft versus host disease (GVHD) (P=0.241) but for patients who obtained complete remission, 22.2% and 75.0% patients complicated with GVHD (P=0.0348) in the 2 groups respectively. Pancytopenia was another kind of toxicity. For patients achieved complete remission, the median duration of absolute neutrophil count less than 500/μL and absolute platelet count less than 20000/μL were longer in CART19 group than in chemotherapy group [22.6 days (4-35 days) vs 16.3 days (8-19 days), p=0.021] and [32.1 days (4-39 days) vs 18.8 days (10-23 days), p=0.023], respectively. Conclusions CART19s induced high complete remission rate both for relapsed patients after allo-HSCT and after chemotherapy in contrast to conventional or salvage chemotherapy. CART19s also induce less incidence of GVHD but longer duration of pancytopenia. Our data suggest that CART19s could provide a novel therapeutic approach for patients with R/R ALL.
Disclosure of conflict of interest

No conflict of interest to declare
Five Years of Therapy with Donor and 3rd Party Derived EBV and CMV Specific Cytotoxic T Cells – Safety After More Than 1,000 Infusions

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Abstract

At Memorial Sloan Kettering Cancer Center (MSK), GMP grade EBV and CMVpp65 viral specific cytotoxic T lymphocytes (CTLs) have been produced for use on specific protocols since 1994 (EBV) and 2005 (CMV), respectively. Experience with adoptive cellular therapy has accelerated over the last 2 decades and we anticipated that this will result in more appropriate referrals for therapy. Over the 5 year period from January 2011 until December 2015 we have treated 164 patients on 5 distinct protocols; in addition, 12 patients who did not qualify for protocol therapy received CTLs on Single Patient Use Emergency INDs. Indications for CMV-CTL treatment were: CMV after hematopoietic cell transplant (HCT), after solid organ transplant (SOT), and in the setting of HIV infection. In the EBV setting, indications were: EBV viremia, and EBV disease arising after HCT and SOT and in the setting of underlying or acquired immune deficiency. In addition, subsets of patients with EBV positive malignancies without defined immune deficiencies have been treated. A total of 1,054 infusions of EBV-CTLs and CMV-CTLs have been administered. Infusions were administered in the inpatient and outpatient setting of the Medicine and Pediatric Stem Cell Transplant Services at MSK. Cumulative safety data collected over this time period is being reported. Of 80 patients treated with donor derived and/or third party derived CMV-CTLs during this time period, 30 patients had severe adverse events (SAEs) reported with 7 patients experiencing 17 possibly related Grade 3 or higher SAEs (1 mental status changes, 1 with diffuse alveolar hemorrhage (DAH) in a patient with a prior history of DAH, 4 hypoxic events and 1 patient with multiple cytopenias). There were no probably or definitely related SAEs. Of 100 patients treated with donor derived and/or third party derived EBV-CTLs during this time period, 34 patients had SAEs reported with 7 patients experiencing 9 possibly related Grade 3 or higher SAEs (1 patient with mental status changes, 1 seizure, 1 nausea, 2 lymphopenia, 3 electrolyte imbalance, 1 febrile neutropenia). There were no probably or definitely related SAEs. No patients developed Graft versus Host Disease (GvHD) related to EBV or CMV-CTL therapy during this time period. Prior to January 2011 one patient developed GvHD after each type of cell therapy (EBV-CTL: Grade 1 skin responding to topical steroids; CMV-CTL: Grade 3 skin and lower GI occurred in the setting of new onset HHV6 viremia required systemic therapy). No patients developed any manifestations of cytokine release syndrome or hemophagocytic lymphohistiocytosis and no patient who had received prior radiation developed radiation recall. In conclusion, adoptive T cell therapy using both using donor derived viral specific cytotoxic T cell lines therapy and third party derived banked viral specific cytotoxic T cell lines has been well-tolerated without infusion reactions or cytokine release syndrome and is associated with minimal GVHD and a low incidence of SAEs with no probably or definitely related Grade 3, 4 or 5 events related to CTLs.

Disclosure of conflict of interest

Drs Doubrovina, Hasan, Koehne and O’Reilly: Atara Biotherapeutics: Consultancy and Research Funding.
TREATMENT OF POST-ALLOGENEIC STEM CELL TRANSPLANT CYTOPENIAS WITH SEQUENTIAL DOSES OF MESENCHYMOAL STROMAL CELLS: RESULTS OF A MULTICENTER PHASE II TRIAL

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Abstract

INTRODUCTION: Post-transplant cytopenias are a complication with remarkable morbidity and mortality for which there is no effective treatment in refractory cases. Mesenchymal stromal cells (MSC) as key regulators of the bone marrow (BM) niche and because of their immunomodulatory properties are a potentially attractive therapeutic tool in this setting. METHODS: Twelve patients were included in this multicenter phase II clinical trial (EudraCT code: 2013-000534-35). Main inclusion criteria were hemoglobin 100x10^9/L] to the day 90, adverse effects and survival was analyzed after infusion four doses of 1x10^6 MSC / kg of recipient body weight on days 1, 4, 11 and 18. OBJECTIVES: Primary objective was to analyze the safety and feasibility of MSC infusion in this setting. Secondary objective was to analyze the effectiveness in terms of blood counts recovery and length of the response. RESULTS: Median age of the 12 patients included was 49 years (range 20-66), and most patients had AML (n=8). Cell source for transplantation included HLA-identical URD (n=7), identical sibling (n=3), UCB (n=1) and haploidentical donors (n=1). Seven patients received RIC regimen. Regarding the type of cytopenia, most patients had isolated thrombocytopenia (n=9), neutropenia (n=1), or both (n=2). Most patients had received one prior treatment for cytopenia (range 1-3), mainly steroids and immunoglobulins. Nine out of the twelve patients (75%) had concomitant GVHD at least a month previous to the first MSC infusion. MSC infusion was performed after a median of 106 days from allo-BMT (range 35-633). There were no adverse effects related to the cell infusions that were performed in the ambulatory setting in some cases. Within the first 90 days, ten out of the twelve patients (83%) responded to cellular therapy and eight of them achieved complete response (CR). On day 90, six patients maintained CR and two maintained partial response (RP), whereas two patients had no response and two were non-evaluable. At the last follow-up, with a median of 213 days (range 76-861), seven patients (3 CR, 4 PR) maintained the response, four patients deceased due to the progression of disease (n=1) or sepsis (n=3). One patient achieved CR with an alternative treatment. Patients that achieve response to MSC therapy seemed to have an advantage in terms of long-term survival. CONCLUSIONS: Treatment of peripheral cytopenias with MSC is feasible, has no adverse effects and is potentially useful in most patients. Achieving either CR or PR to MSC therapy seems to favor long term survival for those patients. Funding: This clinical trial was funded by the EC11-389 grant from Ministerio de Sanidad, Spain.

Disclosure of conflict of interest

None
Adoptive transfer of CMV-specific T cells for persistent CMV infection after haploidentical stem cell transplantation: association between antiviral immunity and the improving of quantity and quality of CMV-specific T cells

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Abstract

Background. Adoptive transfer of cytomegalovirus (CMV) specific T cells has been developed as a safe and effective treatment strategy for CMV infection. However, little experiences had been reported about the CMV-specific T cells adoptive therapy post haploidentical stem cell transplantation (haplo-SCT). Meanwhile, the mechanisms in driving sustained antiviral immunity by adoptive transfer remain undetermined. Aims. (i) To evaluate the safety and antiviral activity of donor CMV-specific T cells for persistent CMV infection in haplo-SCT recipients; (ii) To identify the association of CMV-specific immunity reconstitution (including quantity and function of CMV-specific T cell) and CMV infection resolution. Methods. There were 40 patients with persistent CMV infection prospectively accepted adoptive CMV-specific T cells therapy after haplo-SCT. Another 40 matched patients with transient CMV infection after haplo-SCT and 10 age-matched health donors were selected as controls. Phenotypical and functional characteristics of CMV-specific T cells were analyzed before and after immunotherapy in the treatment group, as well as in the control group. Single pools of overlapping 15-mer peptides for CMV pp65 and HLA class I CMV pentamer-matching peptides were used. Surface staining was performed with the following antibodies: CD3, CD4, CD8, CD45RA, CCR7 and PD-1. For intracellular staining, fixed cell was incubated with IFN-γ, IL-2, TNFα and Granzyme B. Proliferation was detected with CFSE and cultured for 7 day with CMVpp65 peptide. Results. All of the 40 treated patients cleared CMV viremia by 12 weeks post adoptive T cell transfer, and no infusion-related side effect was observed. In the treatment group, 33 patients (33/40, 82.5%) had CMV viremia clearance within 4 weeks post T cell transfer without recurrence. A massive in vivo expansion of CMV-specific CD4+ IFN-γ+ as well as CD8+ IFN-γ+ T cells, especially the effector memory and effector T cell subpopulation, was detected in these patients. With regard to the function of CMV-specific T cells, we observed impairment of CMV-specific T cells in patients with persistent CMV infection after haplo-SCT. To be encouraging, we found that adoptive transfer of CMV-specific T cells could decrease expression of inhibitory molecular PD-1 on CMV-specific T cells and improve cytokine production and proliferation ability of CMV-specific T cells. However, the remaining 7 patients who still had CMV recurrence after 4 weeks post transfer, neither quantity nor function of CMV-specific T cells were reversed. Conclusion. Adoptive transfer of CMV-specific T cells is safe and efficient in eliminating persistent CMV infection after haplo-SCT. Adoptive transfer of CMV-specific T cells would be help for prompt CMV-specific T cell quantitative and functional recovery against persistent CMV infection after haplo-SCT.
Disclosure of conflict of interest

The authors declare no competing financial interests.