Broad spectrum antiviral T cells for viral complications after hematopoietic stem cell transplantation

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Abstract: Major complications of hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT), such as graft rejection and graft-versus-host-disease (GvHD), are countered by suppressing the host immune system via chemotherapy and radiation, immunosuppressive drugs, or conditioning regimens such as *in vivo* or *in vitro* T-cell depletion. While immunocompromised, the patient is rendered susceptible to a number of viral infections and reactivations mainly caused by endogenous herpes viruses like cytomegalovirus (CMV) and Epstein-Barr virus (EBV) and by lytic agents such as adenovirus (ADV). In the paper entitled "Activity of broad-spectrum T cells as treatment for ADV, EBV, CMV, BKV, and HHV6 Infections after HSCT" published recently in *Science Translational Medicine*, Anastasia Papadopoulou and colleagues reported a suitable technology for rapid generation of antiviral T cells with a broad specificity in a single-culture for clinical application. In a small clinical trial with 11 patients they demonstrated safety and efficacy of adoptive multivirus-specific T-cell transfer.

Keywords: Stem cell transplantation; viral infections; adoptive immunotherapy; T-cell therapy; antiviral T lymphocytes; multi virus-specific T cells

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Viral complications in immunocompromised patients after transplantation

Infection with and reactivation of human cytomegalovirus (CMV), epstein-barr virus (EBV), adenovirus (ADV), polyoma virus BK (BKV) and human herpesvirus 6 (HHV6) are frequent and severe complications in immunocompromised recipients after hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT), which are associated with significant morbidity and mortality. Intensive immunosuppressive therapy for prevention or treatment of graft rejection and graft-versus-host disease (GvHD) puts the patients at risk of opportunistic infections due to an ablated or severely compromised T-cell immune response. Such invasive conditioning procedures lead to a lack of immunological competence, which results mainly in a decrease in the number of CD3+ T lymphocytes in the patient's peripheral blood. Lymphopenia increases the patient's risk of de novo infection or reactivation of latent viruses. Classical virostatic medications may succeed in the temporary control of viral replication and novel promising virostatic drugs are currently in clinical testing. However, insufficient responses to antiviral treatment or intolerable side effects are frequent and elimination of virus often relies on an effective cellular antiviral immune response.

Donor lymphocyte infusions (DLIs) can be used to treat both viral infections and leukemia relapses after transplantation but (I) are associated with potentially life-threatening GvHD, (II) not suitable in high risk patients with seronegative donors and (III) not available for patients receiving cord blood in HSCT or cadaveric transplants in SOT and (IV) attended with impaired functionality of antiviral memory T cells in granulocyte colony-stimulating factor- (G-CSF-) mobilized stem cell donors. The shortcomings of conventional therapies have increased the

interest in an immunotherapeutic approach to treat viral disorders. In the last decade it was shown that the adoptive transfer of antiviral cytotoxic effector T cells (CTLs) isolated from seropositive donors can rapidly reconstitute antiviral immunity after stem cell and organ transplantation without significant toxicity and with limited increase in GvHD (1,2).

On June 25, 2014, a paper entitled "Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 Infections after HSCT" was published in *Science Translational Medicine* (3). This paper describes an impressive work on the generation of 48 clinical-grade multiple virus-specific T-cell lines (mVSTs) with specificities to kill cells infected by 5 different viruses (CMV, EBV, ADV, BK virus, HHV6) in a single cell culture using overlapping peptide pools spanning the entire protein sequences of 12 immunodominant viral proteins. These mVSTs were infused prophylactically (n=3) or as treatment for active infection/reactivation (n=8) in a small patient cohort (n=11 patients) for up to four viruses. The authors showed that the adoptive transfer of mVSTs is safe without a correlation between the cell dose infused (0.5×10^7–2×10^7 cells/m^2) and either antiviral T-cell responses or safety. In all patients with viral reactivation expansion of the mVSTs and clinical responses were observed and those patients who received the mVSTs prophylactically remained virus-infection free for >3 months.

Generation of multiple virus-specific T cells in one step

The adoptive transfer of antiviral T cells is emerging as an effective and non-toxic immunotherapeutic strategy for immediate and long-term immune protection after HSCT or SOT. The presence of CD8+ and CD4+ antiviral T cells was reported to be essential in controlling viral infection and reactivation by restoring cellular immunity. Since the first promising results began to emerge in the early 1990s, different strategies to generate virus-specific T lymphocytes for clinical use have been described. In 1995, Walter and colleagues demonstrated that CMV reactivation after HLA-identical allogeneic HSCT can be prevented by adoptive transfer of CMV-specific cytotoxic T cells, which were generated *in vitro* from the transplant donor and transferred to the patient (4). To be suitable for clinical applications, the cells used for adoptive T-cell transfer must be virus-specific T cells generated by *in vitro* induction and expansion from a small number of precursor cells, over a short period of culture, under highly reproducible conditions, and in accordance with good manufacturing practice (GMP). Most protocols for the expansion of virus-specific T cells use peptide-loaded monocyte-derived dendritic cells (DCs), artificial antigen-presenting cells (aAPCs), or virus-infected cells [DCs or EBV-transformed B-cell lines (EBV-LCLs)] as stimulator cells and defined CD4+ and/or CD8+ T-cell responses to whole viral lysates, virally infected cells, recombinant proteins and various HLA-restricted viral peptides (1,5-14). In the present study a small aliquot of 3×10^7 PBMCs isolated from healthy allogeneic stem cell donors was used to generate mVSTs within 9-11 days with an average 13-fold cell expansion. Stimulation of T cells were performed with a mixture of GMP-grade peptide pools spanning the entire sequence of the following 12 viral proteins: EBV-LMP2, -BZLF1 and -EBNA1, ADV-penton, -hexon, CMV-pp65, -IE1, BKV-VP1, -large T and HHV6-U11, -U14, -U90. The usage of synthetic peptide pools consisting of overlapping peptides spanning an entire immunodominant protein is not restricted by HLA restrictions and enables the generation of CD4+ and/or CD8+ T-cell responses to multiple epitopes. mVSTs generated by this strategy mainly consisted of CD3+ T cells (98±0.2%) containing helper CD4+ as well as cytotoxic CD8+ T cells. It is known that specific CD4+ T-cell help is required to elicit and promote an efficient CD8+ CTL response to viral antigens. CD4+ T cells secrete various cytokines to regulate and coordinate the function of T cells and other immune cells. They are also known to be the most effective cell population in clearing infections, such as ADV (7,9). In addition, the authors performed an extensive immunophenotyping of the mVSTs and determined mainly CD45RO+ CD62L+ central memory (TCM) and smaller numbers of CD45RO+ CD62L- effector memory (TEM) T-cell subsets. Recently it was shown, that although TEM have proliferative potential in vitro, these cells fail to survive *in vivo* (15). These results have implications for the types of T cells that should be selected for adoptive transfer.

So far, infusions of peripheral blood-derived T-lymphocyte lines initially enriched in single or triple virus (CMV, EBV ADV)-specific T cells were found to reproducibly control infections by all three viruses after allogeneic HSCT. These results formed the basis of future adoptive immunotherapy trials in patients at risk of multiple infections as described in the study. The authors tried to generate T-cell lines with specificities against 5 different virus strains. Indeed, by testing the antiviral specificity of the 48 mVSTs by IFN-γ enzyme-linked immunospot

assay (ELISpot) they found 29% of mVSTs with reactivity against all 5 viruses (pentavalent), 19% were tetravalent, 25% were trivalent and 22% were bivalent. Only one mVST was found to be monovalent and one mVSTs failed to recognize any of the targeted viruses. The robustness of the described method was indirectly described as none of the mVSTs generated from CMV-seronegative donors was found to have reactivity against CMV. Recently it was show that ADV-specific T-cell responses are less frequent and a donor response to a certain peptide may not be determined on day 0 without restimulation (9,16). In this study all mVSTs showed reactivity against both ADV protein hexon and penton, indicating that the described technology is applicable in the generation of antiviral T cells especially in case of low-frequency memory T cells present in the starting material.

It is important to remember that GvHD remains a dreaded side effect and there is a particularly risk of alloreactivity, especially in partially-HLA matched donor/patient settings t was reported that alloreactivity from virus-specific memory T cells is far more common than predicted with approximately 45% of virus-specific T-cell clones found to be allo-HLA crossreactive (17). Interestingly, when tested in a cytotoxicity assay against recipient or haploidentical phytohemagglutinin blast (PHA blasts) mVSTs did not show any signs of alloreactivity and met the clinical release criterion with less than 10% lysed unloaded target cells.

Adoptive T-cell therapy using mVSTs to treat viral complication after transplantation

The objectives of this study were to determine feasibly and safety of the mVSTs to prevent or treat viral infection (primary objective) and to determine the effect of mVST infusion on viral load, immune reconstitution and clinical response (secondary objectives). The authors have safely applied the antiviral T cells restricted against 4 (and potentially 5) different viruses in a small cohort of 11 patients and performed an up to 3-month comprehensive monitoring of T cell's *in vivo* expansion. Three patients received mVSTs prophylactically and remained virus infection-free for >3 months after infusion. Therefor transfer of mVSTs can effectively prevent the clinical manifestation of viruses with no acute toxicity or increased risk of GvHD.

Interestingly, 4/8 patients who received the cells as treatment for viral infection or reactivation subsequently reactivated virus other than initially treated for. Prior to T-cell transfer a progressive increase in viral loads were detected, which sometimes reached the upper limit of the detection assays (e.g., BK virus $>1\times10^{10}$ copies/µg DNA). Although in some patients endogenous antiviral T cells preexisted (e.g., against BK virus, EBV, CMV) these T cells did not control the disease and the patients suffered from infection or reactivation with the respective virus. After administration of the mVSTs a decrease in viral load and sometimes eradication was reported in all patients who corresponded with an increase in circulating antiviral T-cell frequencies. It was suspected, that long term culture, *ex vivo* stimulation and manipulation may lead to functional T-cell impairment; however, but for the described T-cell doses, this worry seems to be negligible. The authors impressively showed that the transfer of the mVSTs is safe without significant increase in the development of GvHD or toxicities. Only one patient developed de novo skin GvHD, which could be successfully treated using topical steroids.

This is the first clinical trial to show that T cells generated by the above-described procedures can be successfully used to treat viral infection, reactivation or virus-induced malignancies after stem cell transplantation. In this study matched related, matched or mismatched unrelated or haploidentical stem cell donors served as T-cell donors. mVSTs were efficiently produced from memory T cells; however, the data implicate that antiviral T-cell lines from seronegative donors may not be easily generated in this system. CMV-seropositive immunocompromised patients (R+) who receive transplants from seronegative donors (D-) are at high risk of developing CMV disease (18,19) thus representing a key target population for antiviral T-cell transfer. In addition, some seropositive stem cell donors may not be eligible for T-cell donation due to medical and/or immunological reasons or just denied consent. It was recently shown, that even seropositive donors may not have sufficient antiviral memory T cells in their blood despite seropositivity. Recent studies have also shown that G-CSF mobilization has a long-term negative effect on the functional activity of T cells (20), suggesting that antiviral memory T cells from stem cell donors may have to be collected before G-CSF mobilization. It will be interesting to know, if this technology might abolish the negative impact of G-CSF and if aliquots for mVST generation were collected before are after G-CSF mobilization. Under these conditions, partially HLA-matched virus-specific T cells from healthy seropositive third party donors could be a successful alternative and could play a significant role in the

prevention and treatment of viral infections in transplant recipients. However, residual alloreactivity most likely precludes T-cell production strategies without separating steps. Studies on the use of HLA-matched T cells from third-party donors for the treatment of stem cell and organ recipients are currently in progress (8,11,21-23).

Summary

The results of this study are promising for all patients with viral infections, who fail to respond to treatment with conventional drugs. Further, the technology described does not additional wearing the T-cell donor. From a small aliquot of peripheral blood antiviral T cells can be enriched by robust techniques resulting in an effective cellular therapeutic for patients at high risk of viral infections and/or reactivations. Often patients undergoing severe T-cell suppressive conditioning or cord blood transplantation suffer from multiple concurrent of sequential viral reactivations, which can be elegantly targeted by this multi-virus specific approach. The frequency of specific T cells required to mediate an antiviral effect in the patients could not be determined and is still not known. It is likely that doses vary widely depending on the target antigen and many other factors, including HLA type as well as quantitative and even more qualitative properties of the effector T cells as well as the host environment. Phenotypic as well as functional features of T cells that are selected or engineered for therapy were confirmed in this study. For the first time this study demonstrated, therapeutic efficacy of T cells directed against HHV6 and BK virus in addition to CMV, ADV and EBV specificity. Larger prospective studies are warranted to further explore the potential of this elegant cellular therapy concept.

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