First-in-Man Clinical Results With Good Manufacturing Practice (GMP)-compliant Polypeptide-expanded Adenovirus-specific T Cells After Haploidentical Hematopoietic Stem Cell Transplantation

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Summary: Adoptive immunotherapy against viral infections is a promising treatment option for patients after hematopoietic stem cell transplantation. However, the generation of virus-specific T cells is either cost-intensive or time-consuming. We developed the first GMP-compliant protocol to generate donor-derived adenovirus (HAdV), cytomegalovirus, and Epstein-Barr virus-specific T-cell lines (TCLs) within 12 days by the use of overlapping polypeptides derived from different viruses in combination with IL-15. Two patients after undergoing haploidentical hematopoietic stem cell transplantation with HAdV viremia displaying rising viral loads despite treatment with cidofovir received 1×10^4 donor-derived short-term expanded HAdV-specific TCLs per kg body weight. In both patients, HAdV-specific T cells could be detected by IFN-γ-ELISpot 30 and 22 days postinfusion, and resulted in complete clearance or >1.5 log reduction of viral load within 15 and 18 days, respectively. This protocol facilitates rapid and cost-effective generation of virus-specific TCLs, which appear to provide an effective treatment option.

Key Words: hematopoietic stem cell transplantation, adenovirus-specific T cells, adoptive immunotherapy, virus-specific polypeptides, good manufacturing practice

(*J Immunother* 2014;37:245–249)

Human adenovirus (HAdV) infections after hematopoietic stem cell transplantion (HSCT) displaying rising viral load in peripheral blood (PB) despite virostatic treatment are associated with mortality rates of up to 100%.[1] Adoptive T-cell therapy is based either on direct magnetic selection of interferon (IFN)-γ^+ or MHC class-I multimer + virus-specific T cells or in vitro expansion of HAdV-specific T cells. Although magnetic selection itself is fast (24 h), the need of large volumes of fresh blood implies additional unrelated donor request, leading to a substantial delay with an average of 80 days after HSCT, before therapy is administered.[2] Purity of the selected cells is frequently low, and low cell numbers impede sufficient quality controls. Long-term (3–12 wk) in vitro expansion is hampered by time-consuming, cost-intensive, and technically demanding protocols requiring gene therapy.

Recently, we developed a short-term protocol using synthetic polypeptides and cytokines to expand virus-specific T cells within 12 days, with a total working time of 15 hours. PB mononuclear cells were stimulated with viral polypeptides and interleukin (IL)-15,[3] without the use of antigen-presenting cells. The generated HAdV-specific, cytomegalovirus (CMV)-specific, and Epstein-Barr virus (EBV)-specific T-cell lines (TCL) were functional, with absent or only low alloreactivity. The 12-day expansion protocol was fully adapted to good manufacturing practice (GMP) conditions.

Two patients with increasing HAdV viremia despite antiviral treatment received this new polypeptide-derived HAdV-TCL product on an "intention to treat" basis.

METHODS

TCL Culture

For the generation of virus-specific TCLs (seVirus-TCLs), cells from HSCT donors and volunteers were obtained upon approval from the local Ethics Committee (EK Nr.514/2011) and after informed consent were obtained. Briefly, 25×10^6 PBMCs (counted using Sysmex KX-21 N) were cultured in ATMP-ready AIM-V$^+$-medium[3] in G-REX devices (Wilson Wolf Manufacturing, New Brighton, MN) for 12 days and stimulated with either HAdV (AdV5), CMV (pp65), or EBV (BZLF-1&EBNA1) PepTivator (Miltenyi, Bergisch Gladbach, Germany; 0.6 nmol per mL) on days 0 and 6, followed by addition of IL-15 (5 ng/mL) on day 9.

HLA-typing

HLA-typing was performed as described.[3]

ELISpot

For ELISpot analysis, PBMCs ($1–3 \times 10^5$) and seVirus-TCL (5×10^4) were stimulated for 16 to 24 hours with or

Received for publication December 17, 2013; accepted February 27, 2014.

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without (negative control) pools of virus-derived polypeptides (1 μg/mL each). IFN-γ was detected using the human IFN-γ-ELISpot kit (MabTech, Nacka Strand, Sweden) according to the manufacturer's instructions. The level of detection was set at one spot-forming cell (SFC) per 10^5 cells exceeding background levels.

Flow Cytometry

For flow cytometric analysis, 2.5×10^5 cells/sample were stained with streptamers (IBA GmbH) or tetramers (Beckman Coulter) and antibodies and transferred to Trucount tubes (all BD Biosciences, CA) for cell enumeration as described.[3]

Cytotoxicity Assay

For the cytotoxicity assay, CFSE-labelled phytohemagglutinin targets (1.25×10^4) were pulsed with the appropriate PepTivator for 2 hours and incubated for 4 hours at a 1:20 ratio with seVirus-T cells. The percentage of killing was determined as described (Fig. 1E).

RESULTS

seVirus-TCLs for HAdV (n = 8), CMV (n = 2), and EBV (n = 1) were generated under GMP-compliant conditions from 11 donors. After 12 days of expansion (total working time 15 h), the median of $CD3^+$ T cells was

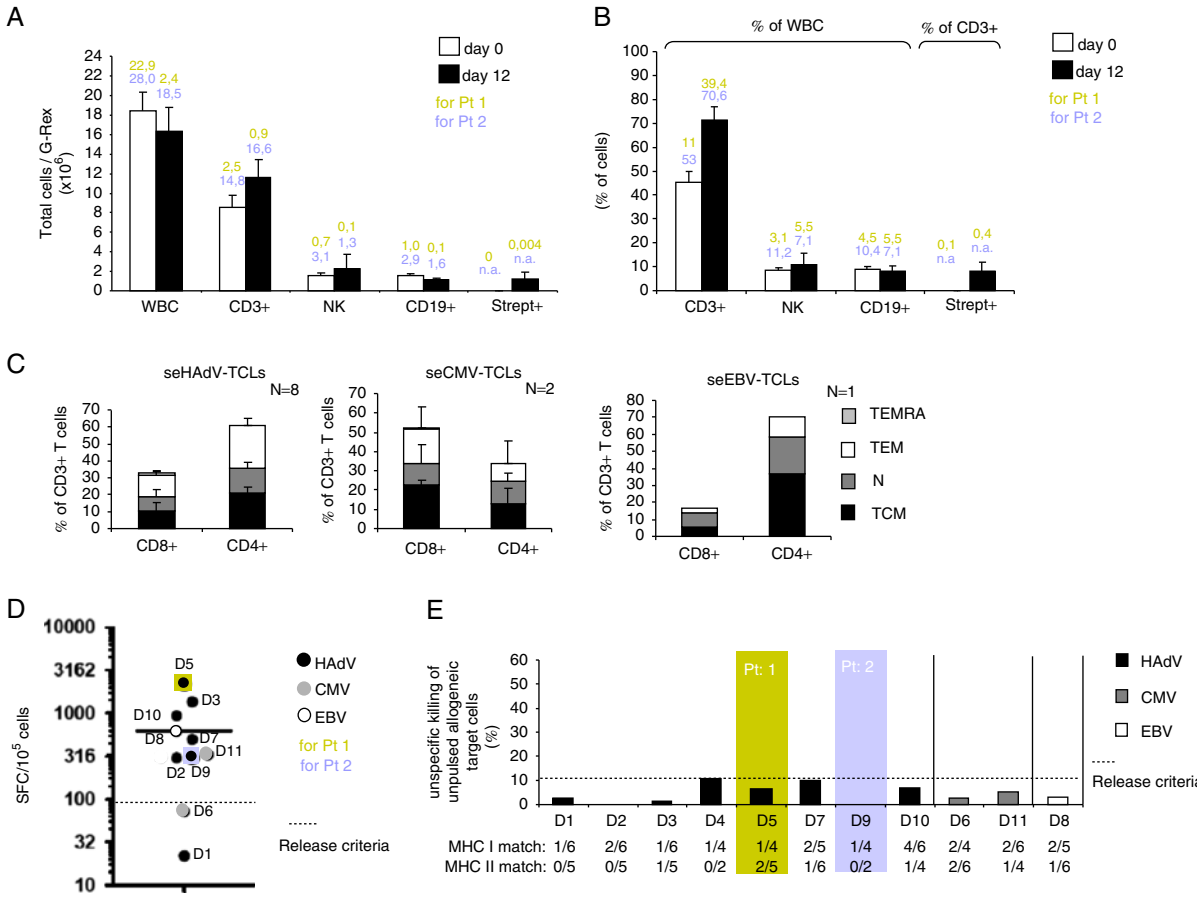

FIGURE 1. Phenotypic and functional analyses of seVirus-T cells against human adenovirus (HAdV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV). PBMCs (25×10^6/G-REX according to Sysmex automatic cell counter) were expanded for 12 days using polypeptide pools and interleukin-15 (IL-15), as described in the Methods section. A, Total number of white blood cells (WBC), $CD3^+$, natural killer (NK) cells, $CD19^+$ and Streptamer$^+$ T cells/G-REX before (white bars) and after expansion (black bars). B, Relative values of those cell populations among WBCs or $CD3^+$ (as applicable). Data from 11 donors include mean + SEM and range, as well as detailed results of the donors of the infused products. C, Percentage values of $CD8^+$ and $CD4^+$ specify the amount of central memory (TCM, black bar), naive (N, dark gray bar), effector memory (TEM, white bars), and effector memory $CD45RA^+$ (TEMRA, light gray bars) T-cell populations within $CD3^+$ cells. D, Number of seVirus-T cells specific for HAdV, CMV, and EBV determined by IFN-γ ELISpot. The dots represent the mean SFC from triplicates/10^5 cells of seHAdV (black), seCMV (gray), and seEBV-T-cells (white). Results of seHAdV-TCLs infused into patients are highlighted in green for patient 1 and in violet for patient 2. Because of lack of appropriate HLA-types, MHC class-I multimer + T cells were not analyzed (NA) for patient 2. E, Relative seVirus-TCL-induced killing of unloaded allogeneic phytohemagglutinin blasts (target cells), representing killing by alloreactive cells. The allogeneic target cells used were differently matched in MHC I and MHC II HLA-antigens (as described). The percentage of lysed target cells was calculated as follows:

$$\text{Allo killing}[\%] = 100 - \frac{(\text{Absolute \# viable allogeneic unloaded target cells} + \text{seVirus-T-cells})}{\text{Absolute \# viable allogeneic unloaded target cells}} \times 100.$$

TCLs indicates T-cell lines.

$11.6 \pm 1.8 \times 10^6$, representing $71.2 \pm 5.5\%$ of all cells. No significant alterations of natural killer and B-cell numbers were observed (Figs. 1A, B). Cell viability was $81.6 \pm 5.2\%$. The seVirus-TCLs comprised $CD8^+$ and $CD4^+$ T-cell subsets including central ($CD62L^+$, $CD45RA^-$) and effector ($CD62L^-$, $CD45RA^-$) memory cells (Fig. 1C). The frequency of virus-specific T cells measured by MHC class-I multimers had increased by 2.5-logs and showed highly specific activity against the appropriate antigen (mean: 662 ± 212 SFCs/10^5 cells) as measured by IFN-γ-ELISpot analysis (Figs. 1A, D). The flow cytometry–based cytotoxicity assay showed killing of unpulsed alloreactive target cells of $\leq 11\%$ for all seVirus-TCLs, reflecting low or even absent alloreactivity (Fig. 1E). Furthermore, seVirus-TCLs showed specific killing of polypeptide-pulsed target cells (mean, $18.4 \pm 5.4\%$; range, 13%–52%). These results are comparable to those reported for medium-term and long-term expansion protocols.[4,5] Nine of 11 seVirus-TCLs fulfilled all release criteria (≥ 50 viability, ≥ 80 SFC/10^5 cells, $\leq 11\%$ allokilling), and 2 of the seHAdV-TCLs were infused into 2 patients with refractory viremia.

Case Reports

Patients #1 (4-year-old girl with relapsed neuroblastoma) and #2 (1-year-old boy with resistant AML) had received CD3/CD19-depleted peripheral stem cells from a haploidentical parent after obtaining informed consent and notification of the authorities. Weekly HAdV screening of stool and PB samples was performed by real-time quantitative PCR.[1] Patients were screened for the presence of HAdV-specific T cells by ELISpot analysis.

Patient #1

HAdV species A and C were detectable in feces from day +6, and only species A in PB beyond day +13. Because of increasing viral load in feces (up to 2×10^{10} copies/g) and PB (up to 7×10^3 copies/mL), despite weekly cidofovir, the patient received seHAdV-TCLs at a dose of 10^4 $CD3^+$ T cells/kg body weight (BW) on day 76. Before infusion, 2 $CD3^+$ T cells/μL PB and no HAdV-specific T cells were detectable. Chimerism was 100% in donor PB and bone marrow. After T-cell transfer, no acute toxic side effects and no GvHD were observed. Thirty days after T-cell transfer, first HAdV-specific T cells (2.4 SFC/10^5 cells) were detectable by ELISpot, and the viral load began to decrease despite severe lymphopenia (53 $CD3^+$ cells/μL PB). Complete clearance of HAdV within the following 15 days was accompanied by increasing frequencies of HAdV-specific T-cells during 61 days. After a short reactivation of HAdV-C (9×10^2 copies/mL PB) on day 182 (data not shown) and HAdV-A (5×10^2) on day 192, which was again immediately cleared, HAdV-specific T cells were still detectable on day 238. One year posttransplant, the patient is alive and well (Fig. 2A).

Patient#2

On day +34, the boy developed febrile HAdV species C enteritis and hepatitis. Despite weekly cidofovir, viral load increased to 7×10^9 copies/g feces and 7×10^6 copies/mL PB on day +89, and HAdV was detectable in urine, tears, and cerebrospinal fluid. Of note, no other viral infection was detectable. Despite 100% donor chimerism, he developed secondary neutropenia, and T cells were not detectable. On day +98, 10^4 $CD3^+$ seHAdV-TCLs/kg were infused (Fig. 2B).

Ten days later, and concomitantly with rising T-cell counts but without detectable HAdV-specific T cells, the patient developed biopsy-proven skin GvHD resistant to treatment with prednisone, mycophenolate mofetil and extracorporal photopheresis, that progressed to intestinal and hepatic GvHD grade IV. On days 22 and 26 after T-cell transfer, 1.4 and 2.7 SFC HAdV-specific T cells were detectable/10^5 white blood cells, followed by a transient decrease of viral load in PB (2×10^4 copies/mL) within the next 18 days. The patient died on day +215 from multiple organ failure associated with HAdV disease and GvHD.

DISCUSSION

We describe the first production of fully GMP-compliant polypeptide-based and cytokine-based HAdV-specific, CMV-specific, and EBV-specific TCLs manufactured within 12 days and without addition of professional antigen-presenting cells (eg, DCs). Release criteria were comparable to long-term (10–14 wk) expanded virus TCLs shown to be safe in other studies.[5,6] A similar protocol—but not fully GMP-compliant and without clinical application—was recently also developed by Gerdemann et al.[7] In contrast to their protocol covering 7 viruses, we focused on the 3 viral infections associated with high mortality in pediatric HSCT.[8] The expansion of seVirus-TCLs from PBMC from 100 mL PB that had been cryopreserved either at the time of (for bone marrow transplantation) or before (for PB stem cell transplantation) HSCT allows the immediate production of targeted T cells upon clinical demand. Especially in case of PB stem cells, the use of G-CSF-mobilized PB as starting material for the isolation of virus-specific T cells is not recommended because of impaired T-cell activation.[9] In both patients, the appearance of circulating HAdV-specific T cells correlated with clearance or transient reduction of viral load after 5 to 6 weeks post T-cell therapy. Although this is 2-week longer compared with what most studies state,[5,10,11] it is in accordance (4–6 wk) with 2 other studies.[12,13]

Because of the fact that HAdV-specific TCLs from the original HSCT donor were infused, direct evidence for T-cell recovery of infused HAdV-specific TCLs was not possible. Of note, also in the past, the direct recovery of infused virus-specific T cells or TCLs could only be proven if third party–derived virus-specific T cells had been infused.[6,10]

One patient developed severe GvHD. In principle, it is possible that the seHAdV-TCLs contributed to GvHD. However, taking into account the early time interval (10 d following the infusion) and the fact that GvHD emerged 12 days before the detection of seHAdV-TCLs, we consider this rather unlikely. Furthermore, the infusion $> 10^4$ nonspecific, potentially alloreactive T cells/kg BW did not induce severe GvHD in other studies.[4,10,14,13]

In summary, we report the first-in-man use of short-term polypeptide-generated HAdV-specific GMP-compliant T cells, with a clinical outcome comparable to other protocols.[5,15] If confirmed in a larger patient cohort, this approach might pave the way to broad clinical implementation of a simple, fast, and cost-effective immunotherapy of life-threatening viral infections. To examine clinical and immunologic effects in a larger patient cohort, a clinical phase I/II study (EudraCT no. 2013-002492-17) has been initiated.

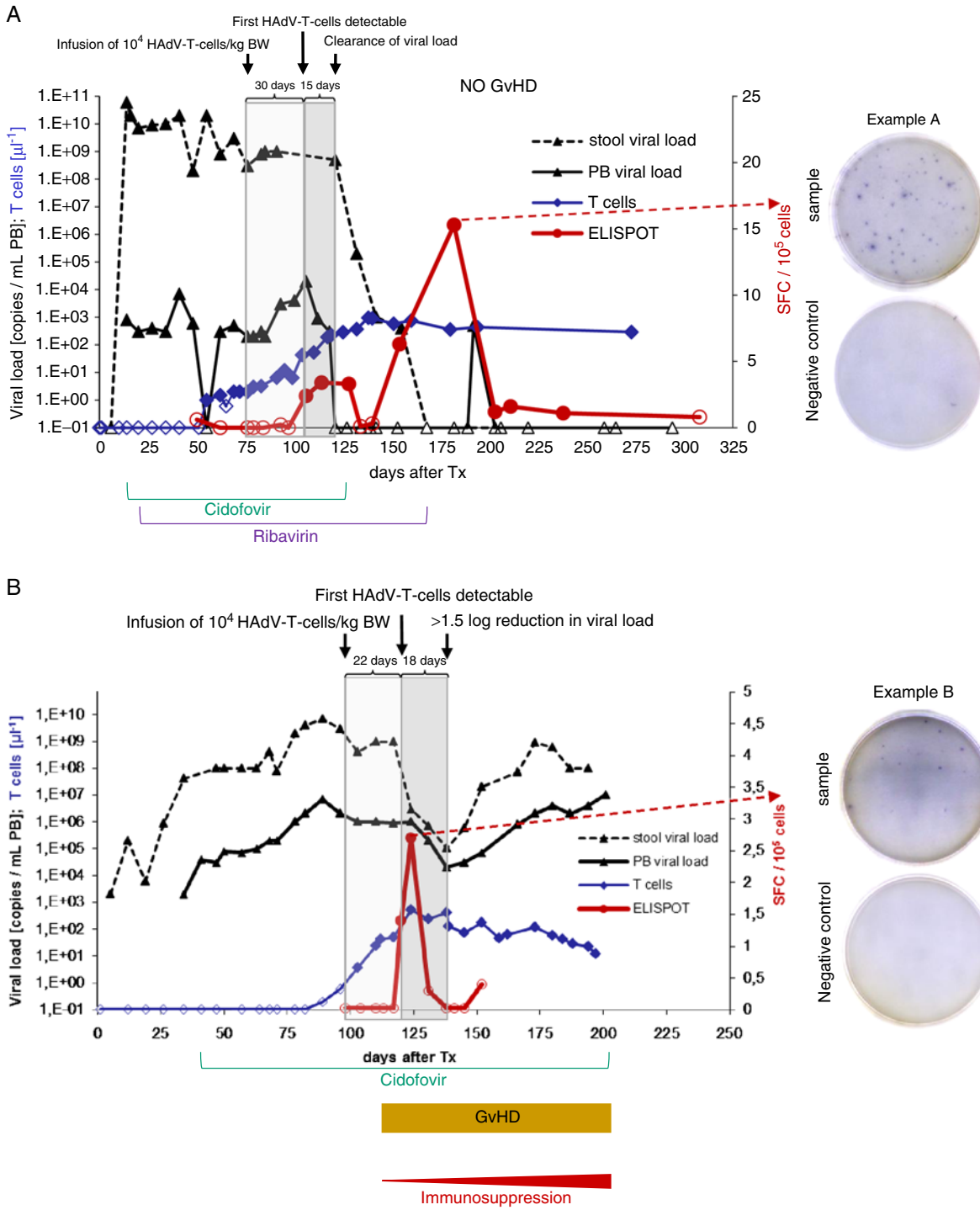

FIGURE 2. In vivo expansion and clinical outcome of seHAdV-T cells in patients. HAdV infection–related copy number per mL PB (black line, open/filled triangle; detection limit ≤100 copies/mL), copy number per gram stool (dotted black line, open/filled triangle; detection limit ≤100 copies/g), SFC/10^5 cells of HAdV-specific T cells (bold red line, open/filled dots), and number of $CD3^+$ T cells/µL blood (blue line, open/filled diamond) of patients 1 (A) and 2 (B). All open dots/triangles or diamonds represent results less than the detection limit. Two images of ELISpot results of both patients are shown, including the negative control and the appropriate sample of the highest number of SFCs. B, Because of severe GvHD commencing 10 days after T-cell therapy, patient 2 was treated with high doses of steroids. GvHD indicates graft-versus-host disease; HAdV, human adenovirus; PB, peripheral blood; SFC, spot-forming cell.

ACKNOWLEDGMENT

The authors thank Lothar Germeroth (IBA GmbH, Göttingen, Germany) for providing technical support.

CONFLICTS OF INTEREST/ FINANCIAL DISCLOSURES

Supported in part by an institutional grant of the Childreńs Cancer Research Institute and by the Eurostars Grant E! 5744 (FFG 829495).

All authors have declared there are no financial conflicts of interest with regard to this work.

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