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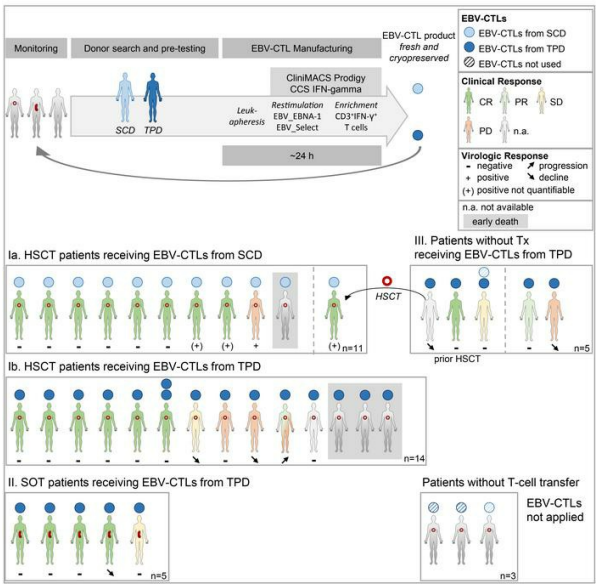

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Patient-tailored adoptive immunotherapy with EBV-specific T cells from related and unrelated donors

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Conflict-of-interest statement

The following authors have declared a potential conflict of interest: GC (honoraria, travel support, data safety monitoring of advisory board), JH (honoraria), HG (leadership or fiduciary role), EJ (honoraria, travel support), NK (leadership or fiduciary role), BMK (leadership or fiduciary role), HCR (grants or contracts, consulting, honoraria, travel support, patents, co-founder of CDL Therapeutics GmbH), RUT (grants or contracts, consulting, honoraria, expert testimony, travel support, leadership or fiduciary role), DW (grants or contracts, consulting, honoraria, travel support, data safety monitoring of advisory board). All other authors declare no conflict of interest.

Abstract

Background: Adoptive transfer of EBV-specific T cells can restore specific immunity in immunocompromised patients with EBV-associated complications.

Methods: We provide results of a personalized T-cell manufacturing program evaluating donor, patient, T-cell product and outcome data. Patient-tailored clinical-grade EBV-specific cytotoxic T-lymphocyte (EBV-CTL) products from stem cell donors (SCD), related third party donors (TPD) or unrelated TPD from the allogeneic T-cell donor registry (alloCELL) established at Hannover Medical School were manufactured by immunomagnetic selection using CliniMACS Plus or Prodigy device and EBV PepTivators EBNA-1 and Select. Consecutive manufacturing processes were evaluated and patient outcome and side effects were retrieved by retrospective chart analysis.

Results: Forty clinical-grade EBV-CTL products from SCDs, related or unrelated TPDs were generated for 37 patients with and without transplantation (Tx) history within 5 days (median) after donor identification. 34 patients received 1-14 EBV-CTL products (fresh and cryopreserved). EBV-CTL transfer led to complete response in 20 of 29 patients who were evaluated for clinical response. No infusion-related toxicity was reported. EBV-specific T cells in patients' blood were detectable in 16/18 monitored patients (89 %) after transfer and correlated with clinical response.

Conclusion: In conclusion, personalized clinical-grade manufacturing of EBV-CTL products via immunomagnetic selection from SCD, related or unrelated TPD is feasible in a timely manner. Overall, EBV-CTL were clinically effective and well-tolerated. Our data suggest EBV-CTL as promising therapeutic approach for immunocompromised patients with refractory EBV-associated diseases beyond HSCT as well as patients with pre-existing organ dysfunction.

Trial registration: Not applicable.

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Introduction

The morbidity and mortality of hematopoietic stem cell (HSCT) and solid organ (SOT) transplant patients is frequently ensued by graft rejection or graft-versus-host disease (GvHD) and increased by infectious complications due to delayed immune reconstitution or immunosuppressive medication (1). EBV is a gamma-herpes virus that infects >90 % of the population worldwide during childhood and persists life-long within the B-cell compartment. Strong $CD4^+$ and $CD8^+$ T-cell responses directed against various lytic and latent EBV-proteins control EBV reactivation and usually prevent EBV-associated diseases in healthy individuals (2). However, in immunocompromised patients, infection and reactivation as well as the development of high-grade secondary malignancies are severe complications.

The most common EBV-associated post-transplant malignancy is termed post-transplant lymphoproliferative disease (PTLD), representing both clinically and histopathologically heterogeneous lymphoproliferations (3-7). The overall incidence of PTLD after allogeneic HSCT is less than 2 %, but was shown to increase after transplantation with T-cell depleted and/or HLA-mismatched grafts (e.g., ≤24 %) (8, 9). During the phase of immune reconstitution, e.g., the first 6 to 12 months after HSCT, incidences can reach up to 40 % (10-12).

In SOT, EBV-seronegative transplant recipients with EBV-seropositive donors and those experiencing primary EBV-infection under post-transplant immunosuppression consequently carry the highest risk of developing EBV-associated PTLD (3, 13-15). Incidences vary from 1 to 20 % depending on the organ type; incidences are high during the first year post-transplant with almost all tumors being EBV-associated but PTLD may occur during the whole period of immunosuppression (3, 5, 16). The type of organ graft, i.e. with respect to its amount of lymphoid tissue as well as the level of immunosuppression needed to maintain immune tolerance, represents another distinctive factor, with highest incidences (≤20 %) detected in lung, small bowel or multiple organ grafts (14, 15, 17-19). Further risk factors for PTLD development are the patient's age at transplantation (esp. <18 and >60 years), the disease initially leading to transplantation, a previous splenectomy, a second transplantation, co-infection with CMV, polymorphisms in cytokine genes, the intensity and duration of the

immunosuppressive regimen, the HLA type and extent of HLA mismatch between donor and recipient, the administration of T-cell depleting antibodies (e.g., anti-thymocyte globulin (ATG), alemtuzumab) as well as the co-existence of multiple risk factors (10, 13-15, 17, 20, 21). A low incidence of PTLD (0.2 %) after HSCT was detected in patients with no risk factors, whereas the incidence significantly increased (8.1 %) if there were three or more risk factors (10).

Treatment of PTLD includes reduction of immunosuppressive medication as tolerated, immunotherapy, and cytotoxic chemotherapy. However, therapy is often complicated by side effects and severe complications are foreseeable in patients with pre-existing organ dysfunction (3, 11, 22).

Besides the reduction of immunosuppression the two main immunotherapeutic approaches to treat EBV-associated PTLDs are: (1) treatment with mAbs (e.g., rituximab) against the B-cell surface molecule CD20 to eliminate EBV-infected B cells and (2) adoptive transfer of functional EBV-specific cytotoxic T lymphocytes (EBV-CTL) from healthy related or unrelated donors. Treatment with anti-CD20 mAb is often associated with a risk of infection in immunosuppressed patients and is sometimes ineffective due to low or absent CD20 expression and antigen loss during treatment.

Adoptive T-cell therapy appears to be an attractive therapeutic option. First developed in the 1990ies, autologous or stem cell donor (SCD)-derived EBV-CTL lines were generated by repetitive in vitro stimulation with antigen-bearing cells (23). More recently, short-term expansion strategies were developed to generate EBV-CTL within two weeks and use them either in the SCD setting (24) or a partially HLA-matched third party donor (TPD) situation (25-29). Some of these study target multiple viral infections (28, 29). In all studies, EBV-CTL infusions were in general well tolerated and effective in the majority of patients.

Although highly attractive, there are several limitations to this treatment approach: Generation of EBV-CTL by in vitro culture is time-consuming and relies on specialized cell growth facilities. Feuchtinger and colleagues developed a different strategy of selecting EBV-CTL directly from peripheral blood by magnetic separation based on EBV-specific cytokine secretion (30). For immunomagnetic selection of EBV-CTL, a donor with sufficient frequencies of EBV-specific T

cells is needed, which often fails in HSCT patients and is impossible to achieve from deceased organ donors. Third, transfer of bystander cells may confer GvHD or allograft rejection.

The current study was designed to retrospectively analyze our program of personalized EBV-CTL manufacturing via immunomagnetic selection from either SCD, related or unrelated TPD from an allogeneic T-cell donor registry (alloCELL) established at the Institute of Transfusion Medicine and Transplant Engineering (Hannover Medical School). In total, 40 clinical-grade EBV-CTL processes were performed for 37 immunocompromised patients with and without transplantation history. Data were analyzed with respect to manufacturing time, cell numbers and transfer frequency, infusion-related side effects, influence on GvHD, as well as clinical, immunological and virologic outcome.

Results

1. Patient Cohort

A total of 40 EBV-CTL products were manufactured between May, 2015, and July, 2019, for 37 patients with refractory EBV infections or EBV-associated malignancies from 21 different hospitals (20 in Germany, one in Finland) intended to receive EBV-CTL (Figure 1, Supplemental Table 1A/B). Three patients (#1/3, #24/36, #28) have been reported before, but were added to this series for completeness reason (31-33). Median age of patients was 37 years (range 2-73 years), 26 patients were male and 11 female (Table 1). Five patients did not have any transplant history before the planned EBV-CTL transfer, four of which had EBV-associated malignancies, and one had chronic EBV-infection without lymphoproliferation due to an inborn immunodeficiency syndrome. Three of these patients were supposed to undergo allogeneic HSCT after EBV-CTL transfer, and in two of those a second transfer from a different manufacturing process after HSCT was planned (#24/36, 2nd transfer received; #33/40, 2nd transfer not received). 28 patients were scheduled to receive EBV-CTL after HSCT, including the two patients mentioned above. Indications for HSCT were malignancy (n=18) and non-malignant disease (n=10) (Supplemental Table 1A). One of the HSCT patients received EBV-CTL from two different manufacturing processes (Supplemental Table 1A/B, #1/3; both from the same unrelated TPD (alloCELL)). For two patients (#24/36, #33/40), EBV-CTL preparations from two different donors were manufactured while 34 patients were scheduled for a single T-cell preparation.

Five patients had a history of SOT prior to EBV-CTL transfer (one heart-, two kidney- and two liver-transplants). All SOT patients received EBV-CTL from TPDs for EBV-associated PTLD; none of them suffered from malignant disease before SOT.

2. Donor selection based on serostatus, HLA match and EBV-specific T-cell frequencies

Donor search and donor pre-testing

Selection of EBV-seropositive donors suitable for generation of EBV-CTL was based on HLA match, EBV-serostatus, and the frequency of EBV-specific T cells as determined by IFN-γ cytokine secretion assay (CSA), which is analog to the clinical-scale Cytokine Capture System (CCS) IFN-gamma process. For patients prior or post HSCT, EBV-CTL may be isolated from the SCD in case of sufficient frequencies of EBV-specific T cells. Alternatively, (partially) HLA-matched related or unrelated TPDs can potentially serve as EBV-CTL donors, which is routinely done in case of SOT or no transplantation history. We here report data from 40 EBV-CTL productions (Supplemental Table 1A/B). In 13 patients, the SCD served as EBV-CTL donor (MSD, n=2; MUD n=9; haploidentical, n=2), for all other patients (67.5 %), EBV-CTL were manufactured from a TPD (related, n=9; unrelated (alloCELL), n=18).

The alloCELL T-cell donor registry records high-resolution HLA types and virus-specific T-cell (VST) frequencies from >3,500 healthy volunteers; 18 of 40 manufacturing processes from the current series were performed with donors from this non-commercial registry. Two of these were from the same donor for one patient (#1/3). Thus in total, 17 donor searches were performed and the results were provided to the requesting clinic within 24-48 h. The median number of HLA-matched, EBV-seropositive potential donors identified for each patient was three (range 1-8 potential T-cell donors, data not shown). All unrelated TPD were high-resolution typed in HLA-A, B, C, DR and DQ. However, to be suited as an EBV-CTL donor, we required at least a 3/6 HLA-match in HLA-A, B, DR with at least one match in class I and class II alleles each, which applied to all donor-recipient pairs (Table 2). On high resolution HLA typing, matching was at least 4 of 10 (in one patient) and up to 8 of 10 (in one patient) with the majority of matches being between 5 of 10 and 7 of 10 (details in Table 2). The median time between donor search result for unrelated TPD and donor identification was 2 days (Figure 2, n=16). The median time between donor pre-testing result and start of the manufacturing process was 5 days and did not significantly differ between unrelated TPD (5 days), related TPD (5 days) and SCD (7 days) (Figure 2, n=34). The process of T-cell manufacturing is an

overnight process. Prolonged times between donor identification and manufacturing resulted from individual pre-treatment regimens with chemo/immunotherapy (#1, #28, #35, #39), these cases are labelled with their individual number in Figure 2 and more information can be retrieved from supplemental table 1A/B. The HLA-match between patients and related TPDs is provided in Table 2.

For SCD as well as related TPDs, EBV serostatus was determined prior to donor pre-testing. Only EBV-seropositive donors were analyzed with respect to frequencies of EBV-specific T cells (34). In order to determine the starting frequencies of therapeutically relevant EBV-specific T cells (30), pre-testing was performed by stimulation using EBV PepTivator EBNA-1 alone and in combination with PepTivator Consensus (referred to as GMP PepTivator EBV Select in manufacturing). Unstimulated cells served as negative control (NC) and values obtained from NC were subtracted from pre-enrichment values. For all donors tested (n=38), stimulation with EBNA-1 and Consensus in combination was done, while for four donors, the amount of PBMCs obtained did not allow for determination of EBNA-1-specific T-cell frequencies alone. The mean frequency of IFN-γ^+ T cells upon stimulation with EBNA-1 was 0.11 % ($CD3^+$), 0.05 % ($CD4^+$), and 0.24 % ($CD8^+$), and the mean frequency of $CD3^+$/IFN-γ^+, $CD4^+$/IFN-γ^+, and $CD8^+$/IFN-γ^+ T cells increased to 0.41 %, 0.17 %, and 0.80 %, respectively, upon stimulation with the combination of EBNA-1 and Consensus (Table 3 and Figure 3A,B). Following magnetic enrichment, the mean frequency of IFN-γ^+ T cells upon stimulation with EBNA-1 was 19.13 % ($CD3^+$), 14.47 % ($CD4^+$), and 24.41 % ($CD8^+$), which increased to 45.36 %, 25.05 %, and 59.39 %, respectively, upon stimulation with the combination of EBNA-1 and Consensus (Table 3 and Figures 3C,D).

3. Manufacturing of clinical grade EBV-CTL products

In total, 40 clinical-grade EBV-CTL products were generated for 37 patients. Thirteen EBV-CTL were derived from the respective SCD, 18 from unrelated TPDs, and nine from related TPDs (Figure 4A). Manufacturing was performed using MACS GMP PepTivator EBV_EBNA-1 in combination with MACS GMP PepTivator EBV_Select and the CliniMACS CCS together

with the CliniMACS Plus (n=13) or CliniMACS Prodigy (n=27) device (Supplemental Table 2) as described before (35). In brief, $1x10^9$ donor white blood cells obtained via leukapheresis were restimulated with MACS GMP PepTivator EBV_EBNA-1 and MACS-GMP PepTivator EBV_Select for four hours, followed by immunomagnetic selection of IFN-γ-secreting cells. The total T-cell numbers ($CD3^+$ and $CD3^+$/IFN-γ^+) obtained were significantly higher when using CliniMACS Prodigy compared to the CliniMACS Plus ($CD3^+$ $p<0.0001$, $CD3^+$/IFN-γ^+ $p=0.0014$; Supplemental Table 2 and data not shown). For all processes (n=40), mean viability of the generated EBV-CTL was 70.5 % and the mean frequency of $CD3^+$/IFN-γ^+ T cells was 40.9 %, which constituted of 39.5 % and 58.8 % IFN-γ^+ cells among $CD4^+$ and $CD8^+$ subsets, respectively (Table 4, Figure 4B,C). The median number of total $CD3^+$ cells was $7.07x10^6$, corresponding to a median number of $2.52x10^6$ $CD3^+$/IFN-γ^+ T cells (Table 4, Figure 4D). There was no significant difference between SCDs, related and unrelated TPDs with respect to T-cell numbers and purity in the final EBV-CTL products (Table 4).

In contrast to the overlapping peptide pool PepTivator EBNA-1, the PepTivator EBV Consensus (referred to as GMP PepTivator EBV Select in manufacturing) contains 32 MHC class I-restricted and 11 MHC class II-restricted peptides, which are derived from 15 lytic and latent EBV proteins. The HLA-A and HLA-B alleles involved in recognition of these peptides as well as their representation in 31 of the EBV-CTL donors are listed in Table 5. Homozygous alleles were considered only once. The HLA restrictions and epitope specificities of the administered T cells were not defined. Manufacturing was performed using a combination of both, PepTivator EBV EBNA1 and PepTivator EBV Select. Hence, it can be assumed that the HLA coverage of the obtained $CD3^+$/IFN-γ^+ T cells is not solely restricted to those HLA alleles covered by PepTivator EBV Select.

4. Patient Follow up

Three patients never received the EBV-CTL product because of death (n=2; #37, #38) or cure (n=1; #39) before end of manufacturing. A fourth patient (#33/40) received TPD-derived EBV-CTL (#33) before HSCT but did not require the already produced SCD-derived EBV-CTL (#40)

after HSCT anymore. These four products were excluded from the analysis of clinical effects and side effects. For all patients included in the analysis, median $CD3^+$ T-cell number of first EBV-CTL transfer was $2.5x10^4$/kg (range $5x10^3$-$2.2x10^5$/kg) and median $CD3^+$ T-cell number of all transfers was $4.2x10^4$/kg (range $5x10^3$-$2.2x10^5$/kg), mean purity of transferred EBV-CTL products measured by percentage of $CD3^+$/IFN-γ^+ was 41.8 % (range 17.7-76.8 %) corresponding to a median number of $7.9x10^3$/kg $CD3^+$/IFN-γ^+ T cells (range $2.2x10^3$-$9.8x10^4$/kg) of first EBV-CTL transfer. Median follow-up was 34.5 months for all patients and 49.5 months (range 11-77) for the patients that were still alive at last follow-up. Details are shown in Supplemental Table 1A/B.

4.1. HSCT patients with stem cell donor used as EBV-CTL donor (group Ia)

For 11 HSCT patients, who received the EBV-CTL product after HSCT, the SCD served as EBV-CTL donor. One patient died within three weeks after EBV-CTL-transfer (multiorgan failure) and was excluded from the long-term follow-up evaluation ("early death", #18). All patients with B-cell PTLD had received prior treatment with rituximab (n=9) or chemotherapy (n=2), one patient with NKT-NHL received PD1-blockade in parallel to EBV-CTL treatment (#23). Median number of EBV-CTL transfers in these patients was 1 (range 1-5), median $CD3^+$ T-cell number of first EBV-CTL transfer was $2.5x10^4$/kg (range $1x10^4$-$2.2x10^5$/kg), mean percentage of $CD3^+$/IFN-γ^+ T cells was 33.8 % corresponding to a median number of $8.5x10^3$/kg $CD3^+$/IFN-γ^+ T cells. One of these patients received EBV-CTL before transplantation for EBV-associated encephalitis and received a total of 14 EBV-CTL administrations (nine before HSCT from an unrelated TPD, #36; five in parallel to or after HSCT from the SCD, #24). Nine of 10 patients achieved complete response (CR) following EBV-CTL transfer (Table 6). In six of these, EBV in peripheral blood became undetectable by PCR (#16, #19, #20, #21, #22, #26; #16 and #21 already had negative EBV-PCR before transfer of EBV-CTL), whereas in three patients EBV-PCR remained positive (#17, #23, #24). Details for EBV-PCR load monitoring in patients with serial measurements are shown in Supplemental Figure 1. Of the patients with CR, three patients died, all of them due to other infections than

EBV (#19, #22, #23). One patient had progressive disease (PD) irrespective of EBV-CTL transfer and finally died four weeks after transfer of EBV-CTL due to progression of EBV-related PTLD (#25).

No graft failure was noticed after EBV-CTL transfer. Three patients in this group of 10 had GvHD before administration of EBV-CTL, two of them were free of GVHD after EBV-CTL transfer (#16, #19), and the third developed chronic GvHD without new GvHD symptoms (#22). Of the seven patients without pre-existing GvHD, three developed GVHD after EBV-CTL transfer. Two of them only had mild to moderate GvHD, one of them directly after reduction of immunosuppression (#17, #20), but the third patient developed grade III GvHD of the liver, skin and oral mucosa (#23). This patient not only received EBV-CTL but also donor lymphocyte infusions (DLI) and pembrolizumab in parallel. The treating physician did not attribute GvHD to EBV-CTL transfer. All three patients with new GvHD after EBV-CTL transfer had only a single transfer, transferred cell numbers were $3.04x10^4$/kg, $2.5x10^4$/kg and $5.0x10^4$/kg $CD3^+$ cells (corresponding to IFN-γ^+ $CD3^+$ T cells $5.4x10^3$/kg, $1.1x10^4$/kg and $2.0x10^4$/kg), respectively.

4.2. HSCT patients with third party EBV-CTL-donor (group Ib)

Fourteen HSCT patients received EBV-CTL from TPDs after HSCT, one of them received EBV-CTL from two different manufacturing processes with the same unrelated TPD (#1/3). Two patients got EBV-CTL from related TPDs (#14, #15), unrelated TPDs were used for the other 12 patients. Three patients died within three weeks after EBV-CTL-transfer (progression of EBV-associated encephalitis, #2; progression of AML and PTLD, #10; multiorgan failure presumably EBV-related, #5). These patients were excluded from the long-term follow-up evaluation ("early death"). All patients had received rituximab prior to EBV-CTL transfer, in three patients additional chemotherapy had been administered. Details are shown in Table 6 and Supplemental Table 1A/B.

The median number of EBV-CTL transfers in the remaining 11 patients was 2 (range 1-6 transfers), median $CD3^+$ T cell number of first EBV-CTL transfer was $1.75x10^4$/kg (range $5x10^3$-

3.69x10^4/kg), mean percentage of CD3$^+$/IFN-γ$^+$ T cells was 45.9 % corresponding to a median number of 5.0x10^3/kg CD3$^+$/IFN-γ$^+$ T cells. Six of 10 patients with outcome data available had a CR after EBV-CTL transfer with resolution of all symptoms; in all these cases EBV became undetectable by PCR in peripheral blood (#1/3, #6, #7, #11, #12, #14; in one patient, #7, EBV-PCR remained low-positive in cerebrospinal fluid). Details for EBV-PCR load monitoring in patients with serial measurements are shown in Supplemental Figure 1. Four of the patients with CR are still alive (#1/3, #11, #12, #14), the remaining two patients (#6, #7) died unrelated to EBV. In one patient (#13), the EBV-associated symptoms remained stable after EBV-CTL transfer, EBV-PCR remained positive but revealed a decrease of viral load. This patient died unrelated to EBV. Three patients had PD following EBV-CTL transfer (#8, #9, #15), although one of them (#9) had negative EBV-PCR; all of them died (two of them EBV-related, the third because of multiorgan failure otherwise not classified). For the last patient (#4), no data was available concerning the clinical response to EBV-CTL, nevertheless EBV-PCR was negative, this patient finally died unrelated to EBV.

No graft failure occurred after EBV-CTL transfer. Nine of 11 patients had a history of GvHD before EBV-CTL transfer. Of these, GvHD persisted at the same level after EBV-CTL transfer in two cases (#7, #14) and in one case acute GvHD developed into chronic GvHD (#4, this patient finally died due to chronic GvHD). Two of the patients with pre-existing GvHD developed new GvHD symptoms following EBV-CTL transfer, but one of them received DLI and the other one nivolumab due to recurrence of underlying malignancy besides EBV-CTL at the same time (#6, #15). Pre-existing GvHD resolved in four cases after transfer of EBV-CTL (#1/3, #9, #12, #13). None of the two patients that were free of GvHD before EBV-CTL transfer developed de novo GvHD thereafter (#8, #11).

4.3. SOT patients (group II)

Five patients had a history of SOT and received EBV-CTL for refractory or high-risk EBV-related PTLD. Four patients with CD20$^+$ PTLD had received rituximab, all patients had received chemotherapy prior to EBV-CTL transfer, which resulted in CR before EBV-CTL transfer in 2/5

patients. Related (n=3; #27, #29, #30, Supplemental Table 1A/B) or unrelated TPDs (n=2; #28, #31, Supplemental Table 1A/B) were used as EBV-CTL donors. Median number of EBV-CTL transfers was 3 (range 1-5), median $CD3^+$ T cell number of first EBV-CTL transfer was $2.5x10^4$/kg (range $1x10^4$-$4.2x10^4$/kg), mean percentage of $CD3^+$/IFN-γ^+ T cells was 35.5 % corresponding to a median number of $9.4x10^3$/kg $CD3^+$/IFN-γ^+ T cells. Four patients showed CR and three of them had negative EBV-PCR following EBV-CTL transfer (#28, #29, #30); however, these three patients already had negative EBV-PCR before transfer of EBV-CTL, the fourth patient with CR still had positive EBV-PCR (#31). The remaining fifth patient showed stable disease (SD) clinically, but EBV-PCR turned negative and PET/CT showed complete metabolic response (#27). None of the SOT-patients developed GvHD or experienced graft loss after EBV-CTL transfer and all of them were still alive at last follow up.

4.4. Patients without history of transplantation (group III)

Five patients received EBV-CTL without any or prior to transplantation for either refractory EBV-infection in suspected/verified immunodeficiency (n=3; #33, 34, 36) or EBV-related lymphomatoid malignancy (n=2; #32, #35, Supplemental Table 1A/B). HLA partially matched related (n=3; #32, #33, #34) or unrelated TPDs (n=2; #35, #36) were used as EBV-CTL donors. In these patients, the median number of EBV-CTL transfers was 3 (range 2-9), median $CD3^+$ T-cell number of first EBV-CTL transfer was $1.0x10^4$/kg (range $1.0x10^4$-$2.5x10^4$/kg) and median $CD3^+$ T-cell number of all EBV-CTL transfers was $4.3x10^4$/kg. Mean percentage of $CD3^+$/IFN-γ^+ T cells was 51.1 % corresponding to a median number of $5.9x10^3$/kg $CD3^+$/IFN-γ^+ T cells for the first EBV-CTL transfer. The three patients with immunodeficiency underwent HSCT afterwards, of these, one patient received EBV-CTL after transplantation as well (#36; see 4.2.; in 4.4 only the EBV-CTL transfers before HSCT were considered).

The first patient showed PD and EBV-PCR remained positive, this patient finally died due to progression of EBV-associated lymphoproliferation (#32). The second patient had SD with increasing EBV load despite EBV-CTL transfer; however, EBV-PCR turned negative after HSCT and the patient did not require any treatment concerning EBV post-transplant anymore

(#33). The third patient initially showed partial response (PR) and a decrease of EBV load and finally achieved CR as well as negative EBV-PCR after HSCT (#34). The fourth patient already had negative EBV-PCR before transfer of EBV-CTL, this patient showed PR clinically whereas in PET/CT complete metabolic response was seen (#35). The fifth patient had CR but EBV-PCR remained positive, therefore this patient got EBV-CTL after HSCT as well (#36). Except for the first, all patients were still alive at last follow-up. None of the patients developed GvHD following EBV-CTL transfer.

5. Detection of EBV-specific T cells in patients' blood

EBV-specific T-cell monitoring in PBMCs after EBV-CTL transfer was performed for 18 of 37 patients. Monitoring results and time points of T-cell transfers for individual patients are displayed in Figure 5 and summarized in Figure 6. EBV-specific T-cell responses were detected in 13 of these (72 %) directly *ex vivo* by using IFN-γ Enzyme-Linked Immune absorbent Spot (ELISpot) assay (Figure 6). From three of the five patients with undetectable EBV-specific T cells via direct ELISpot assay, PBMCs were restimulated once using EBV peptide pools and expanded for seven days in the presence of low dose IL-2. This allows for a more sensitive detection of low-frequency virus-specific T cells and at the same time indicates their functionality as defined by the ability to proliferate and secrete IFN-γ upon antigen recognition. In all of them, EBV-specific T cells were detected after expansion resulting in a total EBV-specific T-cell detection rate of 89 % (3/4 positive after expansion, 16/18 positive in total). In these 18 patients, the median time between first EBV-CTL application and monitoring for EBV-specific T cells in recipient blood was 3 weeks.

From the 20 patients with clinical CR, monitoring was performed for 13 patients (Figure 6A). Of these, ten patients had detectable EBV-specific T cells (77 %, 3/3 positive after expansion, total 100 %). The patient showing PR was monitored and had detectable EBV-specific T cells (100 %). From the three patients showing SD, two were monitored and in one of them, EBV-specific T cells were detected (50 %, no expansion performed). Two of the four patients with PD were monitored and one of them did have detectable EBV-specific T cells (50 %, no

expansion performed). In summary, we were able to detect EBV-specific T-cell responses in all patients with PR or CR after EBV-CTL transfer.

For analysis of EBV-specific T-cell responses in patients based on donor origin, one patient was excluded because this one received EBV-CTL both from an unrelated TPD and the respective SCD (#24/36). For eight patients receiving EBV-CTL from an unrelated TPD, T-cell monitoring was performed and EBV-specific T cells were found in six of them (75 %, expansion performed for 1/2, total 88 %; Figure 6B). T-cell monitoring from three patients receiving SCD-originated EBV-CTL showed that in one of them, EBV-specific T cells were detectable (33 %, 2/2 after expansion, total 100 %). For six of the nine patients receiving EBV-CTL from a related TPD, T-cell monitoring was performed. By direct IFN-γ ELISpot assay, EBV-specific T cells were detected in five of them (83 %, no expansion performed). Thus, irrespective of the donor type, functional EBV-specific T cells were detectable in the majority of patients after transfer of EBV-CTL.

Discussion

In this case series we describe the manufacturing of 40 individualized EBV-specific T-cell products isolated by magnetic separation after peptide pool stimulation and IFN-γ secretion and the intended adoptive transfer into 37 patients with EBV-associated diseases. EBV-CTL products were generated from either SCD (n=13) or TPD (n=27) individually for each patient within 5 days (median; range 1-159 days) after donor identification. Prolonged intervals were mainly due to individual pre-treatment regimens to reduce tumor burden. In HSCT patients, the majority demonstrated clinical response with at least SD in 70 % (TPD) and 90 % (SCD) of patients. While the CR rate was higher in patients receiving EBV-CTL from SCD (90 %) as compared to TPD (60 %), there was no significant difference in the virologic response rate with 73 % clearing EBV from the peripheral blood in the TPD group and 60 % in the SCD group. Few patients in both groups demonstrated re-occurrence or worsening of pre-existing GvHD, while induction of de novo GvHD was observed exclusively in two patients of the SCD group, most likely unrelated to EBV-CTL transfer (°I skin plus II° intestine GvHD; °III liver, skin and oral mucosa GvHD – the first patient had reduction of immunosuppression directly before occurrence of GvHD and the other patient received DLI and pembrolizumab in parallel to EBV-CTL). No GvHD induction was observed in patients receiving EBV-CTL products either for EBV-associated PTLD after SOT or for EBV-complications in immunodeficiency. In this group all patients received TPD EBV-CTL, and 70 % of patients showed partial or complete remission. One patient relapsed and died of lymphoproliferation.

Our series is the largest patient cohort reported to date that received EBV-CTL manufactured by IFN-γ cytokine secretion approach. Previously, Moosmann *et al.* pioneered this approach manufacturing EBV-CTL against known peptide antigens for six patients after HSCT, two of which at early stage PTLD benefitted and demonstrated sustained EBV-specific T-cell expansion after adoptive transfer (36). Subsequently, Icheva reported this approach in ten patients with EBV-associated complications after HSCT, all manufactured from the SCD with EBNA-1 protein or overlapping peptides as the sole target antigen (30). Seven patients showed clinical and/or virologic response. In this case series, we extended this approach to 1) include

multiple EBV antigens for several HLA-types by introducing the EBV_Select peptide pool, 2) extending the approach to patients, in whom the SCD is not available for T-cell donation, and 3) administering multiple infusions of EBV-specific CTL if needed.

We demonstrate that donor identification and EBV-CTL manufacturing is feasible in a timely manner for most patients in need. From the alloCELL registry, we could provide suitable T-cell donors for patients with EBV-seronegative SCD or SCD unavailable for T-cell donation (37-39). Previous studies of *ex vivo* expanded EBV-CTL transferred to partially HLA-matched patients with EBV-associated diseases have demonstrated efficacy in the majority of patients (25-29). Our approach provides an alternative to these previously published strategies of using banked expanded VSTs for rapid use on a best HLA-match basis without the need for long-term in vitro expansion or manipulation. So far, no direct comparison of these two approaches has been conducted, but outcome and side effects observed in our cohort appear comparable to data from adoptive transfer of expanded EBV-CTL.

Although this cohort reports the results of consecutive EBV-CTL manufacturing and adoptive transfer, it is a heterogeneous case series without the power of a prospective clinical trial. Despite this limitation, we attempted to address clinical and virologic efficacy by retrospective chart review. EBV-CTL transfer led to CR in 20 of 29 patients who were evaluated for clinical response. 18 patients are still alive while 12 patients died, with four of them related to EBV (two in the group of HSCT/TPD and one each in the groups of HSCT/SCD and no Tx/EBV-lymphoma). From the remaining fatalities, four died due to other infections, one due to chronic GvHD, one because of primary malignant disease relapse, one because of second malignancy and one because of multiorgan failure not otherwise classified. Thus, both SCD- and TPD-derived EBV-CTL led to disease remission in the majority of treated patients, with a higher CR rate in patients receiving SCD-derived EBV-CTL products. From these data, however, we cannot conclude whether EBV-CTL manufactured from SCD or TPD are more potent; prospective trials are needed to address this question.

All HSCT patients had received and were refractory to rituximab therapy prior to EBV-CTL administration. Exact documentation of response to all prior treatments was, unfortunately, not

available, a limitation of this retrospective series. Some patients in both donor groups (SCD, n=4; TPD n=5) continued to receive antibody (rituximab, brentuximab), immune modulatory (bortezomib, checkpoint blockade) or cytotoxic therapy in parallel to EBV-CTL treatment according to the treating physician's discretion (details in Supplemental Table 1A). We are unable to dissect the effects of individual treatment components in this retrospective analysis, however, parallel treatments were equally applied in both donor groups. Decline in EBV-load in patients responding to EBV-CTL therapy was in close timely context with administration of EBV-CTLs (Supplemental Figure 1A). None of these "responders" died of PTLD. In contrast, persistence of EBV in peripheral blood was associated with treatment failure in four closely monitored patients (Supplemental Figure 1B), three of whom ultimately died of PTLD. With the limitation of not being complete for all patients and differences in local EBV-PCR assay techniques the data support a close correlation between administration of EBV-CTLs, decline of EBV load, and control of PTLD.

No infusion-related side effects were reported in our patient cohort. Similarly, no graft failure (HSCT) or rejection (SOT) in context of EBV-CTL administration were observed. Induction or aggravation of GvHD is a major concern when administering allogeneic T cells, especially since immunosuppressive treatment is usually reduced to a minimum at the onset of refractory viral infections. Only three patients developed de novo GvHD following adoptive transfer of EBV-CTL, two of them only mild GvHD, the third patient suffered from severe GvHD. This case was reviewed by the treating physician, who denied an association with transfer of EBV-CTL but rather with the parallel administration of DLI leading to GvHD. All three patients had undergone HSCT before EBV-CTL transfer and in all cases the SCD served as EBV-CTL donor. Transferred EBV-CTL cell count in all three patients with new GvHD was within the medium range of EBV-CTL cell count of all patients, thus there was no relation between cell dose and occurrence of GvHD. It may be speculated on potentially better engraftment of SCD-derived compared to TPD-derived CTL; however, numbers are too small and patient groups too heterogeneous to fully address this idea in this retrospective patient evaluation. Two HSCT patients in the TPD group had no GvHD before CTL transfer, none of them developed de novo

GvHD after EBV-CTL transfer. In this group, GvHD symptoms aggravated in 3 of 9 patients with pre-existing GvHD, two of them attributed to either checkpoint inhibitor treatment (n=1) or sorafenib (n=1). Further, in none of the patients who developed GvHD, GvHD could be clearly attributed to transfer of EBV-specific T cells.

Monitoring of EBV-specific T cells after adoptive transfer was performed in a subset of patients and most patients had detectable EBV-specific T cells by IFN-γ ELISpot analysis. Numbers are too small and time points of monitoring to diverse to correlate the magnitude of T-cell responses and clinical or virologic outcome. EBV-specific T-cell responses detected shortly after EBV-CTL infusion might be indicative of the development of endogenous T-cell responses rather than a direct effect of EBV-CTL transfer. There is an open discussion on whether the transferred EBV-CTL directly lead to therapeutic effect or whether this is mediated by induction of endogenous T-cell responses. In a number of case reports it was shown that in patients lacking virus-specific T cells, adoptive transfer of virus-specific T cells isolated using the cytokine capture system resulted in detectable virus-specific T cells (31, 40, 41). Further, in a cohort of pediatric solid organ graft recipients, an increase of EBV-specific T cells upon reduction of immunosuppression and treatment with rituximab was observed, indicating that the endogenous immune responses can be boosted by release of viral antigens due to rituximab-mediated cell lysis (42), which might apply to the mechanism of adoptively transferred virus-specific T cells as well. We recently reported that EBV antigens released from EBV-transformed B lymphoblastoid cell lines promote EBV-specific memory T-cell responses (43). Furthermore, we have previously shown by T-cell receptor (TCR) sequencing in a patient receiving TPD-derived EBV-CTL that both persistence and expansion of transferred cells as well as induction of endogenous EBV-specific T-cell responses, thereby broadening the antigenic repertoire, can occur (31). Single cell sequencing studies are now starting to elucidate TCR sequences that confer therapeutic efficacy and protective anti-EBV immunity (44). Future studies including immune monitoring prior to EBV-CTL infusion as well as discrimination between donor- and recipient-derived T cells after transfer are required to elucidate the mechanism as well as the therapeutic efficacy of adoptive T-cell transfer. In a

broader perspective, TCR transfer in autologous T cells may be an alternative though elaborate option for patients lacking a suitable T-cell donor.

In conclusion, our data support the notion that adoptive transfer of EBV-CTL enriched by the CliniMACS CCS IFN-gamma is feasible, clinically effective and safe from both SCD and related or unrelated TPD. Using patient-specific directly manufactured EBV-CTL circumvents the need for prolonged in vitro expansion and GMP-compliant banking of EBV-CTL products despite rapid availability. This treatment seems promising for immunocompromised patients with refractory EBV-associated diseases even beyond HSCT. Limited side effects and low organ toxicity make this approach attractive for patients with pre-existing organ dysfunction. However, prospective clinical trials are required to address questions regarding best available donor, best manufacturing strategy, optimal cell dose and dosing intervals as well as the mode-of-action and persistence of the transferred T cells.

Methods

T-cell donor registry (alloCELL)

The allogeneic T-cell donor registry (alloCELL) established at the Institute of Transfusion Medicine and Transplant Engineering (Hannover Medical School, Hannover, Germany) currently records >3,500 HLA-typed donors with known VST frequencies against common human viruses. Following written informed consent antiviral T-cell frequencies were determined by IFN-γ ELISpot assay (see patient follow-up) using residual blood samples originating from platelet apheresis disposable kits used for routine platelet collection from regular healthy blood donors of the Institute of Transfusion Medicine and Transplant Engineering (ethics committee votes 3331-2016, 3639-2017).

Donor pre-testing

Donor EBV serostatus was determined by analysis of anti-EBV IgG antibodies in serum samples using a line immunoassay (recomLine, Mikrogen). IFN-γ cytokine secretion assay (CSA, Miltenyi Biotec), which is largely analogous to the clinical-grade manufacturing procedure, was performed to determine the EBV-specific T-cell frequencies in selected donors and to predict the expected efficiency in the manufacturing process (35). PBMCs were isolated by density gradient centrifugation and seeded into 24-well cell culture plates with $1x10^7$ cells per well in TexMACS media (Miltenyi Biotec). Following an overnight resting period, cells were stimulated with PepTivator EBV_EBNA-1 alone or in combination with PepTivator EBV_Consensus (both from Miltenyi Biotec) for four hours. As negative control (NC), cells were kept unstimulated. CSA was performed according to manufacturer's instruction. Activated IFN-γ-producing T cells were captured during the magnetic cell enrichment process using anti-IFN-γ Phycoerythrin (PE) antibodies and paramagnetic anti-PE microbeads. Aliquots of the respective cell fractions collected before and after enrichment were used for analysis of IFN-γ^+ T-cell subsets by multicolor flow cytometry using the following antibodies: anti-CD3-FITC (SK7), anti-CD4-AlexaFluor700 (RPA-T4), anti-CD8-Allophycocyanin (APC, SK1), anti-CD45-APC-H7 (2D1, all BD Biosciences), anti-IFN-γ-PE (Miltenyi Biotec). For discrimination of alive

and dead cells, samples were incubated with 7-aminoactinomycin D (7-AAD, BD Biosciences) directly prior to analysis. Samples were acquired at a 10 color BD FACSCanto (BD Biosciences) and analyzed using BD FACSDiva (version 8.0.1, BD Biosciences).

Generation of clinical grade EBV-CTL products

Donor leukapheresis products were enriched for IFN-γ-secreting cells in compliance with EU good manufacturing practice (GMP) starting with $1x10^9$ leukocytes in response to MACS GMP PepTivators EBV_EBNA-1 and EBV_Select (GMP grade product consisting of the same peptides as EBV_Consensus) using CliniMACS CCS IFN-gamma and CliniMACS Plus or Prodigy device (all Miltenyi Biotec). The enrichment process was performed according to the manufacturer's instructions for both devices (35, 37). Aliquots of the leukaphereses and in-process samples (pre-enrichment, final product, negative fraction) were taken for quality control using flow cytometry (35). All products (n=40) fulfilled the specification criteria. The final products were divided into portions according to the dosage. For cryopreservation, products were supplemented with 7.5 % DMSO, processed in a controlled-rate freezer, and finally transferred to the vapor phase above liquid nitrogen for storage. Moreover, leukaphereses and final products were tested for sterility by using a fully automated microbial detection system. Aliquot samples of cryopreserved T-cell products were subjected to quality control as described.

Clinical data collection and response criteria

Clinical data was approved by the Institutional Review Board of Hannover Medical School (ethics committee vote 3207-2016) and was exhibited by standardized questionnaire. Follow up ranged from four weeks to 77 months (median 34.5 months) after EBV-CTL transfer. Data collection contained reason for transfer, local histology report, numbers of EBV-CTL transfers, GvHD before and after transfer, virologic response and clinical response. Response data were collected in all patients who had a follow up of at least three weeks after first CTL transfer. Complete clinical response (CR) was defined as disappearance of all lesions on imaging if

present before treatment and resolution of PTLD-related symptoms. Partial clinical response (PR) was defined as ≥ 25 % reduction in tumor volume and no appearance of new lesions. Stable disease (SD) was defined as no change in tumor volume greater than 25 %.

EBV-PCR was carried out according to the respective local laboratory standards but was consistent within individual patients. Complete virologic response was defined as disappearance of EBV load by PCR. Partial virologic response was defined as reduction in viral load by at least one $\log_{10}$ but still measurable. All other situations were defined as virologic non-response.

Monitoring of EBV-specific T-cell responses after EBV-CTL transfer

For determination of EBV-specific T-cell frequencies in patient blood, IFN-γ ELISpot Assay was performed as described (45). Briefly, PBMCs isolated from patient blood by density centrifugation were allowed to rest overnight in RPMI (Lonza) supplemented with 10 % human AB serum (c.c.pro). Rested PBMCs were cultured in anti-IFN-γ pre-coated ELISpot plates (Lophius Biosciences) for 16-18 h at a density of $2.5x10^5$ or $5.0x10^5$ cells/well and stimulated with PepTivators EBV_EBNA-1 or EBV_Consensus (both 1 µg/ml per peptide, Miltenyi Biotec). Unstimulated cells served as negative control and cells supplemented with 1 µg/ml staphylococcal enterotoxin B (SEB, Sigma-Aldrich) served as positive control. Following overnight incubation, IFN-γ secretion was detected using an AID iSpot Reader System and AID ELISpot Software Version 8.0, both AID GmbH). IFN-γ-positive cells were counted and expressed as the number of spots per well (spw). The mean number of spots in the negative control was subtracted from the mean number of spots in the antigen wells.

To determine low frequency EBV-specific T cells after adoptive T-cell transfer, isolated PBMCs were cultured in presence of PepTivators EBV_EBNA-1 or EBV_Consensus (both 1 µg/ml per peptide, Miltenyi Biotec) for seven days in the presence of 50 IU/ml IL-2 (Peprotech). Subsequent to this expansion period, cells were harvested and subjected to ELISpot assay as described.

636 Data analysis

637 Data were analyzed by Microsoft Excel 2010 (Microsoft Corporation, Redmond). Summarizing
638 graphs were generated using GraphPad Prism 8.2.2 (GraphPad Software, San Diego). For
639 display of flow cytometric data, FlowJo v10 (FlowJo™ LLC, BD Biosciences) was utilized.

640

641 Statistics

642 Descriptive statistics were used to determine median, mean, and range data. Details of further
643 applied statistical tests are stated in the respective Figure Legends.

644

645 Study approval

646 Written informed consent was obtained from donors of the allogeneic T-cell registry (alloCELL)
647 established at the Institute of Transfusion Medicine and Transplant Engineering (Hannover
648 Medical School, Hannover, Germany) (ethics committee votes 3331-2016, 3639-2017).
649 Clinical data collection was approved by the Institutional Review Board of Hannover Medical
650 School (ethics committee vote 3207-2016).

651

652 **Author contributions**

653 BEV and BMK designed and supervised the program, AB, STZ generated and evaluated the
654 donor selection, manufacturing and immune monitoring data, BL collected and analyzed the
655 clinical data. AB was mainly responsible for manuscript writing and data analysis with respect
656 to TPD and patient monitoring. BL was mainly responsible for data analysis with respect to
657 clinical outcome. STZ was mainly responsible for data analysis with respect to SCD. RSF, RB,
658 GB, GC, BF, JG, LH, JH, MH, PH, EJ, KK, OK, NK, SM, RM, MN, MP, HCR, ES, MS, CS, RS,
659 NKS, RUT, MV, DW treated patients and provided clinical data, LG, HGH, LA, RB
660 manufactured EBV-CTL, SR participated in immunological analysis. AB, STZ, BL, BEV and
661 BMK wrote the manuscript. All authors read and approved the final version of the manuscript.

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References

1. Bollard CM, and Heslop HE. T cells for viral infections after allogeneic hematopoietic stem cell transplant. *Blood.* 2016;127(26):3331-40.
2. Callan MF. The evolution of antigen-specific CD8+ T cell responses after natural primary infection of humans with Epstein-Barr virus. *Viral Immunol.* 2003;16(1):3-16.
3. Dharnidharka VR, Webster AC, Martinez OM, Preiksaitis JK, Leblond V, and Choquet S. Post-transplant lymphoproliferative disorders. *Nat Rev Dis Primers.* 2016;2:15088.
4. Al Hamed R, Bazarbachi AH, and Mohty M. Epstein-Barr virus-related post-transplant lymphoproliferative disease (EBV-PTLD) in the setting of allogeneic stem cell transplantation: a comprehensive review from pathogenesis to forthcoming treatment modalities. *Bone Marrow Transplant.* 2020;55(1):25-39.
5. Bollard CM, Rooney CM, and Heslop HE. T-cell therapy in the treatment of post-transplant lymphoproliferative disease. *Nat Rev Clin Oncol.* 2012;9(9):510-9.
6. Hussein K, Tiede C, Maecker-Kolhoff B, and Kreipe H. Posttransplant lymphoproliferative disorder in pediatric patients. *Pathobiology.* 2013;80(6):289-96.
7. Evens AM, David KA, Helenowski I, Nelson B, Kaufman D, Kircher SM, et al. Multicenter analysis of 80 solid organ transplantation recipients with post-transplantation lymphoproliferative disease: outcomes and prognostic factors in the modern era. *J Clin Oncol.* 2010;28(6):1038-46.
8. Fujimoto A, Hiramoto N, Yamasaki S, Inamoto Y, Uchida N, Maeda T, et al. Risk Factors and Predictive Scoring System For Post-Transplant Lymphoproliferative Disorder after Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant.* 2019;25(7):1441-9.
9. Sundin M, Le Blanc K, Ringden O, Barkholt L, Omazic B, Lergin C, et al. The role of HLA mismatch, splenectomy and recipient Epstein-Barr virus seronegativity as risk factors in post-transplant lymphoproliferative disorder following allogeneic hematopoietic stem cell transplantation. *Haematologica.* 2006;91(8):1059-67.
10. Landgren O, Gilbert ES, Rizzo JD, Socie G, Banks PM, Sobocinski KA, et al. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. *Blood.* 2009;113(20):4992-5001.
11. Dierickx D, Tousseyn T, and Gheysens O. How I treat posttransplant lymphoproliferative disorders. *Blood.* 2015;126(20):2274-83.
12. Shaffer DR, Rooney CM, and Gottschalk S. Immunotherapeutic options for Epstein-Barr virus-associated lymphoproliferative disease following transplantation. *Immunotherapy.* 2010;2(5):663-71.
13. Omar H, Hagglund H, Gustafsson-Jernberg A, LeBlanc K, Mattsson J, Remberger M, et al. Targeted monitoring of patients at high risk of post-transplant lymphoproliferative disease by quantitative Epstein-Barr virus polymerase chain reaction. *Transpl Infect Dis.* 2009;11(5):393-9.
14. Jagadeesh D, Woda BA, Draper J, and Evens AM. Post transplant lymphoproliferative disorders: risk, classification, and therapeutic recommendations. *Curr Treat Options Oncol.* 2012;13(1):122-36.
15. Green M, and Michaels MG. Epstein-Barr virus infection and posttransplant lymphoproliferative disorder. *Am J Transplant.* 2013;13 Suppl 3:41-54; quiz
16. Schober T, Framke T, Kreipe H, Schulz TF, Grosshennig A, Hussein K, et al. Characteristics of early and late PTLD development in pediatric solid organ transplant recipients. *Transplantation.* 2013;95(1):240-6.
17. Mynarek M, Schober T, Behrends U, and Maecker-Kolhoff B. Posttransplant lymphoproliferative disease after pediatric solid organ transplantation. *Clin Dev Immunol.* 2013;2013:814973.
18. Santarsieri A, Rudge JF, Amin I, Gelson W, Parmar J, Pettit S, et al. Incidence and outcomes of post-transplant lymphoproliferative disease after 5365 solid-organ transplants over a 20-year period at two UK transplant centres. *Br J Haematol.* 2022;197(3):310-9.

19. Dierickx D, and Habermann TM. Post-Transplantation Lymphoproliferative Disorders in Adults. *N Engl J Med.* 2018;378(6):549-62.
20. Heslop HE. How I treat EBV lymphoproliferation. *Blood.* 2009;114(19):4002-8.
21. Nalesnik MA. Clinicopathologic characteristics of post-transplant lymphoproliferative disorders. *Recent Results Cancer Res.* 2002;159:9-18.
22. Allen UD, Preiksaitis JK, and Practice ASTIDCo. Post-transplant lymphoproliferative disorders, Epstein-Barr virus infection, and disease in solid organ transplantation: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant.* 2019;33(9):e13652.
23. Savoldo B, Goss JA, Hammer MM, Zhang L, Lopez T, Gee AP, et al. Treatment of solid organ transplant recipients with autologous Epstein Barr virus-specific cytotoxic T lymphocytes (CTLs). *Blood.* 2006;108(9):2942-9.
24. Leen AM, Christin A, Myers GD, Liu H, Cruz CR, Hanley PJ, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood.* 2009;114(19):4283-92.
25. Haque T, Wilkie GM, Jones MM, Higgins CD, Urquhart G, Wingate P, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood.* 2007;110(4):1123-31.
26. Tzannou I, Papadopoulou A, Naik S, Leung K, Martinez CA, Ramos CA, et al. Off-the-Shelf Virus-Specific T Cells to Treat BK Virus, Human Herpesvirus 6, Cytomegalovirus, Epstein-Barr Virus, and Adenovirus Infections After Allogeneic Hematopoietic Stem-Cell Transplantation. *J Clin Oncol.* 2017;35(31):3547-57.
27. Prockop S, Doubrovina E, Suser S, Heller G, Barker J, Dahi P, et al. Off-the-shelf EBV-specific T cell immunotherapy for rituximab-refractory EBV-associated lymphoma following transplantation. *J Clin Invest.* 2020;130(2):733-47.
28. Jiang W, Clancy LE, Avdic S, Sutrave G, Street J, Simms R, et al. Third-party CMV- and EBV-specific T-cells for first viral reactivation after allogeneic stem cell transplant. *Blood Adv.* 2022;6(17):4949-66.
29. Pfeiffer T, Tzannou I, Wu M, Ramos C, Sasa G, Martinez C, et al. Posoleucel, an Allogeneic, Off-the-Shelf Multivirus-Specific T-Cell Therapy, for the Treatment of Refractory Viral Infections in the Post-HCT Setting. *Clin Cancer Res.* 2023;29(2):324-30.
30. Icheva V, Kayser S, Wolff D, Tuve S, Kyzirakos C, Bethge W, et al. Adoptive transfer of epstein-barr virus (EBV) nuclear antigen 1-specific t cells as treatment for EBV reactivation and lymphoproliferative disorders after allogeneic stem-cell transplantation. *J Clin Oncol.* 2013;31(1):39-48.
31. Schultze-Florey RE, Tischer S, Kuhlmann L, Hundsdoerfer P, Koch A, Anagnostopoulos I, et al. Dissecting Epstein-Barr Virus-Specific T-Cell Responses After Allogeneic EBV-Specific T-Cell Transfer for Central Nervous System Posttransplant Lymphoproliferative Disease. *Front Immunol.* 2018;9:1475.
32. Mika T, Strate K, Ladigan S, Aigner C, Schlegel U, Tischoff I, et al. Refractory Epstein-Barr Virus (EBV)-Related Post-transplant Lymphoproliferative Disease: Cure by Combined Brentuximab Vedotin and Allogeneic EBV-Specific T-Lymphocytes. *Front Med (Lausanne).* 2019;6:295.
33. Meedt E, Weber D, Bonifacius A, Eiz-Vesper B, Maecker-Kolhoff B, Delecluse S, et al. Chronic Active Epstein-Barr Virus Infection controlled by allogeneic stem cell transplantation and EBV-specific T-cells. *Clin Infect Dis.* 2023.
34. Schulze Lammers FC, Bonifacius A, Tischer-Zimmermann S, Goudeva L, Martens J, Lepenies B, et al. Antiviral T-Cell Frequencies in a Healthy Population: Reference Values for Evaluating Antiviral Immune Cell Profiles in Immunocompromised Patients. *J Clin Immunol.* 2022;42(3):546-58.

35. Priesner C, Esser R, Tischer S, Marburger M, Aleksandrova K, Maecker-Kolhoff B, et al. Comparative Analysis of Clinical-Scale IFN-gamma-Positive T-Cell Enrichment Using Partially and Fully Integrated Platforms. *Front Immunol.* 2016;7:393.
36. Moosmann A, Bigalke I, Tischer J, Schirrmann L, Kasten J, Tippmer S, et al. Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells. *Blood.* 2010;115(14):2960-70.
37. Tischer S, Priesner C, Heuft HG, Goudeva L, Mende W, Barthold M, et al. Rapid generation of clinical-grade antiviral T cells: selection of suitable T-cell donors and GMP-compliant manufacturing of antiviral T cells. *J Transl Med.* 2014;12:336.
38. Eiz-Vesper B, Maecker-Kolhoff B, and Blasczyk R. Adoptive T-cell immunotherapy from third-party donors: characterization of donors and set up of a T-cell donor registry. *Front Immunol.* 2012;3:410.
39. Sukdolak C, Tischer S, Dieks D, Figueiredo C, Goudeva L, Heuft HG, et al. CMV-, EBV- and ADV-specific T cell immunity: screening and monitoring of potential third-party donors to improve post-transplantation outcome. *Biol Blood Marrow Transplant.* 2013;19(10):1480-92.
40. Lindemann M, Eiz-Vesper B, Steckel NK, Tischer S, Fiedler M, Heinold A, et al. Adoptive transfer of cellular immunity against cytomegalovirus by virus-specific lymphocytes from a third-party family donor. *Bone Marrow Transplant.* 2018;53(10):1351-5.
41. Hansen BT, Bacher P, Eiz-Vesper B, Heckl SM, Klapper W, Koch K, et al. Adoptive Cell Transfer of Allogeneic Epstein-Barr Virus-Specific T Lymphocytes for Treatment of Refractory EBV-Associated Posttransplant Smooth Muscle Tumors: A Case Report. *Front Immunol.* 2021;12:727814.
42. Wilsdorf N, Eiz-Vesper B, Henke-Gendo C, Diestelhorst J, Oschlies I, Hussein K, et al. EBV-specific T-cell immunity in pediatric solid organ graft recipients with posttransplantation lymphoproliferative disease. *Transplantation.* 2013;95(1):247-55.
43. Tischer-Zimmermann S, Bonifacius A, Santamorena MM, Mausberg P, Stoll S, Doring M, et al. Reinforcement of cell-mediated immunity driven by tumor-associated Epstein-Barr virus (EBV)-specific T cells during targeted B-cell therapy with rituximab. *Front Immunol.* 2023;14:878953.
44. Lammoglia Cobo MF, Welters C, Rosenberger L, Leisegang M, Dietze K, Pircher C, et al. Rapid single-cell identification of Epstein-Barr virus-specific T-cell receptors for cellular therapy. *Cytotherapy.* 2022.
45. Bieling M, Tischer S, Kalinke U, Blasczyk R, Buus S, Maecker-Kolhoff B, et al. Personalized adoptive immunotherapy for patients with EBV-associated tumors and complications: Evaluation of novel naturally processed and presented EBV-derived T-cell epitopes. *Oncotarget.* 2018;9(4):4737-57.

Figures

Figure 1

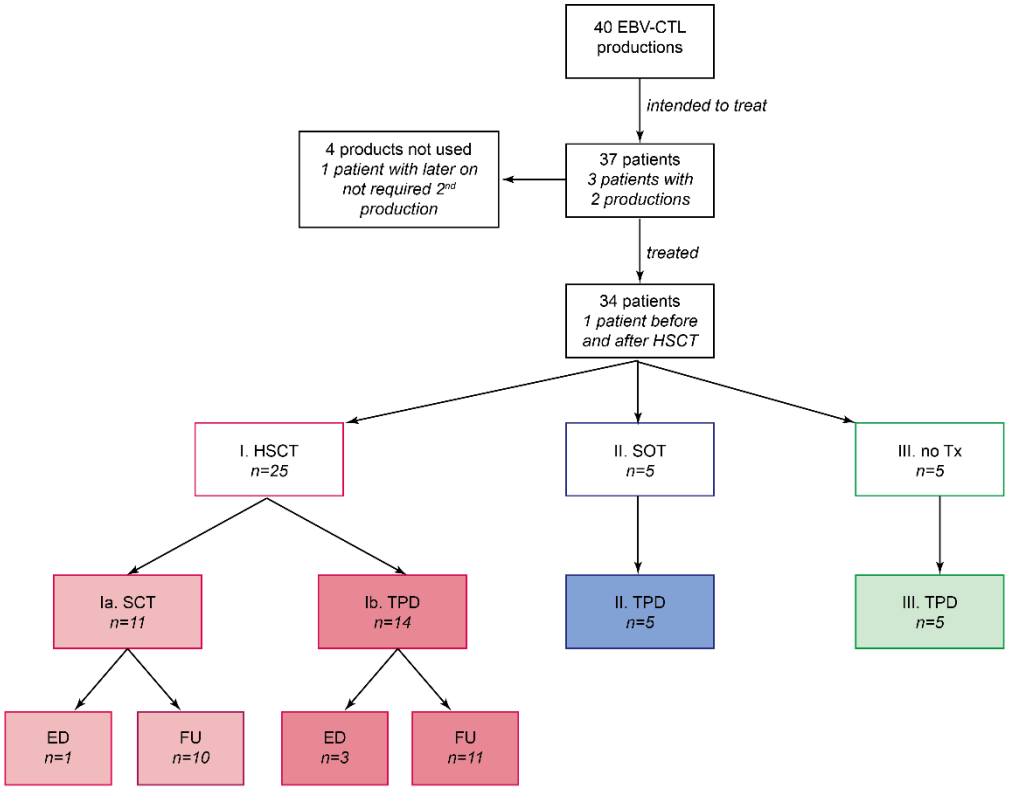


Figure 1: Patient cohort for planned EBV-CTL transfer. EBV-CTL productions and intended/treated patient population. One patient received EBV-CTL before and after HSCT and is therefore recorded in groups IB and IIIA. One patient in group IA received EBV-CTL from two separate production from the same donor. pt, patient; HSCT, hematopoietic stem cell transplantation; SOT, solid organ transplantation; Tx, transplantation; SCD, stem cell donor; TPD, third party donor; ED, early death; FU, follow up.

Figure 2

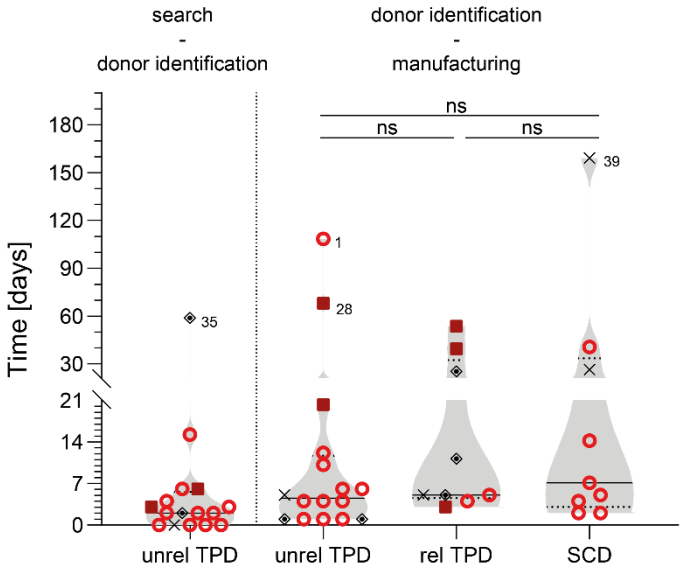


Figure 2: Time between donor search/pre-testing and manufacturing. Shown is the time between donor search and identification for unrelated TPD (left) and the time between donor identification and start of manufacturing for each donor origin (right). Statistical significance was calculated using Kruskal-Wallis test, followed by Dunn's multiple comparison test. ns not significant ($p>0.05$). Violin plots show median, each symbol represents one patient. Red circle: HSCT patient (Ia/Ib); red square: SOT patient (II); black rhombus: no Tx (III); black cross: EBV-CTL not applied.

Figure 3

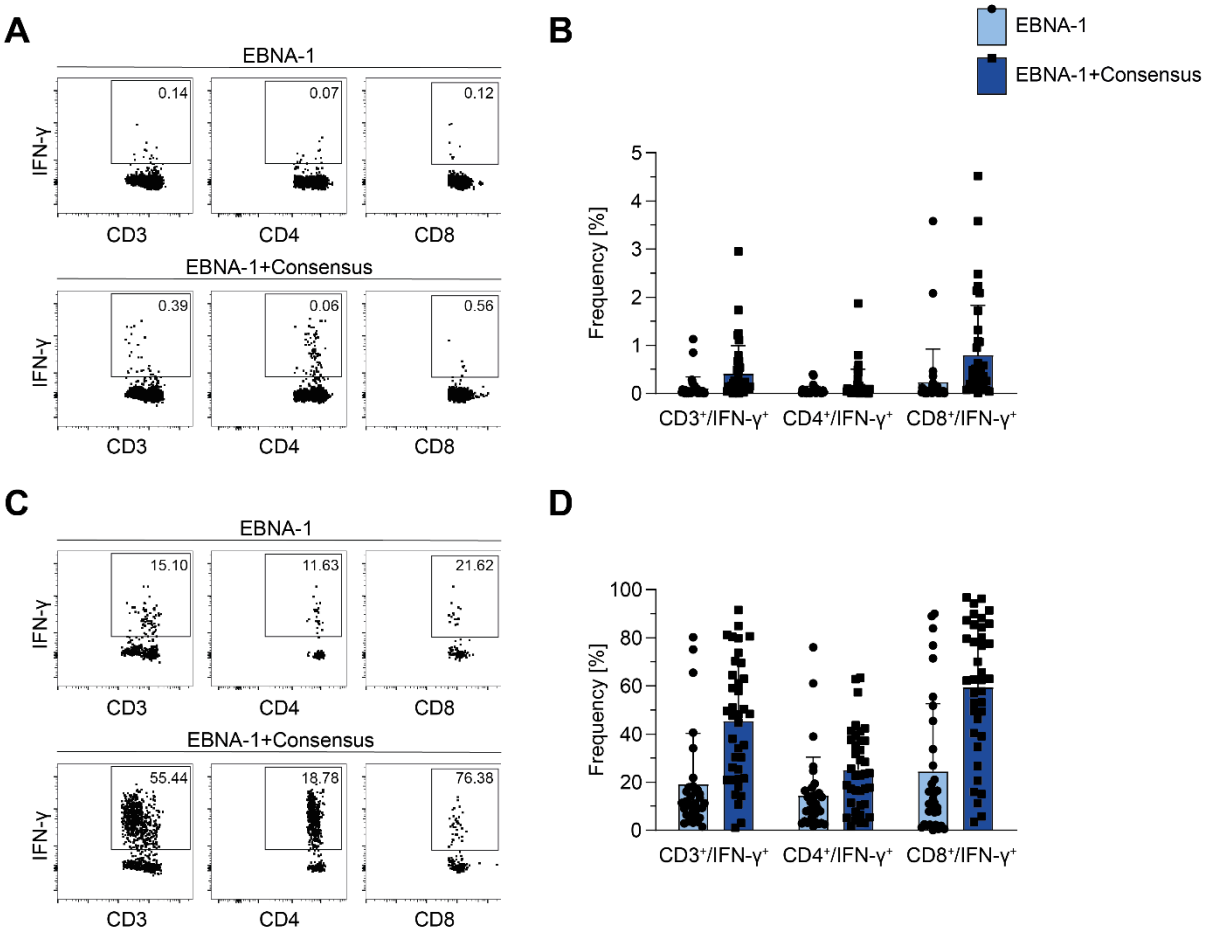


Figure 3: Frequencies of EBV-specific T cells in T-cell donors before and after magnetic enrichment by IFN-γ CSA. Stimulation of donor PBMCs was done with PepTivator EBNA-1 alone (n=34) and combination of PepTivator EBNA-1 and PepTivator Consensus (n=38). Differences in the number of donors tested are due to the amount of PBMCs obtained which did not allow for testing the frequency of EBNA-1-specific T cells alone in 4 out of 38 donors. Exemplary FACS plots are pre-gated on viable $CD3^+$ (left), $CD3^+/CD4^+$ (middle), and $CD3^+/CD8^+$ (right) lymphocytes. (**A,B**) Representative FACS plots and summarizing graphs show frequencies of IFN-γ^+ cells among $CD3^+$, $CD4^+$, and $CD8^+$ T cells before magnetic enrichment as indicated. (**C,D**) Representative FACS plots and summarizing graphs show IFN-γ^+ cells among $CD3^+$, $CD4^+$, and $CD8^+$ T cells after magnetic enrichment as indicated. Bar graphs depict mean+SD and each dot represents data from one donor.

Figure 4

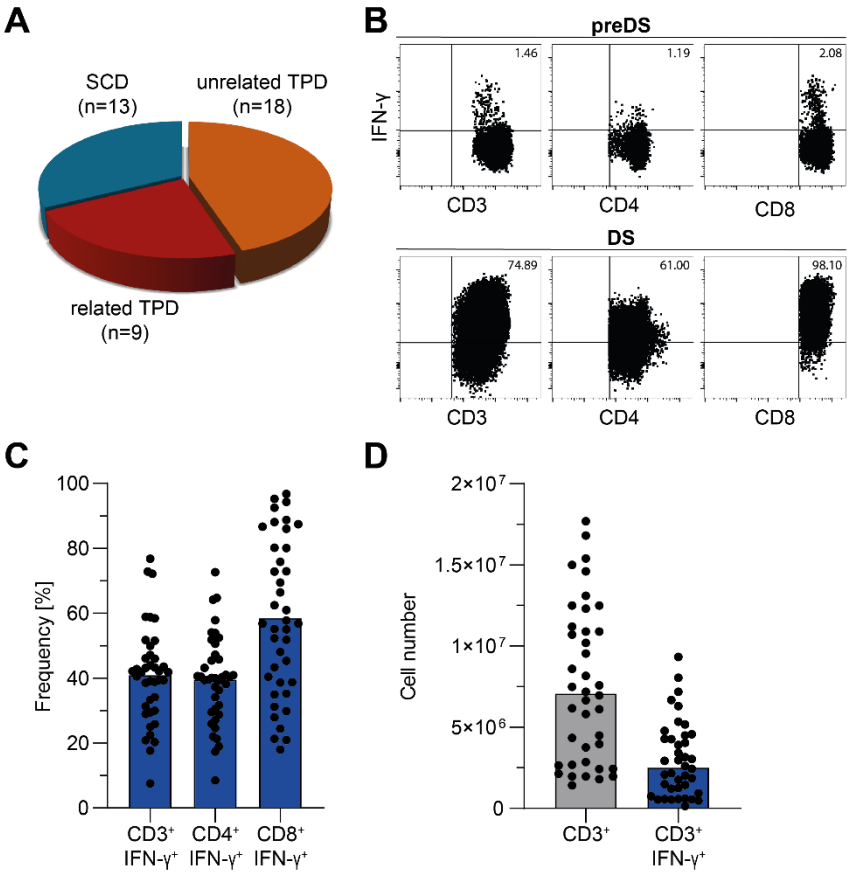


Figure 4: Clinical-grade EBV-CTL manufacturing. Enrichment of IFN-γ-secreting, EBV-specific $CD3^+$, $CD4^+$, and $CD8^+$ T cells after incubation with GMP-grade PepTivators EBV EBNA-1 and EBV Select in combination using the CliniMACS CCS and CliniMACS Plus or Prodigy device. (**A**) Donor origin. (**B**) Representative FACS plots. Gates were set according to Fluorescence minus one (FMO) control. preDS, drug substance before magnetic enrichment; DS, drug substance after magnetic enrichment (**C,D**) Frequencies and numbers of total $CD3^+$ and IFN-γ-secreting, EBV-specific $CD3^+$ $CD4^+$ and $CD8^+$ T cells after stimulation with GMP-grade PepTivators EBNA-1 and EBV Select and enrichment using the CliniMACS CCS and CliniMACS Plus or Prodigy device. Bar graphs depict mean (**C**) or median (**D**) and each dot represents data from one manufacturing process (n=40). SCD, stem cell donor; TPD third-party donor.

Figure 5

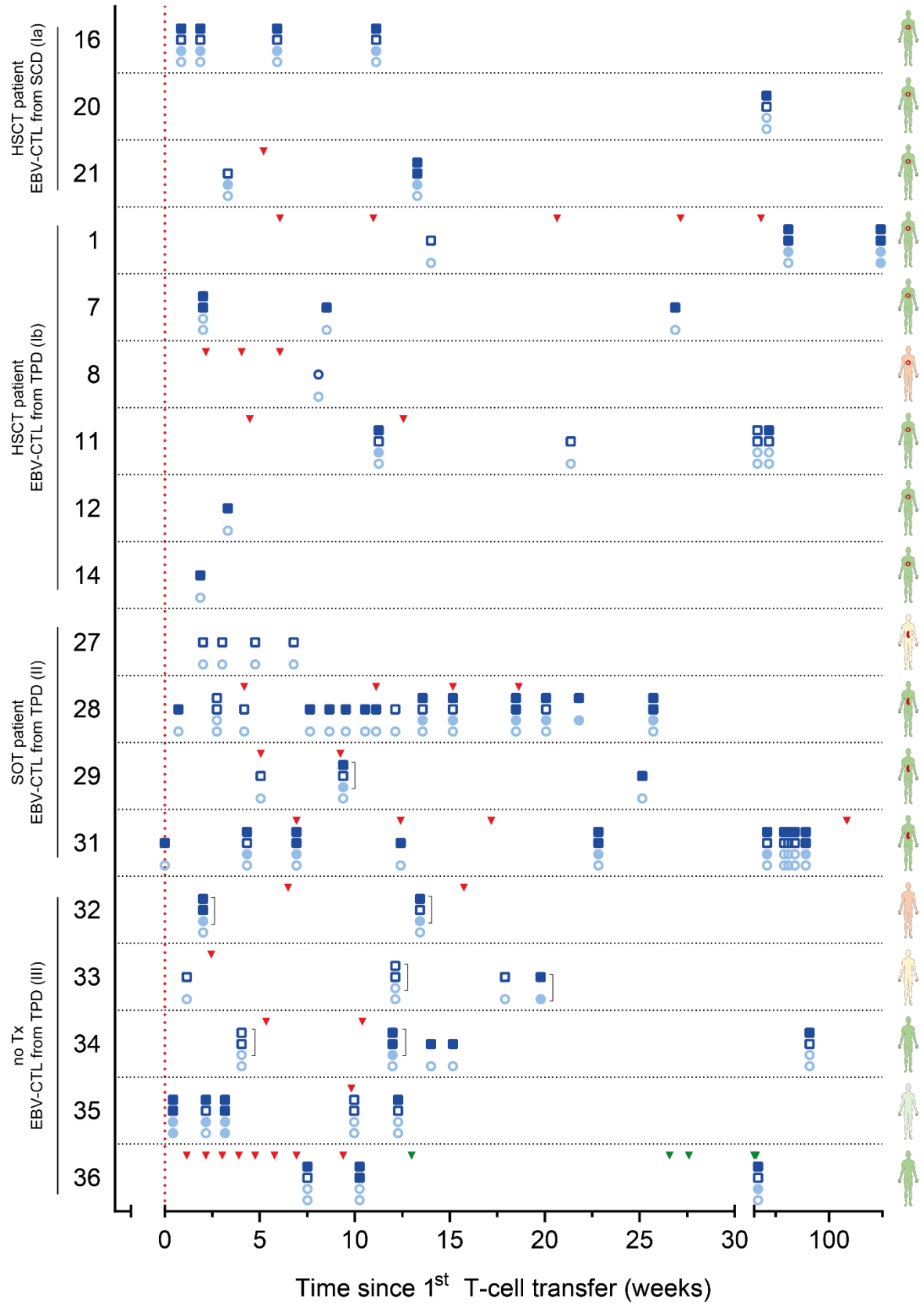
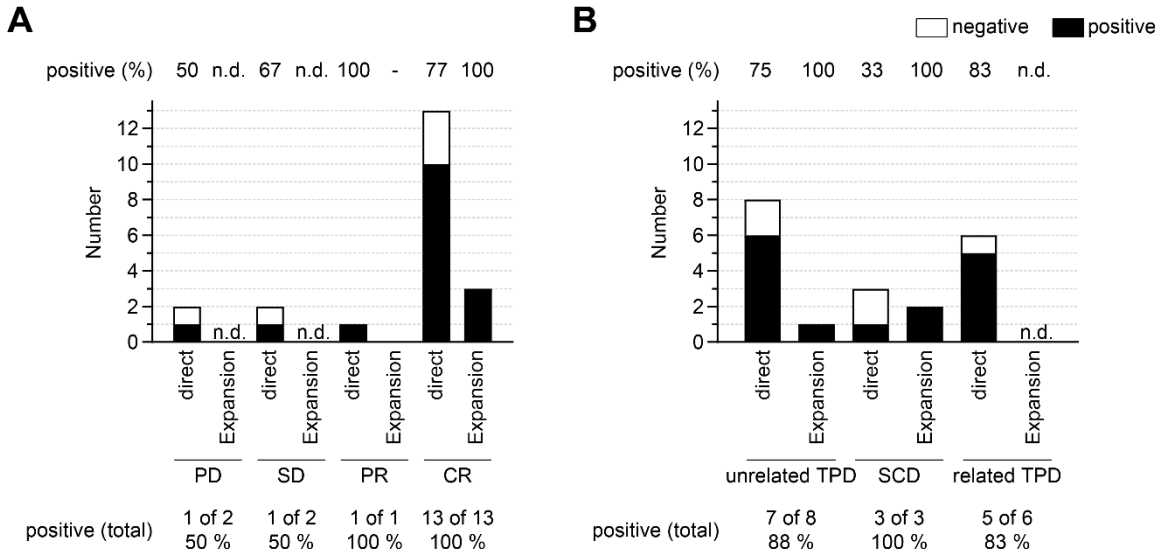

HSCT patient
EBV-CTL from SCD (Ia)
16
20
21
HSCT patient
EBV-CTL from TPD (Ib)
1
7
8
11
12
14
SOT patient
EBV-CTL from TPD (II)
27
28
29
31
no Tx
EBV-CTL from TPD (III)
32
33
34
35
36
0
5
10
15
20
25
30
100
Time since 1st T-cell transfer (weeks)

Figure 5: Immune monitoring in individual patients by IFN-γ ELISpot assay. Peripheral blood samples obtained from patients (ID indicated on y-axis) at different time points post first ÈBV-CTL transfer (indicated on x-axis, in weeks) were subjected to IFN-γ ELISpot assay using EBV_EBNA-1 (light blue) and EBV_Consensus (dark blue) to restimulate EBV-specific memory T cells. Lower circles indicate results from direct EBV_EBNA-1 ELISpot, upper circles indicate results from EBV_EBNA-1 ELISpot after expansion. Lower squares indicate results from direct EBV_Consensus ELISpot, upper squares indicate results from EBV_Consensus ELISpot after expansion.] indicates combined stimulation with both EBV_EBNA-1 and EBV_Consensus. Empty symbols indicate that no specific T cells were detected, while filled symbols indicate that specific T cells were detected. Vertical dashed line and triangles indicate time points of T-cell transfer. #33: green triangles indicate T-cell transfer from 2nd manufacturing process (#24). Symbols on the right indicate clinical response (see graphical abstract). SCD, stem cell donor; TPD third-party donor.

865 **Figure 6**

866



867
868 **Figure 6: T-cell monitoring results.** Detection of IFN-γ-secreting T cells in patient PBMCs after stimulation with
869 PepTivators EBV_EBNA-1 or EBV Consensus using IFN-γ ELISpot assay. Positive: Spots in at least one of the
870 EBV peptide pools. Negative: No spots. Results shown for "Expansion" include only those patients, which did not
871 show detectable EBV-CTL via direct IFN-γ ELISpot assay. Numbers and frequencies (bottom) indicate in how many
872 patients of total tested patients EBV-CTL were detected via either direct IFN-γ ELISpot or after expansion. (**A**) T-
873 cell monitoring results based on clinical response. (**B**) T-cell monitoring results based on donor origin. PD,
874 progressive disease; SD, stable disease; PR, partial response; CR, complete response; SCD, stem cell donor; TPD
875 third-party donor; n.d. not determined.

Tables

Table 1: Patient characteristics and source of EBV-CTL.

			Number (%)
Sex	Male		26/37 (70 %)
	Female		11/37 (30 %)
Transplantation	allogeneic SCT[a]	MSD	4/28 (14 %)
		MUD	21/28 (75 %)
		Haplo	3/28 (11 %)
	SOT		5/37 (14 %)
	no or autologous transplantation or EBV-CTL transfer prior to transplantation[a]		6/37 (16 %)
EBV-CTL donor	SCD		13/40 (33 %)
	related TPD		9/40 (23 %)
	unrelated TPD		18/40 (45 %)
			Range (median
Age [years]			2-73 (37.0)

[a] two patients: 2 EBV-CTL productions for transfer prior to SCT and afterwards; one of them only got EBV-CTL prior to SCT

Table 2: HLA matching between patient and EBV-CTL donor. HLA matching between patient and unrelated TPD (upper) or related TPD (lower), irrespective of SOT or HSCT. HLA-A, -B, -C, DR, DQ (of 10) or HLA-A, -B, -DR (of 6). Homozygous alleles are considered in columns "Recipient – TCD" and "TCD – Recipient". Production runs 1 and 3 were from the same donor for the same patient. n.a., not available.

Donor	Patient	Total HLA-matches (of 10; HLA-A,B,C,DR,DQ)	Total HLA-matches (of 6; HLA-A,-B, -DR)	Recipient – EBV-CTL donor (of 10)		EBV-CTL donor – Recipient (of 10)	
				Matches	Mismatches	Matches	Mismatches
unrelated TPD	1 (=3)	8		8	2	8	2
	2	5		6	4	5	5
	4	6		6	4	6	4
	5	5		5	5	5	5
	6	7		7	3	7	3
	7	4		5	5	5	5
	8	7		7	3	8	2
	9*		6				
	10	5		5	5	5	5
	11	6		6	4	6	4
	12	5		5	5	5	5
	13	5		5	5	5	5
	28	5		7	3	7	3
	31	6		6	4	7	3
	35	5		6	4	5	5
	36*		3				
	37	5		5	5	5	5
related TPD	14	6	3	6	4	6	4
	15	10	6	10	0	10	0
	27	5	3	5	5	5	5
	29	5	3	5	5	5	5
	30	5	3	6	4	5	5
	32	6	4	9	1	6	4
	33	6	3	6	4	6	4
	34	7	4	9	1	7	3
	38	5	3	5	5	5	5

* only HLA-A, B, DR, DQ known (recipient)

Table 3: Results from donor pre-testing. PBMCs from healthy donors were stimulated with PepTivator EBNA-1 alone (EBNA-1, left, n=34) and with combination of PepTivator EBNA-1 and PepTivator Consensus (EBNA-1+Consensus, right, n=38), respectively. Frequencies of IFN-γ$^+$ T cell subsets before and after magnetic enrichment by IFN-γ CSA were determined by flow cytometry. Shown are the frequencies of IFN-γ$^+$ cells within the indicated T cell subset (CD3$^+$, CD4$^+$, CD8$^+$). Differences in the number of donors tested are due to the amount of PBMCs obtained which did not allow for testing the frequency of EBNA-1-specific T cells alone in 4 out of 38 donors.

Stimulation		**EBNA-1 (n=34)**			**EBNA-1+Consensus (n=38)**		
Population		CD3$^+$/IFN-γ$^+$	CD4$^+$/IFN-γ$^+$	CD8$^+$/IFN-γ$^+$	CD3$^+$/IFN-γ$^+$	CD4$^+$/IFN-γ$^+$	CD8$^+$/IFN-γ$^+$
before enrichment*	Mean	0.11	0.05	0.24	0.41	0.17	0.80
	Median	0.04	0.02	0.04	0.19	0.07	0.36
	SD	0.24	0.09	0.69	0.58	0.33	1.04
after enrichment	Mean	19.13	14.47	24.41	45.36	25.05	59.39
	Median	11.31	10.06	12.19	47.93	23.09	62.57
	SD	21.15	15.99	28.21	25.18	16.74	27.57

*Values were obtained after subtracting the values of the NC

Table 4: Clinical-grade EBV-CTL manufacturing. Enrichment of IFN-γ-secreting, EBV-specific $CD3^+$, $CD4^+$, and $CD8^+$ T cells after incubation with GMP PepTivators EBNA-1 and EBV Select and enrichment using the CliniMACS CCS and CliniMACS Plus or Prodigy device.

		Viability [%]	$CD3^+$ [10^6]	$CD3^+$/IFN-γ^+ [10^6]	$CD3^+$/IFN-γ^+ [%]	$CD4^+$/IFN-γ^+ [%]	$CD8^+$/IFN-γ^+ [%]
Total (n=40)	Mean	70.47	7.62	3.00	40.94	39.46	58.83
	Median	72.00	7.07	2.52	41.79	40.05	56.83
	SD	10.46	4.77	2.30	14.83	13.56	23.86
SCD (n=13)	Mean	66.88	8.21	2.89	35.61	34.66	45.47
	Median	71.78	7.58	2.93	38.81	36.30	43.33
	SD	15.52	4.44	1.66	9.21	8.96	17.51
related TPD (n=9)	Mean	70.40	5.71	2.22	39.67	38.59	61.61
	Median	71.50	3.76	1.44	41.81	40.87	75.88
	SD	7.46	4.89	2.12	15.52	14.56	14.56
unrelated TPD (n=18)	Mean	73.09	8.16	3.46	45.41	43.36	66.43
	Median	72.36	7.89	2.60	43.72	40.73	61.74
	SD	6.15	4.95	2.75	16.96	15.21	22.18

Table 5: Occurrence of HLA-A and HLA-B types relevant for PepTivator EBV Consensus Pool among EBV-CTL donors. Shown is the occurrence (number and frequency) of the indicated HLA types that peptides inside the PepTivator EBV Consensus pool (referred to as GMP PepTivator EBV Select in manufacturing) are restricted to.

	Occurrence [n]	Frequency [%]
HLA-A*02:01	13	41.94
HLA-A*03:01	10	32.26
HLA-A*11:01	1	3.23
HLA-A*24:02	7	22.58
HLA-A*26:01	1	3.23
HLA-B*07:02	7	22.58
HLA-B*08:01	7	22.58
HLA-B*15:01	3	9.68
HLA-B*18:01	1	3.23
HLA-B*27:01	2	6.45
HLA-B*35:01	8	25.81
HLA-B*40:01	3	9.68
HLA-B*44:02	3	9.68

Table 6: Outcome of EBV-CTL transfer in HSCT patients.

			Range (median)	
EBV-CTL donor			**TPD**	**SCD**
Age [years]			5-68 (36.0)	22-66 (54.5)
Number of EBV-CTL transfers per patient[a]			1-6 (2.0)	1-5 (1.0)
$CD3^+$ T-cell count of first EBV-CTL transfer per patient [/kg body weight][a]			$5x10^3$-$3.7x10^4$ ($2.5x10^4$)	$1x10^4$-$2.2x10^5$ ($2.5x10^4$)
$CD3^+IFN\text{-}\gamma^+$ T-cell count of first EBV-CTL transfer per patient [/kg body weight][a]			$2.2x10^3$-$2.2x10^4$ ($5.2x10^3$)	$2.6x10^3$-$5.1x10^4$ ($8.5x10^3$)
			Number (%)	
EBV-CTL donor			**TPD**	**SCD**
Sex	male		7/11 (64 %)	8/10 (803 %)
	female		4/11 (36%)	2/10; 20%
SCD	MSD		3/11 (27 %)	1/10 (10 %)
	MUD		8/11 (73 %)	7/10 (70 %)
	haplo		0/11 (0 %)	2/10 (20 %)
GvHD	before EBV-CTL transfer	total	9/11 (82 %)	3/10 (30 %)
		preexisting GvHD worsened after EBV-CTL transfer/ new symptoms of GvHD	2/9 (22 %)	0/3 (0 %)
		preexisting GvHD stable/chronic after EBV-CTL transfer	3/9 (33 %)	1/3 (33 %)
		preexisting GvHD ameliorated after EBV-CTL transfer	4/9 (44 %)	2/3 (67 %)
	first occurrence of GvHD after EBV-CTL transfer		0/11 (0 %)	3/10 (30 %)
Clinical response	complete response (CR)		6/10 (60 %)	9/10 (90 %)
	partial response (PR)		0/10 (0 %)	0/10 (0 %)
	stable disease (SD)		1/10 (10 %)	0/10 (0 %)
	progressive disease (PD)		3/10 (30 %)	1/10 (10 %)
	no data available		1	0
Virologic response (EBV-PCR)[b]	negative	total	8/11 (73 %)	6/10 (60 %)
		negative before transfer of EBV-CTL	1/8 (13 %)	2/6 (33 %)
	positive	total	3/11 (27 %)	4/10 (40 %)
		decrease of viral load	2/3 (67 %)	2/4 (50 %)
		stable viral load	0/3 (0 %)	1/4 (25 %)
		increase of viral load	1/3 (33 %)	1/4 (25 %)
Outcome	alive after EBV-CTL transfer		4/14 (29 %)	6/11 (55 %)
	death after EBV-CTL transfer	total	10/14 (71 %)	5/11 (45 %)
		death within 3 weeks after first EBV-CTL transfer (early death)	3/10 (30 %)	1/5 (20 %)
		death associated with EBV (including progression of EBV-associated underlying disease)[2]	1/10 (10 %)	1/5 (20 %)

Patients with “early death" excluded.

[a] one patient already got EBV-CTL prior to HSCT; only EBV-CTL applications after HSCT were considered

[b] EBV-PCR after end of EBV-CTL transfers