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Durable immunity to EBV after rituximab and third-party LMP-specific T cells: a Children’s Oncology Group study

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Key Points

- LMP-TC bank comprising 14 products with 11 distinct HLA restrictions was sufficient to match a diverse national pediatric population.
- Durable LMP-TC responses were observed in ND pediatric patients with PTLD after incomplete response to rituximab.

Posttransplant lymphoproliferative disease (PTLD) in pediatric solid organ transplant (SOT) recipients is characterized by uncontrolled proliferation of Epstein-Barr virus–infected (EBV+) B cells due to decreased immune function. This study evaluated the feasibility, safety, clinical and immunobiological outcomes in pediatric SOT recipients with PTLD treated with rituximab and third-party latent membrane protein–specific T cells (LMP-TCs). Newly diagnosed (ND) patients without complete response to rituximab and all patients with relapsed/refractory (R/R) disease received LMP-TCs. Suitable LMP-TC products were available for all eligible subjects. Thirteen of 15 patients who received LMP-TCs were treated within the prescribed 14-day time frame. LMP-TC therapy was generally well tolerated. Notable adverse events included 3 episodes of rejection in cardiac transplant recipients during LMP-TC therapy attributed to subtherapeutic immunosuppression and 1 episode of grade 3 cytokine release syndrome. Clinical outcomes were associated with disease severity. Overall response rate (ORR) after LMP-TC cycle 1 was 70% (7/10) for the ND cohort and 20% (1/5) for the R/R cohort. For all cohorts combined, the best ORR for LMP-TC cycles 1 and 2 was 53% and the 2-year overall survival was 70.7%. vβ T-cell receptor sequencing showed persistence of adoptively transferred third-party LMP-TCs for up to 8 months in the ND cohort. This study establishes the feasibility of administering novel T-cell therapies in a cooperative group clinical trial and demonstrates the potential for positive outcomes without chemotherapy for ND patients with PTLD. This trial was registered at www.clinicaltrials.gov as #NCT02900976 and at the Children’s Oncology Group as ANHL1522.
Introduction

Posttransplant lymphoproliferative disease (PTLD) is a major complication after pediatric solid organ transplantation (SOT). \(^1,2\) The risk of PTLD varies based on the organ transplanted, the immunosuppressive regimen required to prevent graft rejection, and the recipient’s Epstein-Barr virus (EBV) status at the time of transplant. Incidences range from 1% to 5% in renal transplant recipients to 15% to 25% in intestinal and lung transplant recipients. \(^3\) The majority of pediatric post-SOT PTLD cases are EBV<sup>+</sup> CD20<sup>+</sup> B-cell proliferations. \(^1\)

EBV is a ubiquitous gamma herpes DNA virus with lifelong persistence in humans. \(^2\) Although 80% to 90% of the US adult population is EBV<sup>+</sup>, many pediatric recipients of SOT are still EBV-naïve at time of transplant and seroconvert within the first 2 years of transplant. \(^3\) The highest risk for EBV<sup>+</sup> PTLD exists in patients with seronegativity who receive a graft from a seropositive donor. \(^3\)

In pediatric recipients of SOT with impaired immune function, EBV<sup>+</sup> PTLD expresses multiple viral antigens associated with latency stage III, including Epstein-Barr nuclear antigen (EBNA) 1, 2, and 3 as well as LMP1 and 2. This renders PTLD a highly immunogenic disease that can be readily targeted by adoptive T-cell therapies (TCs), especially in the post–hematopoietic stem cell transplant setting using donor-derived EBV TCs. \(^4-17\) In the SOT setting, donor-derived EBV TC products are usually not an option because PTLD is typically of recipient origin, and graft donors (usually cadaveric) are not generally available, and even if available, donors are mostly not HLA matched to recipients.

The goal of this study was to evaluate the use of third-party anti-EBV TCs from volunteer donors in pediatric recipients of SOT to demonstrate the feasibility of administering an “off-the-shelf” cell therapy product across multiple institutions in a cooperative clinical trial group setting.

Methods

Patients

ANHL1522 (NCT02900976) was a pilot study of pediatric recipients of SOT with EBV<sup>+</sup> CD20<sup>+</sup> PTLD. Eligibility criteria included age <30 years at enrollment, evaluable and biopsy-proven newly diagnosed (ND), relapsed or refractory (R/R), polymorphic or monomorphic PTLD that is CD20<sup>+</sup> and EBV<sup>+</sup>. Patients had to be unresponsive to or ineligible for reduction of immune suppression. The study was originally designed for ND patients. An expansion to allow eligibility for a R/R cohort defined as patients having a first or later relapse for which no treatment had been initiated or patients with refractory disease after having received at least 3 doses rituximab without complete response (CR) prior study enrollment. Ineligible patients included those with prior EBV-specific TC within 90 days, patients with central nervous system and/or bone marrow involvement >25% and/or fulminant PTLD, and patients with Burkitt morphology (Supplemental Methods).

ANHL1522 was approved by the National Cancer Institute and by institutional review boards at Children’s Oncology Group (COG) member institutions before patient enrollment. Informed consent was obtained from parents or guardians according to the Department of Health and Human Services guidelines.

Treatment

All patients in the ND cohort and R/R cohort received a 3-week induction cycle consisting of 3 weekly doses of rituximab at 375 mg/m<sup>2</sup>. At the end of induction, patients underwent disease evaluation by computer tomography and or positron emission tomography using the International Pediatric Non-Hodgkin Lymphoma Response criteria. \(^18\) Patients in ND cohort who achieved a CR were assigned to receive an additional 3 weekly doses of rituximab at 375 mg/m<sup>2</sup> (arm RTX). Patients in ND cohort with partial response (PR), stable disease (SD), or progressive disease (PD) and all patients in R/R cohort were assigned to receive latent membrane protein–specific T cells (LMP-TCs; LMP-TC arm) after rituximab induction. R/R cohort-refractory patients were assigned to arm LMP-TC at study enrollment and did not receive rituximab induction. (Figure 1)

Arm LMP-TC consisted of a 42-day cycle of LMP-TC at a dose of 2x10<sup>7</sup> cells per m<sup>2</sup> administered on days 0 and 7 with a potential second cycle based on the response. Responses were assessed at the end of each cycle by imaging as mentioned earlier.

Third-party LMP-TC cell bank

LMP-TC products were produced from eligible healthy donors with serologically positive EBV status (as previously described) \(^19,20\) (Figure 2; supplemental Methods). Release criteria for administering the LMP-TC product to patients included viability >70%, negative culture for bacteria after 14 days and fungi after 21 days, endotoxin testing ≤5 Endotoxin (EU)/mL, negative result for Mycoplasma, <2% CD19<sup>+</sup> B cells, <2% CD14<sup>+</sup> monocytes, and HLA identity. LMP-TC products were cryopreserved and stored in the third-party virus–specific T-cell bank at Children’s National Hospital. LMP-TC products were further characterized for polyclonality, polyfunctionality, and epitope specificity (Supplemental Methods).

Correlative biology

Measurement of EBV viremia by EBV polymerase chain reaction and EBV immunity by ELISpot assays. Blood samples were prospectively collected on days 1 and 21 of each rituximab cycle and days 1, 14, and 41 of each LMP-TC cycle as well as month 1, 2, 3, 6, 9, and 12 months after study completion to evaluate for EBV viremia in the whole blood and for EBV-specific T-cell responses using interferon gamma enzyme-linked immuno-spot (ELISpot; Millipore, Burlington, MA; supplemental Methods).

T-cell receptor sequencing. Immunosequencing of the CDR3 regions of human T-cell receptor (TCR) Vβ chains was performed to select third-party LMP-TC products and patient peripheral blood mononuclear cells (before infusion and 3 follow-up time points) using the immunoSEQ Assay (Adaptive Biotechnologies, Seattle, WA) as previously described. \(^21\) EBV-specific T-cell clones were identified using VDJdb, a database of T-cell receptor sequences with known antigen specificity (VDJdb). \(^22\) Total EBV-related sequences as well as sequences unique to the LMP-TC product were quantified before infusion and at follow-up time points. Shannon entropy was calculated for each TCRVβ repertoire to evaluate diversity using the R package Vegan. The amino acid CDR3 sequences are coded to numerical input, and diversity score is calculated and tracked over multiple time points (supplemental Methods).
Statistical analysis

The primary end point of the study was the percentage of patients assigned to arm LMP-TC who (1) had a suitable HLA-compatible third party LMP-TC product available, (2) were treated with LMP-TC product within 14 days of study enrollment for patients with refractory disease or call back for all other patients, and (3) received both weekly doses of LMP-TCs. Secondary end points included estimation of progression-free survival, event-free survival (EFS), overall survival (OS), response rate (RR), and toxicity rates in the study population and validation that absence of EBV viremia correlates with RR, EFS, and OS. (supplemental Methods).

Methods of analysis. For the primary end point, the point estimate of the proportion of successfully matched and treated was the uniformly minimum variance unbiased estimator, and an exact 1-sided binomial 95% confidence interval (CI) was used to get a lower bound for the actual rate of successful treatments as defined in the primary objective.23

The proportion of eligible patients who were successfully matched was assessed using the uniformly minimum variance unbiased estimator for the proportion and an exact one-sided binomial 95% CI (adjusted for the interim analysis by the method of Jung and Kim) to get a lower bound for the actual rate.23 EFS and OS were assessed using Kaplan-Meier estimates, both for all patients combined and also separately in each cohort. Toxicities were graded according to Common Terminology Criteria of Adverse Events (CTCAE) v5.0 and described using descriptive statistics.

Results

LMP-TC bank

A total of 14 LMP-TC products were fully characterized and available for use in a third party bank for this protocol. The products were polyclonal and comprised predominantly of CD3+CD8+ T cells (supplemental Figure 1A). Products demonstrated specificity to LMP1 and LMP2, with higher rates of specificity to LMP2 (supplemental Figure 1B).

Clinical characteristics

Eighteen patients with PTLD (12 who were ND patients, 1 patient who relapsed, and 5 patients who were refractory) were enrolled from 13 Children’s Oncology Group FACT-accredited bone marrow transplant (BMT) sites between March 2017 and October 2020. Ages ranged from 1 to 21 years (median, 9.5 years;
There were 10 males and 7 females in a racial and ethnically diverse population (44.4% white; 27.8% African American; 22.2% Hispanic; and 5.6% Asian). Most patients (77.8%) developed PTLD within 2 years of SOT (range, 3.5-120 months). Most patients (77.8%) had monomorphic PTLD, whereas 16.7% had polymorphic PTLD, and 11.1% had monomorphic and polymorphic PTLD. Five of 6 patients with R/R disease had monomorphic PTLD, and 1 had monomorphic and polymorphic disease. Fourteen patients received calcineurin inhibitors (tacrolimus or cyclosporine); 3 patients received mammalian target of rapamycin (mTOR) inhibitors, and 1 patient received mercaptopurine monotherapy.

**LMP-TC product matching, delivery, and infusion**

Of 18 patients, 15 patients were assigned to the LMP-TC1 arm, and all had suitable LMP-TC products available. Products were shipped to the local site for infusion with a mean time of 8.5 days of call back for ND patients or enrollment for patients who were refractory. Thirteen of 15 patients (86.7%; 95% CI, 59.5-98.3) received both doses of LMP-TC within the prescribed time frame and were considered successes for the primary aim of this study. However, the study was closed to enrollment because of slow accrual despite extending eligibility to patients with R/R disease. Thus, the primary aim was not completed.

Two patients did not receive both doses of LMP-TC within the prescribed time frame. One patient developed an infection delaying the first infusion. The second patient experienced grade 3 cytokine release syndrome (CRS) and hypoxia after the first T-cell infusion and stopped protocol therapy before receiving the second dose of LMP-TCs.

Ten distinct products were infused to 15 patients. One patient who had >1 product available and received 2 cycles of LMP-TC switched to a different product during the LMP-TC2 cycle, receiving 2 distinct LMP-TC products. The 10 LMP-TC products used for this protocol had 11 distinct HLA restrictions or alleles through which antiviral activity was detected (supplemental Table 2). The most common allele match was at HLA A2 (4 products), followed by HLA C7 (2 products; (supplemental Table 3). Twelve patients had products selected with matched HLA class 1 alleles, through which antiviral activity was detected, and the remaining 3 were matched with products that had nonspecific HLA class 2 restrictions.

**Treatment responses**

**Rituximab induction.** Twelve patients in the ND cohort and 1 patient in the R/R cohort received induction therapy. After rituximab induction, there were 2 CRs, 5 PRs, and 5 SDs in the ND cohort. The 2 patients in the ND cohort who achieved a CR after rituximab...
induction alone were treated with additional rituximab monotherapy. The 1 patient in the R/R cohort achieved a PR after rituximab induction but was taken off protocol therapy because of relocation (Figure 1). The overall response rate (ORR) to rituximab induction alone were treated with additional rituximab monotherapy. The 1 patient in the R/R cohort achieved a PR after rituximab induction alone were treated with additional rituximab mono-

Table 1. Summary of patient data, CNI, and mTORi

<table>
<thead>
<tr>
<th>Age range</th>
<th>1-21 y (median, 9.5 y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (55.6)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (44.4)</td>
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<tr>
<td>Race/ethnicity, n (%)</td>
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</tr>
<tr>
<td>White</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>African American</td>
<td>5 (27.8)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Aillograft, n (%)</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>12 (66.7)</td>
</tr>
<tr>
<td>Liver</td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>Kidney</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Heart/kidney</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Immunosuppression, n (%)</td>
<td></td>
</tr>
<tr>
<td>CNI</td>
<td>13 (72.2)</td>
</tr>
<tr>
<td>mTORi</td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Time from transplant to protocol enrollment, n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 24 mo</td>
<td>14 (77.8)</td>
</tr>
<tr>
<td>&gt;24 mo</td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>Disease status</td>
<td></td>
</tr>
<tr>
<td>ND</td>
<td>12 (66.7)</td>
</tr>
<tr>
<td>Relapsed</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Refractory</td>
<td>5 (27.8)</td>
</tr>
<tr>
<td>Pathology, n (%)</td>
<td></td>
</tr>
<tr>
<td>Polymorphic</td>
<td>3 (15.7)</td>
</tr>
<tr>
<td>Monomorphic</td>
<td>14 (73.7)</td>
</tr>
<tr>
<td>Both polymorphic and monomorphic features</td>
<td>2 (10.5)</td>
</tr>
<tr>
<td>CNI, calcineurin inhibitor; mTORi, mTOR inhibitor.</td>
<td></td>
</tr>
</tbody>
</table>

The ORR after LMP-TC1 for the ND cohort was, therefore, 70% (Figure 3A).

Of the 5 patients with R/R PTLD, 1 patient (20%) had SD and received LMP-TC2. The remaining 4 (80%) experienced disease progression during LMP-TC1 and were taken off protocol therapy. The ORR after LMP-TC1 for the R/R cohort was therefore 20% (Figure 3B), and the study-wide ORR was 53%.

Four patients (3 in ND cohort and 1 in R/R cohort) received a second cycle of LMP-TC. Two patients in the ND cohort were evaluable after LMP-TC2: 1 achieved PR and 1 maintained SD. The third patient in the ND cohort developed graft rejection at the beginning of the cycle and was taken off protocol therapy without response evaluation. The patient in the R/R cohort maintained SD through the end of LMP-TC2. The best ORR for the entire study population across both cycles, therefore, was 53%.

**Responses and LMP-TC products.** There was no correlation between outcome and degree of HLA matching of the LMP-TC product and the recipient, nor was there any correlation between outcome and the degree of HLA matching of alleles with known EBV-specific activity. In the 4 patients in the ND cohort with a sustained CR after LMP-TC1, HLA matches ranged from 2 to 5 of 10 alleles (supplemental Table 3). Two of the 4 patients had 1 shared allele with confirmed EBV restriction (A4 and A5), 1 patient had 2 shared alleles with known EBV restriction (A3), and 1 patient was matched at 3 alleles with the T-cell product but had no known antiviral activity via the shared alleles (A11). In patients with PD, HLA matching ranged from 2 to 6 of 10, and all patients had at least 1 shared allele with known EBV restriction.

**Survival outcomes.** EFS was defined as the time to the first occurrence of relapse, second/secondary malignant neoplasm, or death. There were 7 events, all occurring within 1 year of enrollment (Figure 3A). Six events occurred during LMP-TC1, as described earlier (2 in ND cohort and 4 in R/R cohort). An additional patient from the ND cohort who achieved a PR after LMP-TC2 developed progressive PTLD 3 months after the completion of protocol therapy.

The overall 2-year EFS was 60.6% (95% CI, 34.6-79) for the entire cohort. In contrast, the 2-year EFS was 74.1% (95% CI, 39.1-90.9) for patients in the ND cohort compared with only 33.3% (95% CI, 4.6-67.6) for patients in the R/R cohort (Figure 3C). The 2-year OS was 70.7% (95% CI, 36.2-88.9) for the entire cohort, 91.7% (95% CI, 53.9-98.8) for ND patients, and 83.3% (95% CI, 27.3-97.5) for patients with refractory disease (Figure 3D). There were 5 reported patient death (A5, A9, C1, C2, and C4; supplemental Table 1); 4 occurred during follow-up and 1 during LMP-TC1 course (C4). All patients had relapsed before death. Four deaths were due to disease progression (A5, C1, C2, and C4), and 1 death in follow-up was due to unrelated causes (A9; Figure 3A-B). All deaths were deemed unrelated to protocol therapy.

**Toxicity.** There were no acute infusion toxicities associated with protocol therapy. Grade 1 or 2 fever possibly related to LMP-TCs was 27.3-97.5) for patients with refractory disease (Figure 3D). There were 5 reported patient death (A5, A9, C1, C2, and C4; supplemental Table 1); 4 occurred during follow-up and 1 during LMP-TC1 course (C4). All patients had relapsed before death. Four deaths were due to disease progression (A5, C1, C2, and C4), and 1 death in follow-up was due to unrelated causes (A9; Figure 3A-B). All deaths were deemed unrelated to protocol therapy.

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Rejection. Three graft rejection episodes in cardiac transplant recipients treated on arm LMP-TC were reported on protocol therapy. Two episodes occurred in patients in the ND cohort during LMP-TC1 and 1 in a patient in the R/R cohort during LMP-TC2. All 3 patients had undetectable or subtherapeutic levels of immunosuppression at the time of rejection. EBV immunity as measured by ELISpot was evaluated in these 3 patients and was considered stable or waning at the time of rejection (Figure 4). Hence, these rejection events were considered unlikely related to an increase in EBV-specific immunity and, therefore, unlikely secondary to LMP-TC treatment but likely related to subtherapeutic levels of immune suppression in all 3 patients. In all 3 patients, the rejection episode resolved with increased immunosuppression, and there was no allograft loss on protocol therapy. All patients were taken off protocol therapy and did not receive LMP-TCs any further.

CRS. Two patients experienced self-resolved grade 1 CRS (CTCAE v5) during LMP-TC. One patient experienced grade 3 CRS after the first infusion of LMP-TCs requiring tocilizumab and was taken off protocol therapy before receiving the second infusion. Within a week, this patient was also diagnosed with disease progression and died of disease, suggesting that the entire episode represented fulminant PTLD.

Clearance of EBV viremia and augmented EBV-specific immunity after EBV-specific LMP-TC

Patients with CR and PR after LMP-TC1 demonstrated EBV-specific immunity while maintaining an undetectable EBV viral load 3 months after infusion (Figure 5). Patients with PD demonstrated waning EBV-specific immunity but had received further therapy (chemotherapy and chimeric antigen receptor T-cell therapy) in the interim, which likely affected the EBV-specific T-cell immune response.
Four patients had a preinfusion and 3 postinfusion peripheral blood samples evaluated for T-cell receptor (TCRβ) sequencing. Of these 4 patients, 2 achieved a CR (A3 and A4), 1 achieved a PR (A7), and 1 had PD (C2). All patients had evidence of EBV-specific TCRβ sequences present before LMP-TC infusion, with the most prevalent observed in the patients A3 and A4, both with CR (171 and 1460 sequences compared with 19 sequences in the patient with PD) (supplemental Table 5). Patients with CR/PR had persistence of EBV-related sequences at all follow-up time points, with a maximum of 1206 sequences at 8 months. However, because few patient samples were evaluated, these observations remain descriptive.

EBV-related sequences that were unique to the product but not present in the patient’s peripheral blood before infusion were also tracked and compared in the 4 patients before vs after LMP-TC. The 2 patients who achieved a CR (A3 and A4) had increased numbers of unique EBV-related TCRβ sequences tracked over time (maximum 46 sequences at 8 months after infusion). The patient with PD (C2) had no detectable EBV-specific sequences unique to the product, and there was a lack of persistence of preinfusion EBV-related TCR sequences. Moreover, TCR repertoire diversity as determined by Shannon entropy index showed increased diversity in the 3 patients with clinical responses (CR/PR), compared with the patient with PD (Figure 6).

Discussion

ANHL1522 demonstrated that delivery of “off-the-shelf” cellular therapy is feasible in a multi-institution cooperative group setting. An LMP-TC bank of 14 products was sufficient to identify a suitable match for all patients referred irrespective of racial and ethnic backgrounds in a very diverse patient population. Overall, ND patients (n = 12) had a favorable outcome with an EFS of 74.1% at 2 years. RRs to LMP-TCs in patients with high refractory SOT with PTLD treated in the R/R cohort were markedly lower (EFS, 33.3%), with all relapses occurring during therapy. There were no immediate infusion-associated severe adverse events and only 1 episode of grade 3 CRS that correlated with the timing of disease progression in a patient with refractory disease. Although there were 3 episodes of graft rejection that were attributed to inadequate immunosuppression, there was no graft rejection episode that was attributed to LMP-TC, and no patient lost their transplanted organ.

The first reported use of third-party EBV virus–specific T-cell products (EBVSTs) was published by the Crawford group, with 70 distinct products covering all common HLA types in the United Kingdom. That bank was sufficient to treat 33 adult and pediatric patients (31 patients after SOT) with EBVST. The degree of HLA antigen matching varied from 2 to 5 of 6 HLA antigens. The Baylor group established a multivirus-specific T-cell bank with 32 products and used 18 products in a 50 patient study including 9 patients treated for EBV-PTLD after BMT. HLA antigen matches ranged from 1 to 4 of 6 HLA antigens. The group from Memorial Sloan Kettering Cancer Center (MSKCC) established a large third party EBVST bank with 330 products used to treat 50 pediatric and adult patients (including 13 patients after SOT). Based on the HLA restrictions in their bank and the prevalence of HLA antigens in >400 ethnically diverse patients referred for BMT in New York City, the group estimated that an EBVST bank with 40 distinct HLA restriction would be sufficient to cover >95% of the ethnically diverse New York City population.

This study is unique because it was restricted to pediatric patients with PTLD after SOT and included predominantly ND patients. Moreover, we demonstrated that a bank comprising only 14 products was sufficient to successfully match all referred patients with a readily available LMP-TC product. Having “off-the-shelf” products available with known HLA type, EBV specificity, and HLA restriction allowed for quick identification, shipping, and treatment of patients at their local site. All patients had a product available for infusion at their local site within 2 weeks of submission of call back.

Although donor lymphocyte infusions have been associated with graft-versus-host disease in BMT recipients, third-party EBV-specific T-cell products have been well tolerated in multiple studies of (predominantly adult) patients with R/R PTLD after BMT or SOT. To our knowledge, this is the first study in a large
Figure 5.
Figure 5. Persistence of EBV-specific immune response. (A) Persistence of EBV-specific immune responses of responders (blue) as compared with those of nonresponders (red) in new diagnosis stratum is evident on day 90 (A). Patients with CR on day 41 demonstrated EBV-specific immune response while maintaining negative EBV viral load through 3 months after infusion (B). Patients with PR maintained EBV-specific immune response (C). Patients with PD had declining EBV-specific immune response; both patients received further therapy after day 41 evaluation with PD (D).

Figure 6. TCR sequences unique to LMP-TC product expanded after infusion and were maintained in responders. Patient A3, with CR and with TCR sequences unique to product persistent at 11 months (A); patient A4, CR with TCR sequences until 8 months (B); patient A7, PR with sequences present until 17 months (C); patient C2, with PD, had declining unique TCR sequences at second follow-up time point, and patient received further therapy with CAR-T. After CAR-T, evidence of expansion of novel TCR sequences were again evident (D). Diversity of TCR clones present in LMP-TC products (E). CAR-T, chimeric antigen receptor T-cell therapy.
cooperative group setting and provides real-world insights, as highlighted by a diverse approach, across centers, to immune suppression management in SOT recipients receiving T-cell therapies. Three episodes of graft rejection suggested the possible attribution to the T-cell therapy. However, further investigation showed absent and waning EBV-specific T-cell immunity at the time of rejection as well as suboptimal immunosuppression, which likely contributed to rejection. Therefore, the protocol was subsequently amended to specify the requirement for therapeutic immunosuppression. No graft loss or deaths due to protocol therapy were reported. Therefore, in future multicenter protocols, close attention must be given to the management of immunosuppression to provide standardized practices to reduce the risk of graft rejection while preserving adequate function of the LMP-TC products in vivo.

This study included ND and R/R polymorphic or monomorphic PTLD in SOT recipients. Results in ND patients treated with a response adapted approach to immunotherapy are encouraging with an EFS of 74.1% at 2 years, comparable with outcomes using low-dose cyclophosphamide, prednisone, and rituximab (ANHL0221; EFS, 71% at 2 years) and the German Ped-PTLD study (EFS, 67% at 2 years).13,34 Waiting confirmation in larger study cohorts, rituximab with third-party LMP-TCs may be a promising approach to avoid exposure to chemotherapy in ND patients with PTLD after SOT. However, LMP-TC therapy alone may not be adequate in a highly pretreated refractory pediatric SOT patient populations. Other groups have reported 45% to 50% RRs in patients with refractory (predominantly adult) mixed BMT/SOT in single institution settings, which may be related to differences in type of transplant, patient selection, age, ethnicity, and immune suppressive regimens used.8,35

Although this study demonstrated the feasibility of administering a third-party T-cell therapy in a cooperative group setting, it also demonstrated some challenges. The protocol was closed early because of lagging patient enrollment. Possible reasons included reduced incidence of PTLD with the use of tailored immunosuppression and early use of rituximab as well as this being the first cellular therapy protocol at a time when many centers had not yet the experience or infrastructure to open the protocol.

ND patients who achieved a CR or PR after LMP-TC therapy demonstrated persistence of EBV-specific immunity while maintaining undetectable EBV viral loads. Hence, having an undetectable viral load with persistent EBV-specific immune responses after the rituximab and LMP-TC immunotherapy regimen may help predict sustained response to this therapy. Further investigations gaining insights on how to promote such sustained responses need to be explored to further enhance the utility of this therapeutic strategy. In contrast, evidence of EBV-specific immunity was not sufficient for response in patients with R/R disease.

TCRvβ sequencing of products and patient follow-up peripheral blood samples demonstrated a higher number of preinfusion EBV-related T-cell receptor sequences as well as greater numbers of TCRvβ sequences unique to the product, which persisted for up to 17 months after LMP-TC infusion. This degree of T-cell persistence, as detected using TCRvβ sequencing, is dramatically longer than the 3- to 4-month maximal persistence reported previously when third-party EBV-specific T-cell products were administered in the post-SOT setting.36 Because the LMP-TC were not gene marked, it is unclear whether the persistence of these T-cell clones beyond 3 to 4 months were all derived from the infused product. Hence, we posit that (1) LMP-TCs may augment the patient’s own endogenous EBV immune response and (2) the presence of even low levels of EBV-specific T cells in the peripheral blood before infusion may enhance the response to LMP-TC treatment. Therefore, the expansion and long-term detection of EBV-related TCR sequences suggests that LMP-TC treatment may boost EBV-specific immunity, including endogenous immunity, in vivo.

In summary, administration of cell-based therapies in a cooperative setting is feasible, and the combination of rituximab and LMP-TCs shows promising results in ND patients receiving SOT with PTLD. Patients with responses to LMP-TCs showed persistence of EBV immunity and persistence of the infused third-party donor-derived LMP-TC product up to 17 months after infusion with expansion of unique product-specific EBV-related TCR sequences. Furthermore, increased TCR diversity was observed in those with clinical responses (CR/PR) compared with in those with PD. Although this was a small study, these promising results suggest that a chemotherapy-free immunotherapy-based approach warrants further evaluation in larger patient populations with PTLD after SOT.

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Authorship

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Conflict-of-interest disclosure: C.M.B. is a co-founder and scientific advisory board member of Mana Therapeutics and Catalaman Bio; board member of Cabaletta Bio; has stock in NexeImmune and Repertoire Immune Medicines; serves as a data safety monitoring board member for SOBI; and has served on advisory boards for Pfizer, Bristol Myers Squibb, and Roche. C.E.A. serves on advisory boards for SOBI and OPNA, not relevant to this study; and receives study support from Genentech/Roche who manufacture rituximab. P.H. is a cofounder and on the board of directors of Mana Therapeutics; and serves on the advisory boards of Celleleve, Cellexos, Discovery Life Sciences, Capsida, and MicroFluidX. The remaining authors declare no competing financial interests.

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