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CTLA4Ig-based reduced intensity conditioning and donor lymphocyte infusions for haploidentical transplantation in refractory aggressive B-cell lymphoma relapsing after an autograft: Early results from a pilot study

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CTLA4Ig-primed donor lymphocyte infusions (DLIs) have been found to promote natural killer (NK) cell-mediated anti-leukemia effect following haploidentical hematopoietic cell transplantation (HCT). Incorporation of CTLA4Ig in conditioning aided long-term remission in myeloma probably by blocking the CD28−CD86 pro-survival pathway when combined with CTLA4Ig-primed DLI. We explored a similar approach in 12 patients (8−65 years) who had refractory aggressive B-cell lymphoma (R-ABCL) following autologous HCT. They received CTLA4Ig-based reduced-intensity conditioning and sequential CTLA4Ig-primed DLIs on days +7, +21, and +35. None developed acute graft-versus-host disease (GVHD). Two patients developed chronic GVHD. Only 3 patients had disease-progression at 100 days posttransplant with a progression-free and GVHD-free survival at 2 years of 75%. A higher expression of CD80 in tumor cells and a greater proliferation of CD56dim CD16+ NK cells were observed at days +30 and +60 in patients with progression-free survival. We hypothesize that CTLA4Ig, with a greater avidity for CD80, probably interferes with the anti-apoptotic effect mediated through this pathway, and together with early proliferation of mature NK cell when used in conjunction with DLI, this approach might provide a curative option for patients with R-ABCL.

The outcome of patients with refractory aggressive B-cell lymphoma (R-ABCL), namely, diffuse large B-cell lymphoma (DLBCL), primary mediastinal (thymic) large B-cell lymphoma (PMBCL) and mantle cell lymphoma (MCL) with a high proliferative index, who relapse after an autologous (auto) hematopoietic cell transplantation (HCT), is extremely poor, even with an allogeneic HCT. The key factors influencing the outcome in relapsed ABCL are sensitivity to chemotherapy and time to relapse following an auto-HCT [1]. In several recent studies, haploidentical HCT with posttransplantation cyclophosphamide (PTCy) has been reported to offer a viable option for patients with relapsed lymphoma [2]. However, the outcome of chemorefractory ABCL remains extremely poor, irrespective of the intensity of conditioning and donor source [3]. Targeting specific B-cell signaling pathways, such as Bruton tyrosine kinase, phosphoinositide 3-kinase, and the Src kinase family, holds promise for the future [4]. The introduction of autologous CAR-T cell therapy for R-ABCL has yielded encouraging results with a progression-free survival (PFS) of 37% at 2 years [5]. Autologous natural killer (NK) cell therapy has also shown promising results

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in R-ABCL, although the durability of the response has been a major stumbling block [6].

Our group had explored a novel approach to adoptive immunotherapy with CTLA4Ig-primed donor lymphocyte infusions (DLIs) following haploidentical HCT in patients with advanced leukemia [7]. This resulted in early and rapid proliferation of mature NK cells, which correlated with PFS. In this study we explored the feasibility and efficacy of this approach in patients with R-ABCL experiencing early progression following auto-HCT. In addition, CTLA4Ig was introduced in the conditioning regimen based on preclinical evidence of its anti-lymphoma effect and its successful application in refractory myeloma [8].

Methods

Patients less than 65 years of age with R-ABCL were enrolled in this study between January 2015 and May 2018, if they possessed a suitable haploidentical family donor and were not deemed fit for myeloablative conditioning. Approval was obtained from the institutional review board of the DNHRC, and informed consents were obtained from all patients and donors in accordance with Declaration of Helsinki.

Treatment protocol

The reduced-intensity conditioning (RIC) regimen consisted of fludarabine 30 mg/m^2/day × 5 days, cyclophosphamide 15 mg/m^2 × 2 days and 3 Gy of total-body irradiation. CTLA4Ig (Abatacept, Orencia, BMS India, Mumbai, India) was administered at 10 mg/kg on days −15, −8, −1, +7, +21, and + 35 (Figure 1). The graft source was mobilized peripheral blood stem cells. A DLI at 1 × 10^6/kg was administered 12 hours following CTLA4Ig on day +7 and at 5 × 10^6/kg on days +21 and +35 in the absence of graft-versus-host disease (GVHD) [7,8]. PTCy was administered on days +3 and +4 at 50 mg/kg, followed by cyclosporine with a trough level of 75–150 ng/mL tapered between days +60 and +90. Use of granulocyte colony-stimulating factor (G-CSF) was avoided in the first 6 weeks after HCT.

Disease evaluation

All patients were evaluated with routine histology and necessary immunohistochemical staining on the biopsied tissue specimen, ^{18}F-labeled fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography–computed tomography (PET-CT), and bone marrow aspiration and biopsy at the first presentation, at first relapse, at relapse post-autograft, and immediately prior to and 3 months after haploidentical HCT.

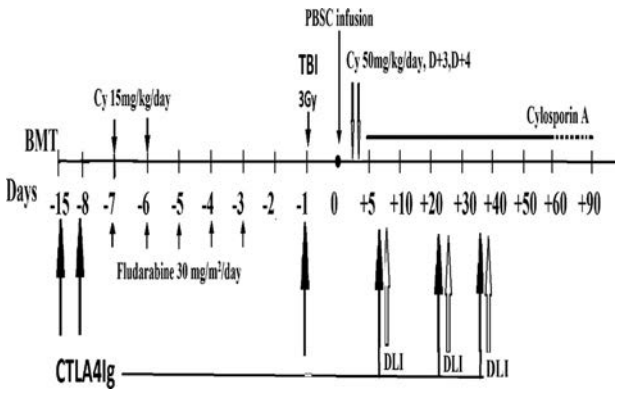

Figure 1. Conditioning protocol for haploidentical HCT in refractory aggressive B-cell lymphoma employing CTLA4Ig-based reduced-intensity conditioning, PTCy, and sequential CTLA4Ig-DLI.

Flow cytometry for immune reconstitution

Flow cytometric assessment of T- and NK-cell subsets was carried out on donor leukapheresis products and peripheral blood samples of patients at days +30, +60, and +90 following the HCT, as described earlier [7,9–11].

The details regarding HLA typing, NK-cell ligand and genotyping, donor selection criteria, and supportive care strategies have been described previously [7,12].

Immunohistochemical staining for expression of CD80 and CD86

Study the correlation, if any, of the expression of CD80 and CD86 on tumor cells with outcome, immunohistochemistry (IHC) was carried out on all the biopsy samples of all patients taken before HCT and in those available at diagnosis. Tissue sections 3.0 μm thick were cut from Formalin-fixed and paraffin-embedded blocks and mounted on slides coated with poly-L-lysine solution. Sections were baked for 30 min at 60°C–62°C to melt the paraffin wax. The slides were then transferred to the fully automated IHC/ISH stainer (Roche Ventana Benchmark–XT, Tucson, AZ) [1,2]. for further processing. Incubation with primary antibodies against CD80—(Rabbit Monoclonal Antibody clone EPR1157 ab134120) and CD86 (Mouse Monoclonal Antibody clone BU63 ab213044) (Abcam, Cambridge, UK) was done for 16 min for CD80 and for 20 min for CD86, respectively, at 37°C. Tissue sections of human reactive lymph nodes were taken as positive controls for expression of CD80 and CD86 and run along with the study cases on the same slides. Negative tissue controls were stained by excluding the primary antibody step.

The presence of brown granular cytoplasmic staining was taken as positive and further categorized as weak, moderate, or strong intensity; the extent of staining was assessed as follows: 0 = non-immunoreactive; + = immunoreactive in 1%–25% of cells, ++ = immunoreactive in 26%–49% of cells; +++ = immunoreactive in 50%–74% of cells; and ++++ = immunoreactive in ≥75%–100% of cells.

Statistics

The primary endpoints of the study were grade 2–4 acute GVHD, nonrelapse mortality (NRM), and disease progression (DP) at 6 and 12 months. The secondary endpoints were chronic GVHD, progression-free and GVHD-free survival at 24 months, and immune reconstitution at days +30, +60, and +90. Binary and continuous variables were analyzed with χ^2 and nonparametric tests. Probabilities of survival were estimated using the Kaplan–Meier product-limit method. An outcome was determined to be significantly different if the observed p value was <0.05. All analyses were performed using the statistical software SPSS Statistics Version 21 (IBM, Armonk, NY).

Table 1. Characteristics of patients with high grade B-cell lymphoma and disease progression post-autologous HCT

							Graft composition		Engraftment (days)				
UPN	Age/ gender	Diagnosis	CD80/CD86 expression	Treatment sequence before HCT/DS at HCT	Interval from auto-HCT (months)	Donor age/ gender	CD34/ TNC $\times 10^6$/kg	CD3 $\times 10^7$/kg	Neutro-phils	Platelets	Acute GVHD/ chronic GVHD	Response at day +100	Outcome
1	19/F	PMBCL BM−	++++/+	CT × 4, RT auto-HCT/Ref	5	33/F	9.00/1287	16.5	12	11	No/yes day +186	CMR	PFS day +1738
2	29/M	DLBCL Non-GCB, CMYC +, BM+	+++/+	CT × 3, RT Auto-HCT/ Ref	4	47/F	7.35/885	16.70	15	14	No/no	CMR BM-MRD negative	PFS day +1475
3	32/M	PMBCL BM−	++++/++	CT × 3, Auto-HCT, RT/ Ref	3	59/F	9.20/1069	10.10	16	14	No/no	CMR	PFS day +1428
4	18/M	DLBCL Non-GCB DE; BM+	++++/+	CT × 3, RT Auto-HCT/ Ref	6	21/M	9.60/987	19.43	13	12	No/no	CMR BM-MRD negative	PFS day +1320
5	30/M	PMBCL BM−	++++/++	CT × 4, RT Auto-HCT/ Ref	8	56/M	8.60/1073	16.45	12	21	No/no	CMR	PFS day +765
6	28/M	DLBCL Non-GCB BM−	++/++	CT × 3, RT Auto-HCT/ Ref	11	56/F	8.35/2207	1.43	18	12	No/no	PD	PD, died day +183
7	65/M	DLBCL Non-GCB BM−	+/+++	CT × 3, RT Auto-HCT CT × 2/ Ref	5	28/M	6.20/615	29.70	15	16	No/no	PD	PD, died day +192
8	16/M	DLBCL Non-GCB CMYC +; BM +	++++/++	CT × 3, RT Auto-HCT/ Ref	4	40/M	7.50/1015	26.00	17	15	No/no	CMR BM-MRD negative	PFS day +455
9	35/M	DLBCL Non-GCB BM−	+/+++	CT × 4, RT Auto-HCT CT x2/ Ref	2	46/M	8.70/1130	9.02	13	13	No/no	PD	PD, alive day +265, lost to follow-up
10	8/M	DLBCL Non-GCB DE; BM+	++++/++	CT × 2, RT Auto-HCT CT x 1/ Ref	6	32/M	6.2/1555	19.8	15	14	No/yes day +270	CMR BM-MRD negative	PFS day +485
11	14/M	DLBCL Non-GCB DE; BM+	++++/+	CT × 3, RT Auto-HCT CT × 1/Ref	9	16/M	7.60/1468	27.00	17	17	No/no	CMR BM-MRD negative	PFS day +385
12	52/F	MCL-Pleomorphic BM+	++++/+	CT × 5, RT Auto-HCT Ibrutinib/ Ref	4	18/M	7.50/714	19.6	13	15	No/No	CMR BM-MRD negative	PFS day +375

Auto=autologous; BM=bone marrow; CMR=complete metabolic remission; CT=chemotherapy; DE=double expressor phenotype; DLBCL=diffuse large B-cell lymphoma; Non-GCB=nongerminal center B-cell-like; GVHD=graft-versus-host disease; MCL=mantle cell lymphoma; PMBCL=primary mediastinal (thymic) large B-cell lymphoma; MRD=minimal residual disease; PD=progressive disease; PFS=progression-free survival; Ref=Refractory.

Results

Patient and donor characteristics

Twelve patients aged 8 to 65 years (median: 29 years) underwent haploidentical HCT on the current protocol for R-ABCL (DLBCL—8 [double expresser cMYC and BCL2: 3, cMYC: 2, Non-Germinal Centre: 3]; PMBCL: 3; and MCL, pleomorphic variant: 1). The disease characteristics are detailed in Table 1. The median International Prognostic Index score was 3 (range: 2–4). All were chemorefractory at the time of HCT, having failed multiple lines of chemotherapy, radiotherapy, and autologous HCT (Table 1). The median number of lines of chemotherapy excluding autologous HCT, prior to haploidentical HCT, was 3 (range: 3–5). All patients received at least one cycle of further salvage chemotherapy with or without radiotherapy and were found to have persistent or progressive disease on PET-CT to be classified as "refractory." The median Karnofsky performance score was 70 (range: 50–90) at the time of HCT.

The median donor age was 37 years (range: 16–59). Only 2 donors were NK alloreactive in the GVH direction, and 7 had the KIR B haplotype. All patients received peripheral blood stem cell (PBSC) grafts. The graft composition in terms of CD34 and CD3 cells is detailed in Table 1. The median number of CD56+CD3− cells in the graft was 16.9×10^6/kg (range: 3.4–44.4).

CTLA4Ig-DLI

All patients received the three scheduled doses of CTLA4Ig-DLI as planned. There were no infusion-related toxic effects. The median dose of CD56+CD3− cells in the DLI was 1.12×10^6/kg (range: 0.16–2.4).

Acute and chronic GVHD

All patients achieved neutrophil engraftment and platelet engraftment at medians of 15 days (range: 12–18) and 14 days (11–21), respectively. None developed grade 2–4 acute GVHD. Two patients (unique patient numbers [UPNs] 1 and 10) developed moderate chronic GVHD of the skin and oral mucosa, which responded to corticosteroids and sirolimus (Figure 2).

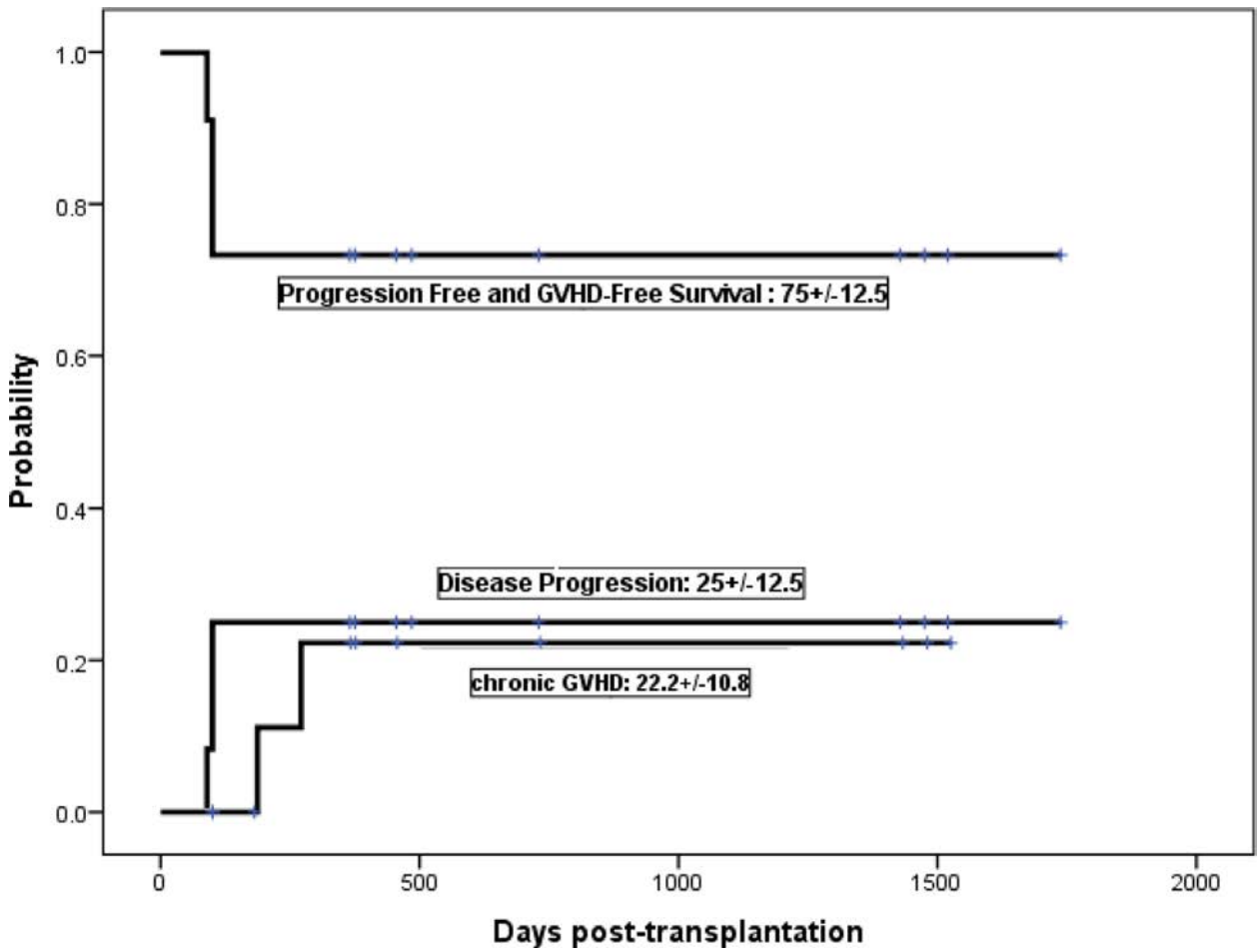

Figure 2. Cumulative incidence of progression-free and GVHD-free survival. Disease progression and chronic GVHD in patients undergoing CTLA4Ig-haploidentical HCT for refractory aggressive B-cell lymphoma.

Disease response and survival

Three patients (UPNs 6, 7, and 9) had disease progression as evidenced by clinical, imaging, and histological assessment at 100 days. Two succumbed to progressive disease, and another showed partial response to DLI and was alive until day +265, when he was lost to follow-up. The rest were disease free at 100 days and all subsequent assessments. At a median follow-up of 765 days (range: 375–1738), 9 patients were alive and free of disease. The actuarial progression-free and GVHD-free survival at 2 years was 75% (95% confidence interval [CI]: 62.5–77.5) (Figure 2). There were no NRMs in this cohort. Four patients had cytomegalovirus reactivation and two patients had late viral pneumonia at 9 and 11 months, respectively. All recovered with treatment.

Immune reconstitution and disease progression

These data are detailed in Supplementary Table E1 (online only, available at www.exphem.org). The median CD4+T-cell count at day 90 was 263 cells/μL (range: 96–760). There was no difference in the T-cell subsets or absolute CD56+CD3− cells at the various time points based on disease progression. However, the recovery of CD56dim CD16+ cells was significantly higher in those without disease progression at day +30 (129 ± 83 μL vs. 17 ± 12 cells/μL, p = 0.004), as well as day +60 (144 ± 80 μL vs. 17 ± 12 cells/μL, p = 0.001) (Figure 3). There was no correlation between the infused NK-cell dose in the DLI and disease progression.

Expression of CD80 and CD86 in the tumor cells

In all the biopsies taken at disease progression before the haploidentical HCT, expression of CD80 the malignant cells was much higher compared with that of CD86. A sequential and comparative analysis in 7 patients revealed an increase in the expression of CD80 at relapse compared with that at the time of diagnosis. It was also noted that the expression of CD80 was higher (+++ to ++++) (Figure 4A) in the tumor cells of those without disease progression after haploidentical HCT than in those with progression (+ to ++) (Figure 4C, Table 1). The corresponding PET scans of two representative patients are provided in Figure 4B and D to illustrate the refractoriness of the disease and its subsequent response in the former and disease progression in the latter.

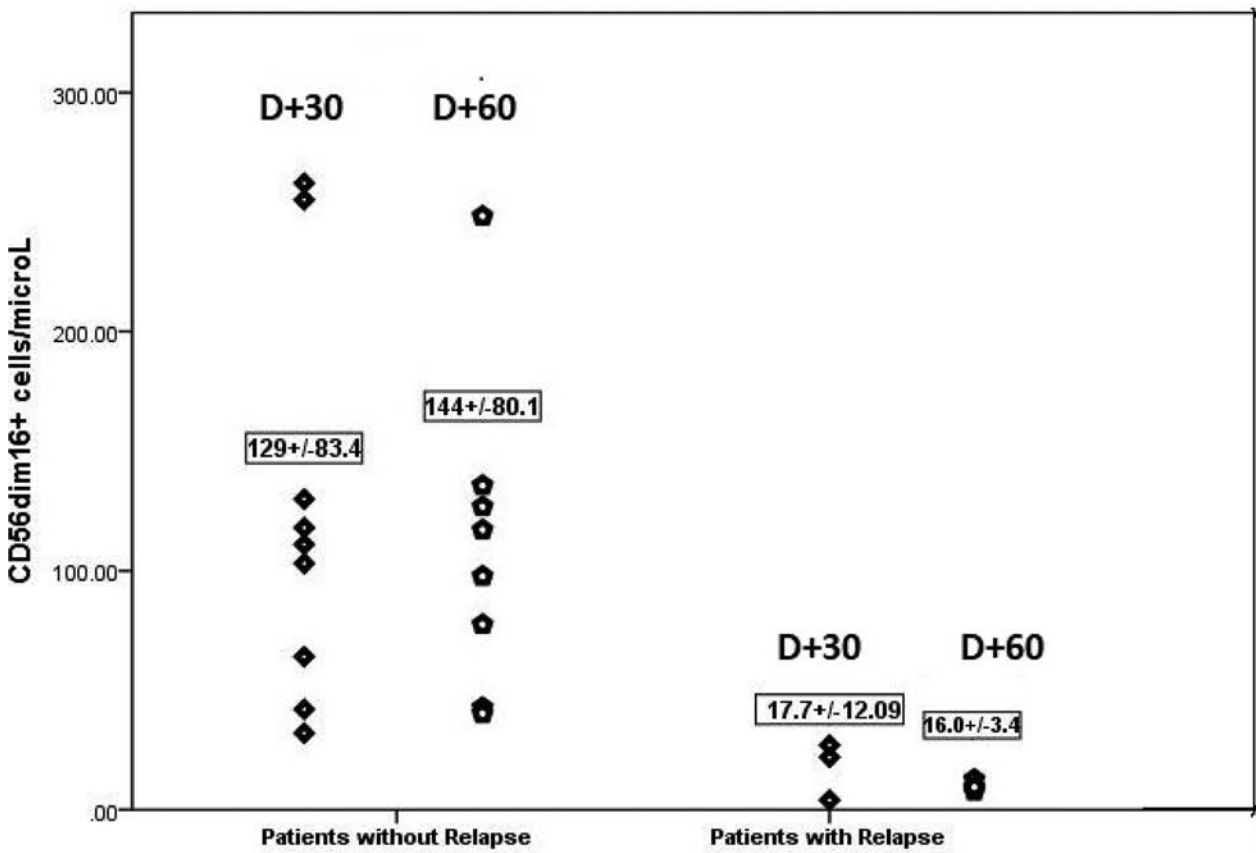

Figure 3. Dotplots revealing recovery of $CD56^{dim}CD16^{+}$ cells (expressed as cells per microliter) at days +30 and +60 in patients undergoing CTLA4Ig-haploidentical HCT for refractory aggressive B-cell lymphoma in relation to disease progression.

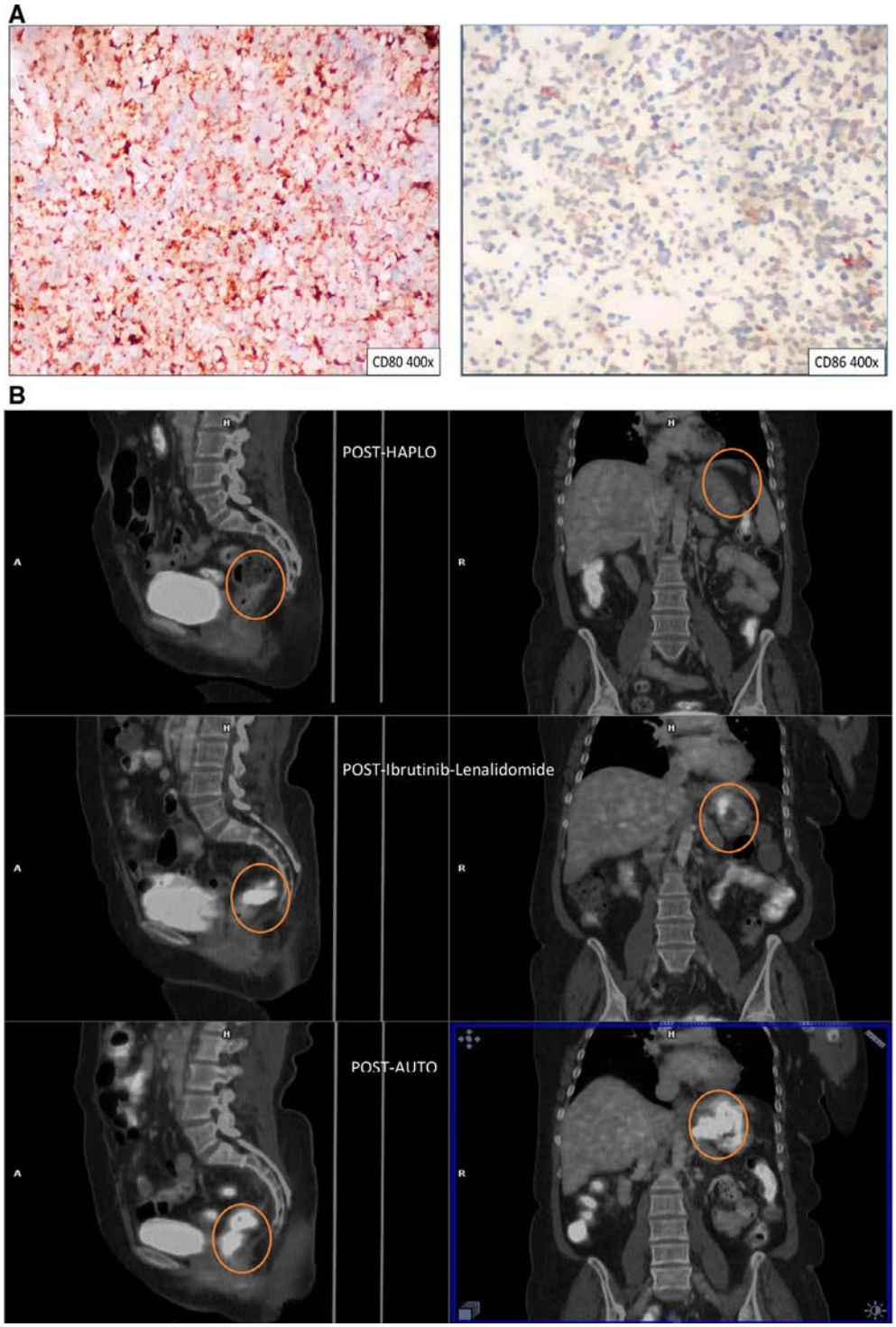

Figure 4. IHC staining for CD80 and CD86 on biopsy samples and corresponding responses on PET-CT in two illustrative cases. **(A)** Strong granular CD80 expression (++++) (left) and patchy moderate granular expression of CD86 (+) (right) in UPN 12, who had refractory pleomorphic MCL. **(B)** Sequential PET-CT in UPN 12. Bottom: Active disease with high FGD uptake in rectosigmoid area and stomach after autograft. Middle: Persistent disease after treatment with Ibrutinib and lenalidomide (Deauville scores 4 and 5). Top: Response on day +100 post-haploidentical HCT with complete anatomical and metabolic response. The areas of active disease are marked in the figure. **(C)** Less intense and patchy granular CD80 expression (+) on the left and a stronger more diffuse granular expression of CD86 (+++) on the right in UPN 9, who had refractory DLBCL, non-GCB type. **(D)** Sequential PET-CT in UPN 9. Active disease with high FDG uptake after autograft and salvage chemotherapy (bottom) persisted after haploidentical HCT at day +100 (top), along with the appearance of new lesions in the upper lobe of the right lung (Deauville score 5). The areas of active disease are marked in the figure.

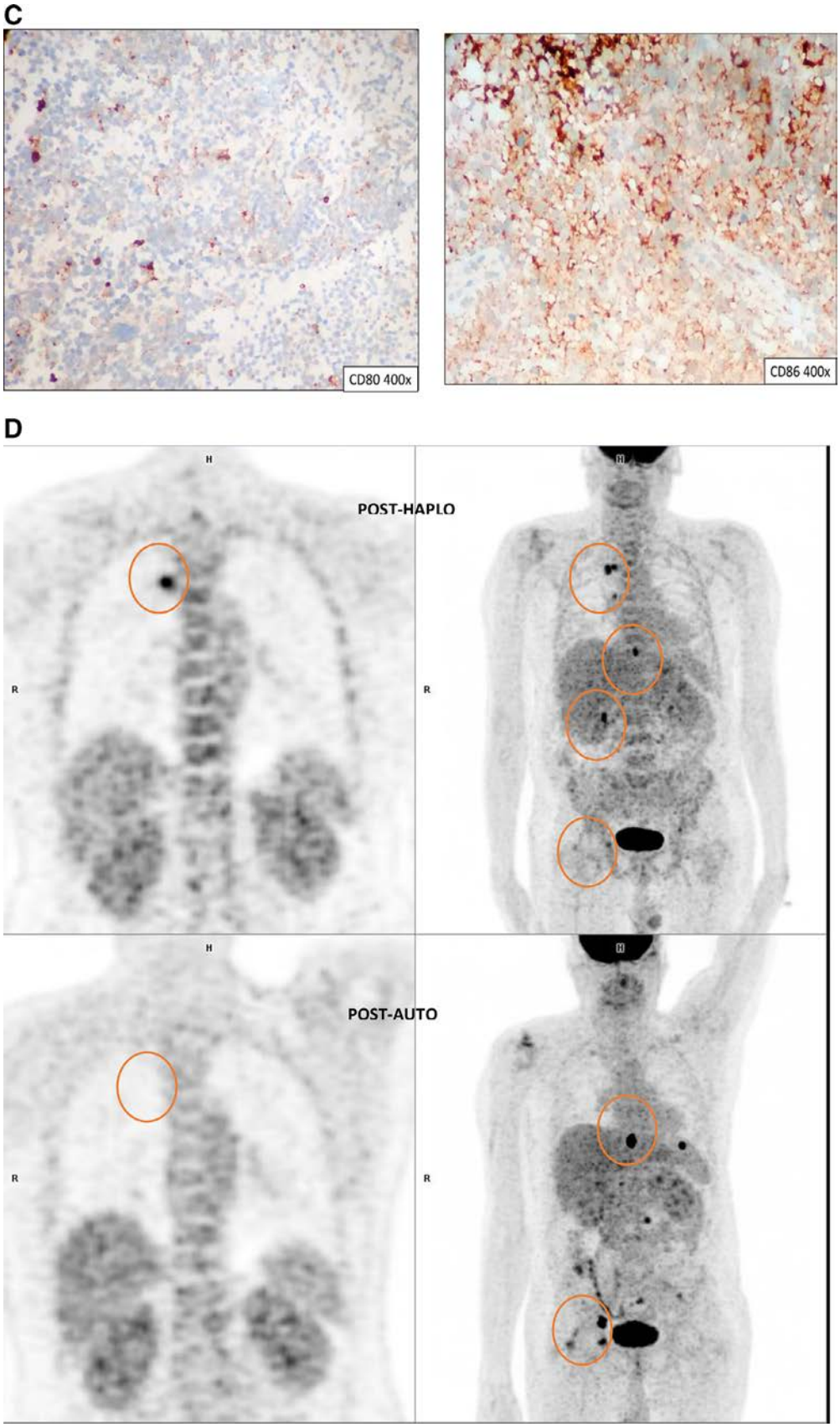

Figure 4. Continued.

Discussion

The outcome of allogeneic HCT in relapsed R-ABCL has been marked by high rates of both NRM and relapse [1,13]. In an analysis of 503 patients with DLBCL who underwent an allogeneic HCT after relapse following an auto-HCT, NRM was 30% and the PFS was 31% [1]. A scoring system based on Karnofsky score, time to relapse, and chemosensitivity revealed that in those with chemorefractory disease relapsing within a year of an auto-HCT, the PFS was 6%–11% [1]. In another study, irrespective of treatment modalities, the median survival for patients relapsing after an autograft in the rituximab era was 8.2 months [14]. Several retrospective registry-based studies on the impact of conditioning intensity observed a NRM rate in excess of 50% with MAC, with a PFS and overall survival (OS) of 18%–25%, irrespective of the conditioning regimen [13,15].

Haploidentical HCT has been increasingly used for lymphoma as evidenced by recent registry-based reports [16]. In one such study, the outcomes following PTCy-based haploidentical HCT and matched sibling donors (MSDs) were similar in patients with a heterogenous group of lymphomas [2]. In an extended study on 1438 patients, PTCy-based haploidentical HCT yielded results equivalent to those for MSD and unrelated donors [3]. However, the outcome of chemorefractory patients remained grim, irrespective of the donor source. Thus, patients with chemorefractory disease, particularly those relapsing after an auto-HCT, have a dismal outcome irrespective of conditioning regimen, donor source, and GVHD prophylaxis.

Conventional approaches to allogeneic HCT are unlikely to improve the outcome in this group of patients. The encouraging results of CTLA4Ig-primed DLI following haploidentical HCT in patients with relapsed/refractory leukemia [7] and myeloma [8] prompted our group to explore this approach in patients with R-ABCL as well. In those with leukemia treated on this protocol, myeloablative conditioning with fludarabine, busulfan, and melphalan was adjudged to be an integral component of the protocol. Similar conditioning in patients relapsing early after an auto-HCT and several lines of prior chemoradiotherapy was not deemed to be feasible. At the same time, an adoptive immunotherapy-based approach dependent on the NK cell-mediated anti-tumor effect would require moderation of the tumor burden for optimization of its efficacy.

We had employed CTLA4Ig in a non-myeloablative conditioning regimen for refractory myeloma after failed auto-HCT based on the concept that the CD28–CD86 pathway is a critical prosurvival pathway for myeloma that can be effectively blocked by CTLA4Ig [8]. The preliminary and yet impressive results of this study led us to explore if CTLA4Ig would have a similar salutary effect on apoptotic pathways in B-cell lymphoma as well. Our primary analysis of 10 patients with newly diagnosed DLBCL and MCL confirmed the fact that there is high expression of CD80 and CD86 on the tumor cells. In a model of mouse AIDS induced in B57BL/6 mice by infection with a replication-defective strain of retrovirus (DU5H) characterized by rapid and uncontrolled lymphoproliferative disease (LPD), the effects of treatment with CTLA4Ig were analyzed [17]. CTLA4Ig-treated mice were shown to have a delayed onset of LPD, and the rate of proliferation was slower than that in untreated animals. Moreover, the loss of in vitro response to mitogens was also reduced. Further data supporting an anti-lymphoma effect of CTLA4Ig emerge from a study by Orbach et al. in which Raji cell lines incubated with CTLA4Ig resulted in inhibition of its growth along with downregulation of anti-apoptotic signals such as cFLIP and increase in pro-apoptotic signals via upregulation of caspase 3 and 9 [18]. The differential effects of CD80 and CD86 were demonstrated in this study with signaling via CD80-inhibited proliferation of the lymphoma cells, but not with CD86. Although some studies have suggested that both CD80- and CD86-mediated signaling induce apoptosis of lymphoblastoid cell lines [19], others have suggested a pro-apoptotic role for CD80 signaling and the reverse for CD86 [20]. CTLA4Ig, having a higher affinity for CD80, probably renders an anti-lymphoma effect by both promoting the pro-apoptotic pathways and inhibiting the anti-apoptotic ones.

Our interim results from the pilot study exploring this approach on the initial cohort of patients with R-ABCL yielded extremely encouraging results with a PFS of 75% with no NRM or acute GVHD. These results are similar to those obtained in larger cohorts of advanced leukemia with CTLA4Ig-DLI. To understand the biology of this response, we investigated the immune recovery as well as expression of CD80 on the tumor cells. Despite the small numbers, the correlation of these findings with the outcome is worth further consideration. A higher expression of CD80 was evident in all the patient samples at relapse, and interestingly, those who failed to show a response had a lower expression of CD80 than the responders. The non-myeloablative conditioning employed in this protocol was unlikely to have any significant anti-lymphoma effect in chemorefractory patients. Incorporation of weekly CTLA4Ig prior to, during, and after conditioning might have had an effect in augmenting the apoptotic pathways as described above. In this regard, expression of CD80 on the tumor cells might be a surrogate for response when CTLA4Ig-based conditioning is employed. However, a functional correlation as demonstrated in in vitro models remains to be investigated in vivo.

CTLA4Ig-DLI exploits the unique property of CTLA4Ig where costimulatory blockade of T-cell activation is accompanied by augmentation of NK-cell proliferation and cytotoxicity [7,21]. Similar to the cohort of acute leukemia [7], we did find a parallel with the recovery of CD56dim NK

cells and disease response, where a rapid surge in this population was associated with long-term PFS and vice versa. We did not find any correlation between the NK-cell dose of the DLI and NK-cell recovery or disease progression. As of now, it remains unclear as to why a small subgroup of patients fail to show early recovery of mature NK cells. In fact, these were the same patients who had lower CD80 expression in the tumor cells. Although this might be a chance occurrence, one might speculate that binding of CTLA4Ig to CD80 receptors on tumor cells to induce apoptosis engages NK cells via the CD86, which is a putative activating receptor mediating anti-tumor cytotoxicity [7,21].

The other aspect of this protocol that warrants consideration is avoidance of postgrafting G-CSF, which has been routinely advocated as a part of PTCy-based HCT. Although the popular concept behind the use of G-CSF has been to hasten neutrophil recovery, this might be counterproductive when a protocol is aimed at NK cell-mediated anti-tumor effect. In vitro studies have demonstrated that G-CSF might reduce the cytotoxic potential of NK cells in terms of expression of activating receptors as well as release of perforin [22]. There was significant augmentation of anti-tumor activity of NK cells in anti-G-CSF-treated mice as well [23]. The detrimental effect of G-CSF in the context of T cell-depleted haploidentical HCT was demonstrated by the Perugia group [24]. Avoidance of GCSF postgrafting was associated with improved functional recovery of T cells. Likewise, absence of G-CSF in our protocol was not associated with delay or failure of engraftment and might have been responsible for hastened NK-cell maturation as well. High CD34 expression and total nucleated cell counts in the graft could have compensated for the lack of G-CSF. Furthermore, the use of sequential CTLA4Ig might have mitigated any deleterious effect in terms of GVHD, which has been associated with higher CD34 and total nucleated cells in the graft [25].

Conclusions

Our pilot study investigated a novel approach to achieve PFS in R-ABCL in the context of haploidentical HCT, employing the probable dual effect of CTLA4Ig on inducing apoptosis of lymphoma cells via direct signaling through the CD80 pathway and furthering NK-cell cytotoxicity without T-cell activation, when used with DLI. These early findings suggest the possible existence of a biological correlate to the clinical response and are speculative and not confirmatory. This report is aimed at instigating further exploration of this approach to further decipher basic biology behind its efficacy as well as clinical validation in a larger cohort of such patients who otherwise have a dismal outcome.

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Author Contributions

SC and SRJ conceptualized, designed, and executed the study. HMA and GR carried out the studies on the biopsy samples. AG and SC analyzed the PET-CT results. SRJ, SC and HMA collected and analyzed the data. SRJ and SC wrote the article. All authors read and approved the final article.

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Supplementary Table E1. Immune Reconstitution At Days +30 +60 And +90 Post-Transplantation

Cells/μl (Median, range)	**(N=12)**
D+30	
CD3+	233 (41-3631)
CD4+	45 (21-222)
CD8+	113 (10-3317)
CD56dimCD16+	88.5 (4-267)
CD56brightCD16-	64 (21-692)
Tregs	4.7 (1.58-12.10)
D+60	
CD3+	1018 (195-2851)
CD4+	125 (85-444)
CD8+	547.5 (103-2430)
CD56dimCD16+	107 (14-306)
CD56brightCD16-	30 (11-163)
Tregs	5.2 (1.75-7.53)
D+90	
CD3+	868 (23-2297)
CD4+	263 (96-760)
CD8+	522 (156-1929)
CD56dimCD16+	85 (11-303)
CD56brightCD16-	25 (6-197)
Tregs	5.2 (2.5-12.6)