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Cryopreserved Cd34 Cells: Measuring Viability Pre-Thaw and Viability over Time Post-Thaw

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Common practices associated with the use of cryopreserved hematopoietic progenitor cells (HPC) include measuring the viability of the thawed cells from a sample derived from the thawed product, and thawing cells at the patient's bedside out of concern for possible toxicity of DMSO in the post-thaw liquid cell suspension. The former practice carries the risk of discovering excess cell death when it is too late to correct, and the concomitant risk of non-engraftment. The latter practice carries all of the risks associated with manipulating cells in a non-controlled environment including risk of contamination and lack of suitable containment in the event of failure of a cryostorage container. In an effort to reduce risks associated with cell cryostorage, we are currently examining the validity of measuring CD34 viability from cells frozen in retention vials concomitantly with product freezing. We are also examining the stability of HPC being held in cryostorage medium from 1 to 24 hours post thaw. We present here our results from a pilot study performed on six bags of cells, previously identified for discard due to death of the intended recipients. Six bags of HPC and associated retention vials, frozen for 5 to 12 years, were selected at random from the pool of discarded products and de-identified per guidelines for discard of medical waste. CD34+ cells were identified using a modification of the ISHAGE method, and dead cells were identified by infiltration with the fluorochrome dye 7-AAD. CD34+ cells stored in retention vials were 94±11 percent viable, and CD34+ cells stored in bags were 96±7 percent viable. A Wilcoxan matched pairs test yielded p=0.6, indicating that these results were not different. Percent viable CD34+ cell recovery, relative to the known number of CD34+ cells per mL originally frozen, was determined immediately following thawing, and following 1, 2, 4 and 24 hours remining in thawed cryostorage medium, with recoveries (mean ± std. dev.) of 88 ± 31, 76 ± 33, 62 ± 28, 88 ± 41, and 49 ± 26 percent respectively. These preliminary results suggest that viable CD34 percent as measured from a retention vial may serve as a predictor of viability at thawing of it's associated product, and also that HPC held in cryostorage medium post thaw retain viability for at least four hours post thawing. Based on these results we are continuing these studies.

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Cytopenias after Chimeric Antigen Receptor T-Cells (CAR-T) Infusion; Patterns and Outcomes

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Patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) have poor treatment outcomes when treated with traditional chemoimmunotherapy. Superior outcomes have been observed with the 2 CAR-T cell products approved by the US FDA; tisagenlecleucel (CTL109) and axicabtagene ciloleucel (Axi-cel) with 12 months overall survival rates of 49% and 59% respectively. The 2 major acute toxicities of these therapies are cytokine release syndrome (CRS) and neurotoxicity. The incidence of grade 3 or higher neutropenia, anemia, and thrombocytopenia was 78%, 43%, and 38% in patients treated with axi-cel. Little is known regarding the patterns and outcomes of these cytopenias. Here we present our preliminary experience on the patterns and outcomes of cytopenias in 32 patients who received commercially-available CAR-T cell therapy at our institution.

We retrospectively reviewed all DLBCL patients receiving either CTL109 or axi-cel between January and September 2018 at our institution. All patients received a conditioning regimen of low-dose cyclophosphamide and fludarabine, followed by CAR-T infusion. Persistent cytopenias were defined as incomplete absolute neutrophil count ($<1 \times 10^9$/L), Hb (<12 g/dL) or platelet count ($<100 \times 10^9$/L) recovery after day 28 of CAR-T cell infusion. Treatment response was assessed with PET-CT imaging and classified according to International Working Group Criteria.

The median age of patients was 59 years (range, 23-80); 63% were male, 4 patients (13%) received CTL109 and 28 (87%) received axi-cel. Twenty-two patients (69%) had a 3 month follow up imaging for assessment of response, of whom 7 patients (32%) achieved complete response (CR), 6 patients (27%) achieved partial response (PR), and 9 patients (41%) had progressive disease (PD). Persistent neutropenia was observed in 3 patients (9%), while persistent thrombocytopenia was seen in 21 cases (65%) and persistent anemia was the most common with a frequency of 72% (23/32 cases). Median time to neutrophil, platelet and Hb recoveries were 11 days (range, 5-218 days), 59.5 days (range, 4-241 days), and 76 days (range, 0-218 days), respectively. Filgrastim was used in 15 (47%) patients to facilitate neutrophil recovery. Twenty-three patients (72%) experienced neurotoxicity, of whom 11 (34%) had grade 3-4 toxicity. The frequency of CRS was 94% (29 out of 32 patients), with grade 3-4 CRS observed in 4 cases (12.5%). We observed no significant association between CRS and persistent cytopenias.

In our preliminary series of 32 patients, persistent anemia and thrombocytopenia were more common than neutropenia after CAR-T cell therapy. However, most of these patients required growth factor support for neutrophil recovery. Longer duration of follow up is necessary to determine the impact of persistent cytopenias on outcomes and survival after CAR-T cell therapy.

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Donor Lymphocyte Infusion from G-CSF-Primed, Unmanipulated Whole Blood Is Safe and Improves Chimerism in HLA-Matched and Haploidentical Transplantation

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Introduction: Donor lymphocyte infusion (DLI) is a useful therapy for mixed chimerism (MC) and measurable residual disease (MRD) after allogeneic transplantation (HSCT). Multiple DLI products can be obtained through apheresis and cryopreservation, but their costs can be prohibitive in developing countries. T-cells in whole blood units can reach $\geq 1 \times 10^6$/kg in healthy donors and are increased after G-CSF exposure. The ideal CD3+ cell dose remains undefined.
Objectives: We aim to assess the safety and efficacy of G-CSF-primed whole blood units (WB-DLI) in peripheral blood HLA-matched sibling (MSD) and haploidentical (Haplo) transplant recipients.
Methods: Adults who received WB-DLI at any time after HSCT due to relapse, MRD, MC or poor graft function were included. Patients with active graft-versus-host disease (GVHD) were excluded. Donors received filgrastim 5 μg/kg QD (days 1-3) followed by a 450 mL whole blood draw on day 4 with crossmatching and lymphocyte quantification. For patients with major/bidirectional ABO mismatch without group switch or a mixed field reaction, a reactive major crossmatch was an absolute contraindication. WB-DLI was infused fresh and unmanipulated in an outpatient basis. Concurrent chemotherapy was allowed. Disease and chimerism were assessed on day 30 and 60, respectively. GVHD prophylaxis included oral cyclosporine/tacrolimus tapered in 60 days.
Results: Fourteen patients received a single WB-DLI, median age was 32 years (range, 16-67), 50% were women. Most common diagnoses were ALL, (n=5) and AML (n=5), followed by MDS (n=2), NHL (n=1) and CML (n=1). Nine had were Haplo grafts, while 4 were MSD. Indication for WB-DLI was relapse in 6 patients, 3 had MRD, 4 MC and 1 had poor graft function. Median chimerism pre-WB-DLI was 72% (range, 38-100%). Median mononuclear cell count obtained was $32 \times 10x^6$/kg (range, 9-74), while median CD3+ cell count was 6.7×10^6/Kg (range, 5.2-19). No immediate severe adverse effects were observed. Febrile non-hemolytic reaction occurred in n=4. No complications were observed in n=4 ABO mismatch cases. Overall 50% responded; chimerism improved in 50%, with a median increase of 28% (range, 9-53%). Median post-DLI chimerism was 92.5% (range, 20-100). Regarding patients treated for relapse or MRD 4/9 responded (33%). Overall 8/14 patients relapsed (57%) with 12-month progression free survival of 27.5% which was significantly lower in relapsed/MRD patients (log rank test p=0.042). Overall survival at 12 months was estimated at 61.2%. Three developed aGVHD, 2 grade III/IV in a median of 13 days (range, 7-38). Four died due to relapse. Median follow-up was 5 months (0.6-20.1 months). The cost of performing WB-DLI was $350 USD per unit.
Conclusions: DLI obtained from GCSF-primed whole-blood is safe and affordable. It improves mixed chimerism, while it is less effective for overt relapse, similarly to DLI obtained through standard methods.

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Effect of Stem Cell Boost and Donor Lymphocyte Infusion on the Incidence of Graft-Versus-Host Disease at Emory University Hospital

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Introduction: Allogeneic hematopoietic stem cell transplantation (SCT) is a widely used to treat refractory and relapsed hematological malignancies. Additional CD34+ selected cells, or stem cell boosts (SCB), and CD3+ T cells, or donor lymphocyte infusions (DLI) are often administered to treat poor hematopoietic graft function or relapse of the underlying malignancy, respectively, following SCT. Although the T cell dose in SCB and DLI vary by more than 3 logs, graft-versus-host disease (GVHD) can be a major cause of morbidity and mortality in patients who receive additional post-transplant donor T cells. The purpose of this study is to test the hypothesis that the incidence of GVHD after infusions of additional donor T cells is associated with the cumulative incidence of GVHD in patients who received DLI vs SCB.
Methods: A retrospective cohort study was conducted on consecutive patients at Emory University Hospital who received a DLI or CD34+ cell-selected SCB from January 2000 to September 2016. Excluding patients with incomplete records, 46 DLI and 15 SCB patients were analyzed for post-infusion GVHD and 2-year post-infusion survival. SPSS Statistics was used to conduct a binary logistic regression on DLI and SCB patients in order to determine significant variation in GVHD incidence per relevant covariates.
Results: The median doses of CD3+ T cells were $18 \times 10E6$ cells/kg and $0.054 \times 10E6$ cells/kg for DLI and SCB recipients, respectively. 73% of SCB patients received prophylactic immunosuppressive drug therapy versus 20% of DLI patients (Table 1). In spite of receiving less than 0.3% of the T cell dose of DLI recipients, SCB recipients had a 60% cumulative incidence of GVHD by 2-years of follow-up versus 37% in DLI patients (Figure 1; p =0.142). Binary regression models showed that GVHD incidence did not vary significantly with the dose of T cells and other covariates, except age in the DLI group (Tables 2 and 3). Overall survival at 2 years was 48% for

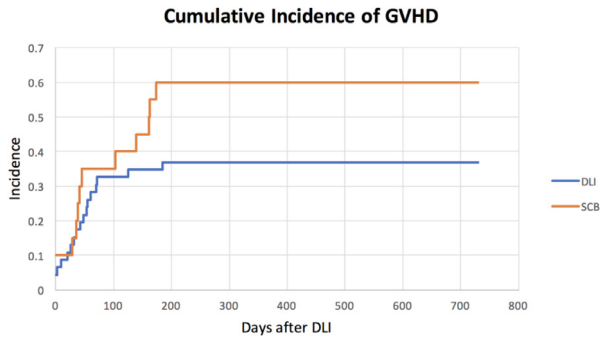

Figure 1. Cumulative Incidence of GVHD in DLI vs CD34+ selected SCB patients.