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PILOT STUDY OF PROPHYLACTIC DOSE-ESCALATION DONOR LYMPHOCYTE INFUSION AFTER T CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANT IN HIGH RISK PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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SCHEMA

High risk patients with hematologic malignancies undergoing T cell depleted Allo-SCT with matched related or unrelated donor consented prior to SCT

Collection of steady-state donor lymphocyte cells and cryopreservation in appropriate CD3 enumerated aliquots. Lymphocytes collected later or from mobilized product acceptable.

Collection of stem cells after G-CSF mobilization. BM collection is acceptable

Allo-SCT (T cell depleted regimens with Alemtuzumab or ATG). Preferably cyto-reduction before conditioning

Withdrawal of immunosuppression at day 60 if no more than Grade I aGVHD

Prophylactic DLI:
Requires no more than Grade I aGVHD and off immune suppression for each DLI. First dose ideally will be at day 75 to day 90 post-SCT, but delayed DLI administration is allowed as long as the subject meets pre-DLI criteria. Subsequent DLI will be given in 4-8 week intervals.

DLI doses by donor:
Matched Related Donor (MRD):
2×10^5/kg ; 5×10^5/kg ; 1×10^6/kg; 2×10^6/kg; 5×10^6/kg

Matched Unrelated Donor (MUD):
1×10^5/kg ; 2×10^5/kg ; 5×10^5/kg ; 1×10^6/kg; 2×10^6/kg

Primary objective: To determine the proportion of patients who receive at least one DLI.

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1. OBJECTIVES

Primary Objective

To determine the feasibility of EDR DLI as measured by the proportion of patients who receive at least one DLI

Secondary Objectives

To assess progression free survival (PFS) at 2 years after stem cell transplant (SCT) for high-risk hematologic malignancies receiving T-cell depleted grafts followed by escalating dose regimen (EDR) prophylactic DLI compared to historical controls not receiving DLI

To assess the safety of EDR DLI for high-risk hematologic malignancies as measured by cumulative incidence of severe grade III-IV acute GVHD

To measure outcomes of grade II-IV acute GVHD, non-relapse mortality, overall survival and chronic GVHD of EDR DLI

To assess the full donor chimerism rate in the CD3 compartment and immune reconstitution after EDR DLI

2. BACKGROUND

2.1 Growth in Stem Cell Transplant:

There has been tremendous progress in the past couple of decades in allogeneic hematopoietic stem cell transplantation (SCT) improving outcomes through incremental improvements. Further, access to SCT continues to expand because of larger number of stem cell donor options to include HLA-matched unrelated donors (MUD), haploidentical related donors, and cord blood units (CBUs), significant improvements in supportive care, and introduction of better tolerated reduced intensity conditioning (RIC) regimens (1-4).

2.1.1 T-cell depletion

T-cell depletion is another approach that enhances tolerability by reducing acute and chronic GVHD, complications which result in tremendous transplanted related morbidity and mortality. We have employed *in vivo* T-cell depletion with alemtuzumab for over 10 years at the University of Chicago and confirmed lower rates of acute and chronic GVHD and similar overall survival (5). Recent observational registry studies (6) and now even prospective studies have further established that T-cell depletion through either ATG or alemtuzumab enables better GVHD free survival (7). Although relapse in general remains highly problematic after transplant (8), particularly for those with active disease at SCT, T-cell depletion further increases rates of relapse. The relapse rate for patients with AML undergoing RIC allo-SCT is in the range of ~25% at one year without T cell depletion and in the range of 40-50% at 4 or 5 years with T cell depletion (4, 5, 9, 10). There has been very little progress in reducing the incidence of relapse following allogeneic SCT. Therefore, the platform of T-cell depletion enables patients to undergo transplant with low rates of GVHD but approaches to harness the immune system later after transplant once conditioning toxicities have resolved requires novel therapies.

2.1.2 Treatment of high-risk hematologic malignances:

Despite the high-rates of relapse if not increased treatment related mortality for patients entering transplant with poorly controlled hematologic malignancies, some of these patients will achieve long-term disease control if not cure after allogeneic SCT. The prognosis for such patients absent a transplant remains dismal although better disease control (e.g., lack of peripheral blood blasts, longer remission duration), a sibling donor, younger recipient age and non-adverse cytogenetics are favorable prognostic factors. Strategies using allo-SCT as a platform hold appeal to improve long-term outcomes. At the University of Chicago, we have developed an interest in improving outcomes for high-risk hematologic malignances. One approach we and other have tested with some success is further intensification using cyto-reductive chemotherapy immediately before reduced intensity conditioning incorporating T-cell depletion with allogeneic SCT Because of the low rate of GVHD from T-cell depletion, patients tolerate these regimens surprisingly well and even those entering transplant with active disease typically achieve remission (11). Although relapse appears to be delayed, most patients eventually develop disease recurrence. This protocol assesses maintenance on this established platform.

2.2 Treatment Options for relapsed AML after SCT:

A general tenant in hematologic malignancies and transplantation specifically is that prevention of relapse is more effective than delaying further treatment until the time of relapse. Strategies that show efficacy at relapse warrant testing as prophylaxis. The current options of management for disease relapse after allo-SCT include withdrawal of immune suppression, chemotherapy, second allogeneic transplant, cytokine and adoptive cell therapy and DLI. Withdrawal of immunosuppression can be performed in all patients irrespective of hematopoietic stem cell source. But the efficacy is very limited. While remission rates approaching 84% were observed in patients with chronic phase CML, they were only 10% for AML, and 0% for ALL and advanced phase CML (12) .

2.2.1 Chemotherapy: For relapsed acute leukemia, both conventional chemotherapy and newer biological agents are able to induce significant remission rates, but long-term survival is very rare. The use of TKI, including dasatinib and nilotinib in patients with Ph+ acute lymphoblastic leukemia (ALL) or 5-azacytidine for relapsed AML may have particular benefit. Sorafenib and other FLT3 inhibitors, such as AC220 have demonstrated preliminary activity in a small number of patients with relapsed FLT3+ AML (13).

In the AML setting, a retrospective analysis from the Fred Hutchinson Cancer Research Center (FHCRC) demonstrated that of those treated for relapse (which is a fraction of the actual number of patients relapsing) around 30% of relapsed AML after allo-SCT could enter CR after chemotherapy with cytarabine (with and without adriamycin), but the median DFS was very short at 9.7 months (14) and the response was highly influenced by time to relapse after allo-SCT. Specifically, 2-year survival estimates for patients relapsing less than 100 days, 100-200 days, and greater than 200 days from allo-SCT were 3%, 9%, and 19%, respectively. Other studies explored the use of DLI after chemotherapy for relapse AML following allo-SCT, and found 1-year survival probability of 10% (95%

confidence interval [CI] 3%-31%) if relapse occurred within 6 months of transplant versus 44% (95% CI 29%- 68%) if relapse occurred later (15, 16). These data led to the current practice standard to offer standard chemotherapy, with and without DLI, only in patients who relapse more than 3 to 6 months after allo-SCT, and refer other patients to clinical trials or palliative care if no trial available. Our own data showed similar results except almost all patients relapsing after T-cell depletion with alemtuzumab were able to receive treatment probably in part from low rates of GVHD and high-tolerability of the regimen (17). Again, this provides further support for the concept that T-cell depletion is a tolerable regimen and platform for subsequent therapy.

Given the relatively futile results of chemotherapy at relapse after allo-SCT, prophylactic chemotherapy after allo-SCT for high risk patients has been attempted, especially using less toxic hypomethylating agents. A phase 1 trial of azacitidine as post-transplant maintenance therapy conducted at the M.D. Anderson Cancer Center demonstrated that azacitidine could be given at 32 mg/m2/day for 5 consecutive days every 4 weeks for at least 4 cycles with limited side effects in 42 patients who underwent reduced-intensity allo-SCT for relapsed/refractory AML (18). Low-dose azacitidine was also used by the M.D. Anderson group to treat relapsed AML and MDS after allo-SCT, and around 20% long-term disease control rate was found in patients with early relapses (18). A German group also used low dose azacitidine followed with DLI in relapsed AML/MDS patients after Allo-SCT with low long-term response rate (19, 20).

2.2.2 Second allogeneic transplant: Patients who have failed an initial Allo-SCT have chemo-refractory disease, making additional chemoradiotherapy unlikely to be curative. Historically, the role of second allogeneic SCT has been limited by unacceptable relapse rates and high mortality rate, depending on previous therapies, age, and time from first transplantation. The efficacy of second allogeneic SCT depends on several factors, such as underlying malignancy, patient age and performance status, type of conditioning regimen employed, and time interval between first and second HSCT. In a large Center for International Blood and Marrow Transplant Research (CIBMTR) retrospective study of patients with hematologic malignancies undergoing second allogeneic HSCT, transplant-related mortality was 30% and the relapse rate was 42%, yielding an overall survival rate of 28% at 5 years post-SCT (21).

Although often suggested, there is no demonstrated benefit to using another donor when performing a second transplant after relapse. Generally, outcomes of second transplant are better for younger patients and for those with a longer time (6-12 months) from transplantation to relapse (i.e, remission duration after SCT). Data from the CIBMTR showed a 5-year survival rate of 51% in patients under age 20 years who relapsed more than 6 months after transplantation and only 3% in older patients who relapsed within 6 months after transplantation (21). The European Group for Blood and Marrow Transplantation (EBMT) reported the best outcomes for patients with late relapse (292 days) in remission at time of second transplantation, with 53% survival at 3 years (22). High-risk patients relapse earlier post-SCT which also translates into lower chances of success with post-SCT therapy.

2.2.3 Novel approaches with immune-therapies

Innovative and novel immunotherapeutic approaches are currently under investigation. Among other approaches, non-specific *ex vivo* activation and expansion through co-stimulation of donor T cells have been used safely, with intriguing GVT responses (23). It also may be possible to generate leukemia-specific cytotoxic T cells to use in adoptive immunotherapy (24). Another strategy of generating CTLs against antigens presented on leukemic cells has been attempted by genetically modifying T cells to introduce antigen receptors capable of recognizing leukemic cells. Cooper *et al.* generated T lymphocytes engineered to express chimeric antigen receptors (CARs) specific for the CD19 molecule that may be able to prevent or treat leukemia relapse in B-ALL patients as these cells almost invariably express CD19 (25). Suppression of negative immune-modulation by anti-CTLA4 antibodies, Ipilimumab, has been explored (26). Vaccine strategies with tumor-specific antigens or modified tumor cells are other promising approaches to generate tumor specific immunity (27, 28). These strategies will likely be most effective in the setting of minimal residual disease.

2.3 DLI for relapse after SCT

Donor lymphocyte infusion (DLI) has been proven to induce remissions post-transplant in patients with relapsed hematologic malignancies most consistently observed in patients with CML, and to a lesser extent in AML, multiple myeloma and myelodysplasia (29).

2.3.1 General principles of DLI: source, effective cell dose, timing and toxicity:

Although G-CSF-stimulated peripheral blood cells for DLI have been used (16), typically donor lymphocyte is obtained by leukapheresis of un-stimulated peripheral blood (a.k.a., steady-state lymphocytes) which will contain other cell types in addition to CD3+ cells, such as dendritic cells, B cells, monocytic cells and natural killer (NK) cells, providing a spectrum of allo-reactive and other accessory cells that might play a role in graft-versus-tumor effect (30).

The optimal CD3 cell dose in DLI is not established. However, some studies have demonstrated that in patients with CML, a dose of $1x10^7$/kg can induce complete donor chimerism and a potent graft versus-leukemia (GVL) effect, in some cases in the absence of clinical GVHD particularly if given at later time points following transplantation (31). On the other hand, Fozza et al demonstrated that the incidence of GVHD was not significantly different in patients receiving less than 1×10^7 CD3+/kg compared with the patients who received doses greater than 1×10^7 CD3+/kg (32).

The timing of DLI and the interval after DLI required to observe a response from the DLI are important factors influencing the effectiveness of this strategy. Patients with relapses occurring more than 6 months post-transplantation have higher chances of responding to DLI (33). In support of the aforementioned data, Choi *et al.* found 55% overall survival at 1 year in patients who were treated for relapse, which occurred greater than 6 months post-transplantation, as opposed to 0% survival at 1 year in patients treated for relapse that occurred within 6 months following SCT (16). Disease response following DLI can be seen between 40 days and up to 1 year following DLI. Another predictor of DLI success is the tumor burden at the time DLI is administered. Patients with evidence of molecular relapse at the time of DLI have better responses even in malignancies not typically viewed as responsive to DLI, such as ALL, which might support careful screening of patients for detection of molecular relapse (33) offer further support for prophylactic approaches

The major complications with DLI are the development of GVHD and cytopenias; marrow aplasia has also been appreciated but is quite rare. Acute GVHD develops in up to 40–60% of patients who receive DLI. The development of GVHD does not always correlate with GVT activity (34). The time interval between SCT and DLI therapy appears to influence the likelihood of developing GVHD. A small dose of 1×10^5 T cells/kg can induce GVHD if administered on the day of transplant (35), yet a dose of 1×10^7 T cells/kg can be given at 12 months post-transplant without GVHD development (31). Other factors which make GVHD more likely to occur include donor sex mismatch (female donor to male recipient), advanced patient age and mismatch at the mHag level (36).

Aplasia is now a relatively infrequent complication of DLI. It is often transient, but in some cases may require hematopoietic stem cell rescue. It was reported historically in 15-20% of treated CML patients with an associated mortality rate of ~ 5%. Aplasia is more common in hematological relapse of CML, possibly due to poor donor myeloid reserve, and is rarely reported in patients with exclusively cytogenetic or molecular relapse (37, 38) or in those treated for low levels of recipient mixed chimerism.

Overall, DLI is an effective form of immunotherapy in patients with CML who relapse following SCT, with remission rates of approximately 80%. The results in patients with acute leukemias and myelodysplasia are disappointing with remission rates in 15–25% of patients and often the responses are not durable.

2.3.2 Strategies to avoid DLI-associated toxicity and improve outcome of DLI

2.3.2.1 Escalated dose regimen (EDR): Administration of DLI as a single bolus of cells collected from a single leukapheresis containing variable numbers of CD3+ T cells is referred to as a bulk dose regimen (BDR) and this approach is associated with a high incidence of GVHD (37-39). The EDR approach is fundamentally different in that the DLI product is quantitated for CD3+, CD4+ and CD8+ T-cell numbers and is then administered in multiple small aliquots with a dose escalation over time. In this way, the minimum cell dose needed to achieve disease remission is administered and with more modest cell doses, the likelihood of GVHD may be reduced (31). One study in CML comparing BDR and EDR approaches demonstrated equivalent remission rates with both schedules, but a significantly lower incidence of GVHD in the EDR cohort (38). It is critical when using the EDR schedule to allow an adequate interval between DLI doses to allow for assessment of response and toxicity. The optimum interval between doses is yet to be defined, but Dazzi et al. report that shorter intervals (rather than total cell dose) leads to a higher incidence of GVHD (38).

2.3.2.2 Manipulation of DLI products:
In order to decrease the incidence of GVHD, many methods have been attempted including depletion of allo-reactive T cells by co-incubation of donor lymphocytes with allogenic recipient stimulator cells followed by targeting with immunotoxin-conjugated antibodies specific for cell-surface activation markers or antibodies (40, 41). CD8+ T cells are thought

to be the primary mediators of GVHD in humans while CD4+ T cells are reported to contribute more to the GVT effect (42). For this reason, a number of groups have explored CD8+ T-cell depletion as a strategy to reduce the incidence of GVHD. CD8+ T-cell depletion of the stem cell graft has been reported to reduce the risk of GVHD without a parallel increase in relapse rates in several studies (43-45).

Other manipulations under study include engineered tumor-reactive T cells expressing either HLA-restricted, heterodimeric TCRs or chimeric antigen receptors (CARs) that recognize native cell-surface antigens. Second generation CARs often comprise an antibody binding motif and a CD28--CD3 dual signaling receptor which facilitates T-cell activation and expansion following stimulation (reviewed in (46)).

On the other hand, Porter et al. demonstrated that infusion of *'ex vivo'* activated donor lymphocytes (using anti-CD3 and anti-CD28 coated beads) in patients with a range of hematologic malignancies led to responses where conventional DLI had been disappointing. A total of 17 patients were evaluated and 8 achieved CR. The incidence of GVHD in this cohort compared favorably with that of conventional DLI (23).

2.3.2.3 DLI preceded by chemotherapy especially in acute leukemia.

Use of chemotherapy appears to improve the results of DLI. Response rates vary from 10% to 60%, with higher response rates than those reported for DLI alone (15, 47). The European Group for Blood and Marrow Transplant (EBMT) reported a retrospective analysis of 399 patients with AML in first hematologic relapse after transplant and found in the DLI subgroup, having less blasts in the BM (<35%), female sex, presence of favorable cytogenetics, and CR at the time of DLI were covariates associated with improved survival (47). The benefit of chemotherapy prior to DLI is suggested here by the 2-year survival >50% for patients that received DLI in CR.

Patients with recurrent AML after allogeneic transplantation have been treated with chemotherapy prior to DLI. Chemotherapy was administered because of rapidly progressive disease or in an attempt to debulk patients prior to DLI. Levine et al conducted a prospective trial of chemotherapy and G-CSF-stimulated DLI. Patients who emerged from the chemotherapy and DLI in complete remission had a 2-year overall survival rate of 41% (15). Studies from Japan and Korea treated patients with relapsed acute leukemia using chemotherapy followed by DLI reported an overall complete response rate of 33% , and 31% estimated overall survival at 24 months respectively (16, 48).

While these results engender some enthusiasm for such a strategy, a number of questions remain unanswered. The optimal timing of adoptive immunotherapy, whether administered during the nadir or after hematopoietic recovery, is unknown. The contribution of the cell therapy to the response rates from chemotherapy alone cannot be determined in the absence of a prospective randomized trial. Our own study in 25 patients with relapsed AML or high-risk MDS after transplant did not demonstrate advantage over chemotherapy alone for patients who received cellular therapy (second transplant or DLI) after chemotherapy, although the sample size was small (17). Our data was consistent with the data from a pediatric study on 49 pediatric patients receiving DLI for relapse following transplantation;

there was no advantage of DLI after adjustment for clinical variables compared with a large cohort of children with relapsed AML who did not receive DLI after transplant (49).

2.4 Prophylaxis DLI after SCT

Because DLI seems to be most effective for patients with minimal residual disease, the role of prophylactic DLI (pDLI) for high risk patients after stem cell transplant has been explored, especially in the setting of T –cell depleted SCT with increased relapse rates. Several studies have examined the utility of pDLI to minimize tumor recurrence in myeloablative (50-55) and in the RIC setting (56-61).

2.4.1 Prophylactic DLI in myeloablative T cell depleted SCT

In the myeloablative setting, the largest series was reported by Montero et al (55). One hundred thirty-eight patients with hematologic malignancies received myeloablative T cell-depleted peripheral blood stem cell transplant from an HLA-identical sibling donor. 112 patients with acute graft-versus-host disease (GVHD) grade <2 received 1 or 2 donor lymphocyte infusions of 10-50x 10^6 CD3+ cells/kg between days 45 and 100. Overall survival (OS), relapse-free survival, relapse, and transplant-related mortality (TRM) were 58%, 46%, 40%, and 20%, respectively, after a median follow-up of 4 years. Fifty-three (39%) and 21 (15%) patients developed grade 2-4 and 3-4 acute GVHD respectively. Forty-two (36%) had limited and 29 (25%) had extensive chronic GVHD. In multivariate analysis, disease risk was an independent factor for OS and relapse, day-30 lymphocyte count for OS and TRM, and chronic GVHD for OS and relapse. PBSCT with early T cell add back leads to comparable rates of chronic GVHD compared with T cell-replete PBSCT. However, this chronic GVHD after T cell add back was associated with less mortality and retains a protective effect in terms of relapse, at least in the standard-risk patients (55).

The patients reported by Schaap received planned DLI at a median of 22 weeks (range: 12–40) post-SCT, provided the post-SCT immunosuppression was discontinued for at least 2 months without evidence of active chronic GVHD and no history of acute GVHD above grade 1 (53). Similarly, Nakamura reported on patients who had planned non-mobilized cryopreserved DLI of 10×10^6 CD3/kg at day +45 and a second infusion of 50×10^6 CD3/kg at day +100 (52). Alternatively, Lee et al. (54) and Ferra et al. (51) selected DLI dosing based on the risk of GVHD and/or relapse risk.

In summary, the risk of relapse ranged from 18 to 69% with TRM occurring in 6–52%. This translated into a DFS that exceeded 40% at 2 years (50-55). Schaap's study (53) compared outcomes of patients receiving DLI with those patients not receiving DLI, relapse rates were lower resulting in improved LFS. Furthermore, the incidence of acute and chronic GVHD and the risk of TRM do not seem to differ from expected outcomes after conventional transplants without DLI.

2.4.2 Prophylactic DLI in non-myeloablative or RIC T cell depleted SCT

Several studies have analyzed outcomes of pDLI after RIC (56-61) . Barge et al. (56) reported on 11 patients, who were given planned DLI at 6 months after RIC MRD SCT with *in vitro* TCD. The DLI dose, given as unselected mononuclear cells, was based on disease status. Patients with relapse or progression at 6 months received $10–100 \times 10^6$

MNC/kg plus IFN-a compared with only $10x10^6$ MNC/kg for patients with stable disease or mixed chimerism. For the 11 patients receiving DLI, 5 responded (CR=3; PR=2) and 1 patient had stable disease. Acute GVHD developed in six patients, chronic GVHD in four patients and GVHD accounted for the death of one patient.

In the report from de Lima et al. (57), 12 patients with anticipated life expectancy of less than 6 months received fludarabine–melphalan conditioning and sibling PBSC allogeneic SCT for active hematologic malignancies, including AML (n=4), myelodysplasia (MDS, n=1), ALL (n=3), CML (n=3) and MM (n=1). All patients were scheduled to receive non-mobilized DLI at days +30, +60 and +90. Six patients received DLI. Of these, four patients achieved a CR, only one of whom was in CR at 14 months after SCT. The other three patients had either died due to TRM or due to relapse.

Schmid et al (59) studied 75 patients with high-risk AML or MDS who received non-myeloablative conditioning with fludarabine, cytarabine and low-dose total body irradiation. Patients were scheduled to receive prophylactic DLI after 120 days if there was no evidence of GVHD and they were off immunosuppression medications. Of the 75 patients enrolled, only 12 patients were able to receive prophylactic DLI due to early relapse, GVHD and other transplant-related complications, demonstrating the challenges of this approach. Another study used prophylactic CD8-depleted DLI after reduced-intensity transplant (60). In that study, 11 of 23 patients were able to receive DLI. Patients receiving CD8-depleted DLI demonstrated accelerated immune reconstitution and minimal GVHD.

Mixed chimerism is common after reduced-intensity regimens and may be associated with higher risks of relapse following transplantation. DLI has been used successfully in some patients with acute leukemia in order to facilitate conversion to full donor chimerism after reduced-intensity transplantation, and seems to lower relapse risk, albeit with a significant risk of acute GVHD (58, 61).

2.4.3 Summary of Feasibility of prophylactic DLI in T-cell depleted SCT.

In the Schmid study after a non-T-cell depleted regimen, only 12 of 75 (16%) received a DLI post-transplant around day 120 because of high rates of aGVHD before planned DLI (59). De Lima showed that 6/12 (50%) could receive pDLI planned at days +30, +60 and +90 (57).

Minimizing aGVHD after T-cell depletion appears to increase the feasibility of giving pDLI. In the setting of TCD myeloablative SCT, Montero et al (55) demonstrated that 112 out of 138 patients (81%) were able to receive prophylactic DLI between days 45 and 100. Several other reports of T-cell depleted regimens demonstrated that 38% to 61% of patients received scheduled pDLI (51,53-54,56,60). No studies have evaluated high-risk patients alone or followed our unique backbone of cyto-reduction prior to transplant conditioning incorporating T-cell depletion.

2.4.4 Rationale for repetitive DLI.

A recent study demonstrated that patients with acute myelogenous leukemia or chronic myelogenous leukemia in remission following SCT exhibited significant numbers of peripheral blood CD8+ T cells that recognized varying combinations of epitopes derived from leukemia-associated antigens. However, these cells failed to proliferate, release cytokines, or de-granulate in response to antigen-specific stimuli. The use of IL-15 or high-dose IL-2, elimination of CD4+ regulatory T cells, and blockade of PD-L all failed to rescue responsiveness of these CD8+ T cells in *in vitro* assays. Rather, the mechanism for CD8+ unresponsiveness after SCT seemed to be replicative senescence (62).

There are multiple sources of chronic stimulation following SCT that may contribute to potential T-cell senescence, including GVHD, GVL activity, infection, persistent stimulation of T cells by residual leukemia cells, and slow reconstitution of CD4+ T cells after SCT during the homeostatic proliferation to repopulate the T-cell pool. On the other hand, population dynamics of the T-cell pool after transplant may be influenced by the intensity of the conditioning regimen before transplant, T-cell dose within the graft, immunosuppressive therapy, and the use of donor lymphocyte infusions (62).

If donor T cells become tolerant or possibly rapidly senescent after SCT as a mechanism leading to relapse, in order to preserve and maintain a competent pool of CD8+ T-cell precursors after allogeneic HSCT, the use of repetitive DLI once a patient achieves remission may be useful.

2.5 Rationale to conduct prophylactic dose-escalation DLI to prevent relapse in hematologic malignancies after T cell depleted allo-SCT.

Since relapse becomes a major issue in non-myeloablative T cell depleted allo-SCT, and DLI is a clinically available and established treatment that is most effective for minimal residual disease, the role of prophylactic DLI for patients in remission to prevent relapse will be assessed in a single arm Phase II study. The study will focus on patients with high risk hematologic malignancies that roughly correlate to having leukemia not in remission, lymphoma not achieving a partial response, or other disease under poor control undergoing allo-SCT. We reviewed the University of Chicago transplant database for patients with high-risk AML or MDS who underwent a T-cell depleted (TCD) allogeneic transplant. Of the 145 patients with AML or MDS transplanted with active disease, relapse occurred at the median of 128 days (24 to 2364 days), with PFS of 137 days (4 to 3272 days) and OS of 214 days (4-3434 days) in the fludarabine/melphalan/Alentuzumab and clofarabine/melphalan/Alentuzumab conditioning regimens (unpublished data). From our published data, using fludarabine/melphalan/Alentuzumab conditioning regimen, patients with high-risk disease had a 39% probability of disease recurrence, a 39% probability of treatment-related mortality, and a 25% probability of progression-free survival at 1 year after stem cell transplant (63). In our clofarabine/melphalan/Alentuzumab protocol (11), 35 out of the total 72 patients accrued had high risk, active disease, and had 1 year PFS of 31% which is similar to the 25% 1 year PFS using fludarabine/melphalan/Alentuzumab, and 1 year relapse rate of 29% for the whole cohort, making the 1 year relapse rate for the high risk patients comparable to 1 year relapse rate of 39% from fludarabine/melphalan/Alentuzumab. The cumulative probability of Grade II-IV acute GVHD was 33% in flu/mel/campath and 22% in clo/mel/campath study at 1 years, and 1 year TRM was 33% and 26% respectively (11, 63). Published data using fludarabine+ bulsufan+ alentuzumab or ATG had comparable results in high risk hematologic malignancies (64, 65).

We have opted against a randomized trial for several reasons. This novel approach requires feasibility testing of the entire process including enrolling high-risk patients, early withdrawal of immune suppression and the ability to escalate DLIs.

3. PATIENT SELECTION

3.1. Inclusion Criteria prior to transplant

1. Age 14 – 75 years
2. The clinical trial will be offered to all high risk (defined in 3below) patients with hematologic malignancies who require stem cell transplants as part of their standard of care using matched related or unrelated donors.
3. Patients with high risk myeloid or lymphoid malignancies at stem cell transplant following ASBMT criteria (http://www.asbmt.org/displaycommon.cfm?an=1&subarticlenbr=35, under disease classification), including but not limited to conditions listed. These criteria apply BEFORE cyto-reductive therapy given within 28 days of planned conditioning:

   Refractory acute myelogenous or lymphoid leukemia
   Relapsed acute myelogenous or lymphoid leukemia
   Myelodysplastic syndromes with 5% or more blasts
   Chronic myelogenous leukemia in chronic phase 3 or more, blast phase presently, or second accelerated phase,
   Recurrent or refractory malignant lymphoma or Hodgkin's disease with less than a partial response at transplant
   High risk chronic lymphocytic leukemia defined as no response or stable disease to the most recent treatment regimen.

   Diseases in response or remission at high risk of relapse at the discretion of the attending physician: Some examples include but are not limited to:
   AML in remission with monosomy 5 or 7, deletion of 5q or 7q, 11q23 MLL rearrangement or complex karyotype (>= 3 chromosome abnormalities)
   NHL in response that is double hit or triple hit (which are characterized by a recurrent chromosome translocation in combination with a *MYC/8q24* breakpoint. These include but not limited to $BCL2^{+}/MYC^{+}$; $BCL6^{+}/MYC^{+}$; $CCND1^{+}/MYC^{+}$; *and* $BCL2^{+/}BCL6^{+}/MYC^{+}$)
   bi-phenotypic lineage leukemia
   CLL with 17p deletion
   ALL with t (4,11) et al.
4. Donors: Matched related or unrelated donor SCT matched at HLA A- B, C, and DRB1 by molecular methods. 7 of 8 matched donor acceptable for related donors.
5. T-cell depletion with ATG (rabbit or horse) or at least 30 mg of Alemtuzumab total in the conditioning regimen. Acceptable conditioning regimens include but not limited to fludarabine/melphalan/Alemtuzumab; fludarabine/busulfan/Alemtuzumab; fludarabine/melphalan/ATG; fludarabine/busulfan/ATG.
6. Immune suppression. Planned post-transplant immune suppression should include tacrolimus or cyclosporin monotherapy (i.e., calcineurin inhibitor or CN) for alemtuzumab regimens and a second immune suppressant for ATG treated patients. Other agents may be used if CN intolerance or toxicity occurs post-transplant.

7. Zubrod PS 0-2 or equivalent Karnofsky PS
8. Eligible for allogeneic transplant in the treating physicians' judgment and by institutional standards.

3.2. Exclusion Criteria prior to Transplant

1. Pregnant or lactating females
2. Hepatitis B with positive viral load prior to transplant conditioning or Hepatitis C virus
3. Human immune deficiency virus
4. Psychiatric illness that may make compliance to the clinical protocol unmanageable or may compromise the ability of the patient to give informed consent
5. Poor organ function (deviations from the following criteria are allowable only with the PIs assent as the risks and benefits must be addressed for patients with incurable hematologic malignancies):
    a. Creatinine >/= 2.0 mg/dL
    b. SGOT and SGPT >/= 5 x ULN. Liver biopsy preferred for such patients.
    c. Bilirubin >/= 3 x ULN (unless Gilbert's syndrome)
    d. DLCO < 50% corrected for hemoglobin
    e. Left ventricular ejection fraction < 40% or equivalent shortening fraction <20% in pediatric patients
6. Unlikely to be able to procure additional donor lymphocytes

3.3. Eligibility to receive DLI post-transplant (patients will be followed on protocol even if DLI not given)

It is recognized that only some subjects will undergo DLI

1. Donor lymphocytes available or able to be collected
2. No evidence of disease by standard morphology. Minimal residual disease or molecular evidence of disease will not exclude.
3. Adequate hematopoietic, renal, and hepatic function at first dose of DLI, defined as:
    - Absolute neutrophil count ≥500/μl
    - Platelet count ≥20,000/μl without transfusion for 7 days.
    - SGOT and SGPT ≤5 x ULN
    - Bilirubin ≤3 x ULN
4. No evidence of Grade II or higher acute GVHD or chronic GVHD at initiation of first DLI.
5. No systemic corticosteroids or immunosuppressive drugs (topical acceptable). Replacement steroids for adrenal insufficiency are not excluded.

3.4. Human Subjects and Inclusion of Women and Minorities

This clinical protocol involves the participation of human patients with hematologic malignancies after stem cell transplant to prevent relapse. Human subjects will be admitted to the protocol on a first-come, first-serve basis, provided eligibility criteria are satisfied. Subjects will be of either sex and of any race. Pediatric patients with hematologic malignancies undergoing SCT who meets the inclusion criteria could be included in this protocol.

4. REGISTRATION PROCEDURES AND ASSIGNMENT

A. The research nurse will ensure that eligibility testing is arranged and the patient is registered with the data management office if the patient is eligible for treatment.
B. A signed informed consent form must be on file before a patient can be registered.
C. Patient eligibility and the existence of a signed consent form will be checked by data management personnel.
D. Treatment on this protocol must occur at the University of Chicago.

5. TREATMENT PLAN

5.1. Study Design

A. This is an open-label, single arm Phase II study aiming to reduce relapse in extremely high risk hematologic malignancies after matched donor stem cell transplant with T cell depleted conditioning regimen by early withdrawal of immune suppression followed by prophylactic DLI. Eligible patients will be consented for this study at the time they are consented for the allogeneic stem cell transplant.

B. The matched related and unrelated donors will be evaluated and cleared in the routine standard fashion and are consented to collection per standard care.

Matched related donors (MRD): Matched donors will be evaluated and collected as per routine standard procedures. The donor will not be consented to this protocol because there is no research procedure performed on donors, and collection of steady state lymphocytes before and/or after transplantation is routine. Clinical consent for collection is required. We will request donors to give steady-state donor lymphocytes prior to stem cell collection. This involves a short-apheresis procedure usually collecting 10 -12 liters of blood. After that, mobilization in standard fashion is started with G-CSF (Neupogen) for 4 days with stem cell collection by leukapheresis on day 5 and/or 6 of a large volume (e.g., 16-24 liters depending on donor weight). Alternatives to pre-mobilization collection include steady-state collection of the donor later closer to the planned DLI infusion or using a fraction of the mobilized stem cell product for DLI. The composition of the DLI differs when obtained from a G-CSF mobilized product, although it is not clear if outcomes differ. Therefore, steady-state (i.e, non-mobilized) lymphocytes are preferred but not mandatory. The steady state donor lymphocytes from the matched donor will be collected and cryo-preserved as needed at our Stem cell lab. They will be enumerated by CD3 count and stored in appropriate aliquots. Typically, one steady state collection will provide an adequate number of CD3 lymphocytes for all the planned DLIs.

Matched unrelated donors (MUD): Steady state collection will likely require approval of the National Marrow Donor Program (NMDP), the organization that oversees collection of unrelated donor products. Ultimately, the decision on when to obtain lymphocytes will be determined by the NMDP, the donor, and the donor center. We will request, steady-state lymphocytes from matched unrelated donor to be collected and shipped to the University of Chicago before the first scheduled DLI infusion and the DLI will be cryo-preserved as above. As for the related donors, alternative options are using a portion of the mobilized stem cell product for DLI or steady-state collection of lymphocytes later after transplant. These methods do not require approval in advance. However, logistical issues may present a barrier to using the mobilized stem cells for DLI as this requires that the MUD product has an adequate stem cell dose by CD34 cells/kg and enough time to obtain the results to cryo-preserve a portion of the product. This protocol will be submitted to the NMDP for approval for the collection of lymphocytes from matched unrelated donors.

C. Treatment:

Immune suppression withdrawal: Rapid taper of immune suppression will begin from day 60 to 75. It is recommended to reduce the dose 25-50% every 5 days over 10-20 days, then discontinue.

Prior to each DLI:
1. Determine peripheral blood chimerism
2. Complete blood count and comprehensive metabolic panel
3. Assess for acute and chronic GVHD. We expect delays especially for people with emerging or resolving GVHD to undergo further evaluation.
4. Three month disease restaging will be done prior to first DLI. Should the results be unavailable, DLI may be given as planned.

DLI after Matched Related Donor (MRD) by recipient weight:

Number	Dose (CD3/kg)
1-MRD	2×10^5
2-MRD	5×10^5
3-MRD	1×10^6
4-MRD	2×10^6
5-MRD	5×10^6

DLI after Matched Unrelated Donor (MUD) by recipient weight:

Number	Dose (CD3/kg)
1-MUD	1×10^5
2-MUD	2×10^5
3-MUD	5×10^5
4-MUD	1×10^6
5-MUD	2×10^6

Patients will get dose escalation DLI at 4 - 8 weeks intervals if there is no grade II-IV aGVHD, no chronic GVHD and patient is not receiving system immune suppression. Topical GVHD therapy is acceptable, including topical GI therapy (e.g., budesonide) with a goal of every 4 weeks. The goal is infuse as close to every 4 weeks as possible. The DLI will be conducted in routine fashion. This is typically an outpatient procedure but can be done as an inpatient if needed.

Timeline of study:

Procedure	Approximate time point after transplant	Interval before next procedure
Withdrawal of immune suppression (e.g., tacrolimus or cyclosporine)	60 days	1 – 3 weeks
Donor lymphocyte infusion (DLI) 1 (Sibling donors 2x 10^5 CD3 /kg Unrelated donors-1x 10^5 CD3 /kg)	80 days	4 – 8 weeks
DLI 2 (Sibling donors- 5x 10^5 CD3 /kg Unrelated donors-2x 10^5 CD3 /kg)	120 days	4 – 8 weeks
DLI 3 (Sibling donors- 1x 10^6 CD3 /kg Unrelated donors-5x 10^5 CD3 /kg)	150 days	4 – 8 weeks
DLI 4 (Sibling donors- 2x 10^6 CD3 /kg Unrelated donors-1x 10^6 CD3 /kg)	180 days	4 – 8 weeks
DLI 5 (Sibling donors- 5x 10^6 CD3 /kg Unrelated donors-2x 10^6 CD3 /kg)	210 days	4 – 8 weeks

After DLI:

1. Clinic visit the day of or within 3 days of planned DLI to assess as above (prior to each DLI).
2. CBC and comprehensive metabolic panel are recommended every 2 weeks after DLI for at least 6 weeks after the last DLI infusion
3. Long-term follow-up of disease will follow standard clinical practice. Our recommended approach has been to repeat disease staging (bone marrow for leukemias and imaging for lymphomas) around day 100, day 180, 1 year and as indicated after stem cell infusion).

D. Number of Patients: 80

E. Accrual: Target accrual will be 1-2 patients every 4 weeks (12-24 patients/year).

5.2. Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for five dose-escalating doses in 5-10 months or until one of the following criteria applies:

- Disease relapse
- Inter-current illness that prevents further administration of treatment
- Grade III-IV acute GVHD
- Grade II acute GVHD lasting more than 8 weeks after last DLI treatment or chronic GVHD. Patients could be continued on the study if GVHD resolves within 8 weeks from the last DLI infusion off steroid unless only for adrenal insufficiency.
- Patient decides to withdraw from the study, or

- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.3. Duration of Follow Up

The patients will be followed according to the current post stem cell transplant guidelines. For this study, the enrolled patients will be followed for 2 years after stem cell infusion, removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.4. Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.2 applies. Patients also may be removed from the study for patient non-compliance, the development of a severe medical condition unrelated to their hematologic malignancies or this treatment, or a decision from the investigator to discontinue the study. The reason for study removal and the date the patient was removed will be documented in the Case Report Form.

5.5. Protocol Management Plan:

For the primary analysis, an intention to treat principle will apply such that all patients consenting will be analyzed. Secondary analysis will include only those patients for whom immune suppression withdrawal was started and DLI planned.

6. DOSING DELAYS/DOSE MODIFICATIONS

The National Cancer Institute Common Toxicity Criteria Scale (version 4.0) will be used to grade toxicities after each DLI infusion.
All Grade 3 and 4 toxicities, require delaying but not necessarily discontinuing treatment. If toxicity occurs, DLI dose can be delayed up to 4 weeks until side effect abates to within eligibility criteria. If a patient requires more than 4 weeks delay due to toxicities, then the patient will be removed from the study.

Evaluation of acute GVHD (aGVHD) on the day of scheduled DLI infusion (4 weeks after last DLI)

No aGVHD	Proceed to scheduled next level dose of DLI
Grade I aGVHD	Proceed to scheduled next level dose of DLI
Grade II aGVHD	Delay DLI, re-evaluate after 4 week. 1. GVHD resolves off steroid or systemic immune suppression; proceed to next level DLI. 2. Still has Grade II aGVHD or higher; off study
Grade III-IV aGVHD	Off study
Active Chronic GVHD	Off study

7. ADVERSE EVENTS MONITORING AND REPORTING:

7.1. Toxicity Monitoring

Patients will be monitored and questioned at every outpatient visit (see test schedule) regarding the occurrence and nature of any adverse experiences. An event is defined as any change in the physiologic or psychological state other than the primary condition that qualifies the patient for the study. More frequent monitoring will be performed in the case that an adverse event is noted.

Allogeneic transplant has substantial expected toxicities which are higher for patients entering transplant with active disease. The transplant approach prior to immune suppression withdrawal and DLI is standard of care and does not require formal comparison or stopping rules. The main toxicity related to this approach is acute and chronic GVHD. We will consider terminating the study based on a high-rate of severe (i.e., grade III-IV) aGVHD. One must recognize aGVHD takes 4-8 weeks after each DLI to develop; one must have approximately 6 months after starting immune withdrawal and DLI to determine if severe aGVHD will develop.

7.2. Toxicity Reporting

Toxicities are common following transplant. Therefore, unexpected, grades 3-5 adverse events (AEs) will be reported to the UCCCC CCTO Quality Assurance (QA) Coordinator by the end of the business day when s/he becomes aware of the event. Events occurring after business hours will be reported to the CCTO by 12pm (noon) the next business day. Each event report must indicate where the event meets the IRB's Unanticipated Problem reporting criteria.

The National Cancer Institute Common Toxicity Criteria Scale (version 4.0) will be used to grade toxicities.

For this study, early withdrawal of immune suppression (around day 60) and prophylactic donor lymphocyte infusions are experimental. The conditioning regimen and transplant itself is not experimental. Adverse event monitoring should occur until 100 days past either of these procedures. Deaths will be reported for at least 200 days after transplant or 100 days past any study procedure, whichever is later.

7.3. **Definitions of Adverse Events (AEs) and Serious Adverse Events (SAEs)**

Adverse Event - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related to the medicinal product.

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction.

Unexpected Adverse Event – An adverse event is "unexpected" when its nature (specificity), severity, or frequency are not consistent with (a) the known or foreseeable risk of adverse events associated with the research procedures described in the protocol-related documents, such as the IRB-approved research protocol, informed consent document and other relevant sources of information such as product labeling and package inserts; and are also not consistent with (b) the characteristics of the subject population being studied including the expected natural progression of any underlying disease, disorder or condition any predisposing risk factor profile for the adverse event.

Expected Adverse Event – Any adverse experience, event, incident, interaction or outcome that is identified in nature, severity or frequency in the protocol-related documents, such as the IRB-approved research protocol, informed consent document and other relevant sources of information such as product labeling and package inserts is considered an expected adverse event. Any event that is previously known or anticipated to result from the underlying disease, disorder, or condition of the human subject or the study population may also be considered an expected adverse event.

Serious Adverse Events – An adverse event that results in any of the following outcomes:

- **Death,**
- **Life-threatening adverse experience,**
- ***Inpatient hospitalization or prolongation of existing hospitalization,**
- **Persistent or significant disability/incapacity or,**
- **Congenital anomaly/birth defect.**
- **Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above**

* The initial hospitalization for transplant procedures or planned hospitalizations after transplant will not be considered an SAE unless the duration of the hospital stay is prolonged beyond what is expected as part of routine care. During the expected initial hospitalization, if toxicities that are not routinely expected after transplant are observed (examples listed in the table below), they will be reported as an SAE. Transplant procedures are expected to result in hospitalization for 10-20 days after stem cell infusion, depending on the stem cell source and patient health. All other hospitalizations unless planned would qualify as an SAE per FDA definitions.

Many toxicities that are grade 3 by CTC are expected and routine for transplant.
Select Examples

Expected Grade 3 -4 Toxicities for Transplant (NOT SAE)	**Not Routinely Expected Grade 3-4 for Transplant(SAE)**
Fever after engraftment without a source requiring several additional hospital days	Intensive care unit admission

Fever/infection requiring IV antibiotics during neutropenia or related to cathether	Infection requiring a major surgical procedure
Confusion requiring additional monitoring in the room	Seizure
Atrial fibrillation or flutter or arrhythmias monitoring on the transplant floor	Arrhythmia requiring monitoring outside of the transplant unit, a pacemaker, or cardioversion
Electrolyte disturbances requiring IV repletion	VOD
Poor nutrition requiring parenteral or enteral nutrition (with myeloablative regimens)	
Acute or chronic GVHD	

REPORTING GUIDELINES FOR ADVERSE EVENTS .

Unexpected Event		**Expected Event**		
Grades 1 and 2	Grades 3-5	Grades 1 - 2	Grades 3-4	Grade 5
Report in Velos as AEs.	Report in Velos within 24 hours of being notified of the event. Report to IRB if meet reporting criteria.	Not reported	Reported in Velos as an AE. No SAE report in Velos or IRB is needed.	Report in Velos within 24 hours of being notified of event.

Attribution – The determination of whether an adverse event is related to a medical treatment or procedure. Treatment is defined as protocol-defined preparative regimen, GVHD prophylaxis, and/or transplant procedure.

Attribution categories:

Definite – The adverse event is clearly related to the study drug/device/procedure/treatment(s).

Probable – The adverse event is likely related to the study drug/device/procedure/treatment(s).

Possible – The adverse event may be related to the study drug/device/procedure/treatment(s).

Unlikely – The adverse event is doubtfully related to the study drug/device/procedure/treatment(s).

Unrelated – The adverse event is clearly not related to the study drug/device/procedure/treatment(s).

Toxicity
Continuous monitoring for severe and unexpected adverse events will occur and regular time-points may be included per protocol.

ALL Serious Adverse Events, whether or not they are considered related to the study agent MUST be reported to the sponsor-investigator and to the University of Chicago Comprehensive Cancer Center (UCCCC). Refer to Section 7.4.1 for reporting guidelines.

7.4. Other Reporting Requirements

7.4.1. University of Chicago Comprehensive Cancer Center (UCCCC)

All serious adverse events (as defined in Section 0) and protocol deviations must also be reported to the UCCCC Cancer Clinical Trials Office (CCTO) in accordance with the UCCCC Data Safety Monitoring Plan.

The Research Nurse or other designated individual should report the SAE/deviation to the UCCCC CCTO Quality Assurance (QA) Coordinator by the end of the business day when s/he becomes aware of the event. Events occurring after business hours will be reported to the CCTO by 12pm (noon) the next business day. Each event report must indicate where the event meets the IRB's Unanticipated Problem reporting criteria.

7.4.2. Institutional Review Board (IRB)

Events meeting current IRB reporting criteria must be submitted by the principal investigator via the IRB's electronic submission system within **the IRB's designated reporting timeframes**. Details of the IRB's current reporting policy and timelines can be found on their website at: http://bsdirb.bsd.uchicago.edu/forms-guidelines/up.html.

The responsible research nurse and/or clinical research associate/data manager are responsible for entering the appropriate information into the IRB's electronic submission system and forwarding the submission to the principal investigator for reporting to the IRB.

7.5. Supportive Therapy

Symptomatic care may be given as required with medications such as anti-emetics and analgesics.

GVHD should be clinically suspected when a patient develops skin rash, diarrhea, and liver abnormalities at any time point after DLI. Diagnosis should be confirmed by skin biopsy, colonoscopy and biopsy of an involved area to confirm a pathologic diagnosis and to rule out other potential causes of the diarrhea; and potential liver biopsy. Should a diagnosis of GVHD be made or suspected, supportive care and non-systemic therapies are favored first. This includes topical steroid for localized skin GVHD, symptomatic anti-diarrheal agents, i. e., loperamide, for diarrhea. Patients with grade 2 to 4 will have standard GVHD treatment according to our protocol with high dose steroids, usually solumedrol at 2mg/kg.

Liver GVHD should be suspected when a patient develops an elevation of transaminases with or without an elevation in the bilirubin level. A hepatology consult and liver biopsy should be obtained to confirm a pathologic diagnosis whenever possible.

8. CORRELATIVE/SPECIAL STUDIES

In addition to routine clinical tests, we will collect additional samples for possible correlative studies.

For the serum Alentuzumab level testing, we will obtain 5-10ml serum/plasma at day 60 at the time of immunosuppression withdrawal and at around day 90 or on the day of first DLI to evaluate serum Alemtuzumab levels according to the published methods (66, 67). The samples will be 10 cc peripheral blood in Red-top tube, and the serum will be stored in our stem cell core facility.

In addition to the standard immune reconstitution studies including lymphocyte subset panel 3 and quantitative immunoglobulin levels, an additional 10 cc heparinized (Green Top) peripheral blood will be will be collected for detection of regulatory T cell panels and potentially other immune markers (e.g., T-cell repertoire) at the time of immunosuppression withdrawal, prior to first, the third, and the fifth DLI, and 1 year, 18 months and 2 years after stem cell transplant.

For MRD detection in myeloid leukemia, CLL and lymphoma patients with bone marrow involvement, bone marrow aspiration study material will be obtained from the bone marrow biopsy prior to stem cell transplant to be used as the baseline sample, since all the patients will have active disease before stem cell transplant. For lymphoma patients without bone marrow involvement, we will retrospectively obtain lymph node biopsy samples at the diagnosis for baseline samples. The follow up MRD samples will be obtained from either bone marrow aspiration if available or peripheral blood at the time of immunosuppression withdrawal, prior to first, the third, and the fifth DLI, and 1 year, 18 months and 2 years after stem cell transplant, or at the off study. The samples will be stored in our stem cell core facility.

We will store 10-20cc blood at the time of immunosuppression withdrawal, prior to first, the third, and the fifth DLI, and 1 year, 18 months and 2 years after stem cell transplant for future potential studies.

The following tests are a list of correlative studies most likely to be performed should funding and samples permit.

A. **Flow cytometry for Treg cells panels.** PBMC will be analyzed by flow cytometry using combinations of anti-CD4, and CD25, and anti-FoxP3 or CD127 monoclonal antibodies to identify the regulatory T cell subpopulation on permeablized cells. By combining the percent-positive cells with the coulter counter data, an absolute number of $CD4^{+}CD25^{+}$ $FoxP3^{+}$ or CD4+CD25+CD127dim/- cells will be determined. We expect these will be done at our Human Immunology Monitoring Facility at University of Chicago.

B. **Measurement of Alemtuzumab level by ELISA.** There is no commercially available test for the Alemtuzumab level in serum or plasma. A group in MD Anderson developed an easy ELISA test (66, 67). Affinity-purified rabbit anti-rat IgG absorbed with human IgG will be used to coat flat-button 96-well microtitre plates. After the plates are blocked using BSA (2%), patient samples will be added in duplicate and incubated. Then, peroxidase-conjugated affinity-purified rabbit anti-human-Fc will be added followed by adding substrate to develop color reaction. After 4–8 minutes, the reaction will be stopped and plates will be red at 450 nm, and a log reading of samples against control will be calculated.

C. **Minimal Residual Disease Monitoring:** The presence of minimal residual disease in myeloid leukemia or MDS will be assessed by monitoring of WT1 transcript levels in blood or bone marrow using a quantitative RT-PCR assay. Briefly, total RNA will be extracted from blood and bone marrow and cDNA synthesized using standard techniques. Amplifications of patient samples, K562 cell line cDNA, and no template controls will be performed in triplicate. WT-1 expression levels will be detected using a transcript specific primer and probe set. In order to compensate for differences in RNA integrity and cDNA synthesis efficiency, the absolute WT1 transcript copy number will be normalized to the endogenous control gene Abl. For the lymphoid diseases, MRD of CLL will be detected using four color flow cytometry (68), and MRD in NHL will be detected using quantitation of IgH or BCL-1/JH copy number using real-time polymerase chain reaction (69).

D. **Additional studies.** There may be additional studies geared toward gaining a better understanding and predictors of disease relapse, GVHD, and immunologic activity of this strategy.

9. CRITERIA FOR STUDY EVALUATION

The proportion of patients receiving one pDLI, proportion receiving all pDLIs, PFS at 2 year after stem cell transplant, highest Grade acute GVHD, relapse rate, and non-relapse mortality at 2 years after stem cell transplant are the relevant endpoints.

Acute GVHD will be scored according to the criteria proposed by Przepiorka et al (70). Limited Chronic GVHD is defined as GVHD with limited skin involvement only or presenting with liver function abnormalities only. All other presentations of chronic GVHD are defined as extensive and will require treatment. The diagnosis and staging of chronic GVHD will be done according to the published NIH Consensus (71).

Relapse will be recorded by the day of initial detection of malignant cells, if these cells were on subsequent testing confirmed to be increasing in number. The molecular detection of MRD will not be taken into account for the definition of clinical recurrence. The diagnosis of disease recurrence will be based on clinical and pathological criteria.

10. STUDY CALENDAR:

The following calendar summarizes the required samples specifically for this study.

Tests and Procedures	Baseline &	IS withdrawal (~Day 60 post SCT) +/- 7 days	Before first DLI (-10 to -1 day)	Day 0 of 1st DLI 1 (-7 to 0 days)	Day 0 of 2rd DLI 2 (-7 to 0 days)	Day 0 of 3th DLI 3 (-7 to 0 days)	Day 0 of 4th DLI 4 (-7 to 0 days)	Day 0 of 5th DLI 5 (-7 to 0 days)	One month after all DLI; 12, and 24 months post-SCT or At time off study (+/-7 days)
Standard tests									
History and exam (including vital signs and PS)	X	X		X	X	X	X	X	
CBC, Plt, diff		X		X	X	X	X	X	
BMP, LDH, LFT		X		X	X	X	X	X	
GVHD Assessment		X		X	X	X	X	X	
Toxicities Assessment		X		X	X	X	X	X	
Disease restaging: Bone marrow biopsy and/or CT imaging with or without PET for lymphoma	X		X*			X[1]		X[1]	X
Cytogenetic analysis	X		X*			X[1]		X[1]	X
Chimerism (blood or Bone Marrow)			X*	X[b]	X[b]	X[b]	X[b]	X[b]	X[a,b]
Immune reconstitution (lymphocyte subset panel and Quantitative Igs levels)		X		X		X		X	X
Research samples (optional but strongly recommended)									
Serum/plasma sample (10cc blood in Red top tube)	X	X		X					
Regulatory T cell panel 1/2# (10cc in Green top tube)[3]	X	X		X		X			X[2]
MRD testing (BM aspiration or peripheral blood or 10cc) [3] (Green top tube) $	X[b]	X[b]	X*	X[b]		X[b]			X[2,a,b]
Extra research sample for proteomics analysis (10cc blood in Green top tube)	X	X		X	X	X	X	X	X[2]

[1] Only clinically indicated. No need to do if the date falls within 2 weeks window of the scheduled bone marrow aspiration and biopsy.
[2] Only need one month after DLI5, and at 1 year post-SCT or at the time of off study.
[3] Samples will be stored and tests will be done in the batch fashion at the end of the study.
[&] Baseline samples will be done prior to cyto-reduction chemotherapy or prior to conditioning chemotherapy.
[*] This will be done -1 to 10 days before the infusion of first DLI. This bone marrow biopsy is required. [a] Using samples from bone marrow biopsy.
[#] Regulatory T cell panel 1: CD4+ CD25+ FoxP3+
Regulatory T cell panel 2: CD4+ CD25+ CD127 dim/-
[$] 10cc bone marrow aspiration will be obtained from the Bone marrow biopsy prior to the cyto-reduction chemotherapy or prior to conditioning chemotherapy. For lymphoma patients without bone marrow involvement, the original lymph node biopsy samples will be retrospectively retrieved for baseline sample for MRD detection.
[b.] From Peripheral blood.

All the patients will get the standard follow-up after the stem cell transplant according to our established transplant guidelines, which includes biweekly clinical visit in first month after discharge from the hospital after SCT, weekly visit in first 3 months.

Transplant patients will have bone marrow aspiration and biopsy at the following time points for cytogenetics analysis, chimerism per standard care. Peripheral blood samples will be obtained at the same time for immune re-constitution by checking lymphocyte subsets and quantitative immunoglobin levels. This is a recommended schedule. Variations to this schedule will not be considered a protocol deviation.

Day 28 (± 1 wk) post-SCT
Day 80-90 (± 1 wk) post-SCT before the first DLI infusion, if first DLI infusion will be delayed, a bone marrow biopsy is strongly recommended prior to the first DLI infusion.
Day 180 (± 1 wk) post-SCT
Day 365 (± 1 wk) post-SCT
Day 730 (± 1 wk) post-SCT
At relapse

11. DATA REPORTING / REGULATORY CONSIDERATIONS

A. The University of Chicago medical records will be utilized for all patients. Data will be entered into a data management file within 3 weeks after each evaluation of the patient. After the patient goes off treatment, follow-up information will be collected and entered into the data management file every 3 months by telephone contact.

B. Pathologic diagnosis and HLA typing will be recorded in a conventional way with a record being placed in the patient's permanent record and data management file. However, investigational correlative assay results will not be made part of the medical record.

C. Data and safety monitoring for this trial will be carried out in accordance with the University of Chicago Comprehensive Cancer Center Data and Safety Monitoring (DSM) Plan. Briefly, accrual, toxicity, and response data will be reviewed weekly at the transplant patient care conference. Adverse events will be reported to the principal investigator, IRB, as described in section 7.2. Decisions will be made regarding study continuation, amendment, or closure at the weekly meeting and a note will be signed by the principal investigator documenting this decision. External monitoring of accrual is performed by the Accrual Monitoring Committee. The study will be independently audited annually by the Cancer Clinical Trials Office of the University of Chicago Comprehensive Cancer Center in accordance with DSM Plan.

12. STATISTICAL CONSIDERATIONS

12.1. Study Design

Patients will be assigned to Matched related donor SCT group or Matched unrelated donor SCT group according to the type of donor for their stem cell transplant.

12.2. Sample Size/Accrual Rate

A total of 80 patients will be accrued with an accrual rate at 10-15 patients per year.

12.3. Statistical Considerations

Statistical analysis:

We expect that this protocol will enroll approximately equal numbers of patients undergoing MRD or MUD SCT with more than 50% of patients from both groups combined will be able to receive at least one DLI treatment. We also intend to analyze the 2 year PFS, OS, and rate of aGVHD among the patients who receive at least one DLI treatment. All the enrolled patients will be followed up at least 2 years from stem cell infusion as long as they are still alive. Baseline characteristics will be summarized using descriptive statistics. Intention to treat analysis will be used to determine the percentage of patients who receive at least one DLI treatment. Progression-free survival (time to relapse or death as a result of any cause) and overall survival will be computed using the Kaplan-Meier product-limit estimate and expressed as probabilities with a 95% CI. Acute and chronic GVHD, and treatment-related mortality will be estimated by cumulative incidence method. Cumulative incidence of treatment-related mortality with relapse of the original disease as the competing risk will be calculated.

Sample size:

We will determine the number of subjects who undergo withdrawal of immune suppression and receive at least one DLI. For this estimate, subjects who consent but do not proceed to transplant will be considered non-evaluable. Based on prior studies outlined in the background with heterogeneous disease risk and regimens, around 40-60% received pDLI after T-cell depleted regimens. We expect to increase the proportion receiving at least one pDLI to around 70% with around 15% having progressive disease after transplant and another 15% having GVHD or other clinical concerns that preclude DLI (e.g., infection, unwillingness to proceed, or lack of available lymphocytes). Thus we will test the null hypothesis of a 50% pDLI rate against a 70% alternative. To achieve 85% power with a two sided test with alpha set at 0.05 requires n= minimum 56 patients.

Should feasibility be established, this regimen will also need to demonstrate adequate disease activity to be pursued further. We will collect preliminary data on the efficacy of this treatment strategy for very high risk disease as measured by improved progression-free survival (PFS) compared to historical data in very similar patients (i.e, similar disease risk, similar regimens but no pDLI) at our institution. PFS was calculated for two major disease categories of AML and Non-Hodgkin Lymphoma treated on Alemtuzumab-based protocols who entered transplant with high-risk disease (not in CR for AML and not in CR or PR for NHL) from our historical institutional experience. There are two populations in which one can calculate PFS. We can evaluate the entire cohort by intention to treat or an enriched cohort of only those who are eligible to begin early immune suppression. We prefer the latter as this tests the efficacy (or biologic activity) of early immune suppression withdrawal and DLI for high-risk diseases. It is unlikely that there will be bias in our comparison as the major criteria for removal--significant aGVHD and disease relapse are unlikely to be affected by the investigators or subjects. Alternative approaches are required to salvage patients with very early relapse or early significant GVHD.

We determined outcomes for AML patients without GVHD and relapse at 80 days post stem cell transplant as these are the patients who would be eligible for early withdrawal of immune suppression and DLI. The 2 year PFS was 32.3% (95% CI 15.4% to 50.5%) for AML/MDS patients and 37.9% (95% CI 10.8% -65.4 %) for NHL patients. These are better than the outcomes of the entire cohort because of censoring as above. Outcomes for higher risk hematologic malignancies tend to be similar to each other (as expected as that is the inherent purpose of “high-risk” classification) thus justifying combining NHL, AML and other high risk diseases. Otherwise too few patients exist to generate a reliable historical control rate. Collapsing AML and NHL into one category, we have a null, two-year PFS rate of 35% based on our historical controls.

Stopping rules:

We will monitor aGVHD regularly and have derived a formal stopping rule. After every 10 patients have passed 6 months of follow up from immune suppression withdrawal, the incidence of aGVHD will be calculated. Patients who relapse or develop significant aGVHD before tapering will not be counted. Overall rates in historical controls after T-cell depleted regimens with alemtuzumab of aGVHD have been very low, at around 15%, with even fewer cases of severe aGVHD. As the regimen tested in this trial may reduce relapse at the expense of higher risks of aGVHD, we will allow a somewhat higher rate to occur. Specifically, if the lower limit of the 95% confidence interval exceeds 25% for severe (grade III-IV) GVHD, the study will halt accrual. Either protocol modification will occur or the study will be terminated. These modifications may include reduced DLI dose or number of infusions, delay of stopping immune suppression, or possibly continued immune suppression even after DLI.

Numbers of severe aGVHD events for every 10 subjects that warrant protocol stopping or revision are:

Severe aGVHD events	Enrolled Subjects	Observed aGVHD (%)	95% CI
6	10	60%	30.3-78.3
9	20	45%	26.0-65.3
13	30	43%	27.9-58.9
16	40	40%	26.9-54.22
19	50	38%	26.5-50.6

However, high rates of aGVHD that do not meet the stopping rules may still warrant protocol modification at the discretion of the investigators.

The pre-defined stopping rule was never met during the enrollment of the first 56 subjects defined by the original protocol. Thus, severe GVHD has been demonstrated to not be a concern for this study. The amended protocol will allow us to enroll more patients to the study for better efficacy evaluation. We expect to close the study after the requested number of 80 subjects has been reached.

While it is acceptable to have significant and severe aGVHD for curative intent approaches, the major problem with high rates of severe aGVHD is that this would make this a poor platform to integrate additional therapies later as severe aGVHD often prevents further immune modulation.

Chronic GVHD also remains of interest but can occur years after aGVHD and thus stopping rules based on this outcome cannot be formulated. We will further monitor the proportion undergoing early immune withdrawal with or without DLI continuously.

13. FUTURE DIRECTIONS

Depending on the results of this study, we will modify or design the future studies. The first possible modification may be to include leukemia patients with high-risk cytogenetics that are under disease control (remission) as outcomes are similar to those with active disease. We have excluded these patients initially as historically cytoreduction is not typically given prior to transplant since they do not have morphologic disease. If high early aGVHD is observed, we will continue immune suppression with DLI, since some data indicate DLI maintains a benefit even if infused with immune suppression. If high rate of acute GVHD occurs with escalated DLI doses, we will not escalate DLI or we will stop DLI after full donor chimerism for patients starting with mixed chimerism. If high rates of chronic GVHD are found, we will continue immune suppression during DLI or give cyclophosphamide after DLI. If there is no change in relapse rates after prophylactic DLI, we will either further escalate DLI doses or add disease specific agents or immune stimulation, such as basiliximab, or ipiluzumab.

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APPENDIX

PRZEPIORKA CRITERIA FOR ACUTE GVHD

Consensus Criteria for Grading of Acute GVHD

Acute GVHD Assessment Worksheet

(Acute GVHD assessment should be completed at onset, on change of treatment or when GVHD resolves. Assessment should follow the CIBMTR guidelines)

Patient Name____________________________ **MRN** ____________________

Immunosuppression therapy ☐Prograf__________ ☐ Steroids__________ ☐ Other ____________________

ORGAN /SYSTEM	STAGE 0	STAGE I	STAGE II	STAGE III	STAGE IV
PERFORMANCE STATUS: ☐	☐ ECOG 0, KPS or LPS 100%	☐ ECOG 1, KPS or LPS 80-90%	☐ ECOG 2, KPS or LPS 70-80%)	☐ ECOG 3, KPS or LPS 60-70%)	☐ ECOG 4, KPS or LPS <60%)
SKIN : (RASH) ____ %BSA	☐ No rash	☐ Maculopapular rash on <25% BSA	☐ Maculopapular rash on <25% to 50%BSA	☐ Rash >50% with generalized erythroderma	☐ Stage 3 rash plus bullae and desquamation
LOWER GI (DIARRHEA) : Stool volume ____ mL	☐ ≤500mL/day or <280mL/m² per day	☐ 501- 1000mL/day or 280- 555mL/m² per day	☐ 1001- 1500mL/day or <556- 833mL/m² per day	☐ >1500mL/day or >833mL/m² per day	☐Severe abdominal pain with or without ileus
UPPER GI:	☐ No protracted nausea or vomiting	☐ Persistent nausea, vomiting or anorexia			
LIVER (BILIRUBIN) : ____ mg/dL	☐ <2mg/dl or <34 µmol/L	☐ 2-3mg/dl or 34-52 µmol/L	☐ 3.1-6mg/dl or 53-103 µmol/L	☐ 6.1-15mg/dl or 104-256 µmol/L	☐ >15mg/dl or >256 µmol/L

Overall grading of acute GVHD

☐ GRADE I	☐ GRADE II	☐ GRADE III	☐ GRADE IV
Stage I or II skin involvement. No gut or liver involvement. Stage 0 or I performance status	Stage I gut with or without Stage I-II skin. Stage I liver GVHD with or without Stage I-II skin. Stage III skin without other organ involvement. Stage II performance status	Stage II-III liver or Stage II -IV gut or Stage III performance status	Stage IV skin rash Stage IV liver or Stage IV performance status with lesser organ involvement.