PROTOCOL SUBMISSION FORM **Amendment Form**	PROTOCOL NO. 09C0224-D	PRINCIPAL INVESTIGATOR (NIH Employee Name, Inst/Br, Telephone and e-mail): Ronald Gress NCI ETIB 301.496.1791 gressr@exchange.nih

PROTOCOL TITLE: Pilot Study of Radiation-Enhanced Allogeneic Cell Therapy for Progressive Hematologic Malignancy After Allogeneic Hematopoietic Stem Cell Transplantation

ABBREVIATED TITLE: Rad Enhanced Allo Cell Therapy Reference Number: 334163

Amendment Version Date: 01/22/2014 Amendment Letter: D

The Following revisions were incorporated into this protocol and approved by:

☒ Expedited Review (risk/ benefit ratio not changed)
☐ Full Board Review (meeting date)

Amendment includes changes required by:

☒ N/A
☐ NCI IRB (CCR)
☐ CTEP
☐ Other
☐ Other Sponsor
☐ FDA
☐ Special Studies IRB (DCEG)

If Other, list:

Amendment required Scientific Review?

☐ Yes ☒ No

ADMINISTRATIVE CHANGES

☐ Protocol Title/ Abbreviated Title
☒ New Principal Investigator
☒ NIH Personnel Change
☐ Non-NIH Personnel Change
☐ Converting to multi-institutional trial

DEC clearance required? ☒ Yes ☐ No

Date submitted to IC DEC: 01/23/2014 Date cleared by IC DEC: 01/24/2014

PROTOCOL CONTENT CHANGES

☐ Precis
☐ Study Objectives
☐ Background and Rationale
☐ Eligibility Assessment and Enrollment
☐ Implementation of Study Design
☐ Supportive Care
☐ Accrual Ceiling Changed to: N/A
☐ Data Collection/Evaluation
☐ Human Subject Protections
☒ Data Reporting
☐ Ionizing Radiation Use
☐ Pharmaceutical Information
☐ Appendices
☐ Transfer of Materials/Data (DCEG Only)

Does the amendment impact the risk/benefit assessment?

☐ Yes ☒ No

INFORMED CONSENT DOCUMENTATION

☐ Text Revisions to Consent(s)
☐ Investigator Contact Information on Consent(s)
☒ No Changes to Consent Form

SIGNATURE

Nancy Hardy - applied signature on 01/30/2014 12:13 PM EST Ronald Gress - applied signature on 01/30/2014 12:29 PM EST
New and Old Principal Investigators - electronic signature and date

APPROVALS

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Protocol Title: Pilot Study of Radiation-Enhanced Allogeneic Cell Therapy for Progressive Hematologic Malignancy after Allogeneic Hematopoietic Stem Cell Transplantation

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Précis

Background:

- The prognosis for patients with cancer who have relapsed or progressive disease after allogeneic hematopoietic stem cell transplantation (allotransplant) is poor. Effective therapies for patients who fail withdrawal of immune suppression and administration of donor lymphocyte infusions (DLI) have not been identified.
- Increasing the efficacy of allotransplant without increasing toxicity is a major goal of transplantation research. A major research effort within the ETIB is to identify ways to build on the allogeneic platform to treat relapse after allotransplant.
- We hypothesize that a single fraction of radiation to tumor prior to administration of donor lymphocytes will increase the potency of systemic graft-versus-tumor (GVT) effects without increasing graft-versus-host disease (GVHD).

Objectives:

- To determine the safety, vis-à-vis GVHD and allograft function, and efficacy, in terms of systemic tumor response, of administering single-fraction, targeted radiotherapy with or without DLI to patients with persistent tumor after allotransplant.

Eligibility:

- Adults with hematologic malignancies that progress or recur after allotransplant, successful donor T cell engraftment, and trial of withdrawal of immune suppression.
- Disease that is amenable to radiation as well as additional measurable disease outside the radiation field.
- Subjects with treatment-refractory acute or chronic GVHD will not be eligible.

Design:

- Subjects will receive radiation in a single, 8-Gy fraction to sites of disease. At least one site of measurable disease will remain untreated with radiation for evaluation of systemic response.
- There will be two arms. Arm A will include subjects with available donor lymphocytes and who have not had GVHD requiring systemic treatment; they shall receive a DLI the day after completion of radiation. Arm B will include those who have previously required systemic therapy for GVHD, are at high risk of significant GVHD, and/or who do not have available donor lymphocytes; they shall receive radiation without DLI.
- Additional disease that is outside the field of radiation will be monitored for systemic effects of the therapy.
- Subjects will be monitored on an outpatient basis for the development or exacerbation of GVHD, excessive hematologic toxicity or other toxicity from radiation, and for tumor responses for at least 60 days.
- Enrollment:
    - Treatment Subjects: The protocol will treat 21 subjects per arm (total 42). There are stopping rules after 8 and 15 patients per arm for excessive GVHD or radiation toxicity.

- DLI Control Subjects; 15 control subjects who receive DLI for persistent disease as part of their care on another NIH protocol, will be included to compare the immunologic effects of radiation followed by DLI (Arm A) with DLI alone.
- Donor Subjects: Related donors of Arm A Treatment Subjects and DLI Control Subjects will be enrolled for collection of clinical DLI product, a portion of which will be used for research (up to 36 Donor Subjects).

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Aims:

To assess the administration of single-fraction, 8-Gy dose(s) of radiation, with or without donor lymphocyte infusion (DLI), in patients with radiation-accessible, relapsed or refractory hematologic malignancies following allotransplant with respect to:

- safety, defined by the development or exacerbation of acute or chronic graft-versus-host disease (GVHD);
- feasibility, with respect to identification of patients with radiation-accessible and evaluable hematologic malignancy; and
- efficacy, defined as tumor responses outside the field of radiation.

1.1.2 Secondary Aims:

- To characterize the quantitative and qualitative effects of single-fraction radiation on circulating allogeneic immune cell populations and blood levels of potential regulatory/inflammatory cytokines. In subjects who will also receive DLI, the effects on circulating immune cells and regulatory/inflammatory cytokines will be compared with those of DLI Control Subjects, consisting of patients that receive DLI as part of standard therapy for relapse on other NIH protocols, without prior irradiation. The number of 15 DLI Control Subjects was specified to allow 81% power to detect a difference equal to one SD (of each group) in a single parameter: the change in proliferating, activated T cells pre- and post- DLI between the Treatment Subjects who receive radiation and DLI and the DLI Control Subjects, who will not be treated on this study, but who receive DLI alone as treatment of persistent disease after allotransplant.
- The early effects of single-fraction irradiation upon irradiated tumor (local effects) and "distant" tumor (systemic effects) will be examined. When tumor accessibility permits, pre- and post-treatment (Day 3) biopsies will be compared with respect to effects on infiltrating immune cell populations, tumor antigen expression (if known) and MHC expression. Additionally, metabolic activity, as a surrogate for inflammatory cell infiltration of the irradiated and distant tumor will be assessed by pre- and post-treatment (Day 3) FDG-PET/CT scans.

1.2 BACKGROUND AND RATIONALE

The hypothesis being tested in this protocol is that radiation-induced tumor damage will increase effective tumor antigen presentation and thereby the antitumor potency of the allogeneic immune response. Specifically, in patients with hematologic malignancies who have progressive tumor after allotransplant, we will assess whether a single fraction of radiation to a discrete (solid-phase) tumor given after allotransplant, with or without an additional DLI, will lead to systemic tumor responses.

Biologic Basis of a Graft-versus-Tumor Effect: Although data support a clinical GVT effect, little is understood about the nature of this effect at the cellular and molecular levels. As detailed above, GVT effects have been described for acute and chronic leukemias, lymphomas, and solid tumors, but the allograft potency against these different cancers remains highly variable. For example, chronic myelogenous leukemia (CML) may be more susceptible than other forms of leukemia to GVT effects.[1] Response rates as high as 90% are observed in CML patients after DLI, while response rates for other malignancies are generally less than 50%.[2] Possible explanations for this observation include the relatively indolent growth kinetics of chronic-phase CML, or presentation of more potent tumor antigens, possibly derived from minor histocompatibility antigens, myeloid antigens, or the *bcr*/*abl* gene product.[3] However, response rates can vary significantly, even among CML

patients. There are significant differences in response to DLI among CML patients based upon the stage of disease, with patients whose disease is in the chronic phase having significantly higher response rates than patients with disease in either accelerated or blastic phases. These observations suggest that growth kinetics and antigen expression may affect response to DLI. There are also significant differences in response to DLI among chronic-phase CML patients. Higher response rates are observed among CML patients whose disease is detectable by molecular techniques as compared to patients with overt relapses in the bone marrow or peripheral blood, suggesting that tumor burden is a major determinant of response to DLI.[2-5] While the GVT effect has been most clearly seen in indolent hematologic malignancies, of which CML is the prototype, dramatic responses to DLI for persistent or relapsed disease indicate that the GVT effect can also occur against aggressive hematologic malignancies, including NHL.[6]

In spite of a GVT effect against lymphoma, however, relapse remains a significant problem after allotransplant. There is a dearth of information on the biology of the GVL effect and its success or failure in controlling malignancy, and a tremendous need for refinement of current therapies and for development of novel strategies for treatment of lymphoma when allotransplant is not curative.[7,8]

Treatment of Relapse after Allotransplant: Allotransplant is a standard therapeutic option for patients with refractory or chronic hematologic malignancies, however, the prognosis for patients who relapse or have disease progression after allotransplant is poor. Transferred immunity is a major therapeutic component of allotransplant for malignancy, and is responsible for the GVL or graft-versus-tumor (GVT) effect. Donor lymphocytes administered at the time of relapse after allotransplant can mediate a durable immunologic GVT effect, as is best described and most reliable in chronic myelogenous leukemia.[1,9,10] While T cell dose[11] and disease kinetics[1,2,12] have a clear impact on response rates for CML, less consistent results for other hematologic malignancies suggest that variability in tumor immunogenicity and/or tumor growth kinetics may contribute as well. In the setting of lymphoid cancers, only the indolent malignancies have reasonable response rates to DLI, perhaps as high as 75 percent.[13-15]

An important limitation to successful therapy with DLI is the risk of GVHD, yet in most studies of DLI, GVHD correlates closely with GVL response. [2,15-19] A large, multicenter, retrospective analysis of DLI, including 48 patients with persistent disease and variable donor engraftment, found 5 of 8 patients who developed GVHD (63%) responded to DLI, compared to 7 of 40 patients without GVHD (18%) (P = .01).[19]

The rate of acute or chronic GVHD from therapeutic DLI is not well defined, with highly variable numbers reported in the literature. Topical reviews on DLI cite GVHD incidence rates from 30–35%[15] up to 40–60%[20] for both acute and chronic GVHD, with similar risk whether the donor is related or unrelated.[20] The variability in reported rates of GVHD - as well as variability in response rates - may largely be explained by heterogeneity among transplant regimens, DLI cell doses and the engraftment status of patients who receive therapeutic DLI.[2,15-19] Greater intensity of conditioning (and similarly, intensity of prior therapy) – postulated to increase DLI response rates and toxicity – may represent surrogates for donor T cell engraftment at the time of relapse and DLI administration. An association between tumor responses and establishment of full donor engraftment is well described.[14,15,17,19,21] Therefore, it seems likely that donor engraftment at relapse would influence rates of tumor response and GVHD after DLI as well. Increased interval from allotransplant to relapse – associated with better survival – may reflect engraftment and initial GVL-mediated tumor control. If loss of GVL reflects exhaustion of the tumor-reactive lymphocyte population, it could potentially be reestablished with administration of additional donor cells. Interestingly, however, in an analysis of Center for International Blood and Marrow Transplant Research, data collected on pediatric patients with relapse after allotransplant, the higher rates of survival seen in late relapse was seen in patients managed with or without additional donor lymphocytes,[18] suggesting that other treatment strategies may be able to rekindle a dwindling GVT response, and/or the biology of late relapse may be quite complex.

In a relatively homogeneous patient population, a retrospective, single-institution study of 83 patients who received matched, related allografts after myeloablative conditioning (73 received grafts that T-cell depleted) found that patients with more than 50% donor chimerism before receiving DLI were 4.5 times more likely to achieve a complete remission and 3.4 times more likely to develop GVHD than patients whose donor

chimerism was less than 50%. Furthermore, patients who achieved full donor chimerism after receiving DLI were 21-fold more likely to achieve a CR than patients who did not.[17]

Given the association with tumor responses and engraftment, the biology of tumor progression and relapse is likely quite different in patients who are fully engrafted compared with those who have mixed chimerism. Therefore, it is difficult to determine that an individual patient's tumor is resistant to the allogeneic immune response until complete donor chimerism is established. For those patients who relapse after full donor engraftment, the data on the risk of GVHD following DLI therapy are very limited, but continue to demonstrate a strong relationship between GVHD and tumor responses.

In a study of 28 patients with indolent lymphomas who received a total of 63 DLI, 13 DLI that were given to patients who had achieved full donor chimerism prior to infusion. This study found no difference in the incidence of significant GVHD after DLI (acute GVHD grade II-IV or extensive chronic) whether or not patients were fully engrafted at the time of administration (8 of 50 DLI to mixed chimeras vs 3 of 13 DLI to full donor chimeras; P = .68). In the same study, 15 patients treated for disease progression were evaluable for both response and GVHD. Of these, 13 patients responded, including 7 without significant GVHD. The two patients who did not respond developed mild acute GVHD (Grade I, skin).[14]

A study exploring the impact of chimerism on outcomes after DLI found similarly high rates of GVHD in the subset of patients who converted from mixed to full-donor chimerism (10 of 13, 77%) and in patients who were full-donor chimeras at the time of DLI (11 of 16, 68%). Among the 16 evaluable patients with full donor chimerism at the time of DLI (all after Day 100 and so GVHD was defined and staged as chronic) there were 11 patients who developed GVHD (4 limited and 7 extensive) and 5 subjects who did not. Whereas only one patient who did not develop GVHD had a tumor response, 9 of 11 patients with GVHD had a tumor response (3PR and 6 CR). From another vantage point, ten of 16 evaluable, fully engrafted patients had tumor responses to DLI; nine of these ten developed GVHD (4 limited and 5 extensive).[22]

Extrapolating from existing data, among patients who are fully engrafted at the time of DLI, the risk of GVHD appears to be correlated with the rate of response, and significant GVHD may occur in 50% of patients whose tumors respond to DLI.

There is no single standard of care for management of tumor relapse after allotransplant.[23] In defining a management approach, important considerations include donor T cell engraftment at the time of progression, the pace of disease progression, whether the patient had any apparent GVL response to allotransplant (potentially suggested by the timing of progression relative to allotransplant, with late relapse showing better outcomes after DLI[4,14,15]), ongoing immune suppression, presence or history of graft-versus-host disease (GVHD), availability of donor lymphocytes and chemosensitivity of relapsed disease. Given the complexity and heterogeneity of patients, diseases and allograft function, the approach must be individualized. Relapse or progression occurring after full donor T cell engraftment has been successfully treated with additional DLI and/or a trial of withdrawal of immune suppression.[24] While the use of monoclonal antibodies and/or chemotherapy in conjunction with DLI or a second allograft may be reasonable approaches in carefully selected patients, durable responses are anecdotal.[24,25]

Mechanisms of Immune Evasion after Allotransplant: Relapse treatment strategies that build on the therapeutic potential of the allogeneic immune system may yield novel, more specific forms of immunotherapy. Several mechanisms of cancer immune evasion from the native immune system have been described,[26-30] including lack of costimulation, loss or down-regulation of HLA, loss of tumor antigens and immunodominance, defective death receptor signaling, lack of access, immunosuppressive cytokines, apoptosis of activated T cells, and regulatory T cells. These mechanisms likely play a role relapse after allotransplant. Approaches that increase tumor immunogenicity *in vivo* may be effective in increasing the potency of the allogeneic antitumor response.

Loss of tumor expression of major histocompatibility antigens or costimulatory molecules, tumor cytokine production inducing down-regulation of immune responses, and absence of a " danger signal" are among the many postulated mechanisms for tumor escape from the native[31,32] as well as the allogeneic immune

response.[33,34] The published literature on specific roles for these mechanisms in the setting of cancer relapse after allotransplant is not definitive.[7,34] The biology of allogeneic graft-versus-tumor responses and mechanisms of immune escape in this immunologic environment are areas of active investigation throughout the transplant community, including lymphoma-specific research. A diverse spectrum of possible mechanisms include tumor-specific donor cell-mediated responses, cytokine-mediated bystander effects of graft-host immune reactions, and that tumor regression is a component of a more general alloreactive immune response.[35] There are data that support and refute each of these possibilities, and it is likely that allotransplant recipients are heterogeneous with respect to which one or more of these or other mechanisms are involved.

While tumor responses are occasionally seen with the current standard therapy for relapse after allotransplant – withdrawal of immune suppression and/or administration of DLI – their observation does not provide insight in to mechanisms of activity. Nor do observations of disease progression after these immunologic maneuvers or the phenomenon of late relapse point to specific factors that account for failure or waning of the potency of the allogeneic immune system to recognize and eliminate malignant cells. To the extent that the efficacy of allotransplant may be the result of tumor- or tissue-specific cellular immune responses, strategies that attempt to change the immunogenicity of the tumor *in vivo* may trigger or boost an allogeneic immune response. Given the responsive and evolutionary nature of the allogeneic immune response, exerting additional pressure on the tumor cell population, e.g., through tissue damage with ionizing radiation, could directly generate novel cellular targets, produce alterations in the tumor microenvironment with secondary tumor changes, and/or generate a release of cytokine signal to recruit an inflammatory cell infiltration. These are among the mechanisms postulated for observations of radiation-enhanced immunotherapy in the autologous setting.[36,37] Either independently or together, these damage-mediated changes could work synergistically with the ongoing allotransplant therapy, through allowing or enhancing antigen presentation and effective tumor targeting of the immune response. Targeting tumor with radiation *in situ* may increase tumor and stromal antigen presentation. In contrast to treatment with multiple fractions of radiation, the use of a single fraction may allow the recruitment of an inflammatory cellular infiltrate, and thereby enhance or permit initiation of an immune response.

Immunologic Effects of Radiation: Systemic effects of local irradiation, including distant tumor responses, have been noted occasionally for several malignancies.[38-43] This phenomenon, termed the "abscopal effect,"[44,45] has prompted investigation into its basis, including effects of radiation on the systemic immune response to tumor and potential use as an adjunct to tumor immunotherapy.[46] Synergistic antitumor effects of radiation and immunotherapy have been described in several murine systems. It was first noted that whole-body radiation was an alternative modality (to cyclophosphamide) that permitted tumor-infiltrating lymphocyte (TIL) antitumor activity in a murine adenocarcinoma model.[47] Subsequent studies in the same murine system demonstrated that synergistic antitumor effects, including tumor outside of the field of radiation, could be achieved with local radiation.[48] In a Lewis lung carcinoma murine model, local irradiation augmented systemic responses and survival after interleukin-6 therapy;[49] similarly, in a murine renal adenocarcinoma (Renca) system, local radiation improved systemic responses to interleukin-2 (IL-2) in a dose-dependent fashion, and with increased tumor cell surface expression of MHC Class I.[50] In this latter model, the tumor is radioresistent, and IV administration results in the development of lung metastasis. After single-lung irradiation plus IL-2 therapy, metastases were similarly reduced in both the irradiated and nonirradiated lungs; this effect was lost when animals were depleted of CD4, CD8 or NK cells prior to radiation and IL-2 treatment. Immunohistochemical examination of irradiated tumor demonstrated an influx of Mac-1^+ cells, increased infiltration of both CD4+ and CD8+ T cells and NK cells. This infiltration was limited to irradiated tumor.[51] From this the authors hypothesized that the combination of radiation and IL-2 treatment resulted in increased antigen presentation and immune cell infiltration at the site of irradiated tumor, with subsequent initiation of a systemic immune response. Interestingly, in a single-tumor murine model, a dose-dependent abscopal effect has been demonstrated after irradiating normal tissue in wild-type mice but not in $p53^{null}$ animals, suggesting that the biological phenomenon of the abscopal effect may be complex, heterogeneous or multifactorial.

The observations that irradiated tumor resulted in changes in the cell surface phenotype of human tumors and resulted in enhanced killing by cytotoxic T cells *in vitro*,[52] more effective ex-vivo antigen loading of dendritic

cells (DC) for anti-tumor therapy,[53] that local tumor irradiation enhances intratumoral dendritic cell vaccine efficacy,[54] results in local and systemic cytokine[55] and antitumor responses,[56] and improves GM-CSF-based tumor vaccine [57] and costimulatory molecule ("TRICOM")-enhanced tumor antigen vaccines[58] raise the question of whether radiation may provide a unique constellation of tumor cell and tissue changes that could enhance antitumor immunotherapy.

Recently, it has been shown that radiation increases cell-surface expression of MHC class I molecules and the intracellular peptide pool, through both protein degradation and synthesis. It was demonstrated that increased protein synthesis resulted from activation of mTOR, and that the increased peptide-MHC-I complex expression reflected increased peptide synthesis derived from both native and novel proteins.[59] These investigators found that irradiation of the colon adenocarcinoma cell line MC38, which expresses gp70, increased their susceptibility to epitope-specific CTL *in vitro*, in an mTOR-dependent fashion (i.e., the effect was lost when cells were treated with radiation *and* rapamycin). This observation is particularly interesting in light of clinical use of rapamycin (sirolimus) for its immunosuppressive and antitumor effects. It may be that rapamycin would interfere with the potential potency of the specific combination of radiation with adoptive cell therapy. This same tumor model was assessed *in vivo*, demonstrating that the combination of tumor irradiation and adoptive CTL transfer resulted in inhibition of tumor outgrowth, and often in complete eradication of tumor, whereas neither irradiation nor adoptive transfer alone did so.

While most of the work to date has been done in murine systems, the combination of radiation and vaccine-based immunotherapy has been used in clinical trials with promising observations.[60] In a recent review of combining radiation and immunotherapy, six clinical reports provide support for radiation-enhanced immune responses. Systemic (abscopal) clinical responses have been described after local stereotactic radiation therapy in metastatic renal cell carcinoma;[61] serological evidence of new antibody responses have been detected in patients with localized prostate cancer after treatment with external-beam radiotherapy or brachytherapy that were not detected after surgical management.[62] Vaccine therapy combined with radiation yielded improved response rates over radiation alone in cervical cancer;[63] treatment of metastatic prostate cancer with combined vaccine and radiation resulted in detectable increase in circulating prostate-specific antigen-specific T cells that was not found after radiation alone, including *de-novo* generation of T cells to prostate associated antigens not present in the vaccine;[64] and a Phase I study of a radiation-enhanced DC vaccine for refractory hepatoma demonstrated increased NK-cell activity and and a Phase I study of a radiation enha[65] While the latter was a Phase I study, clinical responses were observed in six of ten patients, and preclinical work in a murine system demonstrated enhanced tumor control with the combination of vaccine plus radiation over vaccine alone (with no responses with radiation alone); and only the combination suggested generation of a memory response, with long-term eradication of tumor in rechallenge experiments.[66]

Safety of Radiation Therapy: Radiation therapy is frequently used for both definitive therapy and palliation. The total dose of radiation delivered and the number of treatments in which this total dose is delivered vary depending on the indication. The dose selected in this trial (8 Gy delivered in a single fraction) is based on the preclinical literature as described above. A potential benefit of a single fraction regimen compared to a multifraction regimen relates to the possibility of sterilizing lymphocytes recruited to tumor with each additional fraction of radiation. By delivering the radiation dose in a single fraction this possibility is avoided. It is also the dose that was used in the previously cited Phase I clinical trial of combined radiation and DC vaccine therapy for hepatoma.[65] The safety of this dose has been demonstrated in a number of trials in patients receiving radiation for the palliation of bone metastases.[67-74] It is particularly helpful that treatment in these series was delivered to a variety of body sites, as that is the likely situation for patients included on this protocol. Each of these series showed that the use of a single fraction of 8 Gy resulted in a favorable acute toxicity profile compared to the previous standard regimen, in many cases 30Gy in 10 fractions. In fact in the Radiation Therapy Oncology Group (RTOG) trial comparing 8 Gy in a single fraction to 30 Gy in 10 fractions acute Grade 2-4 toxicity was significantly higher in the 30 Gy arm and late toxicity is rare with either regimen (Hartsell et al., JNCI 2005). Based on the safety and tolerability of 8 Gy delivered in a single fraction, this regimen is now considered standard by the RTOG for the management of bone metastases.

A systematic study of the safety of radiation therapy after allogeneic transplantation has not been reported in the literature. We have had 27 NCI protocol patients with progressive disease after allotransplant who were treated with radiation therapy, including four patients who required more than one site irradiated. The incidence of severe hematopoietic toxicity and GVHD was reviewed for these patients. The following findings are limited by several factors but remain of value in light of the dearth of published information. The patients had a variety of malignancies, including Hodgkin's lymphoma, NHL, multiple myeloma and metastatic breast cancer; additionally these patients were treated on different protocols with different GVHD prophylaxis regimens, including single-agent cyclosporine. The dose varied by site of disease and treatment intent, as determined by contemporary standard of care. Two patients experienced GVHD toxicity to which it was felt that radiation may have contributed, including two acute GVHD, both of which occurred during radiation treatments. The first reached Grade II (skin stage 3, gut stage 1 and liver stage 0); and the second reached Grade III (skin stage 2, gut stage 3 and liver stage 0). Both cases of acute GVHD occurred, by definition, within first 100 days after transplant. Nonetheless, we could not exclude the possibility that radiation may have contributed, particularly in light of the fact that the GVHD arose during the period of radiation. There was one exacerbation of chronic GVHD (likely related to radiation, as area of flare fell within the radiation field). A fourth patient developed and eventually succumbed to radiation necrosis after whole-brain radiation. In this case, it was necessary to radiate after the patient had received several doses of intrathecal methotrexate; it was not thought likely that allotransplant contributed significantly to this severe adverse event. In all, this group of patients tolerated radiation with reasonable toxicity, within the range expected for a heavily treated patient population with advanced malignancy.

Feasibility of Planned Approach: We carried out a preliminary feasibility assessment on ten arbitrarily selected patients with lymphoma that had been treated on NCI allotransplant protocols, to determine whether the sites of disease relapse would be amenable to this radiation approach while allowing for systemic disease response. A review of clinical and imaging data was performed by two attending physicians in the NCI/Radiation Oncology Branch to assess the feasibility of treating one or more sites as outlined in this protocol. Independently, both physicians felt that all of these patients would have been able to receive radiation safely and reproducibly based on the imaging and clinical data available. In no case was there concern that it would be unsafe or not feasible to deliver a prescribed dose of 8 Gy with the targeting and planning guidelines included in this protocol.

Summary

The full therapeutic potential of allotransplant remains unmet. Those patients who do obtain a GVT effect often relapse. While additional donor lymphocytes can control some indolent tumors, there are no proven effective options for treating patients who do not respond or who have more aggressive tumors. Identifying novel ways to build on the GVT effect remains a critical goal of transplantation research, yet the biologic basis of GVT is not well understood. The co-incidence of GVHD in many, but not all patients who manifest GVT suggests a complex and variable biology. In patients undergoing allotransplant for malignancy, the GVT effector populations, target antigens and the relationship to GVHD are heterogeneous, and likely depend upon several factors acting interdependently. Alloreactivity is highly unpredictable and can dramatically alter the balance between the immune response to tumor and mechanisms of tumor escape. If the GVT effect can be systematically separated from GVHD, the tumor microenvironment seems a likely source of opportunity for enrichment of tumor-specific reactivity after allotransplant. Immune therapy strategies that build on manipulation of the tumor microenvironment *in vivo*, such as single-fraction radiation, might improve systemic antitumor efficacy, possibly through enhanced tumor-associated antigen presentation, without increasing nonspecific alloreactivity.

Animal models suggest that single-fraction radiation may augment the antitumor effects of adoptive cell therapy in the autologous setting, and might improve the tumor recognition of cell therapy in the allogeneic setting as well. The 8-Gy dose of single-fraction radiation has shown promise in animal models and has been safely delivered to humans. Radiation has been administered to patients after allotransplant with reasonably limited toxicity. The 8-Gy dose will be used in this study.

This protocol will enroll subjects with progressive or recurrent hematologic malignancies after allotransplant, despite successful donor T cell engraftment and an attempt to reduce immune suppression. Since the complexity of progressive tumor after allotransplant requires individualized patient management, and proven curative treatment options are not available in this setting, additional prior treatment requirements will not be specified for eligibility. Instead, potential subjects will be presented at a multidisciplinary case conference to determine whether and when enrollment on this and/or other relapse treatment protocols so that the interests of the patients are best served.

All patients will receive irradiation administered as a single fraction of 8 Gy to the maximum tumor volume that can safely be irradiated, while leaving tumor outside of the radiation field to permit evaluation for a systemic response. Patients without significant GVHD and who have access to donor lymphocytes will subsequently receive a DLI, with dosing that is standard for treatment of persistent disease. Patients with a history or high risk of significant GVHD and/or who do not have donor lymphocytes available will receive radiation alone. Subjects will be followed for toxicity, with the 50% incidence of GVHD in patients who respond to DLI as the standard for safety evaluation. Subjects will also be followed for any increased local and/or systemic radiation toxicity. We will be evaluating for evidence of systemic GVT responses after radiation, using FDG-PET/CT and tumor staging of disease that is outside the field of radiation. Additionally, we will study the tumor and circulating lymphocytes before and after radiation, comparing lymphocyte populations with those of donors and of control DLI recipients who will not have received radiation, to better understand the mechanisms and effectors of GVT and the determinants of its success or failure.

Resources: We have unique resources available to investigate post-allotransplant therapies, including a mission to develop cutting-edge therapeutics for cancer, a large and dynamic immunology community with the technical expertise to address knowledge gaps in our understanding of GVT and mechanisms of immune escape, extensive experience and expertise within the NCI Radiation Oncology Branch in safe and innovative approaches to administration of radiation therapy, and protocols that are actively enrolling patients to treat refractory hematologic malignancies with allotransplant. Additional collaborative relationships have been established within the NIH community and outside investigators in order to maximize the opportunities for understanding the mechanisms and limitations of allogeneic cell therapies.

Ongoing NCI Trials of Allotransplant for Hematologic Malignancy: Several studies of allotransplant for hematologic malignancies are underway. The following protocols are currently recruiting and could potentially yield a population of patients with persistent or progressive disease after allotransplant who might benefit from strategies that take advantage of the allogeneic platform for disease control.

- 04-C-0055 (Sirolimus in Preventing Graft-Versus-Host Disease in Patients With Hematologic Malignancies Who Are Undergoing Allogeneic Hematopoietic Stem Cell Transplantation)
- 07-C-0195 (Pilot Trial of Targeted Immune-Depleting Chemotherapy and Reduced-Intensity Allogeneic Hematopoietic Stem Cell Transplantation Using HLA-matched Unrelated Donors and Utilizing Two Graft-versus-Host Disease Prophylaxis Regimens for the Treatment of Leukemias, Lymphomas, and Pre-malignant Blood Disorders)

It is anticipated patients with relapsed or refractory hematologic malignancies after allotransplant and DLI, who were treated at other institutions, including National Heart, Lung and Blood Institute (99-C-0050, 01-H-0162. 04-H-0112) and extramural oncology programs, will be enrolled on this study. Patients who have been treated with alternative donor allotransplant (including matched unrelated and unrelated umbilical cord blood donors), for whom donor lymphocyte therapy is rendered difficult or impossible due to issues of donor access, will also be eligible for enrollment.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria: Treatment Subjects

2.1.1.1 Patients must have received allotransplant (related or unrelated donor) for hematologic malignancies and have disease progression with a component of solid-phase disease. Eligible diagnoses will include any acute or chronic leukemia with a solid-phase component, Hodgkin's lymphoma, any non-Hodgkin's lymphoma, including mantle cell lymphoma, multiple myeloma. Pathology slides from patients' pretransplant diagnoses will be reviewed by NCI/CCR Department of Pathology.

2.1.1.2 Patients must have at least two distinct sites of disease.

- At least one site must be in solid phase and amenable to irradiation, determined by Radiation Oncology evaluation.
- In addition to the target(s) of irradiation, there must be disease that is discrete from local effects of the radiation that can be evaluated for systemic response to therapy, as detailed in Appendix C.

2.1.1.3 Patients must have disease that has failed to respond after a minimum of four weeks to:

- Evidence of complete donor-T cell engraftment (>90% chimerism) of the circulating T cells
- A trial of tapering immunosuppressive therapy, including trials that are discontinued due to development or flare of GVHD

2.1.1.4 Patients must be 18 – 75 years of age. (Note: an amendment is planned to include pediatric patients if safety is established as outlined in Section 6.)

2.1.1.5 ECOG performance status ≤ 3 (Karnofsky performance status $\geq 50\%$).

2.1.1.6 Life expectancy $\geq$ [redacted] month.

2.1.1.7 Arm A

2.1.1.7.1 Patients with minimal to no clinical evidence of acute GVHD (Grade 0-I) or mild- chronic GVHD (Appendix B, GVHD Score of no more than 1 in no more than two organ systems)[75] while off of systemic immunosuppressive therapy.

2.1.1.7.2 Available source of clinical donor lymphocyte cell product, including stem cell-mobilized product.

2.1.1.7.3 Patients whose related allotransplant donor is available, eligible and enrolled on this or another NIH/CC protocol that permits collection of a clinical donor lymphocyte cell product, and donors are first-degree relatives with genotypic identity at 5-6/6 HLA loci (HLA- A, B, and DR. Haploidentical (<5/6 genotypic identity) allotransplant recipients will not be eligible, due to risk of severe GVHD with DLI.

2.1.1.7.4 Patients whose related or unrelated allotransplant donors are unavailable or ineligible, but who have cryopreserved donor lymphocyte cell products available for use on this trial.

2.1.1.8 Arm B

2.1.1.8.1 Patients with history of GVHD. Specifically:

a. Patients who have a past history of resolved grade III acute GVHD or moderate/severe chronic GVHD and who are no longer requiring systemic therapy to treat GVHD.
b. Patients who require continued prophylaxis with steroid-sparing agents, e.g., cyclosporine. Due to concerns that sirolimus (rapamycin) could interfere with the potential efficacy of radiation-enhanced allogeneic cell therapy[59] (Section 1.2, Immunologic Effects of Radiation), patients on sirolimus as part of GVHD control must be switched to another agent two weeks prior to enrollment.
c. Patients with GVHD controlled with local therapy, e.g., topical steroids, budesonide.
d. Patients with controlled acute GVHD (Grade I-III) or chronic-moderate/severe GVHD on a stable (at least four weeks) or tapering dose of systemic immunosuppression will be eligible for enrollment.

2.1.1.8.2 Patients who do not have donor lymphocytes available for use on this trial, including recipients of unrelated donor allografts.

2.1.1.8.3 Patients whose allotransplant was from a haploidentical (<5/6 genotypic identity) related donor.

2.1.1.8.4 Provision for a Durable Power of Attorney.

2.1.1.8.5 Ability to give informed consent.

2.1.2 Inclusion Criteria: Donor Subjects

2.1.2.1 Donors are the same individual whose cells were used as the source for the enrolling Arm A Treatment Subject or DLI Control Subject's original allotransplant.

2.1.2.2 Age 18 – 90.

2.1.2.3 Adequate venous access for peripheral apheresis, or consent to use a temporary central venous catheter for apheresis.

2.1.2.4 Donors must be HIV negative, hepatitis B surface antigen negative, and hepatitis C antibody negative.

2.1.3 Inclusion Criteria: DLI Control Subjects

The DLI Control Subjects will serve as a comparison for Arm A, and eligibility criteria are intended to enroll subjects who are similar with respect to allotransplant characteristics.

2.1.3.1 Patients must be 18 – 75 years of age.

2.1.3.2 Patients who have received an allotransplant to treat malignancy and who are going to receive an unmanipulated or stem-cell mobilized DLI to treat persistent tumor as part of their treatment program on another NIH/CC protocol.

2.1.3.3 ECOG performance status $\leq$ 3 (Karnofsky performance status $\geq$ 50%).

2.1.3.4 Life expectancy $\geq$ 1 month.

2.1.3.5 Patients with minimal to no clinical evidence of acute GVHD (Grade 0-I) or mild- chronic GVHD (Appendix B, GVHD Score of no more than 1 in no more than two organ systems)[75] while off of systemic immunosuppressive therapy.

2.1.3.6 Available source of clinical donor lymphocyte cell product, including a stem cell-mobilized product.

2.1.3.6.1 Patients whose related allotransplant donor is available, eligible and enrolled on this or another NIH/CC protocol that permits collection of a clinical donor lymphocyte cell product, and donors are first-degree relatives with genotypic identity at 5-6/6 HLA loci (HLA- A, B, and DR. Haploidentical (<5/6 genotypic identity) allotransplant recipients will not be eligible, for consistency with Arm A Subjects.

2.1.3.6.2 Patients whose related or unrelated allotransplant donors are unavailable or ineligible, but who have cryopreserved donor lymphocyte cell products available for clinical use on this trial.

2.1.3.7 Patients must have disease that has failed to respond after a minimum of four weeks to:

2.1.3.7.1 Evidence of complete donor-T cell engraftment (>90% chimerism) of the circulating T cells.

2.1.3.7.2 A trial of tapering immunosuppressive therapy, including trials that are discontinued due to development or flare of GVHD.

2.1.3.8 Adequate venous access for peripheral apheresis, or consent to use a temporary central venous catheter for apheresis or consent to a large-volume (70cc) blood draw.

2.1.3.9 Permission from their treating transplant physician or designee to participate on study.

2.1.3.10 Ability to give informed consent.

2.1.4 Exclusion Criteria: Treatment Subjects

2.1.4.1 Tumor-directed therapy within two weeks of DLI.

2.1.4.2 Patients with rapid disease progression or aggressive tumor histology which, in the opinion of the PI, is likely to require urgent therapy within 60 days in order to preserve organ function or quality of life, and there is an available standard therapy to which the patient has a reasonable chance of responding.

2.1.4.3 Progressive disease that, in the opinion of the PI, requires urgent standard therapy, e.g., threatened organ function, acceptable quality of life, etc.

2.1.4.4 Uncontrolled GVHD, i.e., either acute GVHD Grade III or chronic-moderate/severe GVHD that has not responded to the current dose of systemic therapy or any history of steroid-refractory acute GVHD, Grade IV acute GVHD, or chronic-severe GVHD.

2.1.4.5 Active infection that is not responding to antimicrobial therapy.

2.1.4.6 Active psychiatric disorder which may compromise compliance with transplant protocol, or which does not allow for appropriate informed consent (as determined by Principal Investigator and/or her designee).

2.1.4.7 Pregnant or lactating. Subjects of childbearing potential must use an effective method of contraception (4.6). The effects of the immunosppressive medications that could be required to treat GHVD are likely to be harmful to a fetus. The effects upon breast milk are also unknown and may be harmful to an infant.

2.1.4.8 Absolute neutrophil count of less than 500 cells/μl. At the PI's discretion, patients with marrow replacement by tumor as the probable etiology of an absolute neutrophil count of less than 500 cells/μl may be eligible for enrollment.

2.1.4.9 In order to prevent delay of potentially stabilizing palliative therapy, the following conditions will exclude eligibility: untreated active leptomeningeal involvement with malignancy, untreated brain metastasis, and other organ-threatening diseases in which palliative treatment options with reasonable probability of efficacy (15% or higher) are available. Patients with these conditions for whom available palliative options have been tried or deemed unacceptable but who otherwise meet eligibility criteria may, at the discretion of the PI, be considered for enrollment.

2.1.5 Exclusion Criteria: Donor Subjects

2.1.5.1 History of a psychiatric disorder that the PI determines might compromise compliance with transplant protocol, or that does not allow for appropriate informed consent.

2.1.5.2 Hypertension that is not controlled by medication, history of stroke, or severe heart disease (donors with symptomatic angina will be excluded). Donors with a history of coronary artery bypass grafting or angioplasty who are symptom free will receive a cardiology evaluation and be considered on a case-by-case basis.

2.1.5.3 History of prior malignancy. However, cancer survivors who have undergone potentially curative therapy and have had no evidence of that disease for at least 5 years may be considered for lymphocyte donation on a case-by-case basis.

2.1.5.4 Anemia (Hb < 11 gm/dl) or thrombocytopenia (platelets < 100,000 per ml). However, potential donors with Hb levels < 11 gm/dl that is due to iron deficiency will be eligible as long as the donor is initiated on iron replacement therapy and the case is individually approved by NIH DTM.

2.1.5.5 Pregnancy. Donor Subjects of childbearing potential must use an effective method of contraception (Section 4.6) until after completion of apheresis. The effects of apheresis are unknown to be safe to a fetus.

2.1.6 Exclusion Criteria: DLI Control Subjects

2.1.6.1 Tumor-directed therapy within two weeks of DLI.

2.1.6.2 Uncontrolled GVHD, as defined in Section 2.1.26.

2.1.6.3 Pregnant or lactating. Subjects of childbearing potential must use an effective method of contraception (Section 4.6) until after completion of apheresis. The effects of apheresis are unknown to be safe to a fetus.

2.1.6.4 History of a psychiatric disorder that the PI determines might compromise compliance with protocol, or that does not allow for appropriate informed consent.

2.1.6.5 Hypertension that is not controlled by medication, history of stroke, or severe heart disease (subjects with symptomatic angina will be excluded).

2.2 RESEARCH ELIGIBILITY EVALUATION

Given potential morbidity within this patient population, a preliminary examination of records and determination of probable eligibility will be performed by protocol investigators from NCI/CCR/ETIB and NCI/CCR/ROB prior to protocol screening, if possible, to facilitate an expedited evaluation of prospective treatment subjects and enrollment and treatment of eligible patients.

2.2.1 Treatment Subjects:

Patients must have discontinued all cytotoxic and tumor-directed immunotherapy at least two weeks prior to planned radiation.

2.2.1.1 Chimerism studies: Demonstration of prior complete donor chimerism after engraftment one or months following allotransplant. Chimerism results from outside institutions will be permitted if performed by a CLIA-certified laboratory. Acceptable evidence of full donor chimerism will include demonstration of >95% donor chimerism of bone marrow or whole peripheral blood, >90% donor chimerism of T-cell peripheral blood lymphocytes. If results are not available, or if less than full-donor chimerism is thought to be a reflection of residual tumor, chimerism analyses may be performed at the CC on the bone marrow and/or PBMC and/or CD3+ subsets (delivery of 3 yellow-top tubes, approximately 30 ml, to NIH CC Department of Laboratory Medicine/Hematology).

2.2.1.2 The following parameters must be performed within 30 days of enrollment to determine eligibility, disease status, and to facilitate assessment of radiation risk and identification of best site of radiation with least risk to patient. At the discretion of the PI/designee, radiology and laboratory studies obtained at outside institutions may be used to determine eligibility.

2.2.1.2.1 Complete medical history and physical examination, including Acute and Chronic GVHD Staging Evaluation (See Appendices A and B)

2.2.1.2.2 Evaluation by Radiation Oncology

2.2.1.2.3 CBC with differential, PT, and PTT, Chem-20, Quantitative lymphocyte subset panel (TNBK), Serum or urine β-HCG in females of childbearing potential, and ABO typing

2.2.1.2.4 Infectious disease screening studies, including hepatitis A, B, and C, T. cruzi (Chagas' agent), CMV, adenovirus, EBV, HSV, and toxoplasma and HIV. (Results of these studies will not affect eligibility but are clinically valuable should subjects require immune suppression. Results obtained from outside institutions as part of the initial transplant evaluation may be substituted).

2.2.1.2.5 Pulmonary function testing (PFT), including DLCO measurement within twelve weeks of enrollment. For patients who will not receive radiation to the lungs or chest wall and who do not have a history of chronic respiratory symptoms or who have had stable pulmonary status, PFTs obtained three or more months after allotransplant and within twelve months prior to enrollment may

be substituted. Results of PFTs will be used in the Radiation Oncology and Chronic Graft-vs-Host Disease assessments.

2.2.1.2.6 CT scans of chest, abdomen, and pelvis; neck if history warrants.

2.2.1.2.7 CT or MRI scan of the head.

2.2.1.2.8 Whole-body FDG-PET/CT scan, at the discretion of the PI or designee. See baseline staging requirements, Section 3.2.1.

2.2.1.2.9 For recipients with multiple myeloma: serum protein electrophoresis with M protein; serum IGG level; 24h collection of urine for urinary protein excretion, protein electrophoresis and M protein; B2 microglobulin; immunofixation if M protein is undetectable; skeletal survey.

2.2.1.2.10 Bone marrow aspiration and biopsy with flow cytometry, cytogenetics, and molecular studies as clinically appropriate and required for restaging. A second bone marrow aspiration obtained through the same skin puncture will be used for research studies, detailed in Section 3.3.3, and be delivered to the ETIB Preclinical Support Service, Building 10, Room 12C216.

2.2.1.2.11 Histologic confirmation of malignant diagnosis and relapse. Specimens/Slides obtained at outside institutions for the initial diagnosis and confirmation of relapse must be submitted for review by NCI Department of Pathology. If indicated, a repeat biopsy will be done to confirm pathology of persistent disease.

2.2.1.2.12 Patients at high risk of leptomeningeal disease or signs/symptoms suggestive of leptomeningeal involvement will have lumbar puncture for evaluation of tumor involvement, with cerebral spinal fluid cytology, cell counts and routine chemistries (i.e. glucose and protein).

2.2.1.3 The following studies may need to be repeated to ensure that they are performed within 5 days prior to enrollment to determine clinical status and continued eligibility.

2.2.1.3.1 CBC with differential and Chem-20.

2.2.1.3.2 Acute and Chronic GVHD Staging Evaluation (See Appendices A and B).

2.2.1.3.3 Serum or urine β-HCG in females of childbearing potential.

2.2.2 Donor Subjects:

Clinical and Laboratory Evaluation of the Donor (of recipients who will receive DLI) must be performed within 28 days of enrollment:

2.2.2.1.1 Complete history and physical examination.

2.2.2.1.2 Infectious disease screening studies in accordance with DTM policy, which currently include hepatitis A, B, and C; HIV 1/2, HTLV, T. cruzi (Chagas' agent), CMV, adenovirus, EBV, HSV, toxoplasma and syphilis.

2.2.2.1.3 CBC with differential, PT, and PTT, Chem-20, Quantitative lymphocyte subset panel (TNBK) and ABO typing.

2.2.2.1.4 Serum or urine β-HCG in females.

2.2.2.1.5 VNTR (PCR) of DNA mini-satellite regions for determination of recipient hematopoietic cell chimerism. If these results are available for use from prior studies, this component of the screening evaluation may be omitted.

2.2.3 DLI Control Subjects:

Clinical and Laboratory Evaluation of the DLI Control Subjects (of recipients who will receive DLI) must be performed within 28 days of enrollment:

2.2.3.1.1 Complete history and physical examination, including Acute and Chronic GVHD Staging Evaluation (See Appendices A and B).

2.2.3.1.2 CBC with differential, PT, and PTT, Chem-20, Quantitative lymphocyte subset panel (TNBK) and ABO typing.

2.2.3.1.3 Serum or urine βHCG in females

2.3 REGISTRATION PROCEDURES

2.3.1 Protocol Entry Date

Protocol "entry date" is considered to be the day that the informed consent form has been signed by the patient. The treatment start date is considered to be the day the Treatment Subjects undergo radiation therapy. As not all subjects will have available donors, the timing of patient enrollment is not contingent upon donor enrollment.

2.3.2 Registration

Authorized staff must register an eligible candidate with Central Registration Office (CRO) no later than 24 hours after the patient has signed the consent form. The patient must be registered prior to beginning this study. A registration Eligibility Checklist is available from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) and must be completed and faxed to 301-480-0757. For questions regarding registration authorized staff should call 301-402-1732 between the hours of 8:30 a.m. and 5:00 p.m., Monday through Friday. Voicemail is available during non-business hours.

2.3.3 Off- Study Procedure:

Authorized staff must notify Central Registration Office (CRO) when a patient is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and faxed to 301-480-0757.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1.1 Overview:

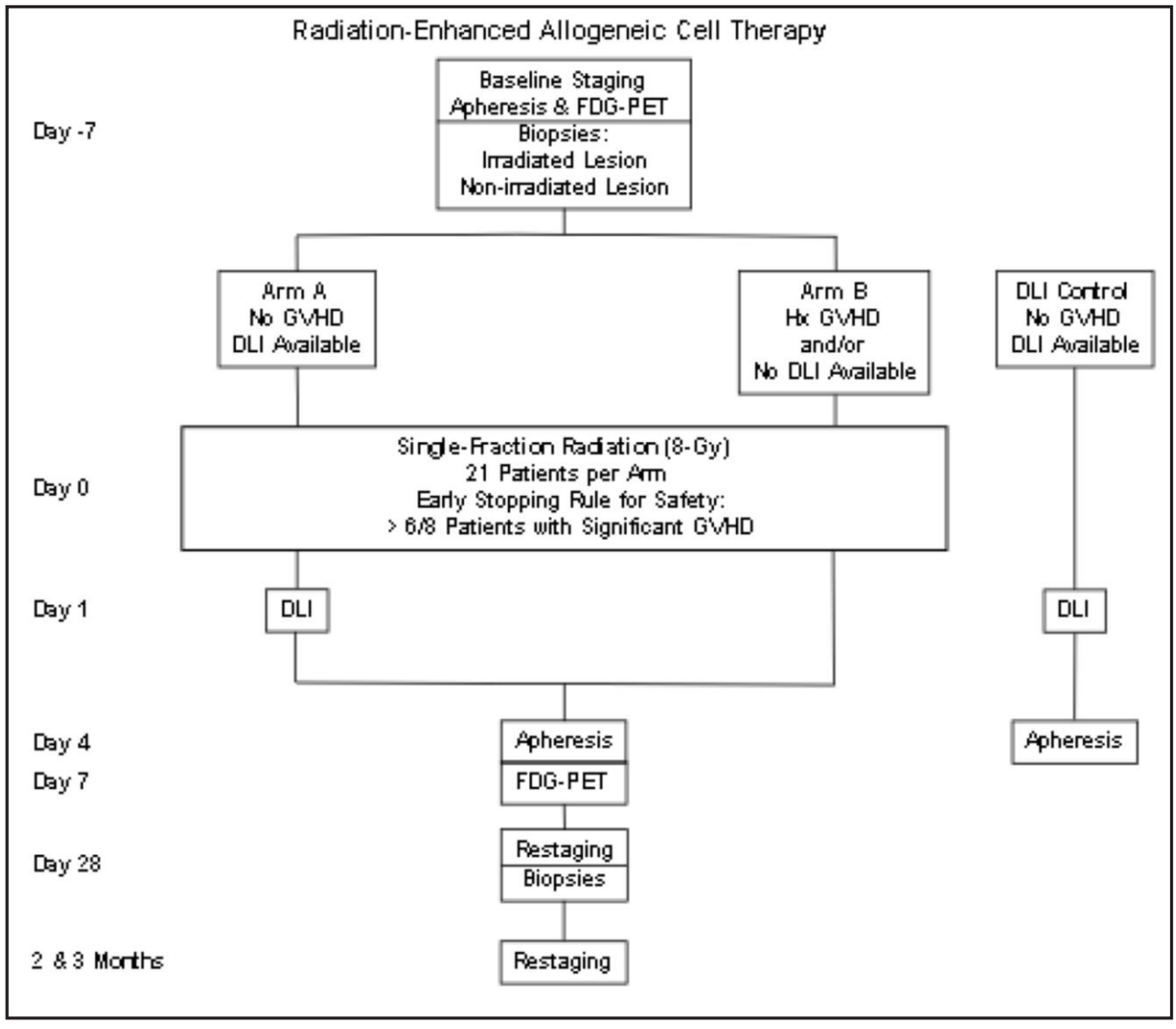
Study Treatment Subjects will be assigned to one of two treatment arms, based on feasibility of administering DLI. All Treatment Subjects receive irradiation in a single, 8-Gy fraction to the maximum number of lesions that can safely be irradiated, while leaving non-irradiated, measurable disease to evaluate a systemic response. Subsequent therapy will be based on Arm:

3.1.1.2 Arm A (DLI): Subjects who have a source of donor lymphocytes available and who are not at high risk of significant GVHD. These subjects will receive a standard DLI on Day 1 after radiation. Administration of a stem cell-mobilized lymphocyte product may be used in subjects whose available lymphocyte product is from a mobilized collection.

3.1.1.3 Arm B (No DLI): Subjects who do not have a source of donor lymphocytes available and/or who have a high risk of significant GVHD, including those who have a history of significant GVHD (Grade II-IV Acute GVHD or Chronic-Severe GVHD (Appendix B, Score 3) and/or those who have received an allotransplant with a haploidentical related donor. These subjects will not receive DLI.

3.1.1.4 DLI Control Subjects will receive standard, unmanipulated DLI on this study; administration of a stem cell-mobilized lymphocyte product may be used in subjects whose available lymphocyte product is from a mobilized collection. They will be assessed clinically and will have blood draws and aphereses for research, in order to provide a control for Arm A subjects in the evaluation for changes in circulating immune cell populations.

3.2 SCHEMA



3.3 STUDY IMPLEMENTATION (APPENDIX E, STUDY TIME POINTS)

3.3.1 Baseline Tumor Evaluation:

Studies that are completed at the Clinical Center within two weeks prior to receiving radiation may be used as baseline measurements.

3.3.1.1 Whole-body FDG PET/CT scan (top of head to bottom of feet)

3.3.1.2 CT scan(s) of areas of measurable disease that were identified on prior imaging and/or PET/CT. These will include CT imaging of sites that will be radiated and the non-radiated index lesion(s) that will be used to monitor systemic response. (The CT images obtained with PET/CT are low-resolution and not intended or reliable for tumor size measurements.) At the discretion of the PI/LAI/designee, in consultation with Radiologist AI/designee, a dedicated CT scan may be omitted if images from PET-CT are adequate for tumor measurements.

3.3.1.3 CT or MRI scan of the head. At the discretion of the PI/LAI or designee, subjects without prior CNS disease, tumors without a high propensity for CNS involvement, no evidence of CNS disease on PET-CT and no symptoms suggestive of CNS involvement, this study may be omitted.

3.3.1.4 For Treatment Subjects with multiple myeloma: serum protein electrophoresis with M protein; serum IgG level; 24h collection of urine for urinary protein excretion, protein electrophoresis and M protein; B2 microglobulin; immunofixation if M protein is undetectable; skeletal survey.

3.3.1.5 Bone marrow aspiration and biopsy with flow cytometry, cytogenetics, and molecular studies as clinically appropriate and required for restaging. A portion of the bone marrow aspirate will be used for research studies, detailed in Section 3.3.3, and be delivered to the ETIB Preclinical Support Service, Building 10, Room 12C216.

The following evaluations must be completed within 2 days prior to radiation:

3.3.1.6 Directed medical history and physical examination, with Performance Status

3.3.1.7 CBC with differential, Chem-20

3.3.1.8 Acute and Chronic GVHD Staging Evaluation (See Appendices A and B)

3.3.2 Apheresis

3.3.2.1 Treatment Subject Research Apheresis

3.3.2.1.1 Baseline: Prior to radiation, all Treatment Subjects will undergo a 2-liter monocyte-enriched mononuclear cell apheresis procedure on a CS-3000 or equivalent machine. Cells will be used for research, as a control for laboratory evaluation of the phenotypic and functional characteristics of circulating cell populations after radiation with or without DLI. The apheresis product will be delivered to the ETIB Preclinical Support Service, Building 10, Room 12C216, where it will be divided into aliquots and cryopreserved. Baseline apheresis may be omitted in subjects who have had collections as part of another NCI ETIB protocol and who have not had intervening changes in cancer or immunosuppressive therapy at the discretion of the PI or designee. At the PI's discretion, in order to expedite on-study treatment, a large-volume (70cc) whole blood collection may substitute for apheresis.

3.3.2.1.2 Post-Treatment: Four days +/- 1 day following radiation, all Treatment Subjects will undergo a 2-liter monocyte-enriched mononuclear cell apheresis procedure on a CS-3000 or equivalent machine. Cells will be used for research, to evaluate the phenotypic and functional characteristics of circulating cell populations after radiation with or without DLI. The apheresis product will be delivered to the ETIB Preclinical Support Service, Building 10, Room 12C216, where it will be divided into aliquots and cryopreserved. At the PI's discretion, if the apheresis procedure would pose significant discomfort to the Treatment Subject, a large-volume (70cc) whole blood collection may substitute for apheresis.

3.3.2.2 Donor Subject Clinical and Research Apheresis

As donor availability permits, and for subjects enrolled on Arm A, lymphocyte collection will be performed in subjects' donors. Donors will undergo a 5-liter apheresis procedure (CS-3000 or an equivalent machine).

3.3.2.2.1 The clinical lymphocyte apheresis product will be divided into aliquots based on an anticipated dosing of 1.0 - 2.0 x 107 cells/kg. Lymphocytes for DLI will be cryopreserved, according to DTM policy and procedure, until needed for potential DLI infusion.

3.3.2.2.2 Up to 25% of the product may be used for research immune studies, as a control for research studies done on patient/recipient tissues. Research samples will be delivered to the ETIB Preclinical Support Service.

3.3.2.2.3 Subjects on Arm A who have available donor-lymphocyte clinical products collected under other clinical protocols may receive these cells provided the products have been stored according to clinical guidelines and necessary approvals, e.g., PI of the protocol under which they were collected, are obtained.

3.3.2.2.4 Stem-Cell Mobilized Collections for Clinical Products: Donor-Subjects may be asked to undergo a stem-cell mobilized mononuclear cell collection, in order to provide a stem-cell mobilized DLI to support Treatment Subject bone marrow function, provided standard DTM donation criteria are met and the Donor-Subject signs the procedural consent at time of additional collection. Procedure for stem-cell mobilized collections is detailed in Appendix D.

3.3.2.2.5 Donors will be permitted to return to the CC to donate additional, standard cell products (either steady-state donor lymphocytes or filgrastim-mobilized collections) if necessary to support further Recipient-Subject treatment, whether said treatment is provided on this or another CC protocol. Donor-Subjects will be evaluated per section 2.2.3 to ensure that they continue to be eligible to donate cells for their respective recipients.

3.3.2.3 DLI Control Subject Research Apheresis

Patients who have received allotransplants and who are receiving unmanipulated DLI as part of their cancer treatment on other NIH/CC protocols (per Sections 2.1.14 - 22) will serve as control subjects for *in-vitro* assays of T cell responses. Three days +/- 1 day following DLI, Control Subjects will undergo a mononuclear cell apheresis procedure on a CS-3000 or equivalent machine. The apheresis product will be used solely for research, as a control for the laboratory evaluation of the phenotypic and functional characteristics of circulating cell populations after radiation with DLI. The product will be delivered to the ETIB Preclinical Support Service, Building 10, Room 12C216, where it will be divided into aliquots and cryopreserved. At the PI's discretion, if the apheresis procedure would pose significant discomfort or inconvenience to the DLI Control Subject, a large-volume (70cc) whole blood collection may substitute for apheresis.

3.3.3 Radiation

3.3.3.1 Subjects will have all lesions that are deemed safe and amenable to irradiation treated with a single, 8-Gy fraction. At least one site will be left non-irradiated for assessment of systemic response.

3.3.3.2 The radiation target(s) will be selected by one of the Radiation Oncology Associate Investigators according to the following criteria, which at the 8-Gy dose, is expected to result in minimal toxicity, i.e., CTCAEv4 Grades 0-2, with treatable Grade 3 possible, but unlikely.

3.3.3.3 The target volume will encompass all sites of gross disease that can be safely irradiated with minimal damage to surrounding normal tissue. Lesions of a size or in a location that would require irradiation of an organ or tissue beyond radiation tolerance will not be considered a target.

3.3.3.4 Radiation of the target volume would not have direct, local effect on all sites of measurable disease maintaining the ability to assess systemic response (i.e., the site chosen as the non-irradiated measurable disease must not be inside the irradiated volume).

3.3.3.5 The target volume will not include immune-privileged sites (brain, eye, testis)

3.3.3.6 The target volume does not include tissue previously or currently affected by Grade 3 or higher acute GVHD or severe chronic GVHD.

3.3.3.7 The following guidelines will be used to determine the target volume:

3.3.3.7.1 The target volume will encompass as many lesions as possible, minus one.

3.3.3.7.2 Exclusion of brain in target volume;

3.3.3.7.3 No more than 10 cm linear length of spinal cord inclusion in the target volume;

3.3.3.7.4 Exclusion of more than 60% of the lung volume from the target volume required;

3.3.3.7.5 No more than 50% of the abdomen can receive the prescription dose (defined as from the top of the diaphragm to the pelvic brim);

3.3.3.7.6 No more than 50% of the liver can receive the prescription dose;

3.3.3.7.7 No more than 60% of the pelvis can receive the prescription dose, with the exception of the situation in which a space occupying lesion pushes normal pelvic tissues such that they are largely excluded from the target volume; and

3.3.3.7.8 No more than 66% of one kidney or the equivalent volume of each kidney (for example 33% of both kidneys) can receive the prescription dose).

3.3.3.8 Simulation and Irradiation: All treatment subjects will be CT-simulated in the radiation oncology clinic with positioning and immobilization as needed to provide maximal normal tissue sparing while providing reproducible patient setup. Tumor volumes will be defined as follows:

3.3.3.8.1 Gross tumor volume (GTV) will be defined as the area of tumor as evident on clinical examination and radiographic imaging.

3.3.3.8.2 The clinical target volume (CTV) will be defined as the GTV with 1 cm margin with corrections for barriers to spread, such as bone and fascial planes. The planning target volume (PTV) will be defined as the CTV with an additional margin of 1 cm. In certain circumstances (i.e. tumor situated in lung or liver), additional margin may be applied at the discretion of the treating radiation oncologist to account for organ motion. This additional margin may be asymmetric and should not exceed an additional 1 cm in any dimension.

3.3.3.8.3 The PTV should be encompassed within the 95% isodose line in the accepted treatment plan. Treatment will be delivered in a single fraction of 8 Gy after portal films or other positioning verification films are obtained.

3.3.4 Donor Lymphocyte Infusion

3.3.4.1 Arm A: Treatment Subjects in Arm A who have no evidence of significant GVHD (i.e., acute Grade II or chronic extensive), verified at their Radiation Day +1 evaluation (Section 3.3.1) will receive an unmanipulated donor lymphocyte infusion. Note: for subjects whose available lymphocyte product is from a stem cell-mobilized collection, or for whom a mobilized product is clinically indicated, a mobilized product may be administered.

3.3.4.1.1 Cell dose will be 1.0 x 10^7 $CD3^+$ cells/kg for subjects who have had matched-related donor allotransplants. If donors are not available, the cell dose will be determined by what is available, but will not exceed 1.0 x 10^7 $CD3^+$ cells/kg. If cells from an unrelated donor are available, the cell dose will be determined by what is available, but will not exceed 1.0 x 10^6 $CD3^+$ cells/kg. Administration of a log lower DLI cell dose is standard practice in recipients of unrelated donor allotransplants, due to the higher risk of GVHD in URD recipients. In cases when the available cell product is stem-cell mobilized (as is the usual case in unrelated-donor recipients and may be the case in related-donor recipients), cell dosing will follow the same guidelines detailed above, with note made of the $CD34^+$ cell dose in the CRIS cell product order. Since cell products are stored in aliquots based on the recipient weight at the time of storage, which may be different from the weight at the time of cell-

product infusion, at the discretion of the AI/designee, the weight of the recipient used to determine cell dose for storage may be used, in order to avoid additional manipulation of the cell product.

3.3.4.1.2 Cryopreserved donor lymphocytes will be thawed and immediately administered intravenously within 24 - 48 hours of completing radiation. Donor cell products collected on study may be administered fresh.

3.3.4.1.3 Cell product infusion monitoring practices are detailed in Section 4.5. No steroids will be allowed for management of DMSO-related toxicities (chills, muscle aches) that may occur after cellular infusion (diphenhydramine and meperidine are allowed).

3.3.4.1.4 Treatment Subjects for whom there is clinical concern for development or flare significant GVHD (consistent with acute Grade II or chronic extensive) at their Day +1 evaluation will be reevaluated at Day +2.

3.3.4.1.4.1 If clinical concern for significant GVHD has resolved, subjects will remain eligible for DLI as specified in Section 2.1.6.

3.3.4.1.4.2 If clinical concern for significant GVHD persists, these Subjects will not receive DLI and will undergo appropriate diagnostic evaluation to ascertain a diagnosis (Section 5.3). These Subjects will remain on study and receive all subsequent evaluations as scheduled.

3.3.4.2 DLI Control Subjects: DLI Control Subjects who have no evidence of significant GVHD (i.e., acute Grade II or chronic extensive), verified with a clinical assessment the day of their scheduled DLI ("Day 0"), will receive an unmanipulated donor lymphocyte infusion. Note: for subjects whose available lymphocyte product is from a stem cell-mobilized collection, or for whom a mobilized product is clinically indicated, a mobilized product may be administered.

3.3.4.2.1 Cell dose will be 1.0 x 10^7 $CD3^+$ cells/kg for subjects who have had matched-related donor allotransplants. If donors are not available, the cell dose will be determined by what is available, but will not exceed 1.0 x 10^7 $CD3^+$ cells/kg. For subjects who have had unrelated donor allotransplants, if cells from an unrelated donor are available, the cell dose will be determined by what is available, but will not exceed 1.0 x 10^6 $CD3^+$ cells/kg. (Administration of a log lower DLI cell dose is standard practice in recipients of unrelated donor allotransplants, due to the higher risk of GVHD in URD recipients.)

3.3.4.2.2 Cryopreserved donor lymphocytes will be thawed and immediately administered intravenously. Donor lymphocytes (mobilized or not) collected on study may be administered fresh.

3.3.4.3 Cell product infusion monitoring practices are detailed in Section 4.5. No steroids will be allowed for management of DMSO-related toxicities (chills, muscle aches) that may occur after cellular infusion (diphenhydramine and meperidine are allowed).

3.3.5 Treatment of progressive disease post-radiation therapy:

If possible, Treatment Subjects should not receive any systemic tumor therapy (including steroids) for a minimum of 12 weeks after radiation. However, if systemic therapy is necessary to preserve organ function or quality of life in the 3-month period following radiation, Treatment Subjects will remain on study so they will continue to be monitored for toxicity.

3.4 CORRELATIVE STUDIES FOR RESEARCH

3.4.1 Clinical Evaluation:

3.4.1.1 History and Physical Examination:

3.4.1.1.1 Treatment Subjects will be evaluated on Days +1, 4, 7, 14, 28, 56, and 84 after radiation. At the discretion of the PI or designee, the Day 7 or the Day 14 clinical evaluation may be performed by

the patient's local physician, e.g., if travel to the NIH poses hardship. Day 7 and 14 evaluations may be performed within two days of the target day if necessary schedule around a weekend/holiday. Subsequent evaluations may be performed within three days of the target day, to facilitate scheduling.

3.4.1.1.2 DLI Control Subjects will be evaluated on the day they will receive their DLI, and Days +3 (+/-1), 7, 14 and 28 after DLI. At the discretion of the PI or designee, the Day 7, 14 and/or the Day 28 clinical evaluation may be performed by the patient's local physician, e.g., if travel to the NIH poses hardship. Days 7 and 14 evaluations may be performed within two days of the target day, and the Day 28 evaluation may be performed within three days of the target day, if necessary to schedule around a weekend/holiday.

3.4.1.2 Safety Evaluation will be a component of all clinical assessments outlined above for Treatment Subjects and DLI Control Subjects, and will include an Acute and Chronic GVHD Staging Evaluation (Appendices A and B), an assessment of toxicity according to CTCAEv4, and clinical blood work, including a CBC with differential and Chem-20.

3.4.1.3 Efficacy Evaluation of Response (Treatment Subjects)

3.4.1.3.1 Systemic responses will be determined by the combined response of measurable disease according to the Clinical Response Criteria and Definitions listed in Appendix C. Systemic response measurements will exclude irradiated sites of disease.

3.4.1.3.2 Response will be measured by disease-appropriate staging studies, as detailed in Baseline Tumor Evaluation (Section 3.2.2.1), and will include a Day +28 clinical FDG-PET/CT scan. Post-treatment restaging will include a bone marrow biopsy and aspirate for all Treatment Subjects at the Day +28 evaluation; subsequent bone marrow studies for restaging will be obtained if indicated for complete disease assessment, but may be omitted in patients without a history of bone marrow involvement.

3.4.1.3.3 Evaluation for clinical response will take place at the following time points: 4 weeks, 8 weeks and 12 weeks (+/- 3 days) after radiation

3.4.1.3.4 Responding patients will continue to be followed as detailed in Section 3.5.1.

3.4.1.3.5 In addition, restaging studies may be performed as clinically indicated

3.4.1.3.6 Responses to radiated lesions will be evaluated by ROB investigators using standard procedure (form in Appendix D).

3.4.1.4 Record Review for GVHD and Response (DLI Control Subjects)

3.4.1.4.1 Medical Records of DLI Control Subjects will be reviewed for development of GVHD and treatment response by 12 weeks post-DLI

3.4.1.4.2 Phone call clarifications may be made to the Control Subjects and/or treating physicians within six months of enrollment

3.4.2 Research Evaluation with FDG-PET/CT (All Treatment Subjects)

3.4.2.1 On Day +7 (up to Day +10 permitted to facilitate scheduling), Treatment Subjects will have an FDG-PET/CT scan performed for research purposes. This PET-CT for research will use a 15 mCi dose of radioactive material, and the total amount of radiation Treatment Subjects will receive for research in this study is 1.75 rem. This is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee. The Day +7 exam will be compared with the Baseline and Day +28 FDG-PET/CT scans, to evaluate whether there are any changes in SUV intensity that would be consistent with a systemic immune response, specifically in non-irradiated tumor or irradiated-tumor-draining lymph nodes.

3.4.2.2 Additional FDG-PET/CT imaging may be obtained at subsequent restaging visits if, in the opinion of the clinical investigator, the information would be helpful in ascertaining a treatment response. These exam(s) will evaluate whether there is any evidence of systemic tumor response using established response criteria (Appendix C).

3.4.3 *In-Vitro* Research Evaluation of Subject Tissue Samples

The PI will report any loss or destruction of samples to the IRB and any new use of the samples, specimens, or data will require prospective IRB review and approval. For all tissues obtain from study subjects, planned *in-vitro* studies fall under the general category of "Immune Characterization." They will focus on characterization of the quantitative, phenotypic and functional properties of distinct cell subsets or tissue/tumor characteristics that may influence an immune response. *In-vitro* assays may include immunohistochemistry, confocal microscopy, flow cytometry, cell proliferation, cytokine production, gene expression, and cytotoxicity. However, the specific assays to be used in the on-going data analyses are subject to be modified, deleted or replaced with evolution of technology and knowledge in the field during the course of the study, without constituting a change in research aims. No change in research subject risk is foreseen from the knowledge acquired from study data. However, if in the judgment of the PI, this should change in the course of the study or if a significant departure from this "Immune Characterization" is contemplated based on accumulated data, then the NCI IRB will be informed to evaluate the eventual need for modification in subject consent process or for re-contacting subjects.

3.4.3.1 Tissue Samples

3.4.3.1.1 Tissue Biopsies

a) Treatment Subjects
The following biopsies will be sent for clinical pathology to confirm tumor histology, and will also be used for research, to determine whether there are changes in the tumor tissues and cell populations that suggest a local and/or systemic tumor-specific immune reaction as a result of the combination of radiation and allogeneic immunotherapy, e.g., IHC for expression of tumor antigens and MHC Class I and Class II, and infiltration of T cell populations (CD4 and CD8). Tumor biopsies will be divided, with portions going to the departments of Pathology and ETIB Preclinical Support Service, Building 10, Room 12C216.
Depending on safety and accessibility of sites of disease, subjects may have tumor biopsies of one radiation target lesion and one non-radiated lesion at up to three time-points (Appendix E): Pre-treatment (up to 2 lesions); 4-7 days post-treatment (up to 2 lesions); and 4 weeks post-treatment (up to 2 lesions). An additional biopsy will be performed if possible to do so with minimal risk or if required for clinical diagnosis, upon development of new tumor lesions (1 lesion). Thus, a total of up to seven biopsies may be taken at up to four occasions. No more than six tumor biopsies will be performed exclusively for research purposes. The pretreatment biopsies will be core-needle biopsies; all subsequent biopsies will be preferably surgical/excisional, but may be core-needle biopsies if sites of tumor are not amenable to surgical excision with minimal risk.

I. Pretreatment Tumor Biopsies (Clinical and Research; up to two sites):
Treatment Subjects with safely accessible tumor will have core needle biopsies of irradiated and non-irradiated lesions within 7 days prior radiation. When feasible, two passes will be made with a core needle to obtain two biopsy specimens within a single tumor site to decrease sampling error.

II. Post-treatment Tumor Biopsies (Primarily Research):

1. 4- 7 days after irradiation (up to two sites). Treatment Subjects with superficial tumors that can be removed under local anesthesia with minimal risk of complication (e.g., cutaneous or subcutaneous tumors) will have excisional biopsies of irradiated and/or non-irradiated superficial tumor.
2. At the 4-week assessment (up to two sites). Treatment Subjects with safely accessible tumors will have biopsies of an irradiated and non-irradiated tumor lesion (the same lesions as pretreatment biopsies, if possible). If accessible through a minimally invasive surgical procedure, samples will preferentially be

obtained by excisional biopsy through the Surgery Consult Service. Thoracotomy or laparotomy will not be performed for research biopsies. If surgical biopsies are not feasible, samples will be obtained with core biopsies through Interventional Radiology. When feasible, two passes will be made with a core needle to obtain two biopsy specimens within a single tumor site to decrease sampling error.

- If it is determined that an excisional biopsy may be possible, the patient will be seen by the surgical consult service and undergo standard preoperative evaluation.
- If it is determined that a core biopsy is necessary, the subjects' clinical status will be evaluated and tumor accessibility reviewed with staff from Interventional Radiology (IR) and undergo standard pre-procedure evaluation.

III. New Lesion Tumor Biopsies (Clinical and Research; up to one site): if post-treatment restaging studies demonstrate new site(s) of disease, Treatment Subjects with safely accessible tumor may undergo biopsy, to confirm diagnosis and compare histologic and immunologic features of the new tumor with radiated and non-irradiated tumor specimens collected on study as detailed above. If accessible through a minimally invasive surgical procedure, biopsy will preferably be obtained by excisional biopsy through the Surgery Consult Service. If surgical excision is not feasible, biopsy will be obtained with core needle through Interventional Radiology. When feasible, two passes will be made with a core needle to obtain two biopsy specimens within a single tumor site to decrease sampling error.

IV. Other Tissues

- Bone Marrow. As part of restaging and in order to evaluate the effect of the therapy on immune cells found in the bone marrow, at the 4-week assessment all Treatment Subjects will undergo a bone marrow aspirate and biopsy. Part of each sample will be collected for research, and be delivered to the ETIB Preclinical Support Service, Building 10, Room 12C216. Additional bone marrow studies may be obtained at subsequent restaging visits if indicated for complete disease assessment, but may be omitted in patients without a history of bone marrow involvement.
- GVHD Biopsies. If Treatment Subjects develop GVHD or other noninfectious inflammatory condition while being treated on this protocol, initial clinical diagnosis shall be supported with tissue biopsy when clinically feasible (Section 3.4.2). If there is sufficient material available, tissue samples will be used in collaboration with other laboratories (ETIB/Gress Laboratory and Clinical Pathology) to identify and characterize phenotypic and functional characteristics of infiltrating cell populations. Samples will be delivered to the ETIB Preclinical Support Service, Building 10, Room 12C216.

b) DLI Control Subjects

1) No tissue biopsies are obtained from DLI control subjects on this protocol.
2) As detailed in the DLI Control Subject Consent Form, tissue from biopsies and/or bone marrow aspirates that are obtained on DLI Control Subjects for clinical or research evaluation as part of another NIH protocol may be used for research studies on this protocol if the protocol specifically allows the use of biopsy specimens for research purposes. These tissues would be used as control samples, to compare with Treatment Subject tissues after radiation with DLI, to determine whether there are changes in the tumor tissues and cell populations that suggest a local and/or systemic tumor-specific immune reaction as a result of the combination of radiation and allogeneic immunotherapy.

3.4.3.2 Research Blood Samples

Subjects may be enrolled on more than one ETIB research protocol simultaneously. ETIB Protocol Research Nurses review the research blood requirements for the protocols on which subjects are enrolled. A research blood log is used to track research sample volumes, to ensure that sampling does not exceed guidelines per MAS Policy 95-9 (currently the smaller of 10.5 mL/kg or 550 mL per 8 week period), unless an IRB-approved exception is in place. When possible, the subject's protocol calendar will be adjusted to avoid exceeding the limits for research blood draws. If a scheduled research blood draw would exceed the guidelines, the protocol PI is notified and research sampling prioritized to keep the research blood volume within the limits.

3.4.3.2.1 Treatment Subject PBMC: (approximately 220 - 240cc total volume of blood over 12 weeks).

Evaluation will explore the effect of administration of irradiation with or without DLI on the composition, phenotype and functional characteristics of circulating immune cell populations. Comparisons will be made between the Arm A Treatment Subjects' pre and post-treatment peripheral blood mononuclear cells (PBMC) and with PBMC from their donors (if available) and from DLI Control Subjects. Research apheresis samples for flow cytometric assessment of PBMC populations, T cell subpopulation phenotype and functional characteristics, and preclinical cell product development, will be obtained as detailed in Section 3.3.2. Research blood samples for flow cytometric assessment of PBMC populations, T cell subpopulation phenotype and functional characteristics will be drawn at the following time points. An additional 70 cc blood volume will be drawn in those subjects for whom a large-volume blood draw is collected in place of an apheresis (Section 3.3.2).

1) On the day of radiation, prior to radiation:
a. 2 red-and-green CPT after apheresis to ETIB Preclinical Support Service
b. CBC diff

2) At 24 hours after irradiation (Arm A: pre-DLI):
a. 2 red-and-green CPT
b. CBC diff

3) At 48 hours after radiation (Arm A: 24 hours post-DLI):
a. 2 red-and-green CPT
b. CBC diff

4) At 48 hours after radiation (Arm A: 24 hours post-DLI):
a. 2 red-and-green CPT after apheresis to ETIB Preclinical Support Service
b. CBC diff

5) At the 4-day assessment after radiation:
a. 2 red-and-green CPT after apheresis to ETIB Preclinical Support Service
b. CBC diff

6) At the 7- and 14-day assessments after radiation:
a. 5 red-and-green CPT after apheresis to ETIB Preclinical Support Service
b. CBC diff

7) At the four-, eight- and twelve-week assessments following irradiation:
a. 2 red-and-green CPT
b. CBC diff

8) At the post-study follow-up assessments as defined in Section 3.5.1:
a. 2 red-and-green CPT
b. CBC diff

9) At first diagnosis of GVHD, if applicable:
a. 2 red-and-green CPT
b. CBC diff

b) Donor Subject PBMC (approximately 20 cc total volume of blood)
1) On the day of their apheresis
a. 2 red-and-green CPT after apheresis to ETIB Preclinical Support Service
b. CBC diff

c) DLI Control Subject PBMC (approximately 210 cc total volume of blood over four weeks)
1) On the day of their clinical DLI, prior to DLI
a. 7 red-and-green CPT to ETIB Preclinical Support Service
b. CBC diff

2) On day 3 +/1 following DLI
a. 2 red-and-green CPT after apheresis to ETIB Preclinical Support Service

b. CBC diff

3) At the 7- and 14-day assessments after DLI:
a. 5 red-and-green CPT to ETIB Preclinical Support Service
b. CBC diff

4) At the four-week assessment following DLI:
a. 2 red-and-green CPT to ETIB Preclinical Support Service
b. CBC diff

3.4.4 *In-Vitro* Research Evaluation of Subject Tissue Samples

3.4.4.1 Additional Studies

3.4.4.1.1 Tissue from biopsies obtained for clinical management may also be used for research, and compared with research biopsy studies described in Section 3.3.3.

3.4.4.1.2 Clinical specimens collected during the course of this protocol may be banked and used for future investigation of questions related to this research, provided the risks of such investigations are addressed in the consent signed at the time of enrollment.

3.4.5 *In-Vitro* Evaluation of Tumor Specific Cell Populations after Radiation

3.4.5.1 As samples allow, biopsy specimens obtained pre- and post-irradiation (detailed in Section 3.3.3) will be evaluated for activation and/or infiltration of a tumor-specific immune response. Depending on quantity of tissue obtained, immunohistochemistry, confocal microscopy and/or flow cytometry will be used to identify and characterize the inflammatory infiltrate and cell-cell interactions.

3.4.5.2 As tissue sample availability permits, assessment of tumor-reactive immune cell populations will include comparisons from Treatment Subjects pre- and post-irradiation (peripheral blood, tumor, bone marrow), from Donor Subjects peripheral blood lymphocytes (PBL) and from DLI Control Subjects pre- and post-DLI (peripheral blood, tumor, if available per Section 3.3.4) will be assessed by examination of proliferative and cytotoxic responses to tumor. Sample collection and distribution are detailed in Sections 3.3 and 3.4.

3.5 TOXICITY MANAGEMENT: GVHD

3.5.1 Prophylaxis:

Subjects may be continued on the GVHD medication they were on at the time of enrollment. No additional GVHD prophylaxis will be added prior to radiation or DLI

3.5.2 Evaluation:

If GVHD is suspected, tissue confirmation will be sought, unless the PI/designee determines that biopsy would unnecessarily compromise the health or well being of the patient. In addition to tissue, standard clinical criteria will be used to establish the diagnosis. GVHD will be graded according to standard criteria detailed in Appendices A and B.

3.5.3 Treatment:

Treatment of Acute GHVD will follow the NIH Transplant Consortium Guidelines, as detailed in Appendix A. Treatment of Chronic GHVD will be based on evaluation and recommendations from the NCI Chronic GVHD Clinic.

3.5.4 Upon Clinical Presentation of GVHD:

Peripheral blood samples and biopsy tissue will be obtained for clinical and research purposes, with samples being sent to the following labs for phenotypic and functional analysis. Research tissue (including blood)

samples, specified in Section 3.3 and 3.4, will be sent to the ETIB Preclinical Support Service, Building 10, Room 12C216.

3.5.5 Criteria for Removal from Protocol Therapy and Off-Study Criteria

Subjects will be removed from protocol for any of the reasons detailed below. Authorized investigators must notified Central Registration when a patient is taken off study.

1. The Treatment Subject refuses to continue with study treatment and/or evaluation
2. Treatment Subject is removed for reasons of noncompliance, intercurrent illness, or rapid disease progression which precludes administration of planned study treatment or return to the Clinical Center for evaluation
3. Progressive disease (Treatment Subject) that, in the opinion of the PI, requires urgent additional therapy, e.g., threatened organ function, acceptable quality of life, etc., prior to administration of planned therapy.
4. Lost to Follow-Up (All Subjects).
5. Treatment Subject receives another investigation cancer treatment within three months of study treatment that would preclude reliable toxicity and efficacy assessments.
6. DLI Control Subject has completed study-specified endpoints.
7. Death of Treatment Subject or DLI Control Subject will result in removal of that subject and respective Donor Subject from study.

3.6 FOLLOW-UP EVALUATION

3.6.1 All Treatment Subjects

Will be followed for a minimum of 12 weeks for toxicity and efficacy, unless they go on to receive another investigation cancer treatment that would interfere with these assessments. Treatment Subjects who develop toxicities that were likely or possibly attributed to the study treatment will continue to be followed every three months or more frequently, as clinically indicated, until resolution of toxicity. If travel to the NIH CC poses a hardship for the Treatment Subject, these evaluations may be obtained by their local health care provider. In this case, an investigator will obtain clinical information from the local provider for data collection purposes.

3.6.2 Treatment Subjects Who have Either Stable Disease or Treatment Responses

Will be seen in follow-up at the Clinical Center at six, nine, and 12 months after completion of radiation therapy, then every six months for up to five years, to continue to monitor disease status (in subjects who had disease stabilization or response to therapy), as defined in Appendix C, and late toxicities related to post-allotransplant radiation therapy. At these times subjects will have disease-appropriate tumor staging studies performed to determine clinical response as defined in Appendix C. Subjects who return for follow-up evaluation will also have blood drawn for research at the above time-points, to continue to follow immunophenotypic characteristics of circulating immune cell populations and identify potential correlates of response (approximately 20 cc total volume of blood per visit). The blood draw for research will include:

1. red-and-green CPT which will be delivered to ETIB Preclinical Support Service, Building 10, Room 12C216
2. Clinical CBC/diff to be obtained with the same blood draw.

3.6.3 Subjects Who Maintain a Treatment Response

Will be followed every 6 months through five years after completion of therapy. Subjects may be evaluated at additional times as clinically indicated.

4 SUPPORTIVE CARE

4.1 INFECTION PROPHYLAXIS

Treatment will follow the NIH Transplant Consortium Guidelines

4.2 BLOOD PRODUCT SUPPORT

4.2.1 Leukocyte filters

Will be utilized for all blood and platelet transfusions with the exception of DLI to decrease sensitization to transfused WBC and decrease the risk of CMV infection.

4.3 CMV STATUS

Patients who are seronegative for CMV and whose donors are also seronegative should receive CMV-negative blood products whenever possible.

4.4 ANTI-EMETICS

Anti-emetics will follow Clinical Center Guidelines as well as consultation from the Pharmacy service.

4.5 HEMATOPOIETIC GROWTH FACTOR SUPPORT

Growth factor support may be given for the treatment of neutropenia or anemia at the clinical discretion of the PI/LAI or designee.

4.6 MANAGEMENT OF CELL THERAPY TOXICITIES

Patients will be monitored for evidence of DMSO-related toxicity, including rigors and muscle aches.

1. Monitoring will follow established practice, described in http://intranet.cc.nih.gov/nursing/practicedocs/procedures_pdf/PROCellThpy%2CInfusProd5_06.pdf, which include having a nurse-patient ratio of one-to-one and physician or nurse-practitioner in the clinical area during cell infusion.
2. Fever and rigors will be treated supportively with acetominophen and meperidine administration, and diphenhydramine will be used to treat manifestations of allergic transfusion reactions such as hives or angioedema, following Clinical Center DTM procedure for evaluation of febrile reactions to blood products.
3. Rash will be evaluated with biopsy and non-GVHD rash treated with topical steroids if clinically indicated at the discretion of the Principal Investigator.

4.7 CONTRACEPTION

4.7.1 Female treatment subjects will be advised to use an effective form of contraception while being monitored for toxicity on this study, for a period of at least three months after treatment and one year after receiving transplant, and to have their male partners use condoms. Male transplant subjects will be advised to use contraception, preferably condoms, while being monitored for toxicity on this study, for a period of at least three months after treatment and one year after receiving transplant. Donor and Control subjects of childbearing potential will be advised to use an effective form of contraception until completion of the aphereis procedures.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION (REFER TO APPENDIX C)

5.1.1 Data will be prospectively collected and entered into the Cancer Central Clinical Data System database (NCI C3D Database; information at http://ccrtrials.nci.nih.gov). Data will also be reported to the International Bone Marrow Transplant Registry.

5.2 RESPONSE CRITERIA

5.2.1 Treatment Subjects will be evaluated for clinical response as detailed in 3.5.

5.2.2 Response will be determined on measurable disease that has not been irradiated, and will be defined according to the response criteria detailed in Appendix B.

5.3 TOXICITY CRITERIA

5.3.1 Graft-Versus-Host Disease Criteria: GVHD will be graded according to criteria in Appendix A.

5.3.2 The NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be used (CTCAEv3). This document can be found at: http://ctep.info.nih.gov/protocolDevelopment/electronic_applications/docs/ctcaev4.pdf.

6 STATISTICAL SECTION

Eligibility for this protocol is as detailed in Section 2.1, is not limited by gender or ethnicity. The age limit of 18 –75 years is intended to be inclusive of the adult populations who have undergone transplantation for hematologic malignancies. Note: a planned amendment will include pediatric patients if safety is established as outlined below.

Statistical Considerations

The primary objectives of this trial are to evaluate safety and systemic efficacy of administering a single, 8-Gy fraction of radiation to residual tumor, with or without additional donor lymphocytes, in patients with radiation-accessible, relapsed or refractory hematologic malignancies following allotransplant.

Patients who have at least two discrete sites of measurable disease will be enrolled into the trial according to the following plan, and evaluated accordingly. There will be two arms to the study:

- Arm A will consist of patients who have a source of donor lymphocytes available and who have never had significant GVHD (Grade II-IV acute or extensive chronic) in whom administration of DLI is reasonable; and
- Arm B will consist of patients for whom a DLI is relatively contraindicated due to high risk of significant GVHD, including those who have a history of significant GVHD or recipients of haploidentical donor allotransplants, and/or patients who do not have an available source of additional donor lymphocytes.

Treatment Subjects in both Arms will receive a single, 8-Gy fraction of radiation to the maximal tumor volume that can be safely irradiated, while leaving measurable disease to evaluate for a systemic treatment response. Subjects on Arm A will receive a DLI one day after radiation and subjects on Arm B will not receive additional donor lymphocytes.

The primary endpoint of the safety evaluation will be the development of significant toxicity from radiation administered in the context of ongoing allogeneic immunotherapy. The specific safety endpoint will be significant GVHD - acute or chronic - defined as requiring initiation of systemic immunosuppressive therapy (see Appendices A and B). Monitoring for other significant adverse events attributable to the study treatment (CTCAEv4), defined as Grade 3 events that do not respond to standard management, Grade 4 events, or Grade 5 events. The primary endpoint of the efficacy evaluation will be clinical response of non-irradiated tumor following radiation to the maximal tumor volume with DLI (Arm A) or without (Arm B).

Within each of the two arms of the trial, 8 patients will be treated and evaluated for 60 days for safety, with respect to both GVHD and radiation toxicities, prior to accrual of additional patients to the respective arms. The statistical properties of the design relative to safety are as follows:

Within Arm A, the first 8 patients with one or more sites of disease amenable to radiation and additional disease outside the field of radiation that can be evaluated for systemic response will have radiation directed at the maximal tumor volume that can be safely irradiated, as defined in Section 3.2.3.

Subsequently, patients in Arm A will receive a DLI. Following the DLI, the patients will be evaluated for up to 60 days for development of *de novo* or exacerbation of acute, late-acute or chronic GVHD. (Since patients are fully engrafted at the time of study treatment, the immunologic effects - therapeutic and toxic - are expected

within this time frame). Published reports on the rate of GVHD after DLI range from 30–60%, with rates of GVHD in patients who have disease responses approximately 50%.[15,20] It would be desirable to determine if this approach to post-transplant immunotherapy produces an incidence and severity of GVHD that is no worse than that of standard DLI.

These initial 8 patients in Arm A will constitute the group on which the first stopping rule for the trial within Arm A is based. If among these first 8 patients in Arm A, 7 or 8 of the 8 are found to have significant GVHD by day 60, then this will be considered unacceptably high, since the lower 90% confidence bound on 7/8 is 59%, which is higher than higher than most published literature indicates would be expected for unmanipulated DLI, even among those who respond to therapy. This rate of significant GVHD in these subjects with maximal sites irradiated will end further accrual to the trial within Arm A. Furthermore, assuming that there are no more than 6 of the 8 subjects initially enrolled in Arm A who are receiving radiation and develop significant GVHD by day 60, then a second evaluation will take place after 15 patients have been enrolled onto this arm. If among these 15 total patients, 12 or more patients have significant GVHD by day 60, then this will be considered unacceptably high, since the lower 90% confidence bound on 12/15 is 61%, which is higher than most published literature indicates would be expected for unmanipulated DLI, even among those who respond to therapy.

Within Arm B, the first 8 patients with one or more sites of disease amenable to radiation and additional disease outside the field of radiation that can be evaluated for systemic response will have radiation directed at the maximal tumor volume that can be safely irradiated, as defined in Section 3.2.3.

The Treatment Subjects in Arm B will not receive DLI. Following radiation, the subjects will be evaluated for up to 60 days for development of *de novo* or exacerbation of acute, late-acute or chronic GVHD. *Significant* GVHD will be defined as in Arm A, above. As reports on the rate of GVHD after DLI range from 30–60%, with rates of GVHD in patients who have disease responses approximately 50%.[15,20] It would be desirable to determine if this approach to post-transplant immunotherapy produces an incidence and severity of GVHD that is no worse than that of standard DLI.

These initial 8 Treatment Subjects in Arm B will constitute the group on which the first stopping rule for the trial within Arm B is based. If among these first 8 subjects in Arm B, 7 or 8 of the 8 are found to have significant GVHD by day 60, then this will be considered unacceptably high, since the lower 90% confidence bound on 7/8 is 59%, which is higher than most published literature indicates would be expected for unmanipulated DLI, even among those who respond to therapy. This rate of significant GVHD in these subjects with maximal sites irradiated will end further accrual to the trial within Arm B. Furthermore, assuming that there are no more than 6 of the 8 subjects initially enrolled in Arm B who are receiving radiation and develop significant GVHD by day 60, then a second evaluation will take place after 15 patients have been enrolled onto this arm. If among these 15 total patients, 12 or more patients have significant GVHD by day 60, then this will be considered unacceptably high, since the lower 90% confidence bound on 12/15 is 61%, which is higher than most published literature indicates would be expected for unmanipulated DLI, even among those who respond to therapy.

If no more than 6 of the 8 subjects, or no more than 11 of the 15, initially enrolled in a given arm and receiving radiation develop significant GVHD by day 60, then enrollment will continue on that arm until 21 patients are treated.

At the conclusion of the trial, the fraction of patients who have significant GVHD within 60 days within each of the two arms will be calculated, and an appropriate confidence interval will be formed. If 21 patients are enrolled within a given arm, then the following are the associated two-sided exact 90% confidence intervals about the observed fractions of patients with significant GVHD:

Fraction with "Significant GVHD"	90% confidence interval
6/21:	13.2% to 48.7%
7/21:	16.8% to 53.6%
8/21:	20.6% to 58.3%
9/21:	24.5% to 62.8%

10/21: 28.6% to 67.2%
11/21: 32.8% to 71.4%
12/21: 37.2% to 75.5%
13/21: 41.7% to 79.4%
14/21: 46.4% to 83.2%
15/21: 51.3% to 86.8%
16/21: 56.3% to 90.1%

Additionally, CTCAEv4-Grade 3 toxicities that do not respond to therapy within 14 days and any Grade 4 toxicity that is determined likely to have been caused by the study intervention(s), will result in a hold of accrual pending review by protocol investigators and the IRB.

Efficacy will be determined within both Arm A and Arm B, provided that adequate safety has been determined in 8 Treatment Subjects in each Arm. For both of these arms, the following efficacy evaluation will take place. The objective will be to determine if the non-irradiated target, evaluable lesion(s) exhibits shrinkage consistent with at least a partial response. A total of twenty-one (21) subjects will be enrolled into each of the two arms (42 total evaluable Treatment Subjects). This number has been selected to determine if at least 20% of patients will demonstrate evidence of a systemic response, a percentage that is consistent with other trials of new therapy for refractory cancers. If there are 0 to 1 clinical responses in a given group, this will be considered inadequate, since the probability of observing 0-1 responses in 21 patients is 87.0% if the true response rate is a very low 3% and the probability of observing 0-1 responses in 21 patients is 5.8% if the true response rate is a modest 20%. Thus, observing 0-1 responses in 21 patients is much more likely to be observed when the true response rate is very low, and will indicate a lack of efficacy in that group. On the other hand, if 2 or more responses are noted in 21 patients, the probability of this occurring if the true response rate were 3% is 13.0% while the probability of this occurring if the true response rate were 20% is 94.2%. Thus, finding 2 or more responders in 21 patients would similarly provide evidence that the true response rate is more consistent with a rate as high as 20% or higher than it would be if the response rate were a low rate such as 3%. These findings would apply to both of the two groups evaluated.

An early stopping rule for lack of efficacy will be implemented as follows. Across both arms taken together, the first 20 Treatment Subjects who are enrolled and evaluable for efficacy evaluation will have their total number of responses determined. Should there be 0 responses in these initial 20 patients across both arms, then further accrual in both Arms A and B will cease, since the upper one-sided 95% confidence interval bound on 0/20 is 13.9%, which would indicate that the true response rate is very likely to be less than 20%. On the other hand, if at least one response is noted in 20 patients, then accrual will be permitted to continue since the upper one-sided 95% confidence interval bound on 1/20 is 21.6%, which would indicate at least marginal consistency with 20%.

The interim efficacy evaluation will take place based on all evaluable patients in Arms A and B combined for whom it has been determined that it is safe to proceed. That is, if safety requirements have been found to be unmet in one of the two arms, but it is safe to accrue in the other arm, then accrual will proceed in the safe arm until a total of 20 patients have been evaluated in both arms combined.

In addition to the treated patients, 15 Control Subjects will also be enrolled in order to compare the change in proliferating, activated T cells pre- and post- DLI between the 21 Treatment Subjects on Arm A and the Control Subjects, who receive DLI as part of treatment of persistent tumor, but are not being treated with radiation on this protocol. With 15 Control Subjects and 21 treated subjects, there is 81% power to detect a difference equal to one SD (of each group) in numbers of circulating T cells with a proliferative phenotype between the two arms, using a 0.05 significance level two-sided t-test. If the data in at least one of the groups is not normally distributed, then the comparison will be made using a Wilcoxon rank sum test.

All other evaluations noted as secondary will be performed using exploratory techniques. No formal adjustment for multiple comparisons will be used since the evaluations are being done to generate hypotheses.

Depending on the proportions and numbers of patients enrolled, up to 21 patients may be enrolled in each of Arms A and B, and up to 21 Treatment-Subject Donors (Arm A), 15 DLI control subjects, and up to 15 Control-Subject Donors. Thus, a total of up to 42 evaluable treatment subjects, 36 donor subjects (Arm A and DLI Control only) and 15 control subjects (total of 93 enrollees) may be entered onto this trial.

It is anticipated that up to 3 years may be required if all 42 Treatment Subjects and 21 Donor Subjects are to be enrolled, but much less time if any of the stopping rules are implemented. In order to allow for a small number of subjects who may not be evaluable, the accrual ceiling will be set at 99 (assuming a maximum of 46 Treatment Subjects, 15 DLI Control Subjects and 38 Donor Subjects).

7 HUMAN SUBJECT PROTECTIONS

7.1 RATIONALE FOR SUBJECT SELECTION:

7.1.1 For patients with hematologic malignancies whose tumors fail to respond or progress after allogeneic HSCT there are no established therapeutic options that predictably result in improved survival. Immune manipulations, including withdrawal of immunosuppressive therapy and administration of DLI, have curative potential for less than half of patients.[13] Patients who receive salvage chemotherapy following failure of immune manipulations achieve significantly lower response rates.

7.1.2 Patient eligibility is limited to patients who have progressive/recurrent hematologic malignancies after therapy with allotransplant and DLI.

7.2 PARTICIPATION OF CHILDREN

7.2.1 Children will not be enrolled on this study. An amendment to allow pediatric patients is planned for after the safety evaluation is completed.

7.2.2 While children with recurrent or progressive hematologic malignancies after allotransplant have extremely limited treatment options and may ultimately benefit from this experimental therapy, the safety and feasibility of this approach should be demonstrated before it is offered to children.

7.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

7.3.1 Treatment Subjects:

The primary risks to Treatment Subjects participating in this research study include radiation injury (site-specific, with an individualized risk assessment provided by the treating radiation oncologist at the time of consent) and potential GVHD and/or graft failure from radiation in the setting of allogeneic cell therapy. The primary risk associated with allogeneic cell therapy is GVHD. The specific risks of administering radiation in the context of ongoing allogeneic cell therapy are unknown, but are hypothesized to be similar to that of unmanipulated DLI. This would suggest that GVHD and tumor recurrence/progression would represent the primary cause of morbidity and mortality after radiation with or without DLI. Possible benefits of radiotherapy in this protocol include transient or prolonged local control of disease at sites that are irradiated that may reduce or prevent symptoms due to mass effect, organ dysfunction, or obstruction. A hypothesis being evaluated in this study is whether tumor-derived lymphocytes will improve tumor-specific alloreactivity. The protocol provides for detailed and careful monitoring of all patients to assess for toxicity and response to treatment. All patients entered on the trial will have relapsed or refractory hematologic malignancies that have not responded to allotransplant or subsequent standard approaches to augment a graft-versus-tumor effect, specifically a trial of withdrawal of immune suppression and, if available, unmanipulated DLI. This protocol provides an opportunity unique to patients that have completed allotransplant, which may have therapeutic benefit. Specific benefits may include local and/or systemic tumor responses. Patients will be treated with therapeutic intent and response to the therapy will be closely monitored. The potential benefits from this therapy are cure, prolonged disease remission, and/or a reduction in cancer-related symptoms. Subjects under 18 years of age will not be included in this study.

The risks and benefits of apheresis are as described for Donors, Section 7.3.2. Apheresis is frequently performed for research purposes after allotransplant and can often be done through catheters the patients already have in place.

7.3.2 Donors:

Apheresis is a safe procedure that is routinely performed in healthy children and adults. The most common side effects of apheresis are pain and bruising at IV sites. Side effects of a temporary central venous catheter, required for occasional donors with poor peripheral intravenous access, include pain, bleeding, bruising, infection, thrombosis, and vascular perforation. Mild side effects from citrate anticoagulant are common and include chills, numbness and tingling sensations ("pins and needles"), anxiety, muscle cramps, and nausea. More serious side effects due to citrate-induced low calcium levels are uncommon and include low blood pressure, seizures, weakness, and tetany. Citrate reactions rapidly resolve away when the collection is slowed down or stopped. Transient mild thrombocytopenia is common after donation. To prevent dilutional anemia, the extracorporeal circuit must be primed with 1 unit of red blood cells for small children. Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. Donors will be closely monitored and procedures to minimize risks and prevent side effects are incorporated into all aspects of the protocol. The ETIB, DTM, and NIH CC have broad expertise to adequately manage side effects.

There are potential benefits to donors who participate on this trial. The most probable is psychological benefit from contributing to medical research designed to improve the health of a family member. Another potential benefits include the diagnosis of previously unknown illnesses (such as viral hepatitis) at the time of donor screening. Finally, donor participation may also help advance scientific knowledge about allotransplantation and lead to improvements in the treatment of cancer.

7.3.3 DLI Control Subjects:

The primary risk associated with DLI is GVHD. The specific risks of GVHD after DLI for the treatment of progressive tumor in the context of full donor engraftment after alltransplant range from 30–60%, with a rate of approximately 50% in patients who have disease responses.[15,20], but are hypothesized to be similar to that of unmanipulated DLI. This would suggest that GVHD and tumor recurrence/progression would represent the primary cause of morbidity and mortality after DLI. DLI Control Subjects are individuals with progressive disease, who are to be treated with DLI regardless of participation on this study. Therefore, the risks of DLI are no different as a result of enrollment.

The risks and benefits of apheresis are as described for Donors, Section 7.3.2. Apheresis is frequently performed for research purposes after allotransplant and can often be done through catheters the patients already have in place.

There are potential benefits to DLI Control Subjects who participate on this trial. The most probable is psychological benefit from contributing to medical research designed to improve the health of other patients with cancer. Another potential benefit is prompt identification and manageent of signs of toxicity from DLI, as a result of close safety monitoring that will occur during the first month after infusion. Additionally, Control Subjects may benefit from the diagnosis of a previously unknown condition at the time of eligibility screening. Finally, Control Subject participation may also help advance scientific knowledge about allotransplantation and lead to improvements in the treatment of cancer.

7.4 RISK/BENEFIT ANALYSIS

Treatment Subjects on this study may be directly benefited by this treatment protocol. The patient population has no curative options available for treatment of their advanced malignancies. Review of the radiation literature, combined with our institutions limited experience in the allogeneic transplant population, suggests that the toxicity associated with this single-fraction radiation approach will be minimal; nevertheless, given the novelty of this treatment regimen, safety will be evaluated prospectively as a primary aim of this study. We hypothesize that the radiation strategy in this protocol will result in systemic tumor responses mediated by the additional donor lymphocytes and/or by the allogeneic immune system that subjects will already have in place.

It is also anticipated that this study will provide scientific information relevant to attempts to mediate graft-versus-tumor effects.

7.5 CONSENT PROCESS AND DOCUMENTATION

7.5.1 The procedures and treatments involved in this protocol, with their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the Treatment Subjects. Similarly, the procedures and treatments involved in this protocol, with their attendant risks and discomforts, will be carefully explained to the Donor Subjects. Likewise, the procedures and treatments involved in this protocol, with their attendant risks and discomforts, will be carefully explained to the Control Subjects. A signed, informed consent document will be obtained from the Treatment Subjects, the Donor Subjects, and the Control Subjects by one of the physician investigators.

7.5.2 Consent forms: The original signed informed consent documents will be kept with patient medical records. Central Registration will also retain a copy of the informed consent document. A copy of their own signed informed consent documents will also be given to Treatment Subjects, Donor Subjects, and Control Subjects.

7.5.3 Central Registration will ascertain the date of IRB approval before registering the first subject.

7.5.4 The Treatment Subject, Donor Subject and DLI Control Subject informed consent forms contain all elements required for consent. In addition, the Principal Investigator, LAI, or their designee will obtain oral consent and will be available to answer all patient questions.

8 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

8.1 DEFINITIONS

8.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted .

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

8.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

8.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

8.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

8.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

8.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

8.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

8.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subject.

8.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.2 NCI-IRB REPORTING

8.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All serious non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

8.2.2 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

8.3 DATA SAFETY AND MONITORING PLAN

8.3.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and if applicable to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8.4 RECORD KEEPING

8.4.1 All subjects (treatment, donor and control) must have signed an Informed Consent. An on-study eligibility checklist will be filled out by the Research RN and faxed to the Central Registration Office (CRO) before a Treatment Subject, Donor Subject or Control Subject is entered on the study.

8.4.2 Complete records must be maintained on all subjects; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on the NCI C3D database from which formal analyses are done. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; flow sheets, specialty forms for pathology, radiation, or surgery; adverse event assessment; and off-study summary sheet, including a final assessment by the treating physician.

9 PHARMACEUTICAL INFORMATION

9.1 PENTASTARCH

Pentastarch for Cryopreservation of Clinical Products (cross-file on BB-IND # 9164):
The NIH Department of Transfusion Medicine will perform cryopreservation of cell products used for this protocol (method described in BB-IND #9164). The NIH DTM is now using this procedure for the cryopreservation of all of their clinical products. This cryopreservation process utilizes a combination of Pentastarch and DMSO.

9.2 DIPHENHYDRAMINE

Supply: Commercially available. Diphenhydramine HCl injection is available in an injectable solution at a 50mg/ml concentration in single dose ampules, syringes and vials as well as multi-dose vials from multiple manufacturers. B) Preparation: Diphenhydramine HCl may be given by direct intravenous injection without additional dilution. Alternatively the prescribed dose may be diluted in a small volume (e.g. 25-50ml) of 5% dextrose in water (D5W) or 0.9% sodium chloride (NS) and infused over 10-15 minutes. C) Storage and Stability: Store commercially available injectable product at controlled room temperature. D) Administration – Diphenhydramine HCl injection may be administered by direct IV injection (IV push) at a rate generally not exceeding 25mg/min. Alternatively, diphenhydramine HCl injection may be diluted and given over 10-15 minutes (see Preparation). E) Toxicities include sedation, sleepiness, dizziness, disturbed coordination, epigastric distress, and thickening of bronchial secretions. Diphenhydramine can provide additive effects with alcohol or other CNS depressants. Diphenhydramine can cause anticholinergic side effects (e.g. dry mouth, fixed or dilated pupils, flushing, urinary retention). Diphenhydramine should be used with caution in subjects with a history of bronchial asthma, increased intraocular pressure, hyperthyroidism, cardiovascular disease or hypertension.

9.3 ACETAMINOPHEN

Supply: Commercially available as 325 mg or 500 mg tablets for oral administration from multiple manufacturers. B) Storage: Store at controlled room temperature. C) Administration: Oral. For analgesia and

antipyresis, the usual dose is 650 to 1000 milligrams every 4 to 6 hours, to a maximum of 4 grams/day. D) Toxicities: No toxicities are anticipated to result from single doses of acetaminophen administered as premedication for cell product infusions.

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11 APPENDICES

11.1 APPENDIX A: GRADING AND TREATMENT OF GVHD

Clinical Grading of Acute GVHD[76]

	Stage			
Grade	Skin	Liver	Gut	PS
0 (none)	0	0	0	0
I	+ to ++	0	0	0
II	+ to +++	+	+	+
III	++ to +++	++ to +++	++ to +++	++
IV	++ to ++++	++ to ++++	++ to ++++	+++

Late-Acute GVHD
Late-acute GVHD will be defined as GVHD that presents with signs and symptoms typical of acute GVHD but presenting after Day 100 post-allotransplant. It will be graded and treated as acute GVHD.

Hyper-acute GVHD
Hyper-acute GVHD will be defined as severe (Grade III or IV) GVHD (defined above) that occurs in the first 14 days post-transplant.

Clinical Grading of Chronic GVHD (Appendix B, Chronic GVHD Score Sheet)[75]
Mild chronic GVHD involves only 1 or 2 organs or sites (except the lung: see below), with no clinically significant functional impairment (maximum of score 1 in
all affected organs or sites).

Moderate chronic GVHD involves (1) at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 will also be considered moderate chronic GVHD.

Severe chronic GVHD indicates major disability caused by chronic GVHD (score of 3 in any organ or site). A lung score of 2 or greater will also be considered severe chronic GVHD.

Treatment of Acute, Late-Acute and Hyper-acute GVHD
This schema is intended to serve as a guideline and to promote consistency in our clinical practice; it may be modified for individual patients as clinical circumstances warrant.

Grade 0-I GVHD or Grade II/Gut-only (+):
Skin: Topical corticosteroids (usually 0.1% triamcinolone; 1% hydrocortisone to face) applied to rash BID.
Gut-only: Topical (enteral) corticosteroids (usually budesonide 3 mg) orally TID.

Grade II-IV GVHD and Hyper-acute GVHD:
1) Methylprednisolone (MP) 62.5 mg/m^2 per dose IV, BID for 4 consecutive days.
2) If no response after 4 days, continue until response (7-day maximum trial).
3) If response within 7 days, taper as follows:

a) 50 mg/m^2 per dose IV BID for 2 days.
b) 37.5 mg/m^2 per dose IV BID for 2 days.
c) 25 mg/m^2 per dose IV BID for 2 days.
d) If clinically appropriate, change MP to oral prednisone 100 mg PO (or oral equivalent of IV dose) daily for 2 days. MP may be converted to prednisone later in the taper at the investigators' discretion.
e) After this, steroids will be reduced by 10% each week until a dose of 10 mg/day is reached. Subsequent reductions will be made at the investigators' discretion.
f) If GVHD worsens during taper, steroids should be increased to previous dose.
g) During steroid taper, maintain cyclosporine at therapeutic levels (Section 3.4).

4) If no response is observed within 7 days of MP treatment:
a) Increase Methylprednisolone to 500 mg/m^2 per dose IV, BID for 2 days.
b) If there is no improvement, consideration will be given to using second-line immunosuppressive therapy, e.g., tacrolimus, mycophenolic acid, monoclonal antibodies, or studies of investigational agents for acute GVHD, if they are available.

Note: Antifungal prophylaxis with agents effective against mould will be started when it is anticipated that patients will be receiving steroids at $\geq$ 1 mg/kg/d of methylprednisolone (or equivalent) for $\geq$ 2 weeks. Voriconazole is the agent of choice, but liposomal amphotericin B (Ambisome) 5 mg/kg/d or amphotericin B lipid complex (Abelcet) 5 mg/kg/d are valid alternatives. During prophylaxis with any of the above agents, fluconazole should be discontinued. In subjects with therapeutic cyclosporine levels at the initiation of voriconazole therapy, the cyclosporine dose should be decreased by approximately 50%.

Treatment Response Assessment

Determination of acute GVHD treatment response should be made within 96 hours of starting the treatment. The following are criteria to determine definitions of response to GVHD treatment:

- Complete response: Complete resolution of all clinical signs and symptoms of acute GVHD.
- Partial Response: 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Maintenance of adequate performance status (Karnofsky Score $\geq$ 70%).
- Non-responder: < 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Failure to maintain adequate performance status (Karnofsky Score $\leq$ 70%).
- Progressive disease: Further progression of signs and symptoms of acute GVHD, and/or decline in performance status after the initiation of therapy.

Chronic GVHD Treatment

Currently, treatment of chronic GVHD is individualized, based on organ systems affected, prior treatment responses and patient clinical status. Treatment subjects who develop *de novo* or flare of chronic GVHD will be referred to the Chronic GVHD Clinic for evaluation and treatment recommendations, as part of their scheduled routine follow-up.

Chronic GVHD Treatment Response Assessment: Subjects will be assessed and GVHD graded using the Chronic GVHD Score Sheet developed and endorsed by the NIH Consensus Development Project (Appendix B, below).

12	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: **KPS ECOG LPS** ☐	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA** ☐ 12.1.1 **involved**	**12.1.2** ☐ No Symptoms	12.1.3 ☐ <18% BSA with disease signs but **NO** sclerotic features	12.1.4 ☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	**12.1.5** ☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus

12	SCORE 0	SCORE 1	SCORE 2	SCORE 3
12.1.6 MOUTH	12.1.7 ☐ No symptoms	*12.1.8* ☐ Mild symptoms with disease signs but not limiting oral intake significantly	*12.1.9* ☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	*12.1.10* ☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
12.1.11 EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	☐ No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN

12	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LUNGS*	12.1.11.1.2 ☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
12.1.11.1 FEV1		☐ FEV1 60-79% **OR** LFS 3-5	☐ FEV1 40-59% **OR** LFS 6-9	☐ FEV1 ≤39% **OR** LFS 10-12
12.1.11.1.1 DLCO	☐ FEV1 > 80% **OR** LFS=2			
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Note: For evaluation of systemic responses, measurements will exclude tumor sites within the local area affected by radiation.

Complete Response (CR)

For Non-Hodgkin's Lymphoma (NHL) or Hodgkin's Lymphoma:

- Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.

1. Typically FDG-avid lymphoma: in patients with no pretreatment PET scan or when the PET scan was positive before therapy, a post-treatment residual mass of any size is permitted as long as it is PET negative.
2. Variably FDG-avid lymphomas/FDG avidity unknown: in patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed on CT to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to ≤ 1.0 cm in their short axis after treatment.

- The spleen and/or liver, if considered enlarged before therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies, and nodules related to lymphoma should disappear. However, determination of splenic involvement is not always reliable because a spleen considered normal in size may still contain lymphoma, whereas an enlarged spleen may reflect variations in anatomy, blood volume, the use of hematopoietic growth factors, or causes other than lymphoma.
- If the bone marrow was involved by lymphoma before treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of > 20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is negative by immunohistochemistry but that demonstrates a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in patient outcome.
- All lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in greatest transverse diameter for nodes > 1.5 cm before therapy).
- Previously involved nodes that were 1.1 to 1.5 cm in greatest transverse diameter before treatment must have decreased to ≤ 1 cm in their greatest transverse diameter after treatment or by more than 75% in the sum of the products of the greatest diameters (SPD).
- In the event that the spleen or other organ is enlarged due to lymphoma involvement prior to therapy, organ must regress in size by CT scan and must not be palpable on physical examination. Any macroscopic nodules in any organs detectable on imaging techniques should no longer be present.

- If bone marrow was involved by lymphoma before treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of the same site.

Complete Response (CR), Continued

For Chronic Lymphocytic Leukemia (CLL):

- Complete resolution of detectable signs and symptoms for at least 2 months, with peripheral blood lymphocytes ≤ 4K/μl, neutrophils ≥ 1.5K/μl, platelets ≥ 100K/μl, hemoglobin > 11g/dl (without transfusion), bone marrow lymphocytes < 30% without lymphoid nodules.

For Chronic Myelogenous Leukemia:

- Hematologic CR – normalization of peripheral blood counts (WBC < 10K/μl, platelets < 450K/μl), no immature cells on peripheral smear (blasts, promyelocytes, metamyelocytes).
- Cytogenetic CR – hematologic CR, with cytogenetic studies negative for Philadelphia chromosome (Ph).
- Molecular CR – hematologic and cytogenetic CR, with PCR studies negative for *bcr-abl*.

For Acute leukemias:

- Hematologic remission is defined as normalization of peripheral blood counts (ANC ≥ 1,500/μl and platelets > 100,000/mm^3) without circulating blasts;
- Bone marrow cellularity > 20% with normal maturation, fewer than 5% blasts in bone marrow, and no detectable Auer rods.
- Extramedullary leukemia may not be present.
- The absence of specific molecular or cytogenetic markers of disease that were previously present further defines molecular or cytogenetic remission, respectively.

For Multiple Myeloma with Plasmacytoma:

- Negative immunofixation on the serum and urine
- Disappearance of any soft tissue plasmacytomas
- Less than or equal to 5% plasma cells in the bone marrow

Partial Response (PR)

For NHL or Hodgkin's Lymphoma:

- At least a 50% decrease in sum of the product of the diameters (SPD) of up to six of the largest dominant nodes or nodal masses. These nodes or masses should be selected according to all of the following: they should be clearly measurable in at least 2 perpendicular dimensions; if possible they should be from disparate regions of the body; and they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
- No increase should be observed in the size of other nodes, liver, or spleen.
- Splenic and hepatic nodules must regress by ≥ 50% in their SPD or, for single nodules, in the greatest transverse diameter.
- With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable and no measurable disease should be present.

For NHL or Hodgkin's Lymphoma, Continued

- Bone marrow assessment is irrelevant for determination of a PR if the sample was positive before treatment. However, if positive, the cell type should be specified (e.g., large-cell lymphoma or small neoplastic B cells). Patients who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders. When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered partial responders.
- No new sites of disease should be observed.
- Typically FDG-avid lymphoma: for patients with no pretreatment PET scan or if the PET scan was positive before therapy, the post-treatment PET should be positive in at least one previously involved site.
- # Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, CT criteria should be used.

For CLL:

- a 50% or greater decrease in SPD of measured lymph nodes, hepatomegaly, or splenomegaly lasting at least 2 months, and;
- one or more of the following: neutrophils ≥ 1.5K/μl platelets > 100K/μl or hemoglobin > 11g/dl (or 50% improvement).

For Chronic Myelogenous Leukemia:

- Hematologic PR – as for hematologic CR, except for (1) persistence of immature cells, or (2) platelets < 50% pretreatment level but > 450K/μl or (3) persistent splenomegaly but > 50% of pretreatment size.
- Cytogenetic PR – hematologic CR, with 1-34% Ph-positive cells (major response).
- Cytogenetic minor response – hematologic CR, with 35-90% Ph-positive cells.

For Acute leukemias:

- All criteria for complete remission are satisfied, except that the bone marrow may contain > 5% but < 25% blasts, or ≤ 5% blasts are present with Auer rods or abnormal morphology.

For Multiple Myeloma with Plasmacytoma:

- A 50% or greater decrease in SPD of all measured plasmacytomas lasting for a period of at least one month;
- No individual plasmacytoma may increase in size, and no new plasmacytomas may appear;
- reduction by ≥ 75% in serum myeloma protein production, with decrease in Bence-Jones proteinuria by ≥ 90%;
- clonal marrow plasmacytosis ≤ 5%;
- no new lytic bone lesions.

Stable Disease (SD)

Response parameters not meeting criteria for CR, PR, or PD.

Relapsed or Progressive Disease (PD)

For NHL or Hodgkin's lymphoma:

- ≥ 25% increase in SPD of all measured lesions compared to smallest prior values, or
- appearance of any new lesion(s).

For CLL:

- ≥ 50% increase in SPD of all measured lesions compared to smallest prior values;
- new lesion(s) or ≥ 50% increase in blood lymphocytes; Richter's syndrome.

For Chronic Myelogenous Leukemia:

- Increase in the number of metaphases demonstrating Ph by cytogenetics or t(9:22) by FISH; return to PCR positivity for *bcr-abl* after previously becoming negative

For Acute leukemias:

- Bone marrow and peripheral blood morphological features consistent with relapse or progression, including rising blast count and re-emergence of specific molecular or cytogenetic markers.

Multiple Myeloma with Plasmacytoma (requires 2 of the following):

- increase in serum M-protein to > 50% above lowest level or rise > 2 g/dl;
- increase in urine light chain excretion to 50% above the lowest value (at least 250 mg/24 hours) or an increase > 2 g/24 hours of light chain excretion;
- increase in soft tissue plasmacytomas by 50% or new or increasing lytic bone lesions;
- the above protein criteria for relapse, plus hypercalcaemia > 12 mg/dl, anemia with hemoglobin decrease > 2 g/dl, increased bone marrow plasma cells by 50%, or generalized bone pain.

12.3 APPENDIX D: DONOR-SUBJECT STEM-CELL MOBILIZED CELL PRODUCT COLLECTION PROCEDURE

Donor-Subjects who agree to provide a mobilized cell product for clinical use by their respective Recipient-Subject will be undergo repeat clinical assessment (Section 2.2.3) to confirm continued eligibility to serve as a donor, and review of risks of G-CSF and apheresis.

Approved donors will receive filgrastim as an outpatient (10 □g/kg/day each morning; subcutaneously) for 5, 6, or 7 days. In cases where it is anticipated that poor mobilization may occur (increased donor age, Caucasian race, low donor weight, high recipient weight), donors may receive filgrastim at an increased dose of 8 □g/kg BID. Donor should take filgrastim upon awakening in the morning. This is especially important on days 5, 6, and 7 of the injections.

A 15- to 25-liter, large-volume, whole-blood apheresis will be performed in the NIH DTM via a two-armed approach or a temporary central venous catheter in the femoral position using the Baxter CS3000Plus, Cobe Spectra, or an equivalent instrument (typically, 4-6 hour procedure). The apheresis procedure will use ACD-A anti-coagulant, or heparin.

Apheresis will typically be performed on days 5 and 6 of this regimen. On some occasions, sufficient numbers of $CD34^+$ cells might be obtained with a single apheresis on day 5; on other occasions, it may be necessary to perform additional apheresis procedures on days 6 and 7 to reach the target $CD34^+$ cell number (usually 3 - 5 x 10^6 $CD34^+$ cells/kg-recipient). Specific $CD34^+$ cell target will be specified by the PI/LAI at time of collection, as it will depend on indication. The donor will be instructed to take filgrastim for the complete 7-day period, unless notified that adequate $CD34^+$ cells were harvested before day 7. If $\geq 3 \times 10^6$ $CD34^+$ cells per kg are harvested after apheresis on days 5, 6, and 7, no further mobilization or apheresis will be performed, and the patient will be eligible to receive the stem cell transplant with that dose of $CD34^+$ cells.

In the event that $< 3 \times 10^6$ $CD34^+$ cells/kg are harvested, and the AI/LAI determines that the collection is inadequate to provide necessary hematopoietic support for the Recipient Subject, Donor Subject retreatment with filgrastim will be permitted, after a two-week rest, (filgrastim 8 □g/kg subcutaneously, BID, for five days) followed by repeat apheresis for peripheral blood stem cell harvesting.

The apheresis product will be cryopreserved and stored at –180° C in Plasmalyte A, Pentastarch, human serum albumin, DMSO, and preservative free heparin (10 U/ml) by the NIH DTM procedure (as defined in BB-IND#9164). The concentration of $CD34^+$ cells in the apheresis product will be determined by flow cytometry, and the number of $CD34^+$ cells in each cryopreserved bag calculated.

If donor and host are ABO incompatible, red blood cells will be depleted from the stem cell product by standard DTM protocols.

The day after collection is completed, Donor-Subjects will have a clinical assessment, including a focused history and physical examination, blood drawn for clinical laboratory evaluation, including CBC/diff and Chem-20, and reminder of clinical symptoms that require evaluation by a local physician or Emergency Room.

FORM NIH-532-10: RADIATION ASSESSMENT FORM

MEDICAL RECORD	Outpatient Progress Notes

Histology: _____ Dates of treatment: _____

Response categories:

R ..... Resolved (0x0, not visible) S ..... Stable D..... Decreasing X ..... Not evaluable
I ..... Increasing N ..... New lesion EXC .....Excised XXX ..... Present but not measured

Evaluation date (time since completion of radiation)

BASELINE (prior to therapy)

Date (mo-yr)	Site	Imaging Modality	Largest diameter x Perpendicular diameter	Code
___-___ #1	_____	_____	_____x_____	R D I S N X
___-___ #2	_____	_____	_____x_____	R D I S N X
___-___ #3	_____	_____	_____x_____	R D I S N X
___-___ #4	_____	_____	_____x_____	R D I S N X

ONE MONTH

Date (mo-yr)	Site	Imaging Modality	Largest diameter x Perpendicular diameter	Code
___-___ #1	_____	_____	_____x_____	R D I S N X
___-___ #2	_____	_____	_____x_____	R D I S N X
___-___ #3	_____	_____	_____x_____	R D I S N X
___-___ #4	_____	_____	_____x_____	R D I S N X

Best Response: CR / PR / SD / Progression Date Achieved: _____ Totals: Longest: LDxPD: _____x_____
Date of Progression: _____ Completed By/Date: _____Verified By: _____

THREE MONTHS

Date (mo-yr)	Site	Imaging Modality	Largest diameter x Perpendicular diameter	Code
___-___ #1	_____	_____	_____x_____	R D I S N X
___-___ #2	_____	_____	_____x_____	R D I S N X
___-___ #3	_____	_____	_____x_____	R D I S N X
___-___ #4	_____	_____	_____x_____	R D I S N X

Best Response: CR / PR / SD / Progression Date Achieved: _____ Totals: Longest: LDxPD: _____x_____
Date of Progression: _____ Completed By/Date: _____Verified By: _____

SIX MONTHS

Date (mo-yr)	Site	Imaging Modality	Largest diameter x Perpendicular diameter	Code
___-___ #1	_____	_____	_____x_____	R D I S N X
___-___ #2	_____	_____	_____x_____	R D I S N X
___-___ #3	_____	_____	_____x_____	R D I S N X
___-___ #4	_____	_____	_____x_____	R D I S N X

Best Response: CR / PR / SD / Progression Date Achieved: _____ Totals: Longest: LDxPD: _____x_____
Date of Progression: _____ Completed By/Date: _____Verified By: _____

NINE MONTHS

Date (mo-yr)	Site	Imaging Modality	Largest diameter x Perpendicular diameter	Code
___-___ #1	_____	_____	_____x_____	R D I S N X
___-___ #2	_____	_____	___ ___.___x___.___ ___.___	R D I S N X
___-___ #3	_____	_____	___ ___.___x___.___ ___.___	R D I S N X
___-___ #4	_____	_____	___ ___.___x___.___ ___.___	R D I S N X

Best Response: CR / PR / SD / Progression Date Achieved: _____ Totals: Longest: LDxPD: _____x_____
Date of Progression: _____ Completed By/Date: _____Verified By: _____

TWELVE MONTHS

Date (mo-yr)	Organ	Imaging Modality	Largest diameter x Perpendicular diameter	Code
___-___ #1	_____	_____	___ ___.___x___.___ ___.___	R D I S N X
___-___ #2	_____	_____	___ ___.___x___.___ ___.___	R D I S N X
___-___ #3	_____	_____	___ ___.___x___.___ ___.___	R D I S N X
___-___ #4	_____	_____	___ ___.___x___.___ ___.___	R D I S N X

Best Response: CR / PR / SD / Progression Date Achieved: _____ Totals: Longest: LDxPD: _____x_____
Date of Progression: _____ Completed By/Date: _____Verified By: _____

Patient Identification	Outpatient Progress Notes 1 NIH-532-10 (8-00) P.A. 09-25-0069 File in Section 2: Progress Notes

ALL: All treatment Subjects, Donor Subjects and DLI Control Subjects
A: Arm A Treatment Subjects
B: Arm B Treatment Subjects
C: DLI Control Subjects
D: Donor Subjects

	Screen	Prior to Day 0	1 – 7 Days prior to XRT	Day 0 *Day of XRT for A & B; DLI for C*	24° Post-XRT *Pre-DLI for A*	48 ° Post-XRT	4D Post-XRT *For C, 3D post-DLI*	7D Post-XRT *For C, post-DLI*	14D Post-XRT *For C, post-DLI*	28D Post-XRT *For C, post-DLI*	2M Post-XRT	3M Post-XRT	F/U
H&P	ALL	C & D *post-pheresis*		A & B	A & B		A & B	A & B & C	A & B & C	A & B & C	A & B	A & B	A & B
Perf. Status	A, B & C		A & B		A & B		A & B	A & B	A & B	A & B	A & B	A & B	A & B
GVHD Ass't	A, B & C		A & B *within 2 D of XRT*	A & B & C		A & B	A & B & C	A & B & C	A & B & C	A & B & C	A & B	A & B	A & B
Screening Bloods	ALL												
Staging Studies	A & B									A & B	A & B	A & B	A & B

	Screen	Prior to Day 0	1 – 7 Days prior to XRT	Day 0 *Day of XRT for A & B; DLI for C*	24° Post-XRT *Pre-DLI for A*	48 ° Post-XRT	4D Post-XRT *For C, 3D post-DLI*	7D Post-XRT *For C, post-DLI*	14D Post-XRT *For C, post-DLI*	28D Post-XRT *For C, post-DLI*	2M Post-XRT	3M Post-XRT	F/U
Bone Marrow	A & B									A & B *all*	A & B *marrow tumor*	A & B *marrow tumor*	A & B *clinically indicated*
Critical Eligibility Bloods (safety)			A & B *within 2 D of XRT*										
Tumor Bx – rad. lesion			A & B *Core Needle*				A & B *Surgical (superficial)*			A & B *Pref. Surgical*			
Tumor Bx – non-rad. lesion			A & B *Core Needle*				A & B *Surgical (superficial)*			A & B *Pref. Surgical*			

	Screen	Prior to Day 0	1 – 7 Days prior to XRT	Day 0 *Day of XRT for A & B; DLI for C*	24° Post-XRT *Pre-DLI for A*	48 ° Post-XRT	4D Post-XRT *For C, 3D post-DLI*	7D Post-XRT *For C, post-DLI*	14D Post-XRT *For C, post-DLI*	28D Post-XRT *For C, post-DLI*	2M Post-XRT	3M Post-XRT	F/U
Tumor Bx – new lesion *(*once only)*										*A & B* Pref. Surgical*	*A & B* Pref. Surgical*	*A & B* Pref. Surgical*	*A & B* Pref. Surgical*
FDG-PET	A & B, *discr'y*		A & B *if not @ screen*					A & B		A & B	A & B *discr'y*	A & B *discr'y*	A & B *discr'y*
Apheresis		D	A & B				A & B & C						
Research Bloods		D	A & B & C	A & B & C	A & B & C		A & B & C	A & B & C	A & B & C	A & B & C	A & B	A & B	A & B
XRT				A & B									
DLI				C	A								
Clinical Bloods	ALL	C & D *after apheresis*			A & B		A & B & C	A & B & C	A & B & C	A & B & C	A & B	A & B	A & B
Toxicity Ass't					A & B		A & B & C	A & B & C	A & B & C	A & B & C	A & B	A & B	A & B

	Screen	Prior to Day 0	1 – 7 Days prior to XRT	Day 0 *Day of XRT for A & B; DLI for C*	24° Post-XRT *Pre-DLI for A*	48 ° Post-XRT	4D Post-XRT *For C, 3D post-DLI*	7D Post-XRT *For C, post-DLI*	14D Post-XRT *For C, post-DLI*	28D Post-XRT *For C, post-DLI*	2M Post-XRT	3M Post-XRT	F/U
Chart Review: GVHD & Response												C	

ALL: All treatment Subjects, Donor Subjects and DLI Control Subjects
A: Arm A Treatment Subjects
B: Arm B Treatment Subjects
C: DLI Control Subjects
D: Donor Subjects

15.1 APPENDIX G: DATA COLLECTION ELEMENTS REQUIRED BY PROTOCOL

All of the following elements will be recorded in the C3D database.

A. Donor Enrollment
   - Date of birth, age, gender, race, ethnicity, pregnancy history, relation to Recipient- or DLI Control-Subject
   - Height
   - Weight
   - Date of Informed Consent signature, consent version and date of registration
   - Baseline History/Physical

B. Patient Enrollment (Recipient-Subjects and DLI Control-Subjects)
   - Date of birth, age, gender, race, ethnicity
   - Height
   - Weight
   - Performance Status
   - Date of original diagnosis
   - Date of Allotransplant
   - Donor Characteristics (relationship, stem-cell sourse, HLA-matching)
   - Conditioning regimen and GVHD prophylaxis
   - Stage at diagnosis
   - Stage at study entry
   - Sites of disease at diagnosis and study entry
   - Tumor histology and date of confirmation
   - Date of Informed Consent signature, consent version and date of registration
   - Baseline History/Physical, including GVHD assessment
   - Baseline Symptoms (at study enrollment to study to radiation)
   - Prior GVHD
   - Prior therapy
   - Prior surgery

C. Study Drug Administration (Radiation and DLI - Recipient-Subjects and DLI Control-Subjects)
   - Date radiation given
   - Radiation dose delivered to each site
   - Date DLI given (Arm A)
   - Cell dose administered (Arm A)
   - Weight at start of radiation
   - Course assessment
   - Type of response to therapy
   - Date of response

D. Laboratory and Diagnostic Test Data

- All Clinical laboratory and diagnostic test results done at screening (all Subjects) and after radiation (and DLI, Arm A and DLI Control-Subjects) administration through 30 days post-radiation (and/or DLI).
- All clinical laboratory and diagnostic tests that support a possible, probable or definite diagnosis of GVHD for 90 days after administration of radiation and to document resolution of adverse events that occurred in the first 30 days after radiation administration. (Recipient Subjects)
- HLA data (all Subjects)
- Serologies: CMV, HSV, EBV, toxoplasmosis, adenovirus (all Subjects)
- TTV data (all Subjects)
- Blood and bone marrow chimerism data (Recipient- and DLI Control-Subjects)

E. Toxicities (Recipient-Subjects and DLI Control-Subjects)
- Grade I-IV toxicities first 30 days from administration of radiation or DLI (Control Subjects only). In Arm A, because DLI are administered in close temporal proximity to radiation, no distinction will be possible between those due to radiation and those due to DLI.
- All adverse events possibly, probably or definitely related to GVHD until 90 days post administration of radiation. GVHD data to include: maximal grade, time of onset, sites of involvement, and response to therapy.
- All hematologic adverse events possibly, probably or definitely related to allograft failure until 90 days post administration of radiation.

F. Concomitant Meds-baseline until 90 days post administration of radiation (Recipient-Subjects)
- Baseline medications (prior to administration of radiation)
- Antibiotics
- GVHD prophylaxis and treatment
- Other therapy for recorded adverse events

G. Treatment of Persistent/Progressive Disease with Standard Therapy (Recipient-Subjects)
- Chemotherapy
- Withdrawal of immune suppression
- Other immunotherapy
- Radiation therapy
- Donor Lymphocyte Infusion

H. Tumor response and measurements (Recipient-Subjects)
- Baseline, post-radiation, four weeks, two, three, six, nine, 12, 15, 18 and 24 months after completion of the radiation, and restaging studies performed as clinically indicated.
- Include PET FDG uptake measurements in index lesion(s)

Storage/Tracking

Normal donor and patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be archived by the ETIB Preclinical Service. All data associated with archived clinical research samples is entered into the ETIB Preclinical Service's Microsoft Excel databases on frozen cells and plasma. These databases are stored on the NCI group drive in the ETIB Preclinical Service folder. Access to this folder is limited to ETIB clinical staff, requiring individual login and password. All staff in the Preclinical Service laboratory has received annually updated NIH/CIT training and maintains standards of computer security.

The data recorded for each sample includes the patient ID , trial name/protocol number, date drawn, treatment cycle/post transplant time point, cell source (e. g. peripheral blood, lymphopheresis, mobilized peripheral blood stem cells, marrow, pleural fluid) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples will receive a unique bar code number, which will be added to the sample Preclinical Service database. Only this bar code will be recorded on the sample vial and the vials will not be traceable back to subjects without authorized access to the Preclinical Service database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.

Samples are stored in locked freezers at -85°C (sera and plasma) or under liquid nitrogen (cells), according to stability requirements. These freezers are located onsite at the Preclinical Service laboratory (12C216) (-85° freezer) or in ETIB common equipment space (CRC/3-3273). Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol or through his/her submission and IRB approval of the NCI IRB Authorization Form (appended) stipulating whether IRB review is not necessary or IRB approval is granted for the pursuit of this new research activity. All researchers are required to sign a form (attached) stating that the samples are only to be used for research purposes associated with objectives of the original protocol for which the samples were collected, or (using only unlinked or coded samples) for an IRB approved protocol as stipulated on the IRB Authorization Form, and that any unused samples must be returned to the Preclinical Service laboratory.

Protocol Completion/Sample Destruction

Once primary research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and patient consent or the IRB Authorization Form stipulating that the activity is exempt from IRB review (see attached authorization form from the NCI IRB).

Samples, and associated data, can only be permanently archived if the subject has provided informed consent. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the Preclinical Service laboratory.

The Preclinical Service staff will report to the Principal Investigators any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher. The Principal Investigators will annually report this information to the IRB.