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Protocol Title: Low Intensity Allogeneic Hematopoietic Stem Cell Transplantation Therapy of Metastatic Renal Cell Carcinoma Using Early and Multiple Donor Lymphocyte Infusions Consisting of Sirolimus-Generated Donor Th2 Cells

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IND Information
Investigator-held IND (Sponsor: Dr. Fowler; IND #11832 for donor Th2.rapa cells). This IND cross-files BB-IND 6675 (in vitro use of anti-CD3, anti-CD28 magnetic beads), IND #4348 (use of recombinant human IL-4), and BB-IND #12541 (Miltenyi Biotec CliniMACS® for CD4 cell isolation).

PRÉCIS

Background:

- Allogeneic hematopoietic stem cell transplantation (HSCT) represents a potentially effective treatment option for patients with metastatic renal cell carcinoma (RCC).
- In a pilot clinical trial in refractory hematologic malignancy subjects, we have found that augmentation of a T cell-replete allograft with donor Th2 cells generated ex vivo in sirolimus (rapamycin; Th2.rapa cells) allows prompt donor engraftment after outpatient-intensity chemotherapy. This transplant approach has been associated with a low incidence of acute GVHD.
- Based on these data, we seek to safely achieve objective clinical regression of metastatic RCC by the following new transplant approach. (1) The allograft will be administered after a low intensity, outpatient induction chemotherapy regimen consisting of pentostatin and cyclophosphamide. This regimen is intended to provide sufficient host immune T cell depletion, and as such, a conventional preparative regimen will not be administered. (2) To avoid mixed chimerism for rapid potentiation of GVT effects, a G-CSF mobilized allograft will be augmented with a donor lymphocyte infusion at day 14 post-transplant consisting of Th2.rapa cells.

Objectives:

- Primary objective: (1) Determine whether this new, low-intensity transplant approach can yield objective partial or complete remission of metastatic RCC, with the goal of ruling out a PR/CR rate of 20% in favor of a rate of 60%.
- Secondary objectives: (1) Evaluate the safety and immune-depleting properties of the pentostatin/cyclophosphamide regimen; (2) Characterize the engraftment kinetics and GVHD profile of this new transplant approach; and (3) Characterize post-transplant immunity in study subjects, including cytokine phenotype, immune reconstitution, and potential anti-tumor effector mechanisms.

Eligibility:

- Adults (18 – 75 years) with metastatic RCC who have an eligible 6/6 HLA-matched sibling donor.
- Must have had one prior therapy with either sorafenib, sunitinib, or temsirolimus, or any other FDA-approved agent for therapy of metastatic renal cell carcinoma.
- Life expectancy $\geq$ 3 months, Karnofsky score $\geq$ 80, relatively normal organ function, and absence of CNS metastases.

Design:

- Patients will receive a 21-day course of pentostatin (intravenous infusion on days 1, 8, and 15; 4 mg/m^2 per dose) and daily oral cyclophosphamide (200 mg per day).
- Patients will receive a mobilized, T cell-replete allogeneic hematopoietic stem cell graft followed by a pre-emptive donor lymphocyte infusion with donor Th2 cells at day 14 post-transplant. GVHD prophylaxis will consist of a short-course of sirolimus plus maintenance therapy with cyclosporine A.
- If $\geq$ 2/5 partial or complete responses are observed within 6 months post-transplant, the therapy will be considered potentially promising, and will be expanded in a Simon two-stage design to evaluate a total of n = 14 subjects. If $\geq$ 5/14 PR/CR are achieved, the therapy will be considered worthy of further investigation.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

1.1.1.1 Using a transplant approach involving Th2.rapa cells, sirolimus GVHD prophylaxis, and a 21-day pre-transplant immune depletion regimen of pentostatin combined with daily oral cyclophosphamide (Cy, 200 mg per day), determine whether clinical regression of metastatic RCC can be safely achieved, with the goal of ruling out a 20% overall PR/CR rate in favor of a 60% PR/CR rate.

1.1.2 Secondary Objectives:

1.1.2.1 To characterize the safety, immune depleting, and immune suppressive effects of the pre-transplant pentostatin and cyclophosphamide regimen.

1.1.2.2 To characterize the engraftment kinetics and GVHD profile of this new transplant approach.

1.1.2.3 To characterize post-transplant immunity, including cytokine phenotype, immune reconstitution, and potential anti-tumor effector mechanisms.

1.2 BACKGROUND AND RATIONALE

1.2.1 Metastatic Renal Cell Cancer: Prognosis and Treatment

The incidence of RCC is increasing, particularly for black Americans, and now accounts for 3% of new cancer cases[1]; in the United States in 2006, it is anticipated that RCC will account for 12,840 deaths. The mean age of diagnosis of RCC is approximately 60 years; as such, the development of low-intensity transplant approaches with an associated low-toxicity profile will be required to more completely address the role of allogeneic transplantation in this patient population. Fully 30% of RCC cases present with metastatic disease; in 20 to 40% of cases, metastatic RCC develops after nephrectomy for clinically localized RCC[2]. The prognosis of metastatic RCC is poor: the median survival is 6 to 10 months and the 2-year survival is only 10 to 20%[3]. Different histologic subtypes of metastatic RCC appear to have similarly poor prognoses[4]. However, it is possible that the different subtypes may respond differentially to immunotherapy, including allogeneic HSCT; as such, the current study will be limited to subjects with clear cell RCC, which accounts for approximately 80% of RCC cases and has been shown to be sensitive to allogeneic HSCT therapy[5].

Until recently, immunotherapy has been the primary therapy for metastatic RCC. High-dose IL-2 is the most effective cytokine therapy for metastatic RCC, as this approach yields a 5 to 7% rate of durable complete remission in eligible subjects[6]. Low-dose IL-2[7] can be utilized in subjects who can not tolerate high-dose IL-2; however, the durable CR rate is significantly reduced relative to high-dose IL-2 therapy. IFN-α is also active as a single-agent, but yields only an approximate three month survival advantage in metastatic RCC (reviewed in reference[8]).

Recently, three new agents (sunitinib, sorafenib, and temsirolimus) have been FDA-approved for therapy of metastatic RCC; in community practice, these agents now represent first-line therapy for metastatic RCC. Both sunitinib and sorafenib are receptor tyrosine kinase inhibitors

(RTKI), whereas temsirolimus inhibits the mammalian target of rapamycin (mTOR). Sunitinib inhibits multiple pathways that play a role in clear cell RCC pathogenesis, including vascular endothelial growth factor receptor and platelet-derived growth factor receptor signals; in the majority of sporadic clear cell RCC cases, these pro-angiogenic pathways are activated through mutation of the von Hippel-Lindau (VHL) tumor-suppressor gene[9]. In a randomized phase III trial involving previously untreated subjects with metastatic RCC[10], sunitinib recipients had increased median progression free survival (PFS; 11 months) relative to IFN-α recipients (5 months). There were no complete responses in either treatment arm; furthermore, responses were relatively transient, as approximately 90% of subjects had progressive disease after 14 months of follow-up. A recent review summarizes the biologic basis and clinical experience of sunitinib therapy[11]. The second RTKI, sorafenib, has recently been shown in a phase III trial involving previously-treated subjects with metastatic RCC to yield an increase in PFS relative to the placebo control group (5.5 month vs. 2.8 month PFS)[12]. A recent review further summarizes the current experience with sorafenib for therapy of metastatic RCC[13]. And finally, temsirolimus has been shown in first line therapy of high-risk metastatic RCC subjects to increase overall survival relative to IFN-α therapy from 7.3 to 10.9 months[14]. Of interest, a recent study indicates that RCC tumors that over-express molecules down-stream to mTOR are more likely to respond to temosirolimus[15]. In light of these clinical data, RTKI therapy or mTOR inhibitor therapy is now commonly utilized in the oncology community as front-line therapy. As such, for this protocol, subject eligibility criteria will require previous therapy with either sorafenib, sunitinib, or temsirolimus; due to the current predominant use of these agents in first-line therapy of metastatic RCC, high-dose IL-2 therapy will not be required for protocol eligibility. These eligibility criteria were discussed with Dr. Peter Bross, who is the FDA clinical reviewer for IND 11832 that will be utilized for this protocol. However, during the consent process, subjects will be counseled with respect to the known capacity of high-dose IL-2 therapy to induce durable remissions in approximately 5 to 10% of subjects with metastatic RCC; high-dose IL-2 therapy may be available on an NCI Surgery Branch protocol (through Dr. Yang), or through a referring physician. Subjects will not be specifically encouraged to receive therapy with low-dose IL-2, which is associated with a nominal CR rate, or IFN-α, which has a negligible CR rate.

1.2.2 Allogeneic HCT in RCC: Review of Previous Results and Overview of Proposed Strategy

The therapeutic mechanism of allogeneic HSCT in the treatment of hematologic malignancy is primarily attributable to a donor T cell-mediated graft-versus-leukemia (GVL) effect[16]. In murine models, allogeneic T cells can also mediate anti-tumor effects against non-hematologic malignancy, thereby generating a graft-versus-tumor (GVT) effect[17-23]. The mechanism of a given GVT effect is variable depending on model utilized, and can involve antigen-specific T cell responses or alloreactive T cell responses, with molecular contribution from inflammatory cytokines and cytolytic effectors[24]. Clinical evidence supports the existence of a GVT effect against solid tumors[25,26]. Of the solid tumors, the best evidence for a GVT effect exists in the setting of metastatic RCC.

The initial study demonstrating a GVT effect against metastatic RCC was performed by Dr. Childs and the NHLBI transplant group[5]. In this study, 10 of 19 subjects achieved an objective tumor regression, including 3 complete remissions. This study demonstrated that tumor regression was temporally associated with the attainment of full donor chimerism and with the

development of GVHD; it was also observed that the treatment of GVHD by immune suppressive therapy was associated with subsequent tumor relapse. GVHD chemoprophylaxis consisted of single-agent cyclosporine. These observations indicate that for therapy of metastatic RCC, it is likely important to attain full donor engraftment without significant delay and also to mediate GVT effects without inducing a severity of GVHD that requires intensive or prolonged anti-GVHD therapy.

The role of immunotherapy, including allogeneic HSCT, in the management of metastatic RCC has recently been summarized[27]. Pooling data from 12 published reports involving 271 patients, an objective remission of metastatic RCC after allogeneic HSCT has been observed in approximately 20% of cases. One component of this pooled data was from the European results of allogeneic HSCT for metastatic RCC, which have recently been published[28]; these data were obtained from several centers and involved several different transplant regimens and GVHD prevention regimens. Tumor responses were observed in 28 of 124 subjects transplanted (23%; n=24 PR, n=4 CR). The cumulative incidence of grade II-IV acute GVHD was 40%, with 33% developing chronic GVHD. Patients who received a transplant from a matched unrelated donor (MUD) and patients who developed GVHD were more likely to achieve tumor regression. Significantly, subjects who were able to receive additional donor T cells in the form of donor lymphocyte infusions (DLI) and subsequently developed chronic GVHD had a 70% 2-year survival (see Figure 1). Patients who achieved donor engraftment more rapidly were more likely to respond. Mixed chimerism was relatively common post-transplant: for example, in 44 subjects who received conditioning with a standard Flu/Cy preparative regimen, median donor chimerism at day 30 post-transplant was only 40% (range, 20 to 80%). In sum, these results indicate that research efforts in metastatic RCC should focus on developing allogeneic transplant approaches that limit host immunity pre-transplant, boost donor immunity through DLI administration, and limit severe acute GVHD (and its associated requirement for systemic immune suppression).

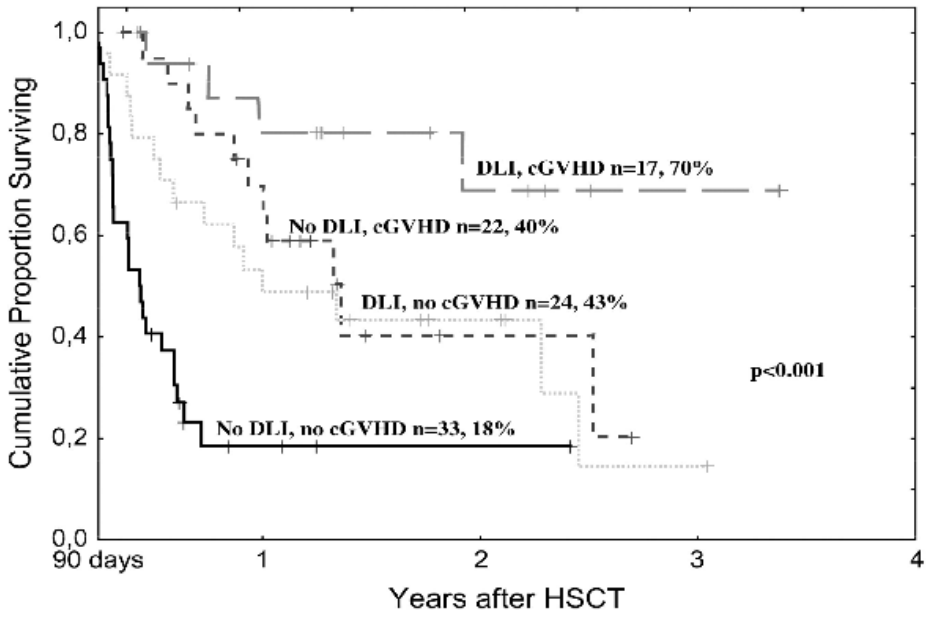

Figure 1. Overall survival in subjects with metastatic renal cell cancer is increased after allogeneic hematopoietic stem cell transplantation in subjects who are able to receive donor lymphocyte infusions (DLI) and subsequently develop chronic GVHD (cGVHD) [graph taken from reference #25, pooled European data, Barkholt et al, Annals of Oncology, 2006].

In the current protocol, we reason that previous attempts to utilize allogeneic HSCT for therapy of metastatic RCC have been limited in part by preparative regimens that yield prolonged mixed donor/host chimerism throughout the first 100 days post-transplant. Mixed chimerism reflects an underlying competition between donor and host T cells, thereby restricting donor anti-host alloresponses[29]. The negative adverse effect of persistent mixed chimerism is suggested by results from a CALGB multi-center study that utilized a fludarabine and cyclophosphamide (Flu/Cy) preparative regimen and post-transplant GVHD prophylaxis with tacrolimus and methotrexate[30]. In that study, a significant incidence and degree of mixed chimerism was observed through day 90 post-transplant (median of 51% donor myeloid elements); no objective responses were observed in 22 subjects with metastatic RCC. Furthermore, in the European analysis of allogeneic HSCT for metastatic RCC, a decrease in the post-transplant time required to achieve full donor chimerism was associated with an improved chance of achieving remission[28]. In light of our previous clinical results[31] using targeted host immune T cell depletion in the setting of hematologic malignancy (where we observed that severe host T cell depletion was associated with early GVL effects), we reason that the high rate of persistent mixed chimerism and variable response rates in published metastatic RCC studies may relate at least in part to inadequate host immune T cell depletion at the time of transplantation. It should be noted that metastatic RCC subjects, unlike subjects with hematologic malignancy, are not typically treated with immune-depleting chemotherapy and therefore are likely to enter transplantation with higher T cell numbers and function; such

residual host immunity thereby predisposes to increased host-versus-graft reactivity and mixed chimerism. As such, the transplant protocol we have designed has incorporated new strategies intended to promote donor chimerism early post-transplant, including: (1) immune-depleting chemotherapy prior to transplantation (combination pentostatin and cyclophosphamide; see Section **1.2.5**); and (2) donor Th2.rapa cells at the time of transplantation and as early DLI at days 14 and 45 post-transplant (see Section **1.2.3**).

Acute GVHD represents a second and related obstacle to the successful use of allogeneic HSCT for metastatic RCC. In the summary analysis of 271 subjects receiving allogeneic HSCT for metastatic RCC[27], the incidence of grade II-IV acute GVHD was 42%. This rate of acute GVHD likely reflects: (1) the inadequacy of GVHD prophylaxis regimens utilized, which have included single-agent calcineurin inhibitors (either cyclosporine or tacrolimus) or calcineurin inhibitors combined with either methotrexate or mycophenolic acid mofetil (MMF); and (2) the existence of both post-transplant mixed chimerism and tumor progression early post-transplant. In this latter setting, investigators will often institute early discontinuation of GVHD prophylaxis in an attempt to augment a GVT effect. Given this information, the transplant protocol we have designed seeks to more effectively prevent acute GVHD through: (1) use of post-transplant sirolimus; and (2) use of donor Th2.rapa cells for allograft augmentation and for DLI.

First, with respect to GVHD, we will utilize post-transplant single-agent rapamycin (sirolimus). Our utilization of rapamycin as GVHD prophylaxis is based on recent studies demonstrating the remarkable clinical efficacy of this agent when combined with a calcineurin inhibitor[32], our murine data showing that Th2.rapa cells are resistant to the immunosuppressive effect of post-transplant rapamycin[33], and from our ongoing clinical trial data on protocol 04-C-0055. On protocol 04-C-0055, only one out of 11 subjects (9%) receiving cyclosporine and short-course rapamycin (administered through day 14 post-transplant) developed significant acute GVHD (one case of grade II acute GVHD); one subject developed grade I acute GVHD requiring only topical therapy, and the remaining nine subjects did not develop acute GVHD. These results compare favorably to our previous results (protocol 99-C-0143) using the same transplant approach with the one exception that GVHD prophylaxis consisted of single-agent cyclosporine: on protocol 99-C-0143, grade II-IV acute GVHD was observed in 63% of cases (12/19 subjects)[34]. The current protocol will evaluate a more prolonged course of single-agent sirolimus (through day 60 post-transplant), with the additional change of achieving higher steady-state post-transplant sirolimus levels. This approach to GVHD prevention has not been reported to date in the literature. However, Dr. John Tisdale of the NIDDK has utilized single-agent sirolimus in a pilot clinical trial at NIH of low-intensity allogeneic HSCT for therapy of sickle cell anemia; in eight subjects treated to date, single-agent sirolimus (with the goal of targeting relatively high sirolimus levels of 15 µg/L) has not been associated with a single case of acute GVHD (Dr. Tisdale, personal communication). As such, it is possible that single-agent, high-dose sirolimus represents a suitable regimen for GVHD prophylaxis in the low-intensity transplant setting. However, it is unknown how these data will translate to the current study, as the subjects treated on Dr. Tisdale's study have been maintained in a prolonged state of mixed chimerism. To address this potential safety concern in our study, in the event that single-agent sirolimus is associated with unacceptable GVHD (as defined by more than 3 cases out of 12 of steroid-refractory acute GVHD), then advancement to the second stage of protocol accrual will not be permitted.

Use of single-agent sirolimus GVHD prophylaxis may be advantageous, as it would avoid the immunosuppressive effect of calcineurin inhibitors; that is, it is important to note that in the setting of malignancy that occurs after solid organ transplantation, a switch from calcineurin inhibition to mTOR inhibition can itself result in clinical tumor regression (reviewed in[35]). In addition, because the mTOR inhibitor temsirolimus shows activity as a single-agent in metastatic RCC, it is possible that high-dose, post-transplant sirolimus may both control GVHD and provide control against RCC progression until alloengraftment is complete and a subsequent immune-mediated GVT effect can be realized. Data are not available in the literature pertaining to the degree of mTOR inhibition in tumor cells that is achieved with rapamycin or rapamycin-analogue therapy. However, with respect to non-malignant T cells, it is known that standard doses of rapamycin (steady state values of 6 to 12 µg/L) are typically associated with only an approximate 80% inhibition in the phosphorylation of p70S6 kinase that lies downstream to mTOR[36]; as such, it is possible to speculate that higher steady-state levels of sirolimus may result in more effective inhibition of mTOR, with subsequent improved immune modulation and anti-tumor efficacy. Also, because sirolimus is not associated with substantial renal toxicity, sirolimus monotherapy may be advantageous relative to the well-known nephrotoxicity induced by calcineurin inhibitors, particularly in the post-nephrectomy metastatic RCC setting. It should be noted that in the setting of solid organ transplantation, the target trough concentration of sirolimus is approximately 10 µg/L when sirolimus is utilized in combination with calcineurin inhibitors (range, 5 to 15) and 25 µg/L when sirolimus is utilized without calcineurin inhibitors (range, 20 to 30)[37]. As such, our protocol will seek to achieve steady-state sirolimus levels of 25 µg/L. One toxicity associated with sirolimus has been hyperlipidemia, which can be ameliorated by pre-emptive therapy with HMG-CoA reductaste (statin) inhibitors[38]; as such, subjects will receive pre-emptive statin therapy during sirolimus therapy. A second toxicity associated with sirolimus has been interstitial pneumonitis, which occurs in approximately 10% of solid organ transplant recipients; it is unclear whether this side effect is dose-related, as it has been observed in subjects with both low and higher trough drug concentrations[39]. The median time to onset of pneumonitis was 8.3 months after initiation of sirolimus therapy; subjects on this study will receive sirolimus for only two months. And third, perhaps uniquely in the setting of allogeneic HSCT, use of sirolimus in combination with calcineurin inhibitors results in thrombotic micro-angiopathy in approximately 10% of cases[40]; it is not known whether single-agent sirolimus therapy would help alleviate this toxicity after allogeneic HSCT.

As for the second point relating to GVHD, we will utilize donor Th2.rapa cells for allograft augmentation and as the DLI inoculum. In animal models, we have characterized acute GVHD primarily as a Th1-driven response that is amenable to regulation by the adoptive transfer of Th2 cells that are generated from the donor[41,42]. In recent experiments, we have found that the generation of Th2 cells in high concentrations of sirolimus results in the generation of Th2.rapa cells that express a T central-memory phenotype, have increased in vivo survival after adoptive transfer, induce marked Th2 polarization in vivo, and are more potent than control Th2 cells for the prevention or therapy of acute GVHD[22,33]. Furthermore, we have found that such Th2.rapa cells are relatively resistant to in vivo rapamycin[33], therefore providing a rationale for using such cells in combination with post-transplant rapamycin drug therapy. Importantly, when allogeneic inocula contained donor Th2.rapa cells in combination with unmanipulated donor $CD4^+$ and $CD8^+$ T cells, a GVT effect could be obtained with reduced GVHD[22].

1.2.3 Potential Role of Th2.rapa Cells for Safely Achieving Rapid Donor Chimerism

As previously discussed, establishment of full donor chimerism early post-transplant may be beneficial for enhancement of a GVT effect against RCC; of note, we have observed that rapid full donor chimerism is associated with potent GVL effects in subjects with refractory lymphoma[43]. It is well established that donor T cells promote full donor chimerism through inhibition or deletion of host T cells that mediate a host-versus-graft reaction[44]. However, donor T cells also initiate GVHD, and identification of a donor T cell dose or T cell surface subset that promotes engraftment without causing GVHD has been elusive[45]. In murine models, we have found that donor T cells of type II cytokine phenotype that secrete IL-4, IL-5, IL-10, and IL-13 promote alloengraftment independent of GVHD[46,47]. Most recently, we found that ex vivo generated donor Th2.rapa cells potently abrogate graft rejection in a fully MHC-mismatched model (see below, Figure 2; manuscript in press, The Journal of Immunology). These data provide a rationale for using donor Th2.rapa cells to promote full donor engraftment after low-intensity regimens such as the pentostatin and cyclophosphamide therapy utilized on this protocol.

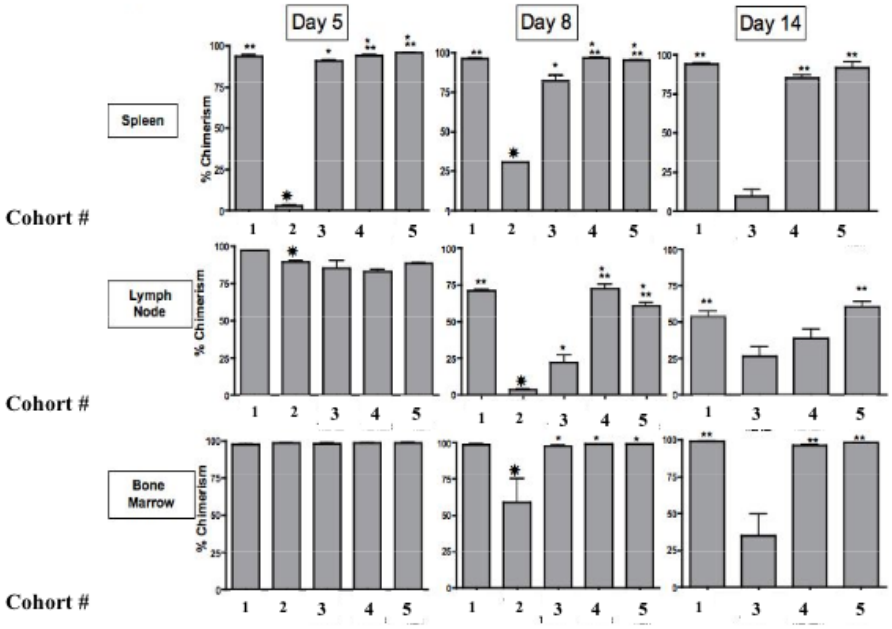

Figure 2. Donor Th2.R cells prevent fully MHC-mismatched graft rejection. B6 bone marrow transplant (SCT) into lethally irradiated BALB/c host (control cohort 1); 0.1 x 10^6 immune competent host T (HT) cells were injected to induce rejection (rejection control cohort 2). Experimental cohorts received BMT, host T cells, and additional donor T cells: control Th2/Tc2 cells (cohort 3) or rapamycin generated Th2/Tc2 cells (cohort 4) or $CD4^+$ purified Th2 cells (cohort 5). Engraftment was evaluated in spleen, lymph nodes, and bone marrow at days 5, 8, and 14 post-transplant. Brisk rejection was observed in rejection controls by day 5 post-transplant; rejection was observed in control Th2/Tc2 recipients by day 14 post-transplant. In contrast, Th2/Tc2.R and Th2.R recipients did not reject.

In addition to these murine data, we now have substantial clinical trial data regarding the use of donor Th2 cells. In an initial pilot clinical trial involving 47 subjects (protocol 99-C-0143), 28 subjects received ex vivo generated donor Th2 cells in the context of a T cell-replete mobilized allograft from an HLA-matched sibling donor[34]. Similar to our current method, such Th2 cells

were generated by co-stimulation and IL-4, IL-2 cytokine addition; however, rapamycin was not included in the initial clinical trial culture method. In the initial trial, we found that donor Th2 cells were feasible to generate at doses of up to 1.25 x 10^8 Th2 cells/kg and were not associated with any specific toxicity. Th2 cell recipients expressed both Th1 and Th2 cytokines post-transplant; however, GVHD was not reduced in Th2 cell recipients. In light of our murine data showing that rapamycin-generated Th2 cells were more potent in vivo than control Th2 cells, we initiated protocol 04-C-0055 to evaluate the strategy of allograft augmentation with donor Th2.rapa cells. For this protocol, we utilized the same induction chemotherapy regimen (EPOCH-F; EPOCH with fludarabine) and the same preparative chemotherapy regimen (concomitant Flu/Cy; total Cy dose, 4800 mg/m^2) as protocol 99-C-0143; however, on protocol 04-C-0055, we also evaluated the effect of GVHD prophylaxis consisting of cyclosporine plus short-course sirolimus through day 14 post-transplant.

In the initial subjects treated on protocol 04-C-0055, five of seven Th2.rapa cell recipients developed severe engraftment syndrome (E.S.) characterized by vascular leak, pulmonary infiltrates, fever, and diffuse erythematous rash concomitant with rapid engraftment (100% donor elements; day 14 post-transplant). In light of our murine data demonstrating a potent pro-engraftment effect of donor Th2.rapa cells, we reasoned that the observed E.S. may have been precipitated by Th2.rapa cell promotion of engraftment in the setting of profound host immune T cell depletion that occurred with protocol induction therapy (EPOCH-F) and preparative Flu/Cy chemotherapy. Based on this reasoning, the initial protocol arms were closed to further accrual, and two new protocol arms were initiated in an attempt to reduce E.S. and provide a safe platform for evaluation of the Th2.rapa cells. In protocol arm IV, which is utilized for subjects who do not achieve a $CD4^+$ T cell count of < 200 cells/µl after EPOCH-F therapy, subjects receive a preparative regimen that is reduced by 75% (total Cy dose, 1200 mg/m^2) and receive Th2.rapa cells on day 14 post-transplant to dissociate any potential Th2.rapa cell toxicity from toxicity related to the preparative regimen. In protocol arm V, which is utilized for subjects who achieve a $CD4^+$ T cell count of < 200 cells/µl after EPOCH-F therapy, subjects receive transplantation after the last cycle of EPOCH-F and without the Flu/Cy preparative regimen. On arm V, subjects receive Th2.rapa cells on day 0 of HSCT.

In the context of EPOCH-F and a reduced preparative regimen (arm IV) or immune depletion with EPOCH-F and no preparative regimen (arm V), donor Th2.rapa cell infusion appears to be safe. As shown in Table I (next page), there has not been a single case of E.S. that has required systemic steroid therapy on arm IV (n=7) or arm V (n=13). By comparison, arms I and II that involved Th2.rapa therapy in the setting of standard Flu/Cy conditioning had severe E.S. in 5/7 cases; in arm III, which utilized standard Flu/Cy conditioning and no Th2.rapa cells, relatively mild E.S. was seen in three of eleven cases.

Table I: 04-C-0055 Protocol Subjects: Engraftment Syndrome

IND Subject #	Engraftment Syndrome	
	Signs Symptoms[1]	Therapy[2]
Arm I, #1	None	None
Arm I, #2	WG, VL, Hy	Levo, Pred, Intub
Arm I, #3	WG, VL, Hy	Levo, Pred, Intub
Arm I, #4	(N.E.)	None
Arm II, #1	WG, Hy	Pred
Arm II, #2	WG, VL, Hy	Levo, Pred, Intub
Arm II, #3	Hy	Pred
Arm IIB, #4	None	None
Arm IIB, #5	(N.E.)	
Arm III, #1	Hy	Pred
Arm III, #2	None	None
Arm III, #3	None	None
Arm IIIB, #4	None	None
Arm IIIB, #5	None	None
Arm IIIB, #6	WG, Hy, DAH	Pred, Bi-Pap
Arm IIIB, #7	None	None
Arm IIIB, #8	None	None
Arm IIIB, #9	WG, VL, Hy, F	Pred, Bi-Pap
Arm IIIB, #10	WG, VL, Hy, F	Pred
Arm IIIB, #11	None	None

IND Subject #	Engraftment Syndrome	
	Signs Symptoms	Therapy
Arm IV, #1	None	None
Arm IV, #2	None	None
Arm IV, #3	None	None
Arm IV, #4	None	None
Arm IV, #5	None	None
Arm IV, #6	None	None
Arm IV, #7	None	None
Arm V, #1	None	None
Arm V, #2	None	None
Arm V, #3	None	None
Arm V, #4	None	None
Arm V, #5	Hy	Intermittent O2
Arm V, #6	None	None
Arm V, #7	None	None
Arm V, #8	None	None
Arm V, #9	None	None
Arm V, #10	WG, F, rash	Topical steroid, diuretics
Arm V, #11	None	None
Arm V, #12	None	None
Arm V, #13	None	None

[1] Abbreviations: WG, weight gain; VL, vascular leak; Hy, hypoxia; F, fever; DAH, diffuse alveolar hemorrhage; N.E., not evaluable (due to cardiac failure from chemotherapy on d -2 of HCT [Arm I, #4] and due to myocardial infarction on d 15 of HCT [Arm IIB, #5])

[2] Abbreviations: Levo, levophed therapy for blood pressure support; Pred, intravenous prednisone therapy; Intub, intubation with ventilatory support; Bi-Pap, Bi-Pap respiratory support.

Results from protocol 04-C-0055 relating to acute GVHD are shown here in Table II. For subjects who were maintained on the intended GVHD prophylaxis, acute GVHD has been observed in only 5 of 25 subjects (20%); however, early discontinuation of all GVHD prophylaxis (for therapy of early progression of refractory disease) resulted in acute GVHD in 7 of 9 subjects (78%). These findings indicate that Th2.rapa cell infusion was associated with a relatively low rate of acute GVHD provided that the intended GVHD prophylaxis regimen was administered. Chronic GVHD has been observed in 16 out of 25 evaluable subjects (64%).

Table II: Protocol 04-C-0055, Acute GVHD According to GVHD Prophylaxis Adherence

Acute GVHD[1]							
Adherence to CSA Prophylaxis				Early CSA Withdrawal for Therapy of Progressive Disease			
IND Subject #	Onset	Grade	Stage	IND Subject #	Onset	Grade	Stage
				Arm I, #1	Day 77	III	0-3-0
Arm I, #2		None					
Arm I, #3	NE; none until death, d 46						
Arm I, #4	NE; none until death, d 12						
Arm II, #1		None					
Arm II, #2		None					
Arm II, #3	NE; none until death, d 26						
Arm IIB, #4	Day 30	I	2-0-0				
Arm IIB, #5	Day 13	II	0-1-0				
				Arm III, #1		None	
Arm III, #2	NE; none until death, d 46						
Arm III, #3		None					
Arm IIIB, #4		None					
Arm IIIB, #5		None					
Arm IIIB, #6	Day 72	II	0-1-0				
Arm IIIB, #7		None					
Arm IIIB, #8		None					
Arm IIIB, #9		None					
				Arm IIIB, #10	Day 56	I	2-0-0
Arm IIIB, #11		None					
Arm IV, #1		None					
Arm IV, #2		None					
Arm IV, #3		None					
				Arm IV, #4	Day 75	IV	2-1-4
Arm IV, #5		None					
				Arm IV, #6	Day 34	I	1-0-0
				Arm IV, #7		None	
Arm V, #1		None					
				Arm V, #2	Day 35	II	3-1-0
Arm V, #3		None					
Arm V, #4		None					
				Arm V, #5	Day 98	III	0-3-0
				Arm V, #6	Day 97	II	0-1-0
Arm V, #7		None					
				Arm V, #8	NE; none until death, d 58		
Arm V, #9		None					
Arm V, #10	36	III	0-1-3				
Arm V, #11	35	III	2-2-0				
Arm V, #12	(None through day 52)						
Arm V, #13	(NE; pre-transplant)						

[1] Day of onset of acute GVHD is noted. Acute GVHD, measured through day 100 post-SCT, was graded using the modified Glucksberg criteria (Overall Grade O to IV). GVHD of skin, intestine, and liver were staged (scale, 0 to 4). Abbreviations: "N.E." = not evaluable (due to either early death or recent HSCT).

Table III below shows the donor engraftment results for protocol 04-C-0055. Subjects who received the standard Flu/Cy conditioning (arms I, II, and III) typically achieved full donor lymphoid and myeloid engraftment by day 28 post-transplant. Arm IV subjects, who received the 75% reduced Flu/Cy dose and delayed Th2.rapa cells at day 14 post-transplant, typically achieved predominantly donor engraftment by day 28 post-transplant. And finally, arm V subjects, who received transplantation without the Flu/Cy regimen but with Th2.rapa cells on day 0 of transplant, also typically had predominant donor chimerism by day 28 post-transplant. For the low-intensity arms IV and V, the median $CD3^+$ donor T cell chimerism at day 28 post-transplant was 91% (range, 48 to 100%). These data indicate that allograft augmentation with donor Th2.rapa cells represents an approach to safely achieve rapid donor engraftment without a conventional preparative regimen provided that the host CD4 count is < 200 cells/µl prior to the final chemotherapy administration; because T cells are depleted approximately 50% with each cycle of EPOCH-F, it can be estimated that the final steady-state CD4 count required to achieve rapid donor T cell engraftment with donor Th2.rapa cell therapy is approximately 100 cells/µl or less.

Table III. 04-C-0055 Protocol Subjects: Alloengraftment (Percent Donor Chimerism[1])

	$CD3^+$ Cells			$CD15^+$ Cells			Total MNC		
IND Subject	d14	d28	d100	d14	d28	d100	d14	d28	d100
Arm I, #1	96	-	-	100	-	-	100	100	100
Arm I, #2	100	-	-	100	-	-	100	100	100
Arm I, #3	(TF)[2]	100	(D)[2]	(TF)	100	(D)	100	100	(D)
Arm I, #4	(D)	(D)	(D)	(D)	(D)	(D)	(D)	(D)	(D)
Arm II, #1	100	-	-	100	-	-	100	100	100
Arm II, #2	NE-55[3]	NE-30[3]	-	100	100	-	NE-83[3]	NE-47[3]	NE-39[3]
Arm II, #3	100	(D)	(D)	100	(D)	(D)	100	(D)	(D)
Arm IIB, #4	96	-	-	100	-	-	100	100	100
Arm IIB, #5	100	-	-	100	-	-	100	100	100
Arm III, #1	67	100	(D)	55	98	(D)	53	97	(D)
Arm III, #2	100	-	-	100	-	-	100	96	100
Arm III, #3	100	-	-	100	-	-	100	100	100
Arm IIIB, #4	91	-	-	100	-	-	100	100	100
Arm IIIB, #5	100	-	-	100	-	-	100	100	100
Arm IIIB, #6	100	-	-	100	-	-	100	100	100
Arm IIIB, #7	74	94	-	100	100	-	100	99	100
Arm IIIB, #8	83	91	-	86	95	-	81	94	100
Arm IIIB, #9	99	-	-	100	-	-	99	100	100
Arm IIIB, #10	99	99	-	100	100	-	100	100	100
Arm IIIB, #11	NE-56[3]	97	-	95	96	-	95	94	100
Arm IV, #1	21	100	99	23	56	98	18	55	98
Arm IV, #2	36	64	93	64	91	100	67	87	100
Arm IV, #3	63	93	-	67	97	-	63	92	100
Arm IV, #4	35	48	100	NE-4[3]	21	43	NE-5[3]	9	46
Arm IV, #5	89	94	93	20	86	70	41	83	70
Arm IV, #6	84	95	(D)	64	93	(D)	44	95	(D)
Arm IV, #7	80	91	93	45	73	96	50	78	93
Arm V, #1	82	90	86	35	58	74	35	52	72
Arm V, #2	95	100	-	96	100	-	92	100	100
Arm V, #3	94	93	100	74	87	100	77	85	100
Arm V, #4	97	100	99	69	87	98	79	89	99
Arm V, #5	52	60	57	56	80	96	57	79	84
Arm V, #6	78	91	95	74	75	98	65	57	98
Arm V, #7	82	79	(P)[3]	52	70	(P)	47	64	(P)
Arm V, #8	52	74	(D)	72	84	(D)	47	80	(D)
Arm V, #9	48	(P)	(P)	64	(P)	(P)	58	(P)	(P)
Arm V, #10	100	-	(P)	100	-	(P)	100	100	(P)
Arm V, #11	41	80	(P)	27	47	(P)	19	40	(P)
Arm V, #12	89	96	(P)	97	95	(P)	93	96	(P)
Arm V, #13	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)	(P)

[1] Donor chimerism was determined by VNTR-PCR analysis on whole blood mononuclear cells ("MNC"), T cells ("$CD3^+$"), or myeloid enriched cells ("$CD15^+$").

[2] Abbreviations: (D), subject died prior to assay; (TF), technical failure of assay; (P) result pending.

[3] Abbreviations: NE-value, chimerism value does not reflect degree of engraftment (NE, not evaluable), as value is reduced due to the presence of circulating malignant T cells or myeloid cells.

Table IVA shows the demographics and overall transplant outcome for subjects accrued to receive Th2.rapa cells in the setting of conventional Flu/Cy conditioning (arms I and II). Four of eight subjects with refractory and bulky hematologic malignancy are alive at 717 to 829 days post-transplant.

Table IVA: Overall Transplantation Outcome
(Arms I and II; Arms not open to accrual; utilized higher-dose preparative regimen)

IND Subject #	Malignant Disease (Study Entry) Disease[1]	Prior Trx[2]	Disease Status	Transplant Outcome (day post-SCT)	1° Cause o Death[3]
Arm I (Flu/Cy-4800 Preparative Regimen) (Th2 Cell Infusion)					
Arm I, #1	DLC	1) EPOCH-R 2) EPOCH-FR 3) IT MTX	Primary Refractory	Died (Day 173)	PD
Arm I, #2	Hodgkin's Disease	1) MOPP 2) MINE/ ESHAP 3) CHOP 4) EPOCH	Refractory	Died (Day 250)	Sepsis, PD
Arm I, #3	TCL-AIB EBV-B	1) EPOCH 2) Campath	Primary Refractory	Died (Day 46)	ARDS
Arm I, #4	DLC	1) PACE 2) EPOCH-R 3) EPOCH-FR	Refractory	Died (Day 13)	Cardiac Failu
Arm II (Flu/Cy-4800 Preparative Regimen) (Th2 Cell Infusion) (Sirolimus GVHD Prophylaxis)					
Arm II, #1	PTCL	1) Hydroxyurea 2) Accutane, IFN-α 3) Ontak 4) Targretin 5) Prednisone	Primary Refractory	Alive + Day 829 Post-SCT	N.A.
Arm II, #2	T-Cell PLL	1) Campath 2) Pentostatin/ Cytoxan 3) Anti-TAC 4) 2-CDA, Cladribine	Primary Refractory	Alive + Day 760 Post-SCT	N.A.
Arm II, #3	Hairy Cell	1) Rituxan 2) 2-CDA 3) BL-22 4) Fludara/Rituxan	Primary Refractory	Died (Day 26)	CNS Hemorrha Platelet Refract
Arm IIB, #1	Hodgkin's Disease	1) ABVD 2) ICE	Responsive Relapse	Alive + Day 717 Post-SCT	N.A.
Arm IIB, #2	CLL	1) Fludarabine 2) Flu/Cy 3) Rituxan 4) Campath 5) Rituxan 6) Pentostatin	Refractory	Alive + Day 717 Post-SCT	N.A.

[1] DLC, diffused large cell non-Hodgkin's lymphoma (NHL); TCL-AIB, T-cell lymphoma, angioimmunoblastic variant; EBV-Epstein-Barr virus related B-cell lymphoma; PTCL, peripheral $CD4^+$ T-cell lymphoma, T-Cell PLL, primary T-cell prolymphocy leukemia; Hairy Cell, hairy-cell leukemia; CLL, chronic lymphocytic leukemia.
[2] Number of regimens prior to study entry; "au" = prior autologous stem cell transplant; IT, intrathecal; BL-22, anti-CD22 immunotox
[3] N.A. = not applicable; PD, progressive disease; ARDS, acute respiratory distress syndrome.

Table IVB shows the demographics and overall transplant outcome for protocol 04-C-0055 recipients who received conventional Flu/Cy conditioning in the absence of Th2.rapa cells (arm III). Eight of eleven subjects are alive with a follow-up range between 556 to 823 days post-transplant.

Table IVB: 04-C-0055, Arm III Overall Transplant Outcome
(Arm III closed to accrual; utilized higher-dose preparative regimen)

IND Subject #	Malignant Disease (Study Entry) Disease[1]	Prior Trx[2]	Disease Status	Transplant Outcome (day post-SCT)	1° Cause of Deat
Arm III (Flu/Cy-4800) (No Th2 Cell Infusion) (Sirolimus)					
Arm III, #1	PTCL	1) CHOP 2) EPOCH-F	Primary Refractory	Died (Day 46)	Sudden Death (Possible Arrhythmia)
Arm III, #2	NHL, DLC, transformed	1) EPOCH-R	Primary Refractory	Alive + Day 766 Post-SCT	N.A.
Arm III, #3	Histiocytic Sarcoma	1) EPOCH-F 2) Prednisone	Primary Refractory	Alive + Day 823 Post-SCT	N.A.
Arm IIIB, #4	Hodgkin's and Non-Hodgkin's	1) CHOP-R 2) BEACOPP	Responsive Relapse	Alive + Day 696 Post-SCT	N.A.
Arm IIIB, #5	NHL, Extranodal Marginal Zone	1) CHOP-R 2) RICE 3) Carmustine, VP-16, Cytoxan, auSCT 4) Rituxan	Refractory	Died (Day 134)	Sepsis
Arm IIIB, #6	NHL, DLC, transformed	1) PACE 2) Rituxan 3) EPOCH-R 4) Rituxan	Refractory	Died (Day 162)	Respiratory Arrest (diffuse alveolar hemorrage)
Arm IIIB, #7	Mantle Cell NHL	1) CHOP-R 2) EPOCH-FR	Refractory	Alive + Day 647 Post-SCT	N.A.
Arm IIIB, #8	NHL, DLC	1) CHOP-R 2) CHOP-R Bleomycin 3) EPOCH-R	Responsive Relapse	Alive + Day 591 Post-SCT	N.A
Arm IIIB, #9	PTCL	1) Hy-CVAD/MTX 2) ICE	Responsive Relapse	Alive + Day 562 Post-SCT	N.A
Arm IIIB, #10	DLC, TCL-enteropathy type, MDS	1) Doxo/Cy/VCR/VP-16 2) Melphalan/TBI (auSCT) 3) EPOCH 4) Gemcitabine 5) Cytarabine/MTX	Responsive Relapse	Alive + Day 568 Post-SCT	N.A
Arm IIIB, #11	TCL-AIB	1) CHOP 2) Mini BEAM	Primary Refractory	Alive + Day 556 Post-SCT	N.A

[1] DLC, diffuse large cell non-Hodgkin's lymphoma (NHL); PTCL, peripheral $CD4^+$ T-cell lymphoma; TCL, T cell lymphoma, enteropathy-type; MDS, myelodysplastic syndrome; TCL-AIB, T cell lymphoma, angio-immunoblastic type.
[2] Number of regimens prior to study entry; "auSCT" = prior autologous stem cell transplant.
[3] N.A. = not applicable.

Table IVB: 04-C-0055, Arm III Overall Transplant Outcome
(Arm III closed to accrual; utilized higher-dose preparative regimen)

IND Subject #	Malignant Disease (Study Entry) Disease[1]	Prior Trx[2]	Disease Status	Transplant Outcome (day post-SCT)	1° Cause of Deat
Arm III (Flu/Cy-4800) (No Th2 Cell Infusion) (Sirolimus)					
Arm III, #1	PTCL	1) CHOP 2) EPOCH-F	Primary Refractory	Died (Day 46)	Sudden Death (Possible Arrhythmia)
Arm III, #2	NHL, DLC, transformed	1) EPOCH-R	Primary Refractory	Alive + Day 766 Post-SCT	N.A.
Arm III, #3	Histiocytic Sarcoma	1) EPOCH-F 2) Prednisone	Primary Refractory	Alive + Day 823 Post-SCT	N.A.
Arm IIIB, #4	Hodgkin's and Non-Hodgkin's	1) CHOP-R 2) BEACOPP	Responsive Relapse	Alive + Day 696 Post-SCT	N.A.
Arm IIIB, #5	NHL, Extranodal Marginal Zone	1) CHOP-R 2) RICE 3) Carmustine, VP-16, Cytoxan, auSCT 4) Rituxan	Refractory	Died (Day 134)	Sepsis
Arm IIIB, #6	NHL, DLC, transformed	1) PACE 2) Rituxan 3) EPOCH-R 4) Rituxan	Refractory	Died (Day 162)	Respiratory Arres (diffuse alveolar hemorrage)
Arm IIIB, #7	Mantle Cell NHL	1) CHOP-R 2) EPOCH-FR	Refractory	Alive + Day 647 Post-SCT	N.A.
Arm IIIB, #8	NHL, DLC	1) CHOP-R 2) CHOP-R Bleomycin 3) EPOCH-R	Responsive Relapse	Alive + Day 591 Post-SCT	N.A
Arm IIIB, #9	PTCL	1) Hy-CVAD/MTX 2) ICE	Responsive Relapse	Alive + Day 562 Post-SCT	N.A
Arm IIIB, #10	DLC, TCL-enteropathy type, MDS	1) Doxo/Cy/VCR/VP-16 2) Melphalan/TBI (auSCT) 3) EPOCH 4) Gemcitabine 5) Cytarabine/MTX	Responsive Relapse	Alive + Day 568 Post-SCT	N.A
Arm IIIB, #11	TCL-AIB	1) CHOP 2) Mini BEAM	Primary Refractory	Alive + Day 556 Post-SCT	N.A

[1] DLC, diffuse large cell non-Hodgkin's lymphoma (NHL); PTCL, peripheral CD4+ T-cell lymphoma; TCL, T cell lymphoma, enteropathy-type; MDS, myelodysplastic syndrome; TCL-AIB, T cell lymphoma, angio-immunoblastic type.
[2] Number of regimens prior to study entry; "auSCT" = prior autologous stem cell transplant.
[3] N.A. = not applicable.

Table IVC shows the demographics and overall transplant outcome for protocol 04-C-0055 recipients who received the 75% reduced dosing of Flu/Cy conditioning and Th2.rapa cells at day 14 post-transplant (arm IV). Three of seven subjects are alive with a follow-up range between 437 to 535 days post-transplant.

Table IV C: 04-C-0055 (Treatment Arm IV Overall Transplant Outcome)
(Arm IV; reduction in Cy Preparative Regimen dosing from 4800 to 1200 mg/m^2 and utilizes day 14 administration of Th2.rapa cells after short-course sirolimus therapy)

IND Subject #	Malignant Disease (Study Entry) Disease[1]	Prior Trx[2]	Disease Status	Transplant Outcome (day post-SCT)	1° Cause o Death[3]
Arm IV (75% Reduced Flu/Cy Preparation) (CSA) (Th2 cells, day 14 administration) (Sirolimus, to day 14)					
Arm IV, #1	MCL	1) CHOP-R 2) Hyper CVAD, Rituxan	Primary Refractory	Died (Day 211)	PD
Arm IV, #2	MDS	1) 5-Azacytidine 2) Thalidomide	Responsive	+ Day 535 Post-SCT	N.A
Arm IV, #3	AML	1) Daunorubicin, VP-16, cytarabine 2) Cytarabine, VP-16 3) Busulfan, VP-16, auSCT 4) Cytarabine/Mitoxantrone/ Asparaginase	Responsive Relapse	+ Day 534 Post SCT	N.A
Arm IV, #4	AML, Transformed	1) Cytara/Idarubicin 2) FLAG/IDA	Responsive	Died (Day 119)	PD; pneumor sepsis
Arm IV, #5	CML (accelerated phase)	1) Hydroxyurea 2) Gleevac	Responsive	+ Day 437 Post SCT	N.A
Arm IV, #6	DLC	1) CHOP-R 2) RICE 3) Hyper-CVAD 4) BEAM au 5) Rituxan	Refractory	Died (Day 91)	PD
Arm IV, #7	AML	(1) Daunorubicin/Ara-C/HIDAC (2) Daunorubicin/Ara-C (3) Ara-C IT/Thiotepa IT Ara-C, FLAG-IDA	Responsive	Died (Day 194)	PD

[1] DLC, diffuse large cell non-Hodgkin's lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia
[2] Number of chemotherapy regimens prior to study entry; "auSCT" = prior autologous stem cell transplant; IT, intrathecal.
[3] N.A. = not applicable; PD = progressive disease.

Table IVD shows the demographics and overall transplant outcome for protocol 04-C-0055 recipients who received transplantation without Flu/Cy conditioning and with Th2.rapa cells administered on day 0 of transplant (arm V). Nine of fourteen subjects are alive with a follow-up range between 5 to 463 days post-transplant.

Table IV D 04-C-0055 (Overall Transplant Outcome)
(Arm V; No Flu/Cy Preparative Regimen Administered)

IND Subject #	Malignant Disease (Study Entry) Disease[1]	Prior Trx[2]	Disease Status	Transplant Outcome (day post-SCT)	1° Cause of Death[3]
Arm V					
(No Flu/Cy Preparation Given) (CSA) (Th2 cells, day 0 administration) (Sirolimus, to day 14)					
Arm V, #1	PTCL	1) CHOP; 2) ESHAP; 3) IT MTX	Responsive Relapse	Day 463 Post SCT	N.A
Arm V, #2	HD	1) ABCD; 2) ESHAP; 3) Mini-BEAM; 4) Gem/ Navelbine; 5) EPOCH-F	Primary Refractory	Died (Day 105)	PD
Arm V, #3	Multiple Myeloma	1) VAD; 2) Cy/Mel auto; 3) Rev; 4) Thal/Dex; 5) VTD/PACE, auHCT; 6) VTD; 7) VTD/PACE; 8) VDR	Refractory	Died (Day 284)	GI hemorrhage/ GVHD
Arm V, #4	DLC/FCL	1) CHOP-R; 2) RICE; 3) Bu/Mel/Thiotepa auto	Responsive	Day 307 post SCT	N.A
Arm V, #5	AML	1) Ara-C/doxo; 2) Ara-C; 3) FLAG	Responsive	Day 226 post SCT	N.A
Arm V, #6	Multiple Myeloma	1) VAD; 2) Rev/Dex	Primary Refractory	Died (Day 168)	Sepsis, GVHD, PD
Arm V, #7	NK/T cell lymphoma	1) CHOP; 2) DHAP	Refractory	Died (Day 58)	PD
Arm V, #8	Multiple Myeloma	1) Thal/Dex	Responsive	Day 159 post SCT	N.A
Arm V, #9	FCL	1) CVP; 2) ESHAP-R; 3) CHOP-R	Responsive Relapse	Day 139 post SCT	N.A
Arm V, #10	DLC	1) CHOP; 2) RICE auto; 3) ESHAP-R, MTX; 4) MINT; 5) Gemz/Cisplat	Refractory	Day 119 post SCT	N.A
Arm V, #11	DLC transformed	1) PACE; 2) CHOP-R	Responsive Relapse	Day 118 post SCT	N.A
Arm V, #12	Burkitts	1) CHOP-R; 2) R-hyper-CVAD; 3) R-IFOS, beam auto; 4) R-Cy-dex	Refractory	Died (Day 81)	PD
Arm V, #13	MCL	1) R-hyper-CVAD; 2) Hy-CVAD, intrathecals	Responsive Relapse	Day 33 post SCT	N.A.
Arm V, #14	Marginal Zone NHL	1) Rituxan; 2) R-CVP; 3) R-CHOP; 4) R-ESHAP; 5) Velcade/Dex	Refractory	Day 5 post SCT	N.A.

[1] DLC, diffuse large cell non-Hodgkin's lymphoma; HD, Hodgkin's Disease; PTCL, peripheral T cell lymphoma
[2] Number of chemotherapy regimens prior to study entry; "au" = prior autologous stem cell transplant.
[3] N.A. = not applicable.

In sum, these clinical data from protocol 04-C-0055 have helped guide the development of this new protocol for metastatic RCC. First, these data indicate that Th2.rapa cells can be administered in a relatively safe manner (without significant engraftment syndrome or other toxicity) provided that the cells are administered with either a reduced dose preparative regimen or no preparative regimen. Because cytoreductive chemotherapy is nominally effective against metastatic RCC, we have elected to develop a treatment strategy that does not involve a preparative regimen. Second, we have shown that predominant donor engraftment can be obtained through administration of Th2.rapa cells even in the absence of a preparative regimen provided that $CD4^+$ T cell depletion has been achieved to a level of < 200 cells per μL prior to the last cycle of EPOCH-F chemotherapy (arm V subjects). Given this information, we have designed an immune depletion regimen of pentostatin and cyclophosphamide that is intended to safely result in similar host immune cell depletion over the 21-day period during which donor Th2.rapa cells are being generated and the donor stem cell product is being harvested.

1.2.4 Potential Role of Th2.rapa Cells for Balancing GVHD and GVT Effects

Although many strategies exist to separate GVHD from GVT effects, clinical dissection of these events has proven problematic in large part due to the shared biology of these two processes (reviewed in Ref. [48]). As this review summarizes, several systemically acting molecular effectors contribute to both GVHD and GVT effects, including IL-2, IFN-γ, fas ligand, TNF-α, and IL-1-α. This pattern of immune activation is characteristic of $CD4^+$Th1 and $CD8^+$Tc1 responses; importantly, such Th1/Tc1 effector mechanisms contribute to anti-tumor responses in experimental renal cell cancer models[49,50]. In our murine studies, we found that donor Th1/Tc1 cells generated potent GVT effects but were limited by lethal GVHD; in contrast, donor Th2/Tc2 cells generated greatly reduced GVHD but also attenuated GVT effects[20]. Based on these results, we developed a new strategy in a murine model that incorporated both unmanipulated donor T cell infusion (which elicits a Th1/Tc1 effector response in vivo) and donor Th2.rapa cell infusion that yields a balanced Th1/Th2 cytokine profile post-transplant and resultant GVT effects with reduced GVHD[22]. Given this information, for transplantation therapy of metastatic RCC, we have designed an allograft strategy comprised of a T cell-replete mobilized hematopoietic stem cell graft and ex vivo generated donor Th2.rapa cells in an attempt to provide a mixed Th1/Th2 cytokine profile post-HSCT.

Post-HSCT cytokine secretion data from protocol 04-C-0055 indicate that Th2.rapa cell infusion in the context of a T cell-replete mobilized allograft indeed is associated with a mixed Th1/Th2 cytokine phenotype (Figure 3). That is, relative to protocol recipients who did not receive Th2 cells (arm III subjects), protocol recipients of Th2.rapa cells (arm I or arm II subjects) had similar post-transplant capacity to secrete the Th1 cytokines IL-2 and IFN-γ and increased capacity to secrete the Th2 cytokines IL-4 and IL-10. Further cytokine data obtained from arm IV protocol subjects (who received Th2.rapa cells as DLI on day 14 post-transplant after the 75% reduced Flu/Cy preparative regimen) indicate that the Th2.rapa cell DLI promotes both Th1- and Th2-type cytokine production post-HSCT (see representative cytokine phenotype, Figure 4). As such, these data indicate that DLI consisting of ex vivo activated donor Th2.rapa cells appear to be capable of activating multiple immune effector mechanisms post-HSCT. Given these data, we now hypothesize that multiple Th2.rapa cell infusions (days 0, 14 and 45 post-HSCT) will promote both Th1 and Th2 immune pathways post-transplant, thereby promoting a GVT effect against metastatic RCC.

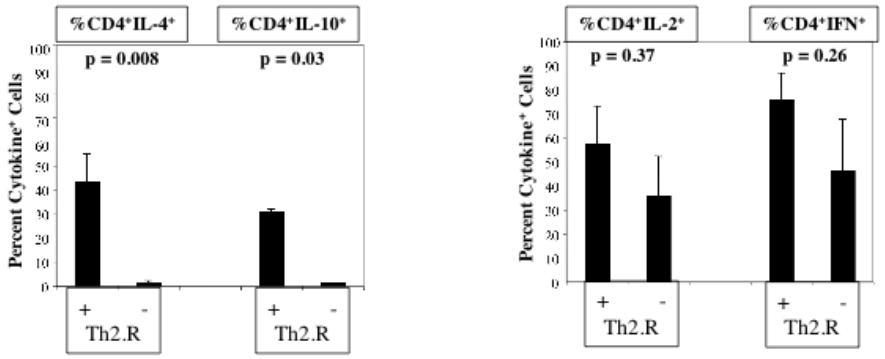

Figure 3. Th2.rapa cell recipients express a balance of both Th1 and Th2 cytokines post-transplant. Protocol 04-C-0055 subjects who received Th2.rapa cells (indicated by "+"; protocol arms I and II) were compared to recipients who did not receive Th2.rapa cells (indicated by "-"; protocol arm III). Peripheral blood was obtained at day 7 post-transplant and co-stimulated overnight. Post-transplant T cells were then harvested and evaluated by flow cytometry for surface CD4 expression and for expression of the Th2 cytokines IL-4 and IL-10 or the Th1 cytokines IL-2 and IFN-gamma by cytokine capture flow cytometry. Results are expressed as the percentage of post-transplant T cells that express a particular cytokine.

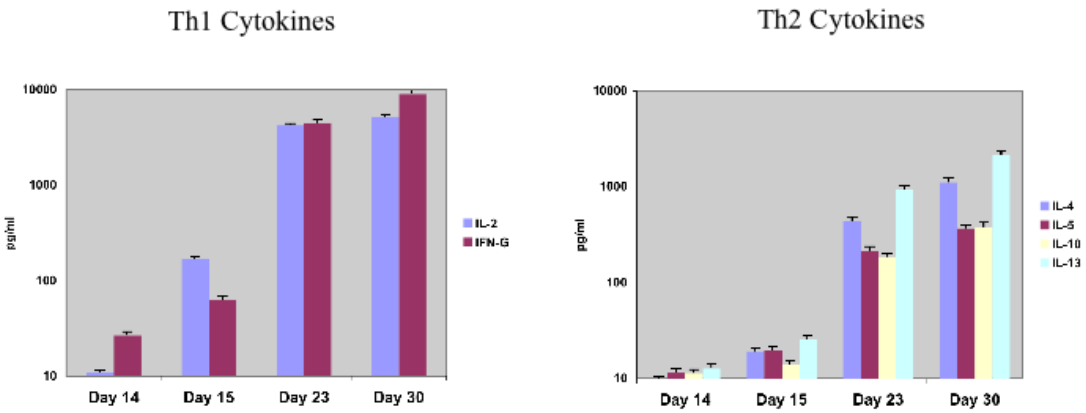

Figure 4. Th2.rapa cell donor lymphocyte infusion (DLI) promotes both Th1 and Th2 cytokines. Post-transplant T cells were harvested at days 14, 15, 23 and 30 from a subject on protocol 04-C-0055 who was treated on arm IV (Th2.rapa cell infusion at Day 14 post-transplant). Post-transplant T cells were co-stimulated, and the 24 hour supernatant was evaluated for cytokine content by Luminex assay (left panel, shown are the Th1 cytokines IL-2 and IFN-gamma; right panel, shown are the Th2 cytokines IL-4, IL-5, IL-10, and IL-13). As this figure shows, at day 14 post-transplant (prior to Th2.rapa cell infusion), the post-transplant T cells had nominal capacity to secrete either Th1 or Th2 cytokines. However, shortly after Th2.rapa cell infusion, the subject had a 10- to 100-fold increase in capacity to secrete IL-2, IFN-gamma, IL-4, IL-5, IL-10, and IL-13.

1.2.5 Host Immune Depletion With Pentostatin and Cyclophosphamide

Host T cells present at the time of transplantation mediate a host-versus-graft rejection response that inhibits alloengraftment, contributes to mixed chimerism (and the resultant delay in onset of GVT effects), and can ultimately result in graft rejection. As such, the number and function of host T cells present at the time of transplantation is an important consideration with respect to alloengraftment; other variables are also important to consider, including donor allograft content and type of post-transplant immune suppression. On arm V of protocol 04-C-0055, we have found that reduction of host immunity to a $CD4^+$ T cell count of < 200 cells/µl prior to the final cycle of EPOCH-F is associated with prompt alloengraftment in the setting of a T cell replete allograft that is augmented with Th2.rapa cells. Because one cycle of EPOCH-F reduces CD4 cells by approximately 50%, it is estimated that arm V subjects would enter transplantation at a CD4 cell steady-state count of approximately 100 cells/µl. Certainly, host $CD8^+$ T cells are known to play a role in mediated graft rejection[51]; indeed, previous studies in the ETIB have found a correlation between host $CD8^+$ T cells and mixed chimerism[52]. Based on these results, we estimate that achieving a $CD4^+$ T cell count of < 100 cells/µl (or, because $CD4^+$ and $CD8^+$ T cell counts are relatively proportional in these recipients, a total $CD3^+$ T cell count of < 200 cells/µl) may be sufficient to ensure prompt alloengraftment in the setting of donor Th2.rapa cell infusion. As such, in the current protocol, we have designed a regimen that we predict will reduce the host's $CD3^+$ T cell count to < 200 cells/µl prior to administration of the allogeneic hematopoietic stem cell and Th2 cell products. Our rationale to develop a novel pentostatin and cyclophosphamide regimen to achieve host immune depletion is based on published clinical data, and our new murine data that indicates pentostatin results in more profound immune T cell depletion than fludarabine.

As previously mentioned, subjects with metastatic RCC are likely to be at higher risk for mixed chimerism and graft rejection relative to hematologic malignancy subjects due to their absence of prior exposure to conventional chemotherapy agents. Metastatic RCC subjects will likely be chemotherapy naïve, and therefore can be anticipated to enroll on study with CD3 cell counts similar to age-matched controls (approximately 1400 CD3 cells/µl)[53]. In our previous protocols involving hematologic malignancy subjects, we utilized EPOCH-F as a regimen to both reduce malignant disease burden and achieve host immune T cell depletion; the number of EPOCH-F cycles was determined in a patient-specific manner depending on the level of immune T cell depletion achieved, and ranged from one to three cycles of therapy (resulting in approximately 21 to 63 days of pre-transplant induction therapy). The EPOCH-F regimen is not a suitable regimen for the current trial in metastatic RCC, as the regimen typically results in only an approximate 30 to 50% reduction in CD3 cell number per cycle. As such, multiple EPOCH-F cycles would need to be administered to achieve the T cell target; such a delay in reaching transplant would be disadvantageous, as the EPOCH-F regimen is most likely not active in metastatic RCC subjects with progressive disease. Therefore, a need exists in the setting of metastatic RCC to develop a new regimen that consistently and quickly achieves significant host T cell depletion (as defined by attainment of host CD3 cell number of ~ 200 cells/µl in a treatment interval of three weeks). The three week time interval of host immune depletion was chosen, as this is the amount of time required to harvest donor cell products and to generate Th2.rapa cells ex vivo. Our plan to administer Th2.rapa cells in the absence of a conventional

Flu/Cy preparative regimen also stems from our desire to avoid the engraftment syndrome toxicity that was observed in that setting.

We will utilize a novel pre-transplant immune T cell depletion regimen that consists of weekly intravenous (i.v.) pentostatin [4 mg/m^2 dose; days 1, 8, and 15; thus, total P dose of 12 mg/m^2] and daily oral, low-dose cyclophosphamide given over a fixed 21-day treatment interval. The rationale for this regimen is derived from the following observations: (1) weekly i.v. pentostatin at a dose of 4 mg/m^2 is effective therapy for many sub-types of human lymphoma, particularly T cell lymphoma[54]; (2) pentostatin depletes non-malignant human T cells prior to allogeneic transplantation; in the only study published on this topic, Dr. Pavletic while at the University of Nebraska found that a total dose of 12 mg/m^2 of single-agent P reduced host CD3$^+$ T cell number by approximately 50%[53]; (3) at a P dose of 4 mg/m^2, it has been determined that the adenosine deaminase (ADA) enzyme, which is the molecular target inhibited by pentostatin, is inhibited for at least one week; (4) analogous to results with fludarabine, more profound depletion of human lymphocytes occurs when ADA inhibition by pentostatin is combined with an DNA alkylation agent, such as cyclophosphamide[55,56]; (5) in humans, a one month regimen of daily oral Cy therapy (100 or 200 mg per day) reduces circulating T cell numbers by approximately 50%[57]; and (6) in murine experiments (see below, Figure 5), we have found that a

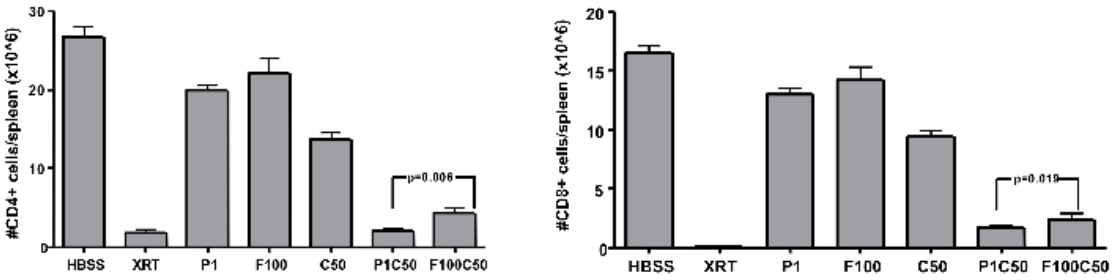

Figure 5. Combination pentostatin + cyclophosphamide results in more profound host CD4$^+$ and CD8$^+$ T cell depletion than combination fludarabine + cyclophosphamide. Cohorts of mice received saline Injection for 3 days ("HBSS"), lethal irradiation (1050 cGy, "XRT"), daily pentostatin x 3 d ("P"; 1 mg/kg/d), daily fludarabine x 3 d ("F"; 100 mg/kg/d), daily cyclophosphamide (Cy) x 3 d ("C"; 50 mg/kg/d), and a combination of either pentostatin + Cy ("P1C50") or fludarabine + Cy ("F100C50"). The pentostatin and fludarabine regimens were equivalent with respect to their induction of host myeloid cell depletion (not shown).

combination of pentostatin and a relatively low-dose of Cy can be identified that modestly reduces the myeloid cell lineage yet results in severe host immune T cell depletion. Importantly, the pentostatin/Cy regimen resulted in greater host T cell depletion than a combination fludarabine/Cy regimen that we have previously evaluated and found to be effective for the prevention of graft rejection[58]. The level of host T cell depletion obtained with combination pentostatin and Cy approached that induced by lethal doses of total body irradiation. These data further demonstrate the great degree of synergy that exists between pentostatin and Cy, as only a nominal level of host T cell depletion was observed with single agent pentostatin therapy. Taken together, these data provide a rationale for developing a novel host immune depletion strategy that utilizes combination pentostatin and cyclophosphamide.

To summarize, we will utilize a 21-day regimen of weekly pentostatin (4 mg/m^2, i.v. on days 1, 8, and 15) and low-dose oral Cy, with the goal of reducing host T cell number from study entry (estimated mean T cell number of 1400 cells/µl) to a level of approximately 200 cells/µl prior to transplantation. Given the synergy between pentostatin and Cy, we believe this target is achievable, as the additive effect of the immune depleting properties of the two agents would be expected to reduce the T cell count from 1400 to 350 (50% reduction each from pentostatin and cyclophosphamide). It is important to note that low-intensity regimens that consisted of single-agent, low-dose pentostatin[59] or pentostatin with 200 cGy of irradiation[53] typically yielded alloengraftment. As such, in the event that host T cell depletion is not as marked as we predict, it is likely that alloengraftment will still occur but with a higher than desirable level of mixed chimerism. If mixed chimerism were to occur and an insufficient anti-tumor response were to develop, then consideration will be given to amend the protocol to evaluate a more intensive preparative regimen.

1.3 RATIONALE FOR 08-C-0088, AMENDMENT C

At the time of amendment C (March, 2010), eight patients have received transplantation on protocol 08-C-0088. Each of the eight patients received the intended outpatient pre-transplant immune depletion regimen without significant adverse event. In each case, the host absolute lymphocyte count was depleted to the target level of less than 200 ALC per microliter; in 8/8 cases, this lymphocyte depletion was accomplished without a single time point of absolute neutrophil count less than 1000 cells. Each patient (8/8) received the HLA-matched sibling allogeneic peripheral blood stem cell graft with subsequent establishment of alloengraftment. A significant degree of mixed donor/host chimerism in the T lymphoid compartment was observed in 7/8 cases: specifically, at day 28 post-transplant, the median percent donor T cell chimerism was 78% (range, 40 to 98%). Each patient tolerated the single-agent, high-dose sirolimus GVHD prophylaxis through the intended period of 60 days post-transplant; through day 100 post-transplant, there has not been a single case of acute GVHD (0/6 fully evaluable patients). Four out of 8 patients have stable disease, with two of these patients at 13 months and 18 months post-transplant; two patients with stable disease are early post-transplant (less than 100 days post-transplant). However, four of 8 patients have died of progressive disease within the first year post-transplant. In sum, these initial clinical trial results indicate that this allogeneic transplantation approach is relatively safe and highly feasible, but is likely limited by relatively modest anti-tumor effects in this population of highly refractory and advanced stage RCC patients. We hypothesize that the apparently modest anti-tumor effects observed in this initial protocol cohort may be due in large part to the post-transplant presence of mixed chimerism in the lymphoid compartment; as a corollary, we also hypothesize that the infusion of a more potent donor T cell product (a modified form of Th2.Rapa cells) will limit mixed chimerism and thereby potentiate anti-tumor effects. In the initial phase of protocol implementation, Th2.Rapa cells were manufactured using a 12-day ex vivo culture interval and infused at days 0, 14, and 45 post-transplant. Recently, we have amended the Th2.Rapa cell IND (#11832) to permit evaluation of a short-term cultured Th2.Rapa cell (6-day culture interval) in the setting of allogeneic HSCT for therapy of refractory hematologic malignancy (NCI protocol 04-C-0055). We hypothesized that modification of the Th2.Rapa cell culture interval from 12-days to 6-days would yield a Th2.Rapa cell product with increased in vivo proliferative potential and resultant increased in vivo effects. At this point in implementation of protocol 04-C-0055, 15 patients

with refractory hematologic malignancy have received the short-term expanded Th2.Rapa cells. This 6-day cultured Th2.Rapa cell product has been well-tolerated, with acute GVHD being observed in 4/15 patients. Preliminary engraftment data suggests that recipients of 6-day cultured Th2.Rapa cells may have reduced mixed chimerism relative to recipients of 12-day cultured Th2.Rapa cells. In addition, partial and complete remissions in patients with chemotherapy-refractory, high-risk hematologic malignancy have been observed in recipients of the short-term expanded Th2.Rapa cells. Because of these initial clinical trial results and the amendment of IND 11832 to evaluate the short-term expanded Th2.Rapa cell product, we propose in amendment C of protocol 08-C-0088 to evaluate this 6-day expanded Th2.Rapa cell product for the allogeneic therapy of metastatic RCC. Specifically, amendment C will utilize the exact same transplant platform that was evaluated in the initial cohort of eight patients (that is: pentostatin plus cyclophosphamide immune depletion; single-agent, high-dose sirolimus GVHD prophylaxis; and Th2.Rapa cell infusion at days 0, 14, and 45 post-transplant). The only difference between the initial cohort and the proposed cohort with amendment C will be the Th2.Rapa cell manufacturing interval (12-days vs. 6-days). In addition, the statistical section will remain the same for the cohort to evaluate the 6-day cultured Th2.Rapa cell. That is, the same safety stopping rules will be employed, and the same efficacy evaluation will be utilized. Specifically, we will evaluate whether therapy with the 6-day expanded Th2.Rapa cell might be associated with a rate of partial or complete remission in metastatic RCC patients that is consistent with a 40% rate in favor of the general 20% rate observed in the literature; to satisfy this anti-tumor objective, at least 3 out of 12 of the initial patients treated with the 6-day expanded Th2.Rapa cells would need to achieve a PR or CR. In such a case, further patient accrual would occur in the second stage of protocol design (to a total of 25 patients).

1.4 RATIONALE FOR 08-C-0088 AMENDMENT F

At the time of amendment F (October, 2011), eleven patients have received transplantation on protocol 08-C-0088. Remarkably, in 11/11 cases, the PC regimen has resulted in host T cell depletion without a single case of neutropenia, thereby confirming our murine model data that demonstrated the PC regimen to be highly selective for lymphocyte depletion; and, in 11/11 cases, the PC-induced host immune depletion was associated with prompt alloengraftment without a single case of graft rejection. As such, the current results indicate that the PC regimen is a novel approach to achieve alloengraftment using an outpatient pre-transplant regimen that does not induce neutropenia.

And, we have found that the high-dose sirolimus regimen, which is continued through day 60 post-transplant, is a very effective approach to GVHD prophylaxis. In fact, our date indicate that this GVHD prophylaxis induces donor T cell tolerance, as indicated by: (a) a failure to generate acute GVHD (0/11 cases of acute GVHD); and (b) stable mixed donor/host T cell chimerism, even in the setting of multiple post-transplant donor lymphocyte infusions consisting of rapamycin-resistant Th2 cells. Consistent with this lack of clinical alloreactivity, we have not seen robust and sustained graft-versus-tumor (GVT) effects in this patient cohort with metastatic renal cell carcinoma. Because there is a tight association between GVHD effects and GVT effects, we have reasoned that the high-dose sirolimus, which is potentially advantageous with respect to reducing tumor cell proliferation and reducing GVHD, is ultimately counterproductive because it tolerizes the adoptively transferred Th2 cells.

These clinical results stand in stark contrast to our results using Th2 cell DLI for the therapy of refractory hematologic malignancy on protocol 04-C-0055. In this protocol, the Th2

cell DLI is administered at day 14 post-transplant after the discontinuation of a short-course of sirolimus and in the context of single-agent cyclosporine GVHD prophylaxis. We have now transplanted more than 80 patients using this approach, and have observed: (1) rapid conversion to full donor lymphoid engraftment after Th2 cell DLI; (2) a rate of acute GVHD of approximately 20% and a rate of chronic GVHD of approximately 50%, with no case of steroid-refractory GVHD; and (3) rapid induction of complete remission in patients with bulky disease, including patients with chemotherapy-refractory and high-grade non-Hodgkin's lymphoma.

As such, on 08-C-0088 and 04-C-0055, we are evaluating the same Th2 cell DLI product in different disease indications (renal cell carcinoma vs. hematologic malignancy, respectively) and in the context of different GVHD prophylaxis (high-dose, prolonged sirolimus vs. short-course sirolimus plus cyclosporine, respectively). At this point, we hypothesize that if we modify the protocol design on 08-C-0088 to mimic the design of protocol 04-C-0055, then we will begin to observe a relatively high-rate of partial or complete responses to the Th2 cell DLI in patients with metastatic renal cell carcinoma. As such, in the amended protocol, we have: (1) maintained the outpatient PC regimen (that aspect of the protocol is not thought to be problematic because prompt alloengraftment has been achieved in each case); (2) changed the GVHD prophylaxis regimen to resemble the regimen used on 04-C-0055 (short-course sirolimus plus cyclosporine); and (3) as in protocol 04-C-0055, adoptively transfer the donor Th2 cell DLI at day 14 post-transplant (after taper of sirolimus; with continuation of cyclosporine GVHD prophylaxis).

Working with our bio-statistician, Dr. Seth Steinberg, we have arrived at a two-stage design that will allow us to rapidly assess whether the proposed new regimen will yield a high-rate of partial or complete remissions in patients with metastatic RCC. Specifically, with only five patients in the first stage of the design, we seek to observe at least 2 out of 5 patients with a PR or CR; this incidence will be considered potentially consistent with a 60% response rate, and therefore merit expansion of accrual to the second stage of the design.

1.5 RATIONALE FOR 08-C-0088, AMENDMENT G

At the time of amendment G (May, 2014), 12 patients have received transplantation on protocol 08-C-0088. The protocol was recently amended to change the transplant platform to more closely mimic the platform that we have developed on protocol 04-C-0055, where we have seen robust anti-tumor responses in lymphoma patients. Specifically, the new transplant platform uses only a short-course of sirolimus, with further immune suppression consisting of cyclosporine; in the lymphoma patients treated on 04-C-0055, this immune suppression strategy (short-course sirolimus and maintenance cyclosporine) has resulted in the conversion of mixed chimerism towards full donor chimerism after pre-emptive T-Rapa cell donor lymphocyte infusion (DLI), with subsequent development of graft-versus-lymphoma effects. As such, this new platform is intended to overcome the primary limitation of the initial version of 08-C-0088 (stable mixed chimerism and RCC tumor progression during long-term, high-dose sirolimus therapy). Only one patient with renal cell carcinoma has been transplanted on this new protocol version of 08-C-0088; an additional four subjects will be accrued to evaluate whether the new platform can generate graft-versus-tumor effects in renal cell cancer patients. This new transplant platform uses: pentostatin plus cyclophosphamide as host preparative chemotherapy; GVHD prophylaxis consisting of cyclosporine plus a short course of sirolimus; and pre-emptive donor lymphocyte infusion with rapamycin-resistant donor T cells manufactured for six days in culture ($T\text{-}Rapa_6$ cells). Also, in protocol 04-C-0055, we have gained experience in using additional doses of T-Rapa cells as DLI in a non-pre-emptive strategy (for therapy of progressive lymphoma after day

60 post-transplant). Three lymphoma patients are evaluable for response to delayed infusion of T-Rapa cells on protocol 04-C-0055. Each patient had bulky disease that was chemotherapy refractory: after T-Rapa cell DLI in this setting, two patients achieved complete remission and one has achieved a partial remission with only minimal evidence of residual disease. Toxicity has been acceptable even though each patient had a history of GVHD prior to the DLI; one patient developed oral chronic GVHD and one patient developed late acute gut GVHD that was steroid responsive. These results suggest that multiple infusions of T-Rapa cells might represent a beneficial post-transplant therapy in patients with refractory, progressive lymphoma; with amendment G of this protocol, we are making one additional change that will bring protocol 08-C-0088 more in line with protocol 04-C-0055. Specifically, with amendment G, patients with persistent renal cell carcinoma tumor post-transplant will be eligible to receive additional infusions of the T-Rapa$_6$ cells. It is our hypothesis that the specialized T-Rapa cell DLI will mediate a more robust GVT effect against RCC than unmanipulated, standard DLI (which we have found to be non-beneficial on previous patients treated on 08-C-0088); in addition, because T-Rapa cells are purified $CD4^+$ T cells with a balanced production of Th1- and Th2-type cytokines, T-Rapa cell DLI may result in less GVHD relative to standard DLI. Standard DLI are associated with a substantial frequency and severity of GVHD; as such, standard DLI is a minimally effective therapy, particularly in the setting of renal cell carcinoma, because it mediates nominal beneficial GVT effects with substantial GVHD-related toxicity. Aside from DLI, there are no known effective therapies for RCC progression post-transplant.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 INCLUSION CRITERIA – RECIPIENT

2.1.1 Diagnosis of metastatic renal cell carcinoma, either clear cell type or non-clear cell type. The diagnosis must be confirmed by the Laboratory of Pathology of NCI (there will be no central pathology review).

2.1.2 The consent process will include a discussion of the potential role of high-dose IL-2 therapy prior to protocol enrollment. High-dose IL-2 therapy is not widely available, but may be available on an NCI protocol (Dr. Yang) . IL-2 therapy may also be administered by any other qualified physician; the Novartis web-site has a list of such physicians. For subjects who are deemed to be eligible for high-dose IL-2 and elect to receive this therapy, such subjects must have progressive disease post-IL-2 to enter this study; such subjects must also have received and have had progressive disease after therapy with one of the agents listed below in Section 2.1.3.

2.1.3 Subject must have progressive disease after therapy consisting of one of the FDA-approved agents for therapy of metastatic renal cell carcinoma, including but not limited to: sorafenib, sunitinib, or temsirolimus.

2.1.4 Patients 18 - 75 years of age. Subjects older than 75 will not be enrolled due to an increased rate of transplant-related complications.

2.1.5 Must have consenting sibling matched at 6/6 HLA antigens (A, B, DR).

2.1.6 Patient must be able to give informed consent.

2.1.7 All previous therapy must be completed at least 2 weeks prior to study entry. Any grade 3 or 4 non-hematologic toxicity of any previous therapy must have resolved to grade 2 or less, unless specified elsewhere in Section **2.1**.

2.1.8 Karnofsky performance status greater than or equal to 80%.

2.1.9 Life expectancy of at least 3 months.

2.1.10 Left ventricular ejection fraction >40% (MUGA or echo) within 28 days of enrollment.

2.1.11 DLCO > 50% of expected value (Hb corrected), obtained within 28 days of enrollment.

2.1.12 Creatinine clearance $\geq$ 40 ml/min. Creatinine clearance will be determined by testing of a 24 hour urine collection and simultaneous serum creatinine value. In previous studies, the creatanine clearance of patients with metastatic renal cell cancer who underwent nephrectomy was 53 ± 19[61].

2.1.13 Serum total bilirubin less than 2.5 mg/dl, and serum ALT and AST values less than or equal to 2.5 times the upper limit of normal. ALT and AST values above these levels may be accepted (up to a maximum of 5 times the upper limit of normal), at the discretion of the PI or study chairperson, if such elevations are thought to be due to liver involvement by malignancy.

2.1.14 Patients with metastatic renal cell carcinoma referred for the study may not be eligible for the experimental protocol therapy due to reasons such as uncertainty about donor HLA typing or need to control malignant disease, infection, or metabolic abnormality such as hypercalcemia on an emergent basis. Should a referred patient present to us in such a scenario, the patient will be referred back to their primary hematologist-oncologist for treatment. However, if referral back to the referring physician is not in the best interest of the patient according to the clinical judgment of the PI, then the patient may receive standard treatment for the malignant disease or complicating conditions (infection, metabolic problems) under the current study. If it becomes apparent that the patient will not be able to proceed to experimental therapy, then he/she must come off study. Recipient-Subjects receiving a standard therapy will be told about the therapy, associated risks, benefits alternatives of the proposed therapy, and availability of receiving the same treatment elsewhere, outside of a research protocol. Because such standard care therapy is not experimental, it is not necessary to complete the eligibility criteria prior to receiving such standard care; however, prior to initiation of the experimental therapy (starting with the PC regimen), the patient must meet each of the eligibility criteria detailed above in sections **2.1.1**through **2.1.13**.

2.2 EXCLUSION CRITERIA - RECIPIENT

2.2.1 Active infection that is not responding to antimicrobial therapy.

2.2.2 Active CNS involvement by malignancy.

2.2.3 HIV infection. There is theoretical concern that the degree of immune suppression associated with the treatment may result in progression of HIV infection.

2.2.4 Chronic active hepatitis B. Patient may be hepatitis B core antibody positive. For patients with concomitant positive hepatitis B surface antigen, patient will require a hepatology consultation. The risk/benefit profile of transplant and hepatitis B will be

discussed with the patient and eligibility determined by the principal investigator and protocol chairperson.

2.2.5 Hepatitis C infection. Patient may have hepatitis C infection. However, each patient will require a hepatology consultation. The risk/benefit profile of transplant and hepatitis C will be discussed with the patient and eligibility determined by the principal investigator and protocol chairperson.

2.2.6 Pregnant or lactating. Patients of childbearing potential must use an effective method of contraception from the time of study entry to at least one year post-transplant; effective methods include intrauterine device (IUD), hormonal (birth control pills, injections, or implants), tubal ligation/hysterectomy, partner's vasectomy, or barrier methods (condom, diaphragm, or cervical cap). Males on the protocol, and their partners of child-bearing potential, must also use an effective form of contraception at study entry and for one year post-transplant. The effects of the chemotherapy, the subsequent transplant, and the medications used after the transplant are highly likely to be harmful to a fetus. The effects upon breast milk are also unknown and may be harmful to the infant; therefore, women should not breastfeed during the interval from study entry to one year post-transplant.

2.2.7 History of psychiatric disorder which may compromise compliance with transplant protocol, or which does not allow for appropriate informed consent (as determined by principal investigator or study chairman).

2.3 INCLUSION CRITERIA – DONOR

2.3.1 Sibling who is 6/6 HLA-matched with recipient.

2.3.2 Ability to give informed consent.

2.3.3 Age 18 years to 80 years. Donors older than 80 will not be eligible due to potentially increased complications from the donation procedure.

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

2.3.5 Donors must be HIV negative, hepatitis B surface antigen negative, and hepatitis C antibody negative. This is to prevent the possible transmission of these infections to the recipient. Donors with a history of hepatitis B or hepatitis C infection may be eligible. However, eligibility determination of such patients will require a hepatology consultation. The risk/benefit of the transplant and the possibility of transmitting hepatitis will be discussed with the patient and eligibility will then be determined by the principal investigator.

2.3.6 A donor who is lactating must substitute formula feeding for her infant during the period of cytokine administration. Filgrastim may be secreted in human milk, although its bioavailability from this source is not known. Limited clinical data suggest that administration of filgrastim or to neonates is not associated with adverse outcomes.

2.4 EXCLUSION CRITERIA – DONOR

2.4.1 History of psychiatric disorder which may compromise compliance with transplant protocol, or which does not allow for appropriate informed consent.

2.4.2 History of hypertension that is not controlled by medication, stroke, or severe heart disease. Individuals with symptomatic angina will be considered to have severe heart disease and will not be eligible to be a donor.

2.4.3 No other medical contraindications to stem cell donation (i.e. severe atherosclerosis, autoimmune disease, iritis or episcleritis, deep venous thrombosis, cerebrovascular accident). Patients with a history of coronary artery bypass grafting or angioplasty will receive a cardiology evaluation and be considered on a case-by-case basis.

2.4.4 History of prior malignancy. However, cancer survivors who have undergone potentially curative therapy may be considered for stem cell donation on a case-by-case basis. The risk/benefit of the transplant and the possibility of transmitting viable tumor cells at the time of transplantation will be discussed with the patient.

2.4.5 Donors must not be pregnant. The effects of cytokine therapy on a fetus are unknown. Donors of childbearing potential must use an effective method of contraception from the time of study entry until at least one year post-transplant.

2.4.6 Anemia (Hb < 11 gm/dl) or thrombocytopenia (platelets < 100,000 per μl). 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 Blood Bank.

2.5 RECIPIENT RESEARCH ELIGIBILITY EVALUATION

2.5.1 The majority of the following clinical, laboratory and radiologic assessments must be performed in the patient (recipient) within 28 days before the enrollment or initiation of induction chemotherapy; some tests, as indicated, can be performed in a window greater than 28 days before initiation of chemotherapy.

2.5.2 Complete medical history and physical examination.

2.5.3 Nutritional assessment (initial consult; within 90 days).

2.5.4 Dental consultation to assess need for teeth cleaning or removal (within 6 months).

2.5.5 Social work consultation.

2.5.6 Typing for HLA-A, -B, and –DR (at any time prior to initiation of therapy).

2.5.7 Antibody screen for hepatitis A, B, and C; HIV, HTLV-I/II, CMV, adenovirus, EBV, HSV, Toxoplasma, and syphilis.

2.5.8 PPD test (if considered to be in a high-risk group).

2.5.9 PT, PTT, and ABO typing.

2.5.10 Urinalysis and 24 hour urine collection for determination of creatinine clearance.

2.5.11 The following items should be obtained at initial screening and also within 48 hours before starting induction chemotherapy:

2.5.11.1 CBC with differential.

2.5.11.2 Acute care panel, hepatic panel, lipid panel, and mineral panel.

2.5.11.3 Urine βHCG in women of childbearing potential.

2.5.12 Chest radiograph (within 90 days).

2.5.13 Electrocardiogram and 2-D echocardiogram (within 90 days).

2.5.14 PET scan and CT scans of chest, abdomen, pelvis, and neck.

2.5.15 CT or MRI of the head.

2.5.16 Pulmonary function test.

2.5.17 Biopsy specimens will be reviewed by the Laboratory of Pathology/CCR/NCI Pathology for confirmation of histologic diagnosis prior to enrollment on study. There will be no central pathology review.

2.5.18 PCR test of DNA mini-satellite regions for future determination of chimerism (at any time prior to initiation of chemotherapy).

2.6 DONOR RESEARCH ELIGIBILITY EVALUATION

2.6.1 Complete medical history and physical examination.

2.6.2 Typing for HLA-A, B, and DR.

2.6.3 Antibody screen for Hepatitis A, B, and C; HIV; HTLV-1/II, CMV, adenovirus, EBV, HSV, toxoplasma, and syphilis.

2.6.4 CBC with differential, PT, PTT, and ABO typing.

2.6.5 Acute care panel, hepatic panel, and mineral panel.

2.6.6 Urinalysis.

2.6.7 Urine Beta-HCG in women of childbearing potential.

2.6.8 Chest radiograph.

2.6.9 Electrocardiogram.

2.6.10 PCR test of DNA mini-satellite regions for future determination of chimerism.

2.7 REGISTRATION PROCEDURES

Protocol "Entry date" is the day that consent forms have been signed by both donor and recipient. "Treatment start date" is the day recipient begins induction therapy.

Authorized staff must register an eligible recipient and an eligible donor with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at CRO, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. Voicemail is available during non-working hours.

2.7.1 Donors Registration (NIH)

To alleviate the burden of additional travel, donors may sign the donor consent form on a provisional basis while awaiting final determination of donor and patient eligibility; thus, the date of donor consent may precede the patient's date of consent by up to four weeks.

However, the patient may not sign consent before both the donor and patient have been determined to be eligible, and neither the donor nor the patient will be registered before both have signed consent. Both the patient and the donor must be registered.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

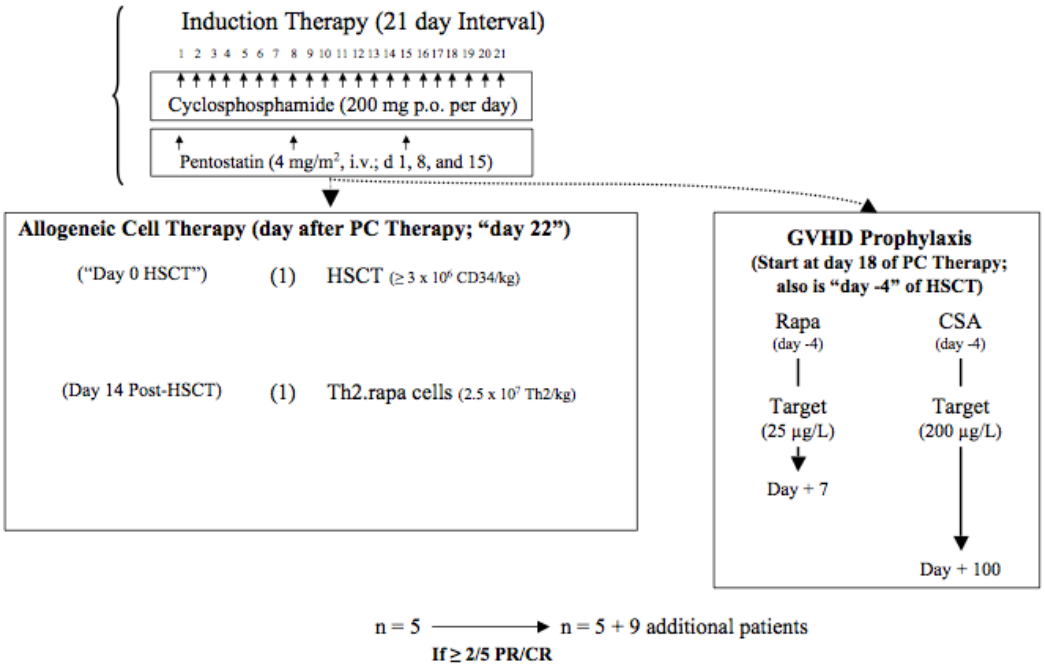

Figure 6. Protocol schema. All subjects will receive induction chemotherapy (pentostatin and cyclophosphamide). Subjects will receive GVHD prophylaxis consisting of short-course sirolimus combined with maintenance therapy with cyclosporine. All patients will receive a mobilized allograft augmented and Th2.rapa cell donor lymphocyte infusion at day 14 post-transplant. Five subjects will be accrued initially; if ≥ 2/5 subjects have a PR or CR, nine additional subjects will be accrued.

3.2 DONOR LYMPHOCYTE HARVEST

3.2.1 Donors will undergo a 10-liter apheresis procedure (CS-3000 or an equivalent machine). The experimental Th2 cells will be generated centrally in the NIH DTM. To accomplish this, donors for recipients will undergo the lymphocyte harvest at the NIH DTM. The donor plasma utilized for ex vivo T cell expansion will be obtained from this apheresis procedure.

3.2.2 The lymphocyte fraction of the product will be enriched for $CD4^+$ T cells by CliniMACS® CD4 enrichment according to the established IND (BB-IND 11832).

3.2.3 The resultant CD4-enriched donor lymphocyte product will be cryopreserved according to the established IND in aliquots of 50 to 200 x 10^6 cells/vial or seeded directly into culture in the NIH DTM (see Th2 culture method, below).

3.3 DONOR HEMATOPOIETIC STEM CELL HARVEST

3.3.1 Following lymphocyte harvest, donors will receive filgrastim as an outpatient (10 ug/kg/day each morning; subcutaneously) for 5, 6, or 7 days. Donors for recipients will undergo stem cell mobilization and harvest 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 ug/kg BID. Donor should take filgrastim upon awakening in the morning. This is especially important on days 5, 6, and 7 of the injections.

3.3.2 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 ($\geq 4 \times 10^6$ per kg). 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.

3.3.3 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.

3.3.4 In the event that $\geq 4.5 \times 10^6$ $CD34^+$ cells per kg is obtained, the volume of the apheresis product that equals 0.5×10^6 $CD34^+$ cells per kg will be set aside for research purposes (specifically, to isolate donor APC populations for immune studies). This sample will be picked up by Dr. Fowler's lab (phone: 301-594-4536) .

3.3.5 In the event that $< 3 \times 10^6$ $CD34^+$ cells per kg are harvested, the donor will be given one to two weeks of rest, and then will be re-treated with filgrastim (8 ug/kg subcutaneously, BID, for five days) followed by repeat blood stem cell harvesting.

3.3.6 A 15 to 25 liter large volume whole blood pheresis will be performed via a 2-armed approach or a temporary central venous catheter in the femoral position using the Baxter CS3000Plus, Cobe Spectra, or an equivalent instrument (typically, 4 to 6 hour procedure).

3.3.7 The apheresis procedure will use ACD-A anti-coagulant, or heparin.

3.3.8 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) according to the method specified in the IND.

3.3.9 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.

3.3.10 If donor and host are ABO incompatible, red blood cells will be depleted from the stem cell product, as specified in the IND.

3.4 IN VITRO GENERATION OF DONOR $CD4^+$ TH2 CELLS

3.4.1 Donor $CD4^+$ T cells will be resuspended to a concentration of 0.3×10^6 cells/ml in X-Vivo 20 media supplemented with 5% heat-inactivated autologous plasma.

3.4.2 To meet the Th2 therapy dose target of 2.5 x 10^7 cells/kg, initial CD4 input into culture should be 300 x 10^6 cells. This input number should allow for expansion of Th2 cells sufficient for Th2 cell DLI at day 14 post-transplant.

3.4.3 Donor $CD4^+$ T cells will be stimulated with anti-CD3/anti-CD28 coated magnetic beads (3:1 bead:cell ratio) in filtered flasks at 37° C in 5% CO2 humidified incubators.

3.4.4 At culture initiation, media will be supplemented with sirolimus (1 uM).

3.4.5 At culture initiation and on day 2 of culture, recombinant human IL-4 (obtained through cross-filing on CTEP IND of Schering IL-4; 1000 I.U. per ml), and recombinant human IL-2 (purchased from Chiron Therapeutics; 20 I.U. per ml) will be added.

3.4.6 After day 2, cells will be maintained at a concentration of 0.25 to 1.0 x 10^6 cells/ml by the addition of fresh X-Vivo 20 media supplemented with autologous plasma (5%), IL-2 (20 I.U./ml), IL-4 (1000 I.U./ml), and sirolimus (1 uM).

3.4.7 The median cell volume and concentration of expanded Th2 cells will be determined using a Multisizer II instrument (Coulter).

3.4.8 There will be no anti-CD3, anti-CD28 restimulation of expanded Th2 cells.

3.4.9 Because the change to a Th2 cytokine phenotype occurs early in cell culture (by day 6 of culture), the Th2 cell product should be harvested and cryopreserved at day 6 of culture.

3.4.10 Th2 cells generated under these conditions express high levels of the IL-2 receptor CD25 and expand more vigorously to higher doses of IL-2 (up to 1000 I.U./ml), with maintenance of the Th2 cytokine phenotype. Thus, if adequate CD4 expansion does not occur in a particular donor, repeat Th2 generation at an IL-2 concentration of 1000 I.U./ml will be utilized.

3.4.11 The following will be the minimal phenotypic requirements of any particular Th2 cell culture to qualify for cryopreservation with subsequent administration. . Cells not meeting these requirements will be discarded.

3.4.11.1 Flow cytometry: > 70% $CD4^+$ T cells, and < 5% contaminating $CD8^+$ T cells.

3.4.11.2 Absence of bacterial and fungal growth.

3.4.11.3 Absence of endotoxin content by the limulus assay.

3.4.11.4 Negative mycoplasma test.

3.4.11.5 After magnetic bead removal, < 100 beads per 3 x 10^6 cells.

3.4.11.6 The functional characteristics of the expanded Th2 cells will be measured and retrospectively correlated to any observed post-transplant effect, such as toxicity or GVHD outcome. However, in this pilot study, this information will not be utilized as formal release criteria for the cell product. Functional studies will include constitutive and co-stimulated cytokine secretion, with evaluation of type II cytokines IL-4, IL-5, IL-10, and IL-13 and type I cytokines IL-2 and IFN-γ, and flow cytometry to evaluate surface CD25, CD28, CD62L, and CD40L expression.

3.5 OPTIONAL TUMOR ACQUISITION

3.5.1 One research objective is to determine whether the therapeutic aspect of transplantation involves an immune component that is specifically directed towards tumor cells. Identification of tumor reactive T cells generated in the allogeneic context may help in the development of new therapies to improve anti-tumor potency.

3.5.2 To facilitate evaluation of tumor specific immune responses, patients may be asked to undergo an optional core biopsy procedure to obtain tumor tissue prior to chemotherapy. The Interventional Radiology Department of NIH, will evaluate each case for their capacity to obtain tumor tissue; only cases that they deem at low risk for complication will be asked to undergo the core biopsy procedure.

3.5.3 Dr. Fowler's lab (4-4536) will pick-up the biopsy specimen from Interventional Radiology, and utilize this material for in vitro assays.

3.6 PENTOSTATIN/CYCLOPHOSPHAMIDE INDUCTION CHEMOTHERAPY REGIMEN

3.6.1 Adequate central venous access is required for all patients on this protocol.

3.6.2 This regimen will be a 21-day treatment interval in all subjects.

3.6.3 Each subject will receive three doses of pentostatin by intravenous infusion on days 1, 8, and 15 of the treatment interval.

3.6.4 Based on published data[62], pentostatin dosing will be as follows:

3.6.4.1 If calculated CrCl is $\geq$ 60 ml/min, give 4 mg/m^2 of pentostatin.

3.6.4.2 If calculated CrCl is between 40 and 59 ml/min, give 3 mg/m^2 of pentostatin.

3.6.4.3 If a subject develops evidence of renal insufficiency during the 21-day interval of pentostatin and cyclophosphamide therapy (increase in serum creatinine level of more than 33%), then the CrCl (performed by estimated CrCl, not by 24-hour urine collection method) should be repeated prior to the day 8 and day 15 dosing of pentostatin.

3.6.4.4 If calculated CrCl decreases during study implementation to between 30 and 39 ml/min, give 2 mg/m^2 of pentostatin.

3.6.4.5 If calculated CrCl falls below 30 ml/min, pentostatin will be held.

3.6.5 Prior to pentostatin infusion, the patient will receive 1 liter of 0.9% sodium chloride over 30 to 60 minutes.

3.6.6 Pentostatin will be reconstituted by pharmacy to a concentration of 2 mg/ml as per vial instructions. The appropriate patient specific dose will then be added to 500 ml of 0.9% sodium chloride and infused over 30 to 60 minutes.

3.6.7 Pentostatin can be highly emetogenic. As such, we provide the following anti-emetic regimen guideline. Variation of this regimen, as described below, is allowed at the discretion of the PI. The neurokinin-1 receptor antagonist aprepitant will be administered in combination with dexamethasone before and after each dose of pentostatin.

3.6.8 Specifically, aprepitant will be administered by IV infusion 60 minutes prior to each dose of pentostatin (days 1, 8, and 15); the dose of aprepitant will be 115 mg. Alternatively,

aprepitant may be administer by P.O. route at a dose of 125 mg given 60 minutes prior to pentostatin.

3.6.9 In addition, oral aprepitant (capsules) will be administered on the next two days following pentostatin therapy (days 2 and 3; days 9 and 10; and days 16 and 17) at a dose of 80 mg each day.

3.6.10 Dexamethasone will be administered by IV infusion 60 minutes prior to each dose of pentostatin (days 1, 8, and 15); the dose of dexamethasone will be 12 mg.

3.6.11 In addition, oral dexamethasone (tablets) will be administered on the next two days following pentostatin therapy (days 2 and 3; days 9 and 10; and days 16 and 17) at a dose of 4 mg each day.

3.6.12 Finally, as a third agent for anti-emesis, ondansetron will be administered at a dose of 8 mg by IV infusion 60 minutes prior to each dose of pentostatin (days 1, 8, and 15).

3.6.13 For the remainder of the 21-day treatment interval, ondansetron will be administered at an oral dose of 8 mg (tablets) every 12 hours on an as needed basis.

3.6.14 Oral cyclophosphamide (Cy) will be given on days 1 through 21 of induction

3.6.15 Because cyclophosphamide can cause cystitis, it is important for patients to stay well hydrated. At a minimum, patients should drink at least 2 to 4 liters of fluid per day to maintain a clear color to the urine. It is also especially important to void the bladder prior to sleeping.

3.6.16 Patient compliance with the oral cyclophosphamide and other oral medications will be assessed by the research nurses at each patient's follow-up visit to NCI .

3.6.17 During the 21-day pentostatin/Cy treatment interval, CBC with differential, chem.-20, should be repeated twice per week (on days 1, 8, 15, and 21; and on one additional time point in-between each of these days).

3.6.18 Urinalysis should be performed on days 1, 8, 15, and 21 of chemotherapy and on one additional time point in-between each of these days (that is, approximately day 4 or 5, day 11 or 12, and day 18 or 19).

3.6.19 The dose of Cy will be 200 mg each day (PO), with some provision for dose reduction as per section **3.6.22**; IV infusion of this same dose may be allowed if a patient is unable to tolerate oral therapy.

3.6.20 In the event that grade 3 neutropenia develops during chemotherapy (absolute neutrophil count [ANC] < 1000 cells per mm^3), G-CSF therapy will be instituted at a dose of 5 µg/kg per day by subcutaneous injection. G-CSF therapy should be discontinued if the neutrophil toxicity has resolved to grade 0 at the next CBC determination (bi-weekly determinations; ANC > 1500 cells per mm^3).

3.6.21 The primary toxicities anticipated with the pentostatin and cyclophosphamide regimen may include hematologic toxicity, nausea, mucositis, and toxicity relating to infectious complications.

3.6.22 The goal of the pentostatin and cyclophosphamide regimen is to uniformly and safely reduce the number of host immune cells prior to transplantation. The target goal for this

immune depletion has defined as an absolute lymphocyte count (ALC) of ≤ 200 cells/μL. It is anticipated that ALC values significantly higher than this may predispose to graft rejection; on the other hand, values significantly lower than this may predispose to engraftment syndrome and GVHD. Given this immune depletion goal, and the desire to minimize toxicity related to cyclophosphamide, the following dose modifications of cyclophosphamide will be utilized. The goal of this dose adjustment schedule will be to ensure that each patient finishes chemotherapy with an ALC < 200, while limiting the chance that patients finish chemotherapy with severe reductions in ALC (< 100).

Dose Adjustment for Cyclophosphamide

Day of Cycle[1]	ALC Value at time of Evaluation[2]	Cyclophosphamide Dose[3]
1	Any	200
4	Any	200
8	≥ 500	200
	251-499	100
	≤ 250	0
11	≥ 400	200
	201-399	100
	≤ 200	0
15	≥ 300	200
	151-299	100
	≤ 150	0
18	≥ 200	200
	101-199	100
	≤ 100	0

[1] Day of cycle: days 4, 11, and 18 represent the mid-point of each of the three weeks (between pentostatin doses). A 24-hour difference in timing may be allowed for logistic purposes (that is, mid-point evaluation may occur for example on days 5, 12, and 19).
[2] ALC values expressed as cells per μL.
[3] Cyclophosphamide dose expressed as mg per day; the indicated dose will be administered daily until the next ALC measurement.

3.6.23 Because pentostatin is rarely associated with neurologic toxicity (seizure, coma), special attention should be paid towards evaluating CNS toxicity. In the event that chemotherapy is associated with any neurologic toxicity of grade 2 or greater severity, the institutional PI should be contacted to discuss whether further pentostatin therapy and further protocol therapy is warranted.

3.6.24 In a previous study, high-dose cyclophosphamide and high-dose total body irradiation combined with pentostatin was associated with cardiac toxicity[63]. To help ensure that cardiac toxicity is not a significant limitation of the low-dose cyclophosphamide regimen utilized in this protocol, the 2-D cardiac echocardiogram will be repeated at day 21 of the chemotherapy cycle (+/- 3 days).

3.7 GVHD PROPHYLAXIS

3.7.1 Patients will receive GVHD prophylaxis consisting of a short course of sirolimus plus maintenance cyclosporine A.

3.7.2 Sirolimus will be initiated on "day -4" of HSCT. Day -4 of HSCT is also characterized as day 18 of the PC regimen; that is, transplantation will ideally occur on 'day 22' of the PC regimen. However, up to a three day delay after the induction regimen may be permitted for logistic reasons (i.e., for inpatient scheduling purposes).

3.7.3 Sirolimus will be administered by mouth as Rapamune tablets at an initial "loading dose" of 16 mg.

3.7.4 On days -3, to +7 post-transplant, sirolimus dosing will be Rapamune tablets, 4 mg, p.o., each day; there will be no sirolimus administered after day 7 post-SCT.

3.7.5 Sirolimus dose should be adjusted to maintain a target therapeutic concentration of 25 μg/L (suitable range, 20 to 30 μg/L). Sirolimus levels will be drawn on a Monday, Wednesday, Friday schedule (if inpatient) or a Tuesday, Friday schedule (if outpatient).

3.7.6 For sirolimus levels, 2.5 cc of blood will be collected into EDTA tubes and analyzed at the NIH Clinical Center .

3.7.7 Cyclosporine will also be initiated at day -4 of HSCT. Dosing will be by oral tablets (initial dosing of 2 mg/kg every 12 hours). Cyclosporine dose should be adjusted to maintain trough concentration of 200 μg/L (acceptable range, 150 to 225). Cyclosporine will be maintained through day 100, and then gradually tapered from day 100 to day 180. However, cyclosporine may be tapered more rapidly in an attempt to control malignancy. In unanticipated cases where oral therapy is not possible, intravenous cyclosporine may be administered.

3.8 ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

3.8.1 On day 0, the patient will receive the peripheral blood stem cell (PBSC) product.

3.8.2 The cryopreserved PBSC product will be thawed and immediately administered intravenously. The target dose of the PBSC is $\geq 4 \times 10^6$ $CD34^+$ cells per kg. However, if donor apheresis on days 5, 6, and 7 yields a total of $\geq 3 \times 10^6$ $CD34^+$ cells per kg, this level of $CD34^+$ cell dose will also be allowed.

3.8.3 If cell transfer results in DMSO-related toxicities (chills, muscle aches), diphenhydramine and meperidine may be administered, but steroids are not allowed.

3.9 TRANSPLANT PROCEDURE: DONOR TH2 CELL ADMINISTRATION

3.9.1 For all subjects, cryopreserved donor Th2 cells will be thawed and administered intravenously on day 14 post-transplant. Th2 cells will be administered using the NIH clinical guidelines for infusing biological products.

3.9.2 The target dose of Th2 cells will be constant for each study recipient (2.5×10^7 Th2/kg). Lower doses of Th2 cells are allowed in the event that Th2 cell manufacturing does not

provide the target dose (doses as low as 2.5 x 10^6 Th2/kg will be permitted; variations in Th2 cell dosing will be reported to the FDA in the annual report.

3.9.3 No steroids will be allowed to treat DMSO-related toxicities (chills, muscle aches) that may occur after cell infusion; diphenhydramine and meperidine are allowed.

3.9.4 Determination of whether a Th2 cell infusion is safe will be based on the presence or absence of hyperacute GVHD, grade 4 or 5 toxicity attributable to the Th2 cells, and whether the alloengraftment goal has been achieved.

3.9.5 For this study, hyperacute GVHD will be defined as grade III or IV acute GVHD that occurs within the first 14 days after Th2 cell infusion.

3.10 TRANSPLANT PROCEDURE: GROWTH FACTOR ADMINISTRATION POST-TRANSPLANT

3.10.1 On day 0 of the transplant, immediately after PBSC transfusion, patients will begin treatment with recombinant human filgrastim at a dose of 5 ug/kg/day s.c.

3.10.2 Filgrastim will continue until the ANC count is greater than or equal to 5000 cells per ul for at least one day or greater than or equal to 1000 cells per ul for three consecutive days.

3.11 DONOR LYMPHOCYTE INFUSIONS CONSISTING OF TH2 CELLS

3.11.1 DLI will be administered on days + 14 (± 2-days to account for weekends and holidays).

3.11.2 DLI will consist of the cryopreserved donor Th2.rapa cells and be administered at a target dose of 2.5 x 10^7 Th2 cells/kg.

3.11.3 The day +14 DLI will not be administered if the subject has developed grade II or greater GVHD or a case of engraftment syndrome of sufficient severity to require systemic corticosteroid therapy.

3.12 TREATMENT OF PERSISTENT OR PROGRESSIVE DISEASE POST-TRANSPLANT

3.12.1 Patients will receive Th2 cell infusion in a pre-emptive manner at day 14 post-transplant.

3.12.2 After day 28 post-transplant, in the event of persistent or progressive disease, a patient may be treated first by early taper of cyclosporine and then by administration of further donor lymphocytes. Additional donor lymphocytes consist of further infusions of ex vivo manufactured Th2 cells, or if additional manufactured Th2 cells are not available, infusion of unmanipulated donor lymphocytes may be used (see below, section 3.12.3).

3.12.3 Donor lymphocytes will be collected by apheresis, either in steady state (no donor therapy) or after G-CSF mobilization; when possible, such G-CSF mobilized, T cell-containing products will consist of a cryopreserved aliquot of cells from the initial mobilized collection (that is, the T cell-containing stem cell product). The donor product may be enriched for lymphocytes by Ficoll-Hypaque procedure as per NIH DTM protocol . Alternatively, in cases where additional donor stem cells are desired, the donor product may be administered without lymphocyte purification. DLI may be sequentially administered, with initial dosing typically at 5 x 10^6 or 2 x 10^7 $CD3^+$ T cells per kg, with subsequent dose increases to 1 x 10^8 T cells per kg.

3.12.4 In cases where DLI has not been successful (no PR or CR), then further DLI may be administered after host immune depletion. Host immune depletion prior to DLI administration is standard practice in allogeneic HSCT and has previously consisted of many different chemotherapy regimens. However, to standardize this aspect of the protocol therapy, any chemotherapy administered prior to DLI on this protocol will consist of a shorter course of the same agents utilized in the pre-transplant regimen (intravenous pentostatin plus low-dose, oral cyclophosphamide; PC regimen). We are currently evaluating either a 7-day or 14-day PC regimen prior to DLI on a separate protocol (11-C-0016); to be consistent across protocols, we will therefore use either a 7-day or a 14-day PC regimen prior to DLI on this protocol. Patients will initially receive the DLI after the 7-day regimen; then, if a satisfactory clinical response has not been obtained (no PR or CR), then patients may be eligible to receive the DLI following the 14-day regimen. The goal of the PC regimen is to perform the DLI with substantial host immune depletion (reduction in the ALC value) and without grade 3 neutrophil toxicity (no reduction of ANC to a value < 1000). The 7-day regimen will consist of: Pentostatin, (4 mg/m^2 iv on days 1 and 5) combined with Cyclophosphamide (200 mg po daily on days 1 through 7). The 14-day regimen will consist of: Pentostatin (4 mg/m^2 iv on days 1, 5, 9 and 13) combined with Cyclophosphamide (200 mg po daily on days 1 through 14). The cyclophosphamide dose may be reduced or omitted if there is any myeloid cell toxicity (reduction in ANC value).

3.12.5 Subjects who fail to achieve stable disease, a PR or CR with this additional DLI therapy may be taken off study. An attempt will be made to refer such subjects for further investigational therapy, such as protocols ongoing or planned in the NCI-CCR. In the event that such therapy is not available, subjects will be taken off study.

3.13 INTERIM EVALUATION AFTER INDUCTION CHEMOTHERAPY (PRE-TRANSPLANT)

3.13.1 Routine chemistry and hematology panels (within 48 hours of transplant).

3.13.2 CT scans of chest, abdomen, and pelvis (within 96 hours of transplant).

3.13.3 2-D Cardiac Echo

3.14 DETERMINATION OF DONOR/HOST CHIMERISM POST-TRANSPLANT

3.14.1 Determination of donor vs. host chimerism will be performed by VNTR-PCR method by a CLIA-certified lab.

3.14.2 Initial baseline determination on donor and recipient will be at time of study entry.

3.14.3 Post-transplant chimerism will be determined at the following time points:

3.14.3.1 Days 7 and 14 post-transplant. We will evaluate T lymphoid, myeloid, and total blood chimerism. For this subset evaluation, send 30 cc blood in yellow ACD tube.

3.14.3.2 Blood chimerism will also be evaluated at days 28, 45, 60, 100 and 180, and 12 months post-transplant. Marrow chimerism will be tested as clinically indicated (i.e., mixed chimerism).

3.14.3.3 If the day 14 chimerism shows > 95% donor elements, these later time points will only evaluate total chimerism. In cases where total chimerism only is being evaluated, send only 10 cc of blood in yellow ACD tube.

3.14.3.4 Chimerism may be measured at other time points, if indicated (e.g., to determine effect of immune modulation in attempt to increase donor chimerism).

3.15 POST-HSCT EVALUATION

3.15.1 After completion of therapy, the patient will be followed for potential complications related to allogeneic HSCT. The patient will be followed at least twice weekly in the outpatient setting until at least day +100 after discharge from the hospital.

3.15.2 The patient will be seen in follow-up to evaluate disease status and late problems related to allogeneic HSCT, at days +28, +60, +100, +145, and +180 (all time points should be plus or minus three days); and 9, 12, 18, and 24 months post-transplant. At these times patients will have the following tests performed:

3.15.2.1 Routine chemistry and hematologic panels;

3.15.2.2 CT scans of chest/abdomen/pelvis/neck (and PET scan if indicated).

3.15.2.3 For any subject that enters a PR or CR, a CT or MRI of the brain must be performed to document continued absence of CNS disease.

3.15.2.4 For patients who enter into PR or CR, a confirmatory CT scan must be obtained one month after the remission observation in order to meet RECIST criteria.

3.16 CONCURRENT THERAPIES

3.16.1 Please see infectious disease prophylaxis as defined in Section **4.1** .

3.17 OFF STUDY CRITERIA

3.17.1 The donor or recipient will be removed from protocol for any of the following reasons:

3.17.1.1 Unacceptable toxicity (> grade 3) for the donor.

3.17.1.2 For the recipient while receiving pentostatin plus cyclophosphamide regimen: irreversible non-hematologic toxicity > grade 3 (does not resolve to ≤ grade 2 toxicity within 14 days).

3.17.1.3 The donor or recipient refuses to continue therapy.

3.17.1.4 In addition, the patient may at any time be removed from protocol at the principal investigator's discretion, if the PI deems the patient to be at unacceptable risk to remain on study. Reasons for this action may include (but are not limited to) disease progression with declining organ function/performance status before transplantation; inadequate family/caregiver support; noncompliance.

3.17.2 Off-Study Procedure

3.17.2.1 NIH Subjects

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

4 SUPPORTIVE CARE

4.1 INFECTION PROPHYLAXIS

(For a full description of infection prophylaxis please refer to the NIH BMT Consortium Supportive Care Guidelines at http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml)

4.1.1 All recipients will receive prophylaxis against *Pneumocystis jirovecii* pneumonia, beginning with the P/C induction therapy and resuming at the time of platelet recovery. PCP prophylaxis will continue until a subject is completely off of immune suppression therapy and the CD4 count is > 200 for 3 months. Trimethoprim/sulfamethoxazole is the preferred regimen.

4.1.2 All subjects will receive fluconazole for prophylaxis against yeast infections. Fluconazole will continue through transplantation until day +100; fluconazole may then be stopped provided that the subject is off of immune suppression therapy. Fluconazole will be stopped during therapy with an alternative antifungal agent.

4.1.3 During the peri-transplant period, subjects will receive prophylactic broad-spectrum antibiotics with ceftazidime in the setting of grade 4 neutropenia (ANC < 500/μl) and a first fever with T $\geq$ 38.3°C). Recipients with allergy to cephalosporin antibiotics will receive an alternative regimen for prophylaxis as recommended by the infectious diseases consultant. Recipients with neutropenia and fever that persists for longer than 4 days despite broad-spectrum antibiotics will receive empiric antifungal therapy with caspofungin, liposomal amphotericin B, or voriconazole, in accordance with standard practices. Empiric antifungal therapy may be started at other times, if clinically indicated. If at all possible, voriconazole should not be administered during the time of sirolimus therapy; if voriconazole must be utilized, it will be necessary to reduce sirolimus dosing by approximately 90%.

4.1.4 All subjects will receive valacyclovir for prophylaxis against herpes simplex virus and varicella zoster virus infection/reactivation. This therapy will start on day 1 of the P/C induction chemotherapy and continue through transplantation until day +100 post-transplant; valacyclovir may be discontinued once a subject has been removed from all immune suppression therapy. Valacyclovir will be discontinued while patients are receiving IV acyclovir, valganciclovir, ganciclovir, or foscarnet.

4.1.5 Subjects with positive pre-transplant serology for Cytomegalovirus (CMV) and/or are to receive transplantation from a CMV-seropositive donor will be monitored for CMV reactivation by clinical evaluation and weekly testing with CMV antigenemia or PCR assays as clinically appropriate. CMV reactivation or disease will be treated according to the NIH BMT consortium web-site. Weekly monitoring will continue through at least 6 months post-transplant, with further monitoring of subjects required in the event that continued immunosuppression therapy is required.

4.1.6 Subjects receiving immunosuppression for chronic GVHD will receive penicillin V for prophylaxis against bacterial infections. All recipients will undergo vaccinations as

beginning 6 months after transplantation, as further detailed in the NIH BMT consortium web-site.

4.1.7 Management of Engraftment Syndrome (ES) - ES may occur at the time of neutrophil recovery. Clinical manifestations include noninfectious fever, rash, and vascular leak causing non-cardiogenic pulmonary edema, weight gain, and renal insufficiency. Diagnostic criteria and treatment schema for engraftment syndrome are included in Appendix A.

4.2 TREATMENT OF GRAFT-VERSUS-HOST DISEASE:

4.2.1 In recipients with suspected GVHD, standard clinical criteria and biopsy findings (when clinically indicated) will be used to establish the diagnosis. Acute GVHD will be assessed according to 1994 Consensus Conference on Acute GVHD Grading criteria. See Appendix B for details concerning the grading and management of acute GVHD. Chronic GVHD will be assessed according to 2005 Chronic GVHD Consensus Project. See Appendix C for details concerning the assessment of chronic GVHD.

4.2.2 Recipients with clinical Stage 1 or 2 (Grade I) GVHD of the skin without any other organ involvement will be treated with a topical corticosteroid cream.

4.2.3 In general, recipients with ≥ Grade II acute GVHD will be treated with high-dose, systemic corticosteroids. However, subjects with gut GVHD who respond rapidly to systemic therapy should be converted to beclomethasone therapy in an attempt to limit the degree of systemic immune suppression.

4.2.4 Recipients who fail to respond satisfactorily to corticosteroids will be considered for second-line immunosuppressive therapy, e.g., tacrolimus, mycophenolic acid, monoclonal antibodies, or experimental GVHD protocols, if they are available.

4.3 MENSES SUPPRESSION AND CONTRACEPTION

(For a full description of menses suppression and contraception please refer to the NIH BMT Consortium Supportive Care Guidelines at: http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml)

4.3.1 Pre-menopausal women who have not undergone hysterectomy will be placed on a monophasic oral contraceptive to provide both menses suppression and contraception. This therapy will begin with the first cycle of induction therapy and continue until platelet recovery after transplantation. (> 50,000/mm^3 without transfusion). Start treatment prior to the onset of thrombocytopenia usually when withdrawal bleeding begins. Once thrombocytopenia occurs therapy is less likely to be effective. As general recommendations:

- Prescribe Lo-Ovral (300mcg norgestrol and 30 mcg ethinyl estradiol)
- 1 tablet daily. Instruct patient not to take placebo tablets.
- If the patient is on another oral contraceptive, switch her to Lo-Ovral
    - If Lo-Ovral is started when the patient is bleeding and bleeding persists for more than 2-3 days, increase the dose to 1 tablet twice daily. If bleeding persists for more than an additional 2-3 days (total 5 to 6 days after starting hormones), consult the NIH Gynecology Consult Service.

4.3.2 Female transplant recipients will be advised to use contraception for at least 1 year after transplantation and to have their male partners use condoms. Male transplant recipients will be advised to use contraception, preferably condoms, for 1 year after transplantation.

4.4 BLOOD PRODUCT SUPPORT

4.4.1 Recipients will receive packed red blood cells and platelets as needed to maintain Hb > 8.0 gm/dl, and platelets > 10,000/mm^3 (or higher, if clinically indicated). All blood products **except for the stem cell product and DLI products** will be irradiated.

4.4.2 Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused leukocytes and decrease the risk of CMV infection.

4.5 NUTRITIONAL SUPPORT

4.5.1 When mucositis or GVHD prevents adequate PO intake expected to last one week or more, parenteral hyperalimentation should be instituted. The dietary service will provide recommendations for macronutrient supplementation and the Pharmacy Department will provide support for the electrolytes and incompatibility issues. Oral intake will resume when clinically appropriate under the supervision of the dietary service of the Clinical Center.

4.6 ANTI-EMETICS

4.6.1 Because of the highly emetogenic nature of pentostatin, an anti-emetic regimen consisting of aprepitant in combination with dexamethasone will be utilized (see Sections **3.6.7** to **3.6.13**).

4.7 HEMATOPOIETIC GROWTH FACTOR SUPPORT

4.7.1 All patients will start filgrastim 5 μg/kg/day on day 0 starting after the stem cell infusion. The dose will be given subcutaneously (SC). Filgrastim will be continued until the absolute neutrophil count (ANC) is ≥ 5000/μl for at least one day or ≥ 1000/μl for three consecutive days. Filgrastim may be resumed if ANC drops below 1000/μl.

5 BIOSPECIMEN COLLECTION

5.1 IMMUNE LAB STUDIES: IMMUNE DEPLETION BY PENTOSTATIN PLUS CYCLOPHOSPHAMIDE (P/C)

5.1.1 Blood samples will be drawn to evaluate the effects of induction chemotherapy on host immune depletion as described in Appendix D.

5.1.2 Every effort should be made to draw the sample on the protocol day listed. However, if this is not possible, it will be permissible to send the sample either 3 days before or 3 days after the protocol day listed.

5.1.3 The following samples will be drawn for this purpose: (1) at the time of study entry; (2) on day 8 of P/C therapy (just prior to pentostatin infusion); (3) on day 15 of P/C therapy (just prior to pentostatin infusion); and (4) after completion of P/C therapy (day 21; or alternatively, day -2 of HSCT).

5.2 IMMUNE LAB STUDIES: EVALUATION OF TYPE I VERSUS TYPE II CYTOKINE EFFECTS POST-TRANSPLANT

5.2.1 Blood will be drawn for cytokine evaluation as described in Appendix D.

5.3 IMMUNE LAB STUDIES: EVALUATION OF IMMUNE RECONSTITUTION POST-TRANSPLANT

5.3.1 Blood will be drawn for evaluation of immune reconstitution as described in Appendix D.

5.3.2 These samples will be utilized to evaluate potential anti-tumor effector mechanisms associated with any observed GVT effects. Such studies will include tetramer analysis for detection of an increase in frequency of potential anti-tumor T cell receptor specificities (experiments to be performed by Dr. Vonderheide; these studies will only be applicable in cases involving HLA-A2+ individuals).

5.3.3 At NIH ONLY: Further potential anti-tumor effector mechanisms will be evaluated by the Rader laboratory in the ETIB and will focus on analysis of post-transplant antibodies that selectively recognize RCC cell surface antigens. Dr. Rader hypothesizes that post-transplant antibodies that selectively recognize RCC cell surface antigens may be capable of mediating antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) and, thereby, contribute to the allogeneic GVT effect. In addition, these antibodies may serve as tools for the discovery of new targets for human monoclonal antibody therapy. The Rader laboratory previously developed a sensitive flow cytometry assay for detection of post-transplant serum antibodies against tumor cell surface antigens expressed in hematologic malignancies. In order to identify these antibodies and subsequently the tumor cell surface antigens they recognize, the Rader laboratory has generated human antibody libraries from post-transplant PBMC for selection on primary tumor cells by phage display. The inclusion of post-transplant serum antibodies and PBMC from patients with solid malignancies in general and RCC in particular provides an important extension of this ongoing study. See Appendix D for timepoints and complete details.

5.3.4 In some cases, it may be necessary to biopsy tissue post-transplant to confirm a diagnosis of malignant disease relapse. In such cases, a portion of the biopsy sample will be sent to Dr. Hakim's lab for research analysis of tumor infiltrating lymphocytes.

5.4 IMMUNE LAB STUDIES: EVALUATION OF IMMUNE CELL POPULATIONS AND MOLECULAR EVENTS IN THE TARGET TISSUES OF CHRONIC GVHD

5.4.1 Buccal mucosal, minor salivary gland (lower lip), and skin biopsy samples collected on subjects undergoing clinically indicated biopsies to diagnose GVHD will be used to evaluate immune cell populations and cytokine profiles associated with the development of chronic GVHD (a portion of each biopsy will be sent to Gress Lab 12C121 301-594-5340). These research studies will be performed only at NIH. To help ensure accurate clinical diagnosis of chronic GVHD, such biopsies should be arranged in consultation with Dr. Paveletic, who is an AI on this protocol and head of the chronic GVHD clinic at NIH.

5.4.2 The pathogenesis of chronic GVHD is poorly understood and no studies rigorously evaluated cellular and molecular changes in target tissues of chronic GVHD. Heterogeneity in patient and treatment factors complicates the group comparisons.

Prospectively designed study with planned sequential sample collection is the ideal format that would give the best chance of success in study of this complex problem.

5.4.3 At NIH only: We will focus on the skin and oral cavity as the two most common target organs of chronic GVHD. An optional biopsy consisting of a standard 6 mm punch biopsies will be performed in the skin and buccal mucosa in patients at the time of initiation of immunosuppressive taper prior to onset of clinical chronic GVHD at day 63+/-3. Another optional biopsy will be performed at the onset of clinical chronic GVHD and at 6 months post transplant in patients who do not develop chronic GVHD by this time point. Whole saliva will be collected at the same time as the buccal mucosal biopsies are performed to evaluate the changes in the salivary proteome at the onset of chronic GVHD. The samples will be analyzed using a variety of methods including immunofluorescence and confocal microscopy, gene expression profiles, and protein based assays in order to better understand the reconstitution of resident immune cell populations following HSCT and how they change with the onset of chronic GVHD (samples to Gress Lab 12C121 301-594-5340).

5.5 SAMPLE MANAGEMENT AND STORAGE

5.5.1 All samples will be coded and the key will be available to a restricted number of investigators. However, the nature of the study requires that clinical correlation be feasible as part of hypothesis generation. 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, NCI IRB will be informed to evaluate the eventual need for modification in subject consent process or for re-contacting subjects.

5.5.2 Coded samples will be stored frozen at -20 to -180 C in a single location under the restricted control of the Clinical Core laboratory of ETIB.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

6.1.1 Data at NCI and participating sites will be prospectively collected and entered in real time into the Cancer Central Clinical Data System database (NCI C3D; information athttp://ccrtrials.nci.nih.gov). It is expected that clinical data be entered into C3D no later than after 10 business days of the occurrence. The NCI PI and research nurse will have access to this data via web access. (See Appendix E for details).

6.1.2 Toxicity Criteria: The NCI Common Terminology Criteria for Adverse Events version 3.0 will be used (CTCAEv3). This document can be found at: http://ctep.info.nih.gov/CTC3/default.html

6.1.3 All grade 3 and 4 adverse events will be recorded except grade 3 and 4 lymphopenia.

6.1.4 The following grade 2 adverse events will be recorded:

6.1.4.1 Toxicities potentially associated with GVHD;

6.1.4.2 Toxicities potentially attributable to the Th2 cells; and

6.1.4.3 Toxicities potentially related to infection.

6.1.5 After Day 100 post-transplant, adverse events will be recorded if potentially associated with the Th2 cells, opportunistic infection, GVHD, or secondary malignancy.

6.1.6 For this study, the development of hyper acute GVHD will be considered a toxicity likely attributable to Th2 cell administration. Hyper acute GVHD will be defined as severe GVHD (grade III or IV) that occurs in the first 14 days after Th2 cell infusion.

6.1.7 Data will also be reported to the International Bone Marrow Transplant Registry.

6.2 RESPONSE CRITERIA

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [JNCI 92(3):205-216, 2000]. Changes in only the largest diameter (one-dimensional measurement) of the tumor lesions are used in the RECIST criteria.

6.2.1 Response Definitions

6.2.1.1 Complete Response (CR): Disappearance of all target lesions.

6.2.1.2 Partial Response (PR): At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.

6.2.1.3 Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

6.2.1.4 Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

6.2.2 If necessary, reference can be made to a publication that further describes the specific application of the RECIST criteria in the setting of metastatic RCC[66].

6.2.3 In RECIST, tumor measurement consists of a one-dimensional measurement of the greatest tumor dimension, with the sum of tumor measurements utilized to determine response.

6.2.4 Ideally, the CT scans used to measure lesions will utilize both oral and intravenous contrast. However, in cases of abnormal renal function, intravenous contrast may be avoided.

6.2.5 Tumor measurements will be made by a single radiologist at NIH ; this radiologist will not be study investigators.

6.2.6 Based upon these radiology measurements, the protocol PI at NCI will be responsible for determining disease response by the RECIST criteria.

6.2.7 Because the effect of the P/C induction regimen on metastatic RCC is not known, the tumor measurements at the completion of the P/C regimen will be utilized as the baseline for determining disease response.

6.2.8 The single best overall response will be determined and utilized for the statistical purpose of determining PR/CR rate.

6.2.9 The following table further defines response rate determination:

Target Lesion	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

6.2.10 Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

6.2.11 In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

6.2.12 Partial or complete responses must be confirmed. The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

6.2.13 To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met.

6.2.14 The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 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 above in Section **6.1**.

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All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2.**, **7.3** and **7.4.**

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.

7.1.2 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.

7.1.3 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.

7.1.4 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.

7.1.5 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.

7.1.6 Disability

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

7.1.7 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.

7.1.8 Protocol Deviation (NIH Definition)

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

7.1.9 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.

7.1.10 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.

7.2 NCI-IRB REPORTING

7.2.1 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.

7.2.2 NCI-IRB Requirements for PI Reporting 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.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an Unanticipated Problem will need to be reported to the NCI IRB.

7.3 IND SPONSOR REPORTING CRITERIA

An investigator must immediately report to the sponsor using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis).

7.4 FDA REPORTING CRITERIA

7.4.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

The Sponsor will notify FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a.
The Sponsor is also responsible for reporting any:

- suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug

- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure

to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

7.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

The PI and Protocol Nurse will provide continuous, close monitoring, and adverse events will be reported as required above.

Prior to the signing of the consent, each subject's record will be reviewed for verification of eligibility by the research nurse, transplant coordinator, and PI or his designee. Documentation of this review will be made on the eligibility checklist and a copy will be kept in each subject's research record.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. 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 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.

7.5.2 Sponsor Monitoring Plan

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.
At least 25% of enrolled patients' will be randomly selected and monitored at least quarterly, based on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

7.5.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date.

Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL CONSIDERATIONS

8.1 PRIMARY OBJECTIVE

The primary Objective of this pilot/phase II study is to determine if the transplant approach consisting of pentostatin and cyclophosphamide host immune depletion and donor allografting augmented with Th2.rapa cells can mediate substantial clinical responses in subjects with metastatic renal cell cancer. Consideration may then be given to further evaluate this approach in a more definitive, subsequent trial.

8.2 INITIAL PROTOCOL ACCRUAL

Initial Protocol Accrual evaluated a Th2.Rapa cell product that was manufactured for 12-days of ex vivo culture. Six of the eight patients accrued are fully-evaluable for anti-tumor effects. The incidence of partial or complete response in this group of patients was 0/6. If the true rate of achieving a PR/CR with this initial approach was 20% (the literature standard), then the chance of achieving our goal of at least 3 cases of PR/CR in the first 12 patients would be only 9.9%, given that 0/6 have responded thus far.

8.3 AMENDMENT C

Because of this low chance of achieving the anti-tumor objective in the initial transplant approach, the protocol was modified in amendment C to evaluate a short-term expanded Th2.Rapa cell (expanded for 6-days). The hypothesis is that the short-term cultured Th2.Rapa cell will be more potent in vivo, thereby facilitating anti-tumor effects. With amendment C, the statistical details below will now pertain to therapy using the short-term manufactured Th2.Rapa cell. The initial data using the 12-day expanded Th2.Rapa cells will not be included in these statistical evaluations, but will be analyzed and reported separately; that is, the initial 12 patients described below will consist of the first 12 patients to receive the 6-day expanded Th2.rapa cells.

8.4 FIRST STAGE OF SUBJECT ACCRUAL

In the first stage of subject accrual, 12 patients will receive induction therapy of cyclophosphamide (200 mg orally per day) and three weekly doses of pentostatin (4 mg/m^2 intravenously for each dose). This immune depletion regimen will be followed by allogeneic HSCT, donor Th2 cell infusions (Th2 cells manufactured by the 6-day culture interval), and GVHD prophylaxis consisting of sirolimus, as detailed in section **3.1**. The safety of transplant-regimens is primarily related to the intensity of host conditioning (this protocol utilizes a low-intensity regimen) and to the rate and severity of acute GVHD. In this protocol, unacceptable toxicity will be defined as the occurrence of severe acute GVHD (grade III or IV) in more that 4 cases out of the initial 12 subjects. Up to four cases of severe GVHD in the initial cohort of 12 subjects is marginally tolerable or better, as the upper one-sided 80% confidence bound on 4/12 is 50%; this means that there is 80% confidence that the true rate of steroid-refractory GVHD would be 50% or lower. On the other hand, if a 5th patient is noted to have severe GVHD at or

before the 12th enrolled patient, this will be considered excessive because the upper one-sided 80% confidence bound on 5/12 is 58%. In addition to severe GVHD, transplant-related mortality (TRM) will be utilized as a stopping rule. TRM will be defined as a subject death that occurs in the first 100 days post-transplant in the absence of tumor progression. Only one case of TRM in the first twelve subjects will be allowed; in the event of a second case, protocol accrual will stop.

8.5 FIRST COHORT

The 12 patients in the first cohort will be part of a standard Simon optimal phase II design (Simon R. Controlled Clinical Trials 10:1-10, 1989). The decision to proceed to the second stage of protocol accrual will be made by the NCI Safety Monitoring Committee after review of the clinical data from the first stage cohort.

8.6 SECOND STAGE OF PROTOCOL ACCRUAL

At a minimum, advancement to the second stage of protocol accrual will require a low level of TRM (no more than one case out of 12), no more than 4 cases out of 12 of severe GVHD, and a potentially promising rate of tumor regression. Since this is intended to be a small study designed to look for potential hints regarding efficacy, alpha will be 0.10 and beta will be 0.20 (thus there is a 10% chance of accepting the strategy as good, and 20% chance of rejecting a good strategy). Clinical responses will be noted to have occurred or not occurred by 6 months post-transplant. In order to rule out a clinical response rate of 20% (p0=0.20) in favor of a rate showing improvement (40%; p1=0.40), three or more of the initial 12 subjects must achieve either a PR or a CR. If 0-2 of the initial subjects attain a PR or CR, then no further patients will be enrolled. If 3 or more out of 12 patients attain a PR or CR, and the TRM and GVHD safety conditions are met, then an additional 13 patients will be accrued in the second stage of the protocol. After the second stage, if only 3 to 7 out of 25 patients attain a PR or CR, then the regimen will not be considered promising. On the other hand, if at least 8 out of 25 patients attain a PR or CR, then the regimen will be considered potentially worthy of further evaluation. Under the null hypothesis, the probability of early termination will be 56%. As such, the study may be terminated early after the interim analysis conducted by the NCI Safety Monitoring Committee (if there are inadequate anti-tumor responses or excessive toxicity, as defined above). Because of this interim analysis, it may be necessary to suspend accrual pending the NCI Safety Monitoring Committee decision.

8.7 RATE OF SUBJECT ACCRUAL

It is expected that 1 patient per month may enroll onto this trial. With 12 patients required in the first evaluation stage, no more than 25 patients should be required address the question posed in amendment C; however, 8 patients (and 8 donors) were initially treated with the Th2.rapa cell product manufactured in 12-days. Thus, 2 years may be required to enroll up to 25 eligible and evaluable patients and 25 donors. To allow for the possibility of a small number of inevaluable patients, the accrual ceiling following amendment C will be set to 28 patients and 28 donors, for a total of 56 newly enrolled subjects on the trial. With the addition of the 8 patients and 8 donors already treated on the protocol using the 12-day manufactured Th2.Rapa cells, the grand total for subjects to be enrolled on this trial would be 56 + 16 (72).

8.8 SUBJECT REMOVED FROM PROTOCOL PRIOR TO TRANSPLANTATION

An assigned patient will not be eligible for the primary endpoint of achieving a PR or CR if that patient does not ultimately receive transplantation (for example, due to chemotherapy toxicity, uncontrolled infection or malignancy, or voluntary withdrawal).

8.9 STATISTICAL SECTION PERTAINING TO AMENDMENT F

As a result of new knowledge gained from treating patients with lymphoma on protocol 04-C-0055, amendment F will further modify the treatment regimen, as detailed in the protocol schema shown in Figure 6. It is hypothesized that this change in the protocol will result in a high rate of clinical responses similar to what we have observed on protocol 04-C-0055. In amendment F, we will seek to explore this potential gain in anti-tumor efficacy in a very small number of patients using a two-stage design. Specifically, we will now seek to rule out a 20% response rate (p0=0.20) and target a higher rate of 60% responses (p1=0.60). To do so requires a very small first stage, with only 5 patients.

At a minimum, advancement to the second stage of protocol accrual will require a potentially promising rate of tumor regression. Since this is intended to be a small study designed to look for potential hints regarding efficacy, alpha will be 0.10 and beta will be 0.20 (thus there is a 10% chance of accepting the strategy as good, and 20% chance of rejecting a good strategy). Clinical responses will be noted to have occurred or not occurred by 6 months post-transplant. In order to rule out a clinical response rate of 20% (p0=0.20) in favor of a rate showing improvement (60%; p1=0.60), two or more of the initial 5 subjects must achieve either a PR or a CR. If 0-1 of the initial subjects attain a PR or CR, then no further patients will be enrolled. If 2 or more out of 5 patients attain a PR or CR, then an additional 9 patients will be accrued in the second stage of the protocol. After the second stage, if only 2 to 4 out of 14 patients attain a PR or CR, then the regimen will not be considered promising. On the other hand, if at least 5 out of 14 patients attain a PR or CR, then the regimen will be considered potentially worthy of further evaluation. Under the null hypothesis, the probability of early termination will be 74%. As such, the study may be terminated early after the interim analysis conducted by the NCI Safety Monitoring Committee (if there are inadequate anti-tumor responses or excessive toxicity). Because of this interim analysis, it may be necessary to suspend accrual pending the NCI Safety Monitoring Committee decision. On protocol 04-C-0055, over 80 patients have been transplanted using a strategy similar to the one detailed in amendment F; in such therapy, we have not observed excessive toxicity. Specifically, we have not observed steroid-refractory GVHD. As such, to help ensure safety in the current protocol design, further protocol accrual will be halted if more than one case of steroid-refractory GVHD is observed.

It is expected that 6-8 patients (and 6-8 donors) per year may enroll onto this trial after implementing amendment F. With 5 patients required in the first evaluation stage after amendment F, no more than 14 total patients should be required to address the question posed in amendment F. However, 8 patients (and 8 donors) were initially treated with the Th2.rapa cell product manufactured in 12 days and 3 patients and 3 donors were enrolled relative to the Th2.rapa cell product manufactured in 6 days. Thus, 11 patients and 11 donors are already enrolled on the study. Approximately 2 years may be required to enroll up to 14 eligible and evaluable patients and 14 donors for amendment F. To allow for the possibility of a small number of inevaluable patients, the accrual ceiling for new subjects following amendment F will

be set to 16 patients and 16 donors, for a total of 32 newly enrolled subjects on the trial. With the addition of the 8 patients and 8 donors already treated on the protocol using the 12-day manufactured Th2.Rapa cells and 3 patients and 3 donors enrolled using the 6-day manufactured Th2.Rapa cells, the grand total for subjects to be enrolled on this trial would be 32 + 22 (54).

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

Patients with metastatic renal cell carcinoma will be the subjects for this study. Both men and women and members of all races and ethnic groups are eligible for this trial. This patient group was chosen because allogeneic hematopoietic stem cell transplantation represents a potentially effective treatment approach for this disease. Current FDA-approved therapies for this disease yield a disease response in only a minority of subjects with typically limited response duration (a few to several months). The one exception to this therapeutic outcome appears to be high-dose IL-2, which can result in durable remissions in approximately 5-10% of individuals. As such, each potential subject will be counseled as to whether IL-2 therapy should be considered prior to transplantation. Metastatic renal cell carcinoma affects all races and genders. In general, the age group of selected patients will be those individuals over age 40 (most likely, median age will be approximately 60 years); because myeloablative preparative regimens are associated with a high degree of morbidity and mortality in this age group, such individuals are most likely to benefit from the low-intensity transplant approach used in this study. Individuals with HIV disease will not be candidates for this protocol, due to the high rate of post-transplant complications in this group. Individuals who are pregnant or lactating will not be candidates for this protocol, due to risk to the fetus or newborn.

9.2 PARTICIPATION OF CHILDREN

Children will not be evaluated on this protocol. Evaluation of this transplant strategy in children would require a separate protocol and a separate disease indication, as renal cell carcinoma is very rare in children.

9.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Patients are unlikely to obtain direct benefit from anti-tumor activity of the induction chemotherapy consisting of pentostatin and cyclophosphamide, as renal cell carcinoma is typically chemotherapy resistant. The main benefit of the chemotherapy will be to reduce host immunity such that the allograft is not rejected. The chemotherapy utilized in this study has the risk of direct drug toxicity; in addition, immune suppression resulting from chemotherapy administration is associated with an increased risk of opportunistic infection. However, it is anticipated that these risks will be lower than the risks associated with traditional allogeneic transplantation utilizing high-dose, myeloablative regimens.

The primary risk associated with allogeneic stem cell transplantation is graft-versus-host disease (GVHD), which remains the primary cause of morbidity and mortality post-transplant. Each patient on this study will receive post-transplant immune suppression to reduce the rate and severity of GVHD. Subjects will receive an investigational regimen consisting of high-dose, single agent sirolimus; it is possible that this regimen will be effective for GVHD prevention, and also more effectively control tumor mass post-transplant, as renal cell tumors are known to

respond to mTOR inhibitors such as temsirolimus. However, it is also possible that this regimen will not effectively reduce GVHD. Other risks of transplant include graft rejection, opportunistic infection, veno-occlusive disease, and medication side effects.

9.4 DISCUSSION OF RELATIVE RISKS AND BENEFITS

Patients on this study may be directly benefited by this treatment protocol. In previous studies, in a low percentage of cases, successful allogeneic stem cell transplantation appears to offer patients an effective therapy for their malignancy. It is hypothesized that the transplant approach used in this study will result in the successful transfer of allogeneic stem cells with reduced morbidity and mortality from the transplant preparative regimen and with reduced graft-versus-host disease. It is also anticipated that this study will provide scientific information relevant to attempts to prevent graft rejection, to limit GVHD, to mediate graft-versus-tumor effects, to reduce transplant-related mortality.

9.5 CONSENT AND ASSENT PROCESSES AND DOCUMENTS

9.5.1 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 recipient. Similarly, the procedures and treatments involved in this protocol, with their attendant risks and discomforts, will be carefully explained to the donor. A signed, informed consent document will be obtained from both the recipient and the donor.

9.5.2 The original signed informed consent goes to Medical Records; a copy will be kept with the research record. A copy of the informed consent documents will also be given to the recipient and donor.

9.5.3 The Clinical Coordinator, Data Management Section, will ascertain the date of IRB approval before registering the first patient.

9.5.4 Informed consent will be obtained from all patients and their donors entered on this trial. The investigational nature and research objectives of this trial, the attendant risks and benefits associated with the procedures and treatments of this trial, and the potential for alternative therapies will be carefully explained to protocol subjects. A signed informed consent document will be obtained prior to entry onto the study by the Principal Investigator and/or Associate Investigator at each participating institution. Each participating institution will be responsible for its own informed consent form, and will obtain IRB approval of the informed consent initially at their institution and then at the NCI IRB.

9.5.5 The attached informed consent contains all elements required for consent. In addition, the Principal Investigator or his designee will provide oral consent and will be available to answer all patient questions.

9.6 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

9.6.1 Stored samples include peripheral blood cells and/or bone marrow cells from the transplant donors and from the transplant recipients both before and after the transplant. Samples obtained from tumor biopsy will be stored as a single cell suspension after tumor digestion. These samples are stored under liquid nitrogen cryopreservation in research

freezers maintained in the laboratory of the study PI, Dr. Fowler, and in the laboratory of research associate investigator, Dr. Fran Hakim. The research purpose of these samples is to perform laboratory experiments relating to the scientific objectives of this protocol, specifically: to understand the role of Th1/Th2 cytokine biology in the modulation of human transplant responses, including graft-versus-host disease, graft rejection, and graft-versus-tumor effects; and to understand the biology of immune reconstitution that occurs after allogeneic hematopoietic cell transplantation. The cells are stored in a coded manner, with only the PI or the AI able to track specific samples to the identity of the donor or recipient. The samples will be stored until the completion of the protocol; at that time, the samples will be destroyed. The PI will report to the IRB any loss or destruction of samples, or any new use of the collected samples.

10 PHARMACEUTICAL INFORMATION

10.1 CYCLOPHOSPHAMIDE (CTX, CYTOXAN, NSC-26271)

10.1.1 Supply – Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied in tablet form.

10.1.2 Preparation – tablets.

10.1.3 Storage and Stability - The vials of cyclophosphamide are stable when stored at room temperature.

10.1.4 Route of Administration - The cyclophosphamide used in this protocol will be given by oral administration on a daily basis on days 1 through 21 of the induction, pre-transplant chemotherapy regimen. The dose of cyclophosphamide will initially be 200 mg per day. Cyclophosphamide may also be given prior to DLI for patients with persistent malignancy post-transplant (See Section 3.12.4). In such cases, the cyclophosphamide will be administered for either 7 days or 14 days.

10.1.5 Mechanism of action: DNA alkylation.

10.1.6 Toxicities:

a) Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].
b) Cytopenias.
c) Hemorrhagic cystitis.
d) Mucositis.
e) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

10.2 FILGRASTIM (G-CSF, NEUPOGEN®)

10.2.1 Supply – Commercially available as filgrastim injection in a concentration of 300μg/ml in 1ml (300μg) and 1.6ml (480μg) vials.

10.2.2 Preparation – For subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5%

in water to a concentration greater than 5μg/ml just prior to administration; storage of the diluted filgrastim is not recommended. Dilution of filgrastim to a final concentration of less than 5μg/ml is not recommended at any time. Do not dilute with saline at any time; product may precipitate. Filgrastim diluted to concentrations between 5 and 15μg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/ml. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes. The dose may be "rounded down" to within 10% of patient's calculated dose to use the drug cost-effectively.

10.2.3 Storage and Stability – Filgrastim for injection should be stored in the refrigerator at 2^{o} to 8^{o}C (36^{o} to 46^{o}F). Avoid shaking.

10.2.4 Administration – Subcutaneous injection is preferred. If clinically indicated, filgrastim may be administered as an intravenous infusion over 4 or 24 hours.

10.2.5 Toxicities – Medullary bone or skeletal pain is the most commonly reported toxicity. In addition, reversible elevations in uric acid, lactate dehydrogenase, and alkaline phosphatase are common laboratory abnormalities. Four cases of splenic rupture have been reported in healthy donors when given filgrastim or other myeloid growth factors for peripheral blood stem cell mobilization; 1 of these cases resulted in fatality. Five additional cases of splenic rupture have been reported in cancer patients undergoing chemotherapy or peripheral blood stem cell mobilization; splenic rupture may have contributed to deaths in 2 of these cases. One additional death due to splenic rupture after filgrastim therapy was reported to the manufacturer without additional information. According to the manufacturer, the reporting rate for splenic rupture with filgrastim is less than 1 in 486,000.

10.3 PENTOSTATIN (NIPENT®; 2'-DEOXYCOFORMYCIN)

10.3.1 Supply – Commercially available. The lyophilized powder will be resuspended according to manufacturer instructions, into a solution of 2 mg/ml concentration (10 mg. vial).

10.3.2 Preparation- The pentostatin dose will be determined by the creatinine clearance value (as determined by the 24 hour urine collection method). The appropriate dose of the reconstituted pentostatin solution will be further diluted with 500 ml of 0.9% sodium chloride and infused over 30 to 60 minutes.

10.3.3 Storage and Stability- upon reconstitution, the pentostatin can be stored at room temperature but should be used with 8 hours of reconstitution.

10.3.4 Administration- subjects will receive one liter of 0.9% sodium chloride by intravenous infusion prior to the pentostatin delivery.

10.3.5 Mechanism of action: inhibition of adenosine deaminase, thereby increasing lymphocyte susceptibility to apoptosis.

10.3.6 Toxicities- pentostatin is cleared by a renal mechanism (90%). As such, the pentostatin dose must be reduced for renal insufficiency (see Section **3.6.5**). The primary toxicity is related to opportunistic infection due to T cell depletion. At higher doses, CNS toxicity

may include seizures, coma, and death. Interstitial pulmonary toxicity has also been described. Other toxicities include nausea, vomiting, and skin rash.

10.4 DIPHENHYDRAMINE

10.4.1 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.

10.4.2 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.

10.4.3 Storage and Stability – Store commercially available injectable product at controlled room temperature.

10.4.4 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).

10.4.5 Toxicities – Sedation, sleepiness, dizziness, disturbed coordination, epigastric distress, 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 patients with a history of bronchial asthma, increased intraocular pressure, hyperthyroidism, cardiovascular disease or hypertension.

10.5 ACETAMINOPHEN

10.5.1 Supply – Commercially available as 325 mg or 500 mg tablets for oral administration from multiple manufacturers.

10.5.2 Storage – Store at controlled room temperature.

10.5.3 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.

10.5.4 Toxicities – No toxicities are anticipated to result from single doses of acetaminophen administered as pre-medication for rituximab infusions.

10.6 VALACYCLOVIR (VALTREX®)

10.6.1 Supply – Commercially available as 500mg tablets and 1gm tablets. Dose adjustment is necessary in patients with significant renal impairment (refer to the manufacturer's labeling for dose adjustment guidelines.)

10.6.2 Pharmacology – Valacyclovir is the hydrochloride salt of L-valyl ester of the antiviral drug acyclovir. After oral administration, valacyclovir is rapidly absorbed from the GI tract and nearly completely converted to acyclovir and L-valine by first-pass intestinal or hepatic metabolism.

10.6.3 Storage and Stability – Oral tablets should be stored at 15° to 25°C (59° to 77°F).

10.6.4 Administration – Oral.

10.6.5 Toxicities – Nausea and/or vomiting, headache, dizziness, abdominal pain, dysmenorrhea, arthralgia, acute hypersensitivity reactions, elevations in liver enzyme laboratory values (e.g. AST). Renal failure and CNS symptoms have been reported in patients with renal impairment who received valacyclovir or acyclovir at greater than the recommended dose. Dose reduction is recommended in this patient population (refer to the manufacturer's labeling for dose adjustment guidelines).

10.7 FLUCONAZOLE (DIFLUCAN®)

10.7.1 Supply – Commercially available as 50 mg, 100 mg, 150 mg and 200 mg tablets, or as powder for oral suspension for reconstitution at a concentration of 10mg/ml or 40 mg/ml. Parenteral fluconazole is available in a solution for injection at a concentration of 2 mg/ml in glass bottles and Viaflex® Plus plastic containers containing either 100ml (200 mg) or 200ml (400 mg).

10.7.2 Preparation – For parenteral administration, the commercial solution for injection is available in its final form for administration (concentration of 2 mg/ml).

10.7.3 Storage and Stability – Oral tablets and oral suspension should be stored at temperatures below 30°C (86°F). Store reconstituted oral suspension between 5° to 30°C (41° to 86°F) and discard unused portion after 2 weeks. Fluconazole for injection in glass bottles should be stored between 5° to 30°C (41° to 86°F). Fluconazole for injection in Viaflex® Plus plastic containers should be stored between 5° to 25°C (41° to 77°F).

10.7.4 Administration – Oral and parenteral. Parenteral doses should be administered by an intravenous infusion at a maximum rate of 200mg/hr.

10.7.5 Toxicities – Nausea, vomiting, headache, skin rash, abdominal pain, diarrhea have been reported at an incidence of 1% or greater in clinical trials. In combined clinical trials and marketing experience, there have been rare cases of serious hepatic reactions during treatment with fluconazole. The spectrum of these hepatic reactions has ranged from mild transient elevation in transaminases to clinical hepatitis, cholestasis and fulminant hepatic failure, including fatalities.

10.7.6 Drug Interactions – Fluconazole is a potent inhibitor of the cytochrome P450 3A4 isoenzyme system. Co-administration of fluconazole with other drugs metabolized by the same enzyme system may result in increased plasma concentrations of the drugs, which could increase or prolong therapeutic and adverse effects. Refer to the package literature or other drug information resources for additional information on identification and management of potential drug interactions.

10.8 TRIMETHOPRIM/SULFAMETHOXAZOLE (TMP/SMX, COTRIMOXAZOLE, BACTRIM, SEPTRA)

10.8.1 Supply – Commercially available as a single strength tablet containing trimethoprim 80mg and sulfamethoxazole 400mg and a double strength (DS) tablet containing trimethoprim 160mg and sulfamethoxaole 800mg. It is also available in a oral suspension at a concentration of 40mg of trimethoprim and 200mg sulfamethoxazole per

5ml. Parenteral TMP/SMX is available in a solution for injection at a concentration of 80mg of trimethoprim and 400mg of sulfamethoxazole per 5ml.

10.8.2 Preparation – For parenteral administration, the commercial solution for injection must be diluted prior to administration. It is recommended that each 5ml of the solution for injection be diluted with 100-125 ml or, if fluid restriction is required, in 75ml of dextrose 5% in water. 0.9% sodium chloride, Inj. may be substituted as a diluent but the resulting solutions have reduced stability. Consult with pharmacy for questions regarding diluent, volume, and expiration.

10.8.3 Storage and Stability – Oral tablets and oral suspension should be stored at 15^{o} to 30^{o}C (59^{o} to 86^{o}F) in a dry place and protected from light. TMP/SMX for injection should be stored at room temperature between 15^{o} to 30^{o}C (59^{o} to 86^{o}F) and should not be refrigerated. Stability of intravenous doses after final dilution is dependent on concentration and diluent. Consult with pharmacy for questions regarding stability and expiration dating.

10.8.4 Administration – Oral and parenteral. Parenteral doses should be administered by an intravenous infusion over 60 to 90 minutes.

10.8.5 Toxicities – The most common adverse effects from TMP/SMX are gastrointestinal disturbances (nausea, vomiting, anorexia) and allergic skin reactions (such as rash and urticaria). Fatalities associated with the administration of sulfonamides, although rare, have occurred due to severe reactions, including Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia and other blood dyscrasias. For TMP/SMX injection, local reaction, pain and slight irritation on IV administration are infrequent. Thrombophlebitis has rarely been observed.

10.9 SIROLIMUS (RAPAMYCIN) (RAPAMUNE®, WYETH-AYERST LABORATORIES)

10.9.1 Supply - For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. For in vitro Th2 cell expansion, oral solution will be purchased by the NIH Clinical Center Department of Transfusion Medicine, Cell Processing Section.

10.9.2 Storage and Stability - Oral tablets should be stored 20-25 C° (68-77 F°). Oral solution should be refrigerated (2-8 C° or 36-46 F°).

10.9.3 Administration: Because fatty food can increase the absorption of sirolimus, the tablets should be administered in the setting of a steady diet that is relatively constant with respect to fat content.

10.9.4 Toxicities: 1) Sirolimus induces immune suppression, which has been associated with opportunistic infection and an increased rate of malignancy, particularly skin cancer. 2) Some individuals may develop hypersensitivity to sirolimus. 3) May cause an increase in cholesterol and triglycerides, which may be associated with pancreatitis. 4) With long-term administration, may result in impaired renal function. 4) Co-administration of voriconazole is strongly discouraged on this protocol, as the combination of voriconazole and sirolimus results in an approximate 10-fold increase in sirolimus blood levels.

10.9.5 Drug Interactions: May increase the in vivo drug levels of simvastatin.

10.10 APREPITANT (EMEND® ORAL CAPSULES)

10.10.1 Supply - For patient administration, oral capsules will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

10.10.2 Storage and Stability - Oral capsules should be stored 20-25 C° (68-77 F°).

10.10.3 Administration: One oral capsule (125 mg) will be administered 60 minutes prior to each dose of pentostatin (days 1, 8, and 15). On days 2 and 3, 9 and 10, and 16 and 17 of the 21-day chemotherapy regimen, one oral capsule (80 mg) will be administered in the morning.

10.10.4 Toxicities: In the setting of emitogenic chemotherapy, there are no known toxicities that are specifically increased by aprepitant administration.

10.10.5 Drug Interactions: Aprepitant is a known inhibitor of CYP3A4, and therefore may reduce the clearance of drugs metabolized by CYP3A4, including: docetaxel, paclitaxel, etoposide, irinotecan, ifosfamide, imatinib, vinorelbine, vinblastine, and vincristine. Aprepitant should not be used concurrently with pimozide, terfenadine, astemizole, or cisapride (drugs metabolized by CYP3A4). Aprepitant will also increase the area-under-the-curve for dexamethasone by approximately two-fold; the recommended dose of dexamethasone to be used with aprepitant is 12 mg (loading) and 8 mg (maintenance).

10.11 DEXAMETHASONE (DECADRON® ORAL TABLETS)

10.11.1 Supply - For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

10.11.2 Storage and Stability - Oral tablets should be stored 20-25 C° (68-77 F°).

10.11.3 Administration: 60 minutes prior to each dose of pentostatin (days 1, 8, and 15), dexamethasone oral tablets will be administered at a total dose of 12 mg. Then, on days 2 and 3, days 9 and 10, and days 16 and 17, dexamethasone will be administered by oral tablets at a dose of 8 mg.

10.11.4 Toxicities: Dexamethasone is relatively contra-indicated in patients with systemic fungal infection. Rarely, anaphylaxis can occur as a result of dexamethasone therapy. Other toxicities include exacerbation of hypertension, fluid retention, and suppression of the hypothalamic-pituitary-adrenal axis.

10.11.5 Drug Interactions: Concomitant use of dexamethasone and non-steroidal anti-inflammatory agents can increase risk of gastro-intestinal bleeding.

10.12 ONDANSETRON (ZOFRAN®)

10.12.1 Supply - For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

10.12.2 Storage and Stability - Oral tablets should be stored 20-25 C° (68-77 F°).

10.12.3 Administration: Oral tablet (8 mg) administered 60 minutes prior to pentostatin therapy on days 1, 8, and 15. Ondansetron may also be administered at a dose of 8 mg orally every 12 hours on an as needed basis throughout the 21-day chemotherapy cycle.

10.12.4 Toxicities: Ondansetron should not be administered to individuals who have a known hypersensitivity to the agent or to other selective 5-HT_3 receptor antagonists.

10.12.5 Drug Interactions: No significant interactions.

10.13 EX VIVO GENERATED TH2 CELLS

10.13.1 In Vitro Use of X-Vivo 20 Media, ACK Lysis Buffer and Hanks Balanced Salt Solution (purchased from BioWhitaker). For all cell culture procedures in this study, X-Vivo 20 media will be utilized. In additon, ACK lysis buffer will be used to initially remove red blood cells from the cell product. Hanks Balanced Salt Solution will be used in the cell processing as a wash buffer. All of these media are ancillary reagents in the Th2 generation process, as they are washed out prior to final Th2 cell cryopreservation.

10.13.2 In Vitro Use of IL-4 (cross-file on CTEP IND: BB-IND 4348). Recombinant human IL-4 is a cytokine produced by recombinant DNA technology. IL-4 has many effects on the immune system. For this protocol, IL-4 will be used in vitro to enhance the growth of T cells in culture (for generation of donor Th2 cells). IL-4 will be used at a dose of 1000 International Units/ml of culture. The target specific activity for IL-4 is 2.67 x 107 IU per mg. IL-4 is supplied by NCI in vials that contain 100 micrograms per ml per vial or 200 micrograms per ml per vial.

10.13.3 IL-4 Drug Ordering and Drug Accountability: NCI supplied agents (IL-4) may be requested by the Principal Investigator (or their authorized designee) at the participating institution. Pharmaceutical Management Branch (PMB) policy requires that drug be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions unless prior approval from PMB is obtained. The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 572 and a CV. If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution. Drug may be requested by completing a Clnical Drug Request (NIH Form # 986) and mailing it to the Drug Management and Authorization Section, PMB, DCTD, NCI, 9000 Rockville Pike, EPN 707, Bethesda, MD, 20892-7422, or FAX the form to 301-480-4612. For questions, call 301-496-5725.

10.13.4 Drug Inventory Records (IL-4): The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all drugs received from DCTD using the NCI Drug Accountability Record (DAR) Form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage).

10.13.5 In Vitro Use of IL-2 (licensed product; purchased from Chiron): Recombinant human IL-2 is a cytokine produced by recombinant DNA technology. IL-2 has many effects on the immune system. For this protocol, IL-2 will be used in vitro to enhance the growth of T cells in culture (for generation of donor Th2 cells). IL-2 will be used at a dose of 20 International Units/ml of culture.

10.13.6 In Vitro Use of Anti-CD3/anti-CD28 coated Beads (cross-file on IND held by Dr.Carl June: BB-IND 6675): Anti-CD3 and anti-CD28 coated beads have been utilized

in clinical trials to expand T cells for re-infusion in the setting of HIV disease and in the setting of autologous transplantation for the treatment of lymphoma, CML, and multiple myeloma and in the setting of allogeneic transplantation for the therapy of lymphoma and ovarian cancer. Tosylated magnetic beads (Dynal) are conjugated with an antibody to human CD3 (clone OKT3) and an antibody to human CD28 (clone 9.3). Such antibody-coated beads are then used to stimulate T cells in culture. In 120 infusions of T cells grown with anti-CD3/anti-CD28 coated beads, there have been no adverse except the development of an asymptomatic HAMA serologic response in one patient.

10.14 CYCLOSPORINE (GENGRAF, SANDIMMUNE, NEORAL)

10.14.1.1 Supply

Cyclosporine will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (25 mg and 100 mg), USP [MODIFIED], oral solution (100 mg/ml), USP [MODIFIED], and as a parenteral concentrate for injection (50 mg/ml). Because cyclosporine will be initiated four days prior to hematopoietic stem cell transplantation (thereby allowing ample time to reach steady-state levels) and will be used in combination with oral sirolimus, initial dosing of cyclosporine should be by oral administration unless the patient is not able to tolerate oral therapy. When oral capsules are prescribed for this protocol, the cyclosporine capsules, USP [NON-MODIFIED] should NOT be used.

10.14.1.2 Preparation

For parenteral doses, in the unusual case where oral therapy is not possible, each milliliter of concentrate (50 mg/ml) should be diluted in 20 to 100ml of dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of cyclosporine will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing (see Storage and Stability, section **10.1.3**). Oral cyclosporine solution may be mixed in orange juice or other beverages, but not milk.

10.14.1.3 Storage and Stability

Capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light. Cyclosporine concentrate for injection that has been diluted to a final concentration of approximately 2mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for sorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.

10.14.1.4 Administration

Cyclosporine may be given intravenously over 2 hours or orally.

10.14.1.5 Toxicities

1) Acute cyclosporine nephrotoxicity is usually manifested by a moderate decline in renal excretory function, which is readily reversible by a decrease in drug dosage. Although some degree of transient renal dysfunction may occur in patients with therapeutic levels of cyclosporine, significant renal toxicity is associated with elevated trough levels. 2) In addition to an increase in BUN and creatinine, hyperkalemic hyperchloremic acidosis, low fractional excretion of sodium and the onset of hypertension with hypomagnesemia are seen with

cyclosporine nephrotoxicity. 3) Hypertension occurs in up to 60% of patients. 4) Hypomagnesemia, which may contribute to cyclosporine neurotoxicity in the form of seizures, cerebellar ataxia, depression, and coma. 5) Dose-related hepatotoxicity, manifested by elevation of serum transaminases and bilirubin, has been reported.

11 APPENDICES

11.1 APPENDIX A: ENGRAFTMENT SYNDROME

Clinical Definition of Engraftment Syndrome

A constellation of clinical symptoms and signs has been observed during neutrophil recovery following HSCT, most commonly termed "engraftment syndrome" (ES), but interchangeably termed "capillary leak syndrome" and "autoaggression syndrome". A diagnosis of ES is established by the presence of all three of the major criteria listed below, or two major criteria and one or more minor criteria; the clinical signs and symptoms should appear within 96 hours of neutrophil recovery.

Major criteria:	• Fever $\geq$ 38.3° with no identifiable infectious etiology • Erythrodermatous rash involving more than 25% of body surface area and not attributable to a medication • Noncardiogenic pulmonary edema, manifested by diffuse pulmonary infiltrates and hypoxia
Minor criteria	• Hepatic dysfunction with either total bilirubin $\geq$ 2 mg/dl or transaminases levels $\geq$ two times normal • Renal insufficiency (serum creatinine > two times baseline) • Weight gain > 2.5% of baseline body weight • Transient encephalopathy unexplainable by other causes

Treatment of Engraftment Syndrome

The mainstay of therapy for ES is high-dose corticosteroids, based upon the literature on diffuse alveolar hemorrhage in the setting of bone marrow transplantation – a complication that many investigators now believe to be part of the spectrum of ES. Our group has adopted the following treatment schema for patients diagnosed with ES, with satisfactory results:

Day 1: Methylprednisolone 250 mg IV Q6 hours x 4 doses
Day 2: Methylprednisolone 250 mg IV Q8 hours x 3 doses
Day 3: Methylprednisolone 250 mg IV Q12 hours x 2 doses
Day 4: Methylprednisolone 125 mg IV Q12 hours x 2 doses
Day 5: Methylprednisolone 60 mg IV Q12 hours x 2 doses
Day 6: Methylprednisolone 30 mg IV Q12 hours x 2 doses
Days 7-8: Prednisone 60 mg PO QD x 2 days
Days 9-10: Prednisone 50 mg PO QD x 2 days
Days 11-12: Prednisone 40 mg PO QD x 2 days
Days 13-14: Prednisone 30 mg PO QD x 2 days
Days 15-16: Prednisone 20 mg PO QD x 2 days
Days 17-18: Prednisone 10 mg PO QD x 2 days
Day 19: Discontinue prednisone

In the event that symptoms or clinical signs of ES recur during the steroid taper, patients should be retreated with methylprednisolone at a minimum dose of 60 mg IV QD. *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.

11.2 APPENDIX B: ACUTE GVHD

Clinical Staging of Acute GVHD

Stage	Skin	Liver	Gut
+	Rash < 25% BSA	Total bilirubin 2-3 mg/dl	Diarrhea 500-1000ml/d
++	Rash 25-50% BSA	Total bilirubin 3-6 mg/dl	Diarrhea 1000-1500ml/d
+++	Generalized erythoderma	Total bilirubin 6-15 mg/dl	Diarrhea >1500ml/d
++++	Desquamation and bullae	Total bilirubin > 15 mg/dl	Pain +/- ileus

BSA = body surface area; use "rule of nines" or burn chart to determine extent of rash.

Clinical Grading of Acute GVHD

	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 ++++	+++

TREATMENT OF 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:

1) Topical corticosteroids (usually 0.1% triamcinolone; 1% hydrocortisone to face) applied to rash BID.

Grade II-IV 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 **3.5.1**).
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.

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

6) Determination of 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:
   a) Complete response: Complete resolution of all clinical signs and symptoms of acute GVHD.
   b) Partial Response: 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Maintenance of adequate performance status (Karnofsky Score $\geq$ 70%).
   c) 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.

11.3 APPENDIX C: CHRONIC GVHD

	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 involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
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

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
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
LUNGS* **FEV1** ☐ **DLCO** ☐	☐ No symptoms ☐ FEV1 > 80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Severe symptoms (shortness of breath at rest; requiring 0_2) ☐ FEV1 ≤39% **OR** LFS 10-12
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

***** AP may be elevated in growing children, and not reflective of liver dysfunction

<u>Other indicators, clinical manifestations or complications related to cGVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact (none – 0,mild -1, moderate -2, severe – 3)</u>

ˆ Esophageal stricture or web___ ˆ Pericardial Effusion___ ˆ Pleural Effusion(s)___
ˆ Ascites (serositis)___ ˆ Nephrotic syndrome___ ˆ Peripheral Neuropathy___
ˆMyasthenia Gravis___ ˆ Cardiomyopathy___ ˆ Eosinophilia > 500µl___
ˆ Polymyositis___ ˆ Cardiac conduction defects___ ˆ Coronary artery involvement___
ˆ Platelets <100,000/µl ___ˆ Progressive onset ____ Others_____

11.4 APPENDIX D: TABLES FOR SPECIMEN COLLECTION AT NIH

Selected clinical specimens, specifically Lymphocyte TBNK and Chimerism, are listed below along with research labs to provide guidance on disposition and time-points (as they relate to research labs). Collection tube types may change, as determined by the lab receiving the specimen; however, the volume of research specimen collected will not exceed those listed below and total volume collected will not exceed 450ml in any given 6-week period. These tables are not comprehensive in that routine clinical labs, outlined elsewhere, are not listed.

Table I. Specimen Collection for Transplant Patients at NIH

Description	Time points	Collection Tubes	Disposition
Anti-Tumor Effector – baseline	Prior to initiation of treatment on Day 1	Four-10ml Green Top Tubes	Rader/Baskar Lab 10/3248
Lymphocyte TBNK (CD3 subtypes, B cells, NK count)	Baseline and Days 8, 15, 21 prior to P/CY infusion	3 ml Lavendar Top tube	Clinical Pathology (accessioning) – CBC/Diff required on same day.
Immune Depletion	Baseline and Days 8, 15, 21 prior to P/CY infusion	Three-8ml Red&Green CPT Tubes	Fran Hakim Lab 12C216
Chimerism	Days 7, 14, 28, 45, 60, 100, 180, And 1-year post.	See section 3.14.3 for determining number of 10ml Yellow Top ACD Tubes to collect (Typically 10ml)	Hematology (NIH Accessioning) Must be in lab by 10am on day of collection at NIH
Immune/Cytokines	Days 7, 14, 21, 28, 45, 60, 100	Four-8ml Red and Green CPT and One-7ml SST Tube	Fran Hakim Lab 12C216
Immune Reconstitution Post Transplant	Days 7, 14, 21, 28, 45, 60, 100; 9, 12, 18, 24 months; annually to 5 yrs	Four-8ml Red and Green CPT (to Fran)	Fran Hakim Lab 12C216
Anti-tumor effector mechanism	Days 7, 14, 21, 28, 60, 100; 9, 12, 18, 24 months; annually up to 5 yrs.	Two-10ml Green Top tubes to Rader's lab (One 10-ml Green Top Tube on Days 21 & 28)	Rader/Baskar Lab Building 10/3-3248
Optional tumor biopsies	At time of cGVHD diagnosis/biopsy	Five-8ml Red and Green CPT Tubes	Fran Hakim Lab 12C216

11.5 APPENDIX E: DATA COLLECTION ELEMENTS REQUIRED BY PROTOCOL

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

A. PATIENT ENROLLMENT

Recipient

- Date of birth, age, gender, race, ethnicity
- Height
- Weight
- Performance Status
- Date of original diagnosis
- 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
- Baseline Symptoms
- Prior therapy
- Prior surgery
- Findings of consultations done at screening

Donor

- Date of birth, age, gender, race, ethnicity
- Height
- Weight
- Baseline History/Physical (Y/N)

B. STUDY DRUG ADMINISTRATION AND RESPONSE FOR EACH COURSE OF THERAPY GIVEN

- Dates study drugs given
- Dose level, actual dose, schedule and route given
- Height, weight, and body surface area at start of each course
- Response assessment for each restaging performed

C. LABORATORY AND DIAGNOSTIC TEST DATA

- All Clinical laboratory and diagnostic test results done at screening (recipient and donor) and until day 100 post-transplant with the following exceptions:
- Diagnostic tests which are not specified in the protocol, and if the results are not needed to document the start or end of an adverse event that requires reporting.

- All clinical laboratory and diagnostic tests done after day 100 that support a possible, probable or definite diagnosis of GVHD, infection or secondary malignancy and those done to document a change in grade and the end of these adverse events.
- All tests done to document resolution of adverse events that occurred in the first 100 days post transplant
- HLA data (patient and donor).
- Blood, bone marrow, and tumor chimerism data

D. ADVERSE EVENTS

- All grade 3 and 4 adverse events will be recorded except grade 3 and 4 lymphopenia.
- The following grade 2 adverse events will be recorded:
  1) Toxicities potentially associated with GVHD;
  2) Toxicities potentially attributable to the Th2 cells; and
  3) Toxicities potentially related to infection.
- After Day 100 post-transplant, adverse events will be recorded if potentially associated with the Th2 cells, opportunistic infection, GVHD, or secondary malignancy.
- For this study, the development of hyper acute GVHD will be considered a toxicity likely attributable to Th2 cell administration. Hyper acute GVHD will be defined as severe GVHD (grade III or IV) that occurs in the first 14 days after Th2 cell infusion.

Data will also be reported to the International Bone Marrow Transplant Registry

E. CONCOMITANT MEASURES

- Baseline medications
- Antibiotics
- GVHD prophylaxis and treatment
- Other therapy for recorded adverse events

F. TREATMENT OF PERSISTENT/PROGRESSIVE DISEASE WITH STANDARD THERAPY

- Chemotherapy
- Immunotherapy
- Radiation therapy
- Donor Lymphocyte Infusion

G. TUMOR RESPONSE AND MEASUREMENTS

- Restaging studies performed at protocol specified time points and as clinically indicated.

H. OFF STUDY

- Date and reason for off study
- Date and cause of death
- Autopsy findings

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