Amendment

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* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: N/A

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Title: Rapamycin-Resistant T Cell Therapy of Multiple Myeloma: Relapse Prevention and Relapse Therapy

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D. Makes decisions about subject eligibility
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Investigational agent: Rapamycin-Generated Autologous Th1/Tc1 Cells

IND # 14337 IND Holder: Rapa Therapeutics

Commercial Agents: Bortezomib, Lenalidomide, Cyclophosphamide, Pentostatin

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PRECIS

Background:

- Autologous Hematopoietic Cell Transplantation (AHCT), which represents the standard of care for newly diagnosed Multiple Myeloma (MM), is not curative therapy. New approaches to prevent relapse after AHCT and to treat relapse are needed.
- In murine models, we used ex vivo culture to generate rapamycin-resistant, Th1/Tc1 polarized T cells (Th1/Tc1.Rapa cells) that were both rapamycin-resistant and apoptosis-resistant with an increased in vivo survival and in vivo function.
- Because Th1 /Tc1 polarized lymphocytes are pivotal in anti-tumor effects, we hypothesize that adoptive transfer of Th1/Tc1Rapa cells will be of benefit to MM patients.

Objectives:

Primary

Dose escalation study

Evaluate the feasibility and toxicity of an infusion of autologous, ex vivo rapamycin-generated, anti-CD3 and anti-CD28 co-stimulated, Th1/Tc1 lymphocytes (Th1.rapa cells) in subjects diagnosed with high-risk multiple myeloma following AHCT.

MM Relapse Prevention and Treatment Cohorts

- For Cohort A, in newly diagnosed MM patients who have received AHCT, evaluate the safety of a defined regimen of Th1/Tc1.Rapa cell therapy and determine progression-free survival.
- For Cohort B, in relapsed MM, determine the PR/CR rate of Th1/Tc1.Rapa cell therapy.

Eligibility:

- For CohortA relapse prevention, patients with MM (normal- or high-risk) who are receiving induction therapy and subsequent AHCT.
- For Cohort B relapse therapy, patients with MM who have measurable disease after at least 2 prior treatment regimens.

Design:

- For Cohort A, patients will receive two infusions of autologous Th1/Tc1.Rapa cells (at one and two months post-AHCT; each infusion preceded by a 7-day course of immune modulating chemotherapy [pentostatin plus low-dose cyclophosphamide; PC regimen].
- For Cohort B relapse therapy, patients will up to four infusions of Th1/Tc1.Rapa cells, with each infusion preceded by either a 7-day or 14-day PC regimen.

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1 INTRODUCTION

1.1 STUDY OBJECTIVE

1.1.1 Primary Objectives

1.1.1.1 Dose escalation study

Evaluate the feasibility and toxicity of an infusion of autologous, ex vivo rapamycin-generated, anti-CD3 and anti-CD28 co-stimulated, Th1/Tc1 lymphocytes (Th1.rapa cells) in subjects diagnosed with high-risk multiple myeloma following AHCT.

1.1.1.2 MM Relapse Prevention and Treatment Cohorts

- For Cohort A, in newly diagnosed MM patients who have received AHCT, evaluate the safety of a defined regimen of Th1/Tc1.Rapa cell therapy and determine progression-free survival.
- For Cohort B, in relapsed MM, determine the PR/CR rate of Th1/Tc1.Rapa cell therapy.

1.1.2 Secondary Objectives

- Characterize the cellular aspects and cytokine phenotype of immune reconstitution in recipients of Th1.rapa cells.

1.2 BACKGROUND AND RATIONALE

1.2.1 Overview of Treatment of MM

Approximately 14,600 new cases of MM are diagnosed each year in the United States. The reported incidence is 5 per 100,000 with a peak at age ~70 years; rates are higher in African Americans and in men. It is the second most common hematologic malignancy in the United States. There are an estimated 45,000 people living with MM in this country and it is estimated that there were 19,900 new diagnoses and 10,790 deaths due to MM in 2007[1]. The median survival of patients with MM was less than one year before introduction of alkylating agents (melphalan) in the 1960s which resulted in improved survival but still in a very limited number of complete responses and in no curative effect.

For many years dexamethasone alone or in combination with oral melphalan (MP) or with vincristine and doxorubicin (VAD)[2] has been the mainstay of induction therapy for newly diagnosed MM. Recently, several agents with novel mechanisms of action have shown superior activity in MM. Thalidomide in combination with dexamethasone was found to be superior to dexamethasone alone as well as superior to VAD in randomized trials[3,4]. Additionally, bortezomib, a proteosome inhibitor, has been shown to be superior to VAD as induction therapy in randomized trial[5]. Two phase III studies show an increased overall survival with oral lenalidomide plus dexamethasone compared to dexamethasone plus placebo in previously treated MM patients[6,7]. Phase II studies in newly diagnosed patients so far corroborate these findings. The multiple improved therapeutic options now available as induction therapy for MM were recently reviewed[8] and are available as standard of care for newly diagnosed MM. Guidelines are described and regularly up-dated by the National Comprehensive Cancer Network (http://www.nccn.org/professionals/physician_gls/f_guidelines.asp)

In the last decade, it has also been shown that high-dose chemotherapy followed by Autologous Hematopoietic Cell Transplantation (AHCT) significantly increases the percentage of complete remissions (CR) to almost 50% in selected patients (*versus* 1–13% CR with conventional dose therapy), but the disease recurs almost without exception[9-11]. High-dose chemotherapy increases the CR rate and time-to-progression and has shown a survival improvement[12,13] though not consistently[14,15]. Double (tandem) AHCT has recently been shown to improve long-term survival in eligible patients less than 60 years old[10,16], but the majority of patients eventually relapse even after the double transplant and the survival advantage, although significant, remains relatively modest.

Of great interest, is the demonstration that effective immunotherapy is achievable in MM. Susceptibility of MM to immunotherapy has been established in the context of allogeneic transplantation (graft versus tumor effect) but the high toxicity of myeloablative allogeneic transplantation has prevented its translation into any survival benefit and myeloablative allogeneic transplantation has been largely abandoned for the treatment of MM. However, the non-myeloablative approach, with its significantly decreased toxicity, offers more promise[17,18]. The recent demonstration that AHCT followed by an non-myeloablative allogeneic sibling transplant yield results superior to tandem AHCT indicates that the introduction of an immune intervention in the context of minimal residual disease post AHCT may be more effective than repeated high dose chemotherapy[19-21]. This suggests that effective autologous immunotherapeutic interventions in the same context (following maximal disease reduction by AHCT) could have a role in overall therapeutic strategies for MM and should be developed since most patients do not have a suitable allogeneic donor.

Therefore, significant progress was made in the last decade in the management of MM with the advent of the new generation of targeted therapies such thalidomide, bortezomib and more recently lenalidomide[22] and their combination in strategies including AHCT. In patients not exposed to this new generation of drugs, the median survival following relapse after AHCT is about 18 months while in patients exposed to these drugs following relapse after AHCT, the median survival has almost doubled to 36 months. Overall, in a recent study from Mayo Clinic, the median overall survival for subjects diagnosed in the last decade was 44.8 months (95% CI; 39.6, 50) compared with 29.9 months (95% CI; 28.3, 31.6) $p < .001$, for those diagnosed earlier[23]. These successes have allowed moving these agents to front line therapy in newly diagnosed subjects with significant improvement in response rates and EFS[24-26].

However, none of these modalities, to this date, provide a significant prospect for cure. Therefore, in spite of these advances, much remains to be done to improve the long term outcome of subjects with either newly diagnosed or relapsed MM.

1.2.2 Rationale for the subject population

Several strategies have been used in order to stratify subjects with newly diagnosed MM into simple risk categories for the prognostic purposes as well as for optimal evaluation of treatment strategies.

1.2.2.1 International Scoring System

The working party of the ISS for MM gathered data on 10,750 patients from 15 Asian, European, and North American institutions and groups[27]. A simple staging system was agreed upon and validated which defined 3 stages in MM as follows:

- stage I: serum β2-microglobulin less than 3.5 mg/l plus serum albumin greater or equal to 3.5 g/dl
- stage II: neither stage I nor III
- stage III: serum β2-microglobulin greater or equal to 5.5 mg/l

1.2.2.2 Definition of a high-risk group in newly diagnosed subjects

Multiple studies have been published describing a large number of cytogenetic abnormalities in MM and correlating them with clinical outcome [28-34]. Several cytogenetic abnormalities have been associated with better or worse outcome in MM, correlating the more common specific abnormalities with overall (OS) and progression free (PFS) survival as follows:

Abnormality	OS	PFS
- p53 del	14.7	7.9
- t(4:14)	18.3	9.9
- 13q del	34.4	20.2
- t(11:14)	37.2	25.2
- none of above	not reached	32.1

Recapitulating the larger body of published data and based on 104 MM cases that had informative FISH results for all four genetic markers, three distinct prognostic categories were proposed with genetic-based risk stratification[32]: intermediate risk and high-risk with any one or more of the genetic abnormalities other than t(11;14) (48% of cases), low-risk patients with none of the genetic abnormalities tested or only t(11;14) (52% of cases). The median PFS was 10 to 20 months for the higher risk group and 32.1 months for the low risk group. The median OS was 18 months to 46 months for the higher risk group and was not reached for the low-risk group.

1.2.2.3 The study population

In the initial phase I aspect of the protocol, accrual was limited to patients with high risk disease with the shortest estimated event-free survival; however, with amendment K, after substantial safety data was obtained, the accrual was expanded to include both high-risk and standard-risk multiple myeloma patients. High-risk disease includes subjects with recurrent or persistent disease following conventional initial therapy. Based on cytogenetic and other high risk features at diagnosis, it is estimated that approximately 50% of subjects with newly diagnosed MM may belong to the high risk group. The high-risk patients are defined as follows:

- Subjects with high-risk cytogenetic features[28,30,34] defined as the presence of any of the following:
  - t(4;14) by FISH[31]
  - t(14;16) or t(14;20) by FISH
  - del 17p13 by FISH
  - del 13 or aneuploidy (by metaphase analysis33)
- Subjects with other high-risk features
  - β2-microglobulin >5.5 mg/dl

- plasmablastic morphology at diagnosis[35]
- circulating MM cells by FACS ($CD38^+/CD45^{low}$) following induction therapy[36]

The "good-risk" group (defined as the absence of high-risk features and the presence of any of these: hyperdipoidy, t(11;14) by FISH[37], t(6;14) by FISH) and the standard-risk group (those patients not falling into the good-risk or high-risk categories) will be considered for the phase II aspect of the study given that multiple myeloma is generally incurable even in these risk groups.

Age will not be a strict exclusion criterion since age is not a bad prognostic feature for AHCT in MM when older individuals are matched for other prognostic factors with younger individuals[38,39]. However, physiologic age and co-morbidity will be thoroughly evaluated before enrolling older individuals.

1.2.3 Th1 immunity and cancer

1.2.3.1 The Th1 immunity and cancer paradigm

Cancer development is associated with a multitude of alterations in immune functions. Such alterations include suppressed cell mediated immunity associated with failure to reject tumors, as well as enhanced humoral immunity that can potentiate tumor promotion and progression[40]. Distinctive $CD4^+$ T cell subsets, Th1 and Th2 T cells, have distinctive function and cross regulate each other[41,42]. Th1 cells produce interleukin (IL)-2 and interferon (IFN)-γ and direct cell mediated immunity responses such as cell mediated cytotoxicity, whereas Th2 cells produce IL-4 and IL-10, and facilitate local humoral immunity responses.

There is increasing evidence that many cancers are accompanied by a Th1 / Th2 imbalance, both in the peripheral blood and at the tumor site, where the proportion of Th2 cells is significantly elevated at the expense of Th1 cells number, as compared to the proportion of Th1 and Th2 cells seen in otherwise healthy patient populations[43,44]. Chronic Th1 / Th2 imbalance in favor of Th2 potentially leads to suppressed cell mediated immunity and angiogenesis, and reduced apoptosis, thereby providing a propitious environment for decreased effective immuno-surveillance and development of malignant disease[40]. For example, as reflux esophagitis progresses to Barrett's esophagus, the inflammatory infiltrate initially composed of Th1 effector cells is largely replaced by infiltration of Th2 effector cells, suggesting that this shift contributes to the increased risk for malignant transformation to esophageal cancer seen in Barrett's esophagus[45]. Likewise, in human cervical carcinomas, $CD3^+$ tumor infiltrating T cells have an enhanced Th2 cytokine profile[46]. A recent study found that $CD3^+$ T cell densities infiltrating colorectal cancer surgical biopsies, as opposed to peripheral blood, represented a better predictor of patient survival than all current histo-pathological staging methods. Moreover, the authors found a positive correlation between the presence of markers for Th1 polarization of cytotoxic and memory T cells and a low incidence of tumor recurrence, suggesting Th1 immunity may mediate rejection of residual tumor cells after surgery in subjects with a favorable outcome[47]. B cells suppress Th1 responses in mice[48,49] and B cell depletion in humans has been associated with decrease tumor burden in patients with colo-rectal carcinoma[49]. Based on the large body of pre-clinical evidence that Th1/Tc1 responses are the main cellular effectors of anti-tumor activity, multiple attempts are being made to induce Th1 polarization in clinical settings such as the use of post-transplantation IL-12 administration for hematologic malignancies[50].

1.2.3.2 Th1 immunity and Multiple Myeloma

Idiotype specific T cell responses are found in most patients with early stage MM. These include Th1 responses with IL-2 and IFN-γ production[51]. Yi et al. reported that Th1-type immunity was found preferentially in cases of indolent disease and that Th2-type responses predominated in cases of advanced MM, demonstrated by Elispot[52]. Defective Th1 immune responses (mediated by IL-6 production) as well as dysregulated cytokine network are found in MM patients[53]. Myeloma idiotype-specific T helper cells derived from MM patients are consistently of non-Th1 phenotype[54]. $CD4^+$ T cells from patients with progressing MM showed a proliferative MHC class II-dependent response when cultured with dendritic cells loaded with the specific MM immunoglobulin. $CD8^+$ T cell reactivity and Th1 activation were consistently low or absent, and Th2 and regulatory cytokines were expressed. MM cells have specific T cell inhibition properties in which TGF-β has a role[55,56]. As TGF-β has been implicated in the Th1 to Th2 shift[57], such a shift toward Th2 response in MM may be mediated by TGF-β secreted by the myeloma cells[55].

As in the previously mentioned solid epithelial tumors, a large body of evidence from animal models points to the critical role of Th1 responses in anti-MM effective immune responses, including cytotoxicity. In multiple animal models, effective *in vivo* anti-myeloma T cell responses are of Th1 nature. This was found to be the case following intra-tumoral dendritic cell immunization[58] and models of adoptive immunotherapy for MM indicate that Th1/Tc1 cells are the effectors of the anti-tumor activity[59,60]. In mice, the therapeutic outcome following combination immunogen vaccination therapy is greatly improved with the addition of IL-12 which results in Th1 polarization of the response[61]. Indeed, a consistent feature in models showing increase efficacy of tumor eradication or protection from tumor is that the specific intervention results in Th1 polarization of the immune response.

1.2.4 Post AHCT immune reconstitution

There is ample evidence that even short courses of standard dose chemotherapy have a significant impact on an individual's residual immune function[62]. The kinetics and the extent of the immune recovery correlate closely with the individual's amount of residual thymic function, children recovering a population of naïve T-cells via a thymic expansion pathway much more readily than adults[63]. Following more severe immune depletion, such as post AHCT, the expansion of peripheral lymphoid progenitors is similarly contingent upon the functionality of the thymus[64]and the return to a normal $CD4^+$ lymphocyte count in adults following AHCT is predicated upon the re-emergence of a pool of naïve T-cells which is delayed 18 to 24 months and may occur only in individuals younger than 50 years[65,66]. Furthermore, a comparative study of immune reconstitution following AHCT in a variety of hematologic malignancies and breast cancer did not find significant disease specific differences in immune reconstitution up to a year following AHCT[67]. The substantial body of evidence accumulated in recent years on the protracted delay in immune reconstitution following AHCT has been recently reviewed[68].

Significant functional abnormalities have been described post-AHCT as well. During the first 6 months following engraftment, post-transplant derived peripheral blood mononuclear cells stimulated with phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) demonstrate statistically significant decrease in production of IL-3, IL-4, GM-CSF and IFN-γ as compared to normal controls. When the overall group of transplant recipients is compared to the control group, there is also a statistically significant lower production of IL-2[69].

Disease free survival post-AHCT correlates with several aspects of impaired immune reconstitution. Several studies have correlated disease-free survival and an early absolute lymphocyte count recovery following AHCT in leukemia[70], breast cancer[71], non Hodgkin's lymphoma and multiple myeloma[72]. Memory cells producing IFN-γ are significantly lower in patients who experienced relapse of original cancer within 1 year following AHCT compared to those who showed no sign of relapse even after 2 years, indicating that efficient reconstitution of Th1-type function of memory T cells is important in maintaining appropriate levels of immune surveillance against residual tumor burden[73]. Moreover, a predominant Th2 immune reconstitution has been documented specifically in MM patients following AHCT[74].

Post-AHCT immunodeficiency is, by itself, associated with poorer disease outcome in cancer patients but also constitutes a barrier to successful subsequent attempts to active immunotherapy, the therapeutic potential of which may be misjudged or altogether overlooked as a consequence. Attempts to enhance immune reconstitution following AHCT with adoptive immunotherapy have been made recently, in particular using anti-CD3 / anti-CD28 poly-clonally activated T lymphocytes[75,76]. Such T cells expressed primarily a Th1/Tc1 cytokine phenotype that in general has been associated with a favorable anti-tumor effector function (section **1.2.3**). The rationale for polyclonal versus antigen-specific T cell expansion is that polyclonally expanded T cells will be poised for *in vivo* activation by infectious and / or tumoral antigenic stimulations, either occurring naturally or following specifically designed immunization strategies, thereby possibly taking advantage of an *in vivo* epitope spreading phenomenon post active immunization. The proof of principle of this approach was provided in a recent study[75] where subjects undergoing AHCT for MM were first immunized pre-AHCT (with polyvalent pneumococcal conjugate vaccine), autologous T cells were then collected by apheresis pre-AHCT and *ex vivo* co-stimulated and expanded, then re-infused post AHCT followed by repeated *in vivo* vaccine boosts. This strategy led to significant acceleration of $CD4^+$ cell count recovery and function restoration with improved responses to the vaccines over the control groups. Of great interest, T cells responses to antigens that were not contained in the vaccine were also enhanced[75]. The study design did not allow statistical evaluation of disease response.

Mid to long-term persistence of such adoptively transferred lymphocytes, however, remains a critical and problematic issue. Clinical studies conducted in the Surgery Branch, NCI show that homeostatic T cell proliferation is important for effective adoptive immunotherapy and pretreatment with immune depleting chemotherapy may enhance the effects of infused T cells and their *in vivo* survival[77]. Rapamycin treatment during the lymphocyte *ex vivo* expansion may also be of critical importance in the life span of the transferred cells (section **1.2.5**).

1.2.5 *Ex vivo* polarized T cell expansion in high dose Rapamycin: ETIB experience

In murine models, we have recently observed that T cells expanded *ex vivo* in high-dose rapamycin (10 μM) can attain either a Th2/Tc2 (type II) cytokine phenotype or a Th1/Tc1 (type I) cytokine phenotype depending upon the cytokine environment (IL-4 added for type II cells; IL-12 added for type I cells)[78]. Both rapamycin-generated type 1 and type 2 T cells expressed a central memory T cell (T_{CM}) phenotype, including expression of the lymph node homing molecule CD62L, or L-selectin; this T_{CM} phenotype has been associated with enhanced *in vivo* engraftment and efficacy upon adoptive transfer in murine anti-tumor T cell models[79]. In our studies, we found that rapamycin-generated Th2/Tc2 cells persisted longer *in vivo* and promoted greater levels of type II cytokines than control Th2/Tc2 cells; similarly, rapamycin-generated

Th1/Tc1 cells persisted longer *in vivo* and promoted greater levels of type I cytokines than control Th1/Tc1 cells[78].

In previous models of murine allogeneic bone marrow transplantation, we have characterized acute graft-versus-host disease (GVHD) as an immune process that is primarily caused by donor Th1/Tc1 cells and inhibited by donor Th2/Tc2 cells; for example, using co-stimulated donor T cells not generated in rapamycin, we found that Th1/Tc1 cells mediated greatly increased GVHD relative to Th2/Tc2 cells[80]. Remarkably, in the more recent experiments, we found that rapamycin-generated donor Th1/Tc1 cells mediated greatly increased GVHD relative to control Th1/Tc1 cells not generated in rapamycin[78]. Of note, the GVHD-related toxicity of the rapamycin-generated Th1/Tc1 cells was greatly ameliorated by *in vivo* rapamycin drug therapy. These data indicate that *ex vivo* exposure to rapamycin represents a new method for enhancing Th1/Tc1 cell therapy; such a strategy may have limited application in the allogeneic transplant setting due to GVHD, and as such, we are developing the current approach of using such cells in the autologous transplant setting for the purpose of enhancing immune reconstitution. In addition, these data indicate that in the event of immune-related toxicity of the rapamycin-generated Th1/Tc1 cells, rapamycin drug therapy may represent a potential therapeutic option.

The majority of our data relating to the study of rapamycin-generated T cells relates to the study of Th2 cells in the context of allogeneic bone marrow or hematopoietic stem cell transplantation. Specifically, we have found that murine allograft augmentation with rapamycin-generated Th2 cells represents a successful strategy to separate graft-versus-tumor (GVT) effects from GVHD[81], and also represents a new approach to prevent fully MHC-disparate graft rejection[82]. In these projects, we have also investigated potential mechanisms accounting for the increased *in vivo* efficacy of rapamycin-generated T cells. We found that rapamycin-generated T cells were indeed rapamycin-resistant, as they were fully blocked at molecules down-stream to mTOR such as 4-EBP1 and S6 kinase[82]; maintenance of proliferation and attainment of effector polarization in the setting of mTOR blockade may be mediated in part due to the PIM-1 and PIM-2 kinases[83] as expression of these molecules was preserved in our experiments. Remarkably, during *ex vivo* expansion, the attainment of rapamycin-resistance was accompanied by the development of apoptosis-resistance; importantly, this apoptosis-resistance was observed in both Th1- and Th2-type T cells generated in high-dose rapamycin. Resistance to apoptosis was associated with marked alteration in the bcl-2 family of mitochondrial proteins, specifically an up-regulation of anti-apoptotic bcl-xL and down-regulation of pro-apoptotic bim and bid. Given these results, it is likely that the improved in vivo function and increased in vivo persistence of rapamycin-generated T cells is due at least in part to an anti-apoptotic phenotype.

Significantly, we have translated this area of research into the clinic through the study of rapamycin-generated donor Th2 cells in the setting of allogeneic hematopoietic stem cell transplantation. In an initial clinical trial (99-C-0143), we performed a phase I study of co-stimulated Th2 cells generated without ex vivo rapamycin; in this 47 patient study, we found that the infused Th2 cells were well-tolerated, but clinical outcome was limited by engraftment syndrome (which occurred in approximately 50% of subjects) and grade II to IV acute GVHD (which occurred in approximately 60% of subjects)[84]. In an ongoing clinical trial (04-C-0055), we are evaluating rapamycin-generated donor Th2 cells. It is difficult to make an attribution to any difference in outcome between these two studies because the protocols differ not only in the method of Th2 cell expansion (without rapamycin [99-C-0143] or with rapamycin [04-C-0055]) but also with respect to GVHD prophylaxis (cyclosporine alone [99-C-0143] or cyclosporine

plus short-course rapamycin [04-C-0055]) and with respect to transplant conditioning (full dose fludarabine plus cyclophosphamide [99-C-0143] or at least 75% reduced dose cyclophosphamide [04-C-0055]). Nonetheless, current results on protocol 04-C-0055 appear favorable, as engraftment syndrome has been eliminated as a significant complication (0 cases out of 35 evaluable) and grade II to IV acute GVHD has been reduced to approximately 20%.

In the allogeneic setting, Th2 cells are not known to directly mediate anti-tumor effects; they are administered in the context of a T cell replete allograft, thereby providing a Th1/Th2 balance that may improve the ratio of anti-tumor effects relative to GVHD effects. In syngeneic tumor models that reflect the autologous T cell therapeutic setting of this current proposal, Th1 cells have been characterized as having more potent anti-tumor effects than Th2 cells. Therefore, given the results described above, we now hypothesize that generation of Th1/Tc1 cells in high-dose rapamycin will generate a T cell population with enhanced *in vivo* persistence and a commensurate increased capacity to restore immune competence after autologous transplantation. As a step towards clinical translation, we evaluated whether human Th1/Tc1 cells might be generated in rapamycin. Using anti-CD3, anti-CD28 co-stimulation, rapamycin significantly blunted human T cell expansion over the 6 days of culture (yield at day 6 of culture was approximately equivalent to the number of input T cells; data not shown). Figure 1 below shows a representative T cell cytokine phenotype of the resultant T cell product; as this figure shows, rapamycin reduced the resultant T cell capacity to secrete the Th1 cytokine IFN-γ. However, the further addition of either IFN- γ or IL-12 to rapamycin facilitated the generation of T cells that secreted high levels of IFN- γ. Each culture showed nominal secretion of type II cytokines such as IL-4 (data not shown). Because IFN- γ is approved for clinical use, whereas IL-12 is not, we have elected to incorporate IFN- γ into the Th1/Tc1 manufacturing method. Finally, using a human-into-murine model of xenogeneic GVHD (xGVHD), we evaluated whether rapamycin-exposure during human Th1/Tc1 cell expansion might alter the in vivo persistence of the expanded human T cells in a manner consistent with the murine T cell results. Remarkably, at day 48 post-transplant, the human Th1/Tc1 cells generated in rapamycin were readily detected in the murine immune-deficient hosts (Figure 2); in marked contrast, the

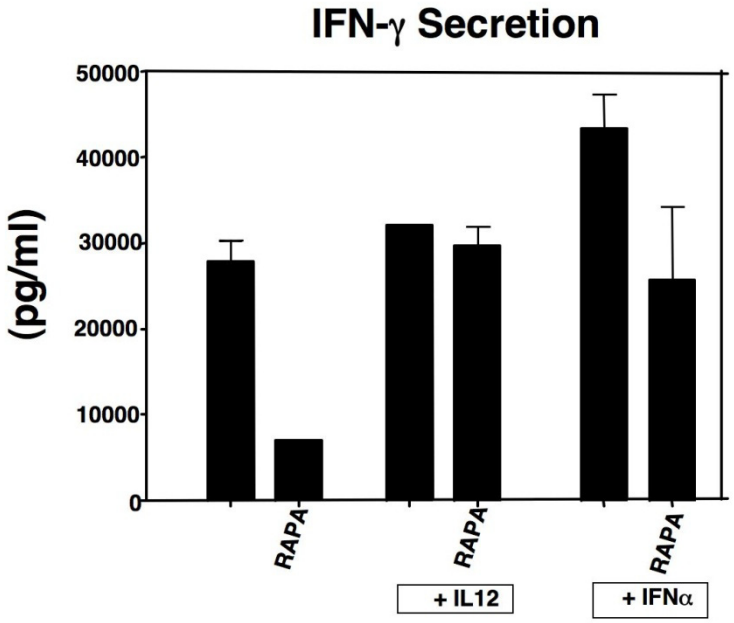


Figure 1. Type I polarization of human T cells in rapamycin. Human T cells were isolated by apheresis, incubated with anti-CD3/anti-CD28 coated magnetic beads, and cultured in X-Vivo 20 media supplemented with 5% autologous plasma, rhu IL-2 (100 I.U./ml), and with or without high-dose rapamycin (RAPA; 1 μg/ml). Further cultures received additional cytokines of rhu IL-12 (2.5 ng/ml; columns 3 and 4) or rhu IFN-α (10,000 I.U./ml; columns 5 and 6). After six days in culture, T cells were harvested and washed, adjusted to 1 x 10^6 cells/ml, and co-stimulated again to generate 24 hour supernatants that were then tested by Luminex assay for IFN-γ content.

control Th1/Tc1 cells were not detected. Furthermore, only the rapamycin-generated Th1/Tc1 cells resulted in a clinical syndrome of xGVHD, as evidenced by dermatitis involving > 50% of the body surface area (not shown) and T cell infiltration of murine organs such as liver (Figure 2). In sum, these results indicate that human Th1/Tc1 cells can be generated in high-dose rapamycin and indicate that, similar to our murine studies, such T cells have an increased capacity to persist in vivo after adoptive cell transfer.

1.2.6 Rationale for Dose and Timing of Th1/Tc1 Cells

In this protocol, we will evaluate a new T cell product (Th1/Tc1.R cells) that has not been previously evaluated in clinical trials. However, pre-clinical experiments using Th1/Tc1.R cells (using a human-into-murine xenogeneic transplantation model) and data from clinical trials using somewhat related T cell products were carefully considered for the design of the current protocol. First, we have performed experiments to directly compare the biology of Th1/Tc1.R cells with control, co-stimulated T cells that were not generated in the presence of rapamycin [85]; such control T cells used in these experiments were somewhat similar to T cell products that were manufactured through co-stimulation (and without rapamycin and without cytokine polarization) and evaluated in previous clinical trials[75,86]. First, relative to T cell production of inflammatory cytokines such as IFN-γ, we found that Th1/Tc1.R cells produced more IFN-γ,

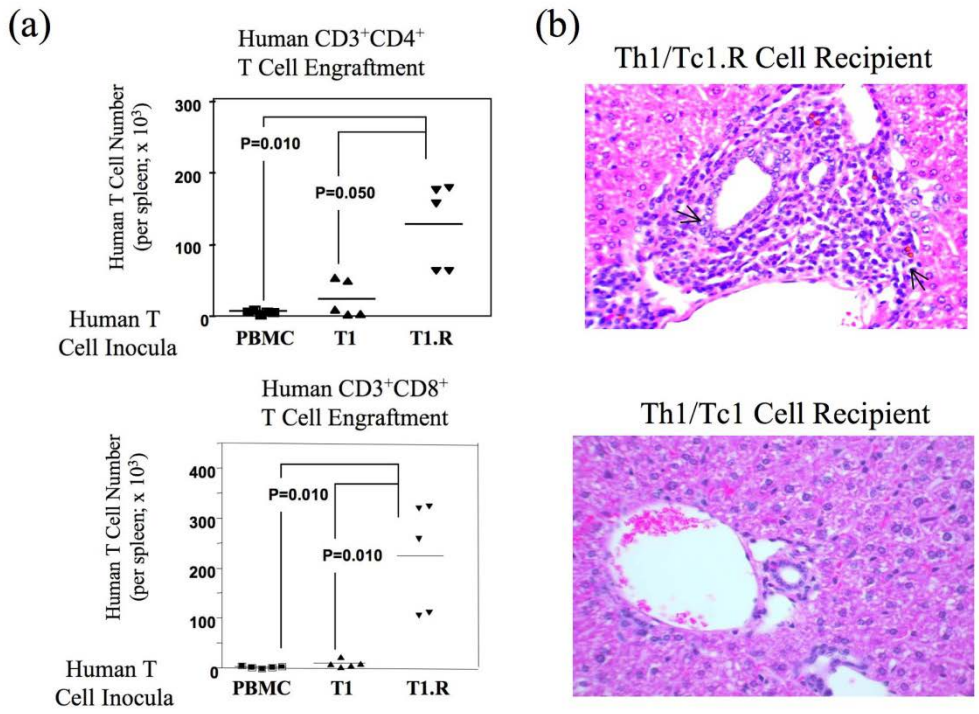


Figure 2. Rapamycin-resistant human Th1/Tc1 cells have increased in vivo persistence after adoptive transfer. Human T1 cells were manufactured as described in Fig. 1 either with ex vivo rapamycin ("T1.R") or without rapamycin ("T1"). Immune deficient mice ($RAG^{-/-}c\gamma^{-/-}$) were utilized to compare the engraftment potential of human T1.R cells, control T1 cells, or control peripheral blood mononuclear cells ("PBMC"). (a) On day 48 after adoptive transfer, the number of human $CD4^+$ T cells (top panel) and $CD8^+$ T cells (bottom panel) persisting in the spleen were enumerated by flow cytometry. (b) Histology was performed at day 48 post-transfer to document persistence of T1.R cells in the liver of the murine hosts (top panel, representative T1.R cell recipient; bottom panel, control T1 cell recipient).

more Tbet transcription factor, and more T central memory markers relative to control, co-stimulated T cells (see Figures 1 and 2 contained in reference [85]). Second, we found that such Th1/Tc1.R cells proliferated and persisted in vivo to a greater degree than control, co-stimulated T cells (see Figures 6 and 10 contained in reference [85]). And third, we found that infusion of Th1/Tc1.R cells resulted in a greater degree of xenogeneic graft-versus-host disease relative to infusion of control, co-stimulated T cells (see Figure 11 contained in reference [85]). In sum, these data indicate that the Th1/Tc1.R cells that we will evaluate have a significantly increased in vivo capacity to produce inflammatory cytokines and to proliferate and persist in vivo after adoptive T cell transfer (relative to control co-stimulated T cells); as such, initial clinical evaluation of the Th1/Tc1.R cell product should utilize a conservative dosing strategy.

The most relevant prior clinical trial data in terms of T cell dosing for this trial can be ascertained from the recent data published by two clinical trials that were also conducted in the setting of autologous therapy of multiple myeloma[75,86]. One rationale for performing T cell therapy in this context is that enhanced recovery of T cells after autologous transplantation for multiple myeloma has been associated with improved survival[72]. These clinical trials were performed using autologous T cells that were co-stimulated using the anti-CD3 and anti-CD28 method developed by Dr. Carl June; this is the exact same method of T cell co-stimulation that we will utilize in the current clinical trial. However, as previously detailed, the current clinical trial T

cell product will differ from these prior studies because the co-stimulated T cells will be rendered rapamycin-resistant and type I cytokine polarized. In the first previous clinical trial[75], the co-stimulated T cells were infused on day 12 after autologous hematopoietic stem cell transplantation at a dose of approximately 1 x 10^9 T cells/kg recipient body weight. It was concluded that the T cell infusions were relatively safe (for summary of adverse events, see Table 1 of reference [75]); however, 23% of recipients developed a skin rash post-infusion that was suggestive of an autologous GVHD-like reaction. In this clinical trial, it was demonstrated that T cell recipients had significantly enhanced B cell and T cell immunity relative to randomized control recipients that did not receive the co-stimulated T cells.

In the second clinical trial that evaluated co-stimulated T cell therapy in the multiple myeloma setting[86], T cells were infused at day 2 after autologous transplantation (rather than at day 12 in the initial clinical trial); the co-stimulated T cell dose remained constant at approximately 1 x 10^9 T cells/kg. The rationale for changing the T cell infusion to day 2 was as follows: at this early post-transplant time point, the levels of T cell homeostatic cytokines are high, and as such, T cell expansion in vivo will be maximized. Indeed, in this clinical trial, it was documented that levels of IL-15 were greatly elevated at the time of T cell infusion, and resultant T cell counts at day 14 post-transplant were elevated. Importantly, a new toxicity was observed in this clinical trial: 'engraftment syndrome' characterized by diarrhea and fever was observed in 16% of T cell recipients; by histology analysis, colitis was observed that was consistent with an autologous GVHD-like reaction. In sum, these two trials indicate that the co-stimulated T cells are relatively safe but that some degree of auto-reactivity can be observed when administered in the autologous transplantation context in multiple myeloma patients; the degree of toxicity appears to increase as the T cell infusion is moved more proximal to the chemotherapy administration.

As such, we have considered both the pre-clinical data (comparing Th1/Tc1.R cells to control co-stimulated T cells) and the clinical trial data (pertaining to co-stimulated T cells in the myeloma setting) in the design of the current clinical trial with respect to both T cell dose and T cell timing. First, because the Th1/Tc1.R cell product is anticipated to be more potent relative to previously evaluated co-stimulated T cells, we will initiate a phase I evaluation with a low starting cells dose of 1 x 10^5 T cells/kg; this starting dose represents only approximately 0.05% of the T cell dose evaluated on the previous clinical trials. Second, to perform the phase I dose escalation in a relatively conservative manner, subsequent dose levels will be evaluated at only a three-fold increase in dose up to a maximum dose of 45 x 10^6 T cells/kg). And third, because of the apparent correlation of co-stimulated T cell in vivo effects with timing of administration, we will delay the Th1/Tc1.R cell infusion until day 42 post-transplant or post standard consolidation if such treatment is deemed necessary by the treating physician (in contrast, the previous trials tested T cell infusion at either day 12 or day 2 of transplant). As such, in this first-in-man evaluation of Th1/Tc1.R cells, we will utilize a low T cell dose, modest increases in T cell numbers between cohorts, and a conservative time point of T cell administration.

1.2.7 Immune depletion in cohort 5 B

1.2.7.1 Rationale

Preliminary evidence of clinically successful immunotherapy in cancer points to the need for a prolonged exposure of tumor cells to the effects of the immunotherapy (such as in the graft versus tumor effect following allogeneic hematopoietic transplantation). Our protocol already attempts to address this by using cells expanded in presence of rapamycin which was

demonstrated in pre-clinical models to considerably prolong T cell *in vivo* survival (see section **1.2.5**). The use of repeated infusions of autologous T1.rapa cells following a targeted immune depletion is the logical progression towards our efforts to improve the therapeutic efficacy of the Th1.rapa cell population.

It has now been well documented in several clinical settings in addition to extensive pre-clinical data that targeted lymphocyte depletion prior to adoptive cell transfer increases the *in vivo* expansion and engraftment of the adoptively transferred cells. These effects are believed to be mediated by the up-regulation of homeostatic cytokines triggered by the lymphopenia. In addition, targeted lympho depletion over a period of days also depletes the host of regulatory T cells which can inhibit anti-tumor T cells. Several regimens of immune depletion are presently exploited in multiple clinical protocols from ETIB, Surgery Branch, LMB and POB.

So far, the Th1.rapa cell infusions have been extremely well tolerated at all tested dose levels. No toxicity attributable to the Th1.rapa infusions has occurred in any of the subjects from cohort 1 through cohort 5 which infuses $5x10^6$ autologous T1.rapa cells per kg at day 42 post-transplant (i.e. remote from host conditioning). Four of the six subjects enrolled on cohort 5 have received Th1.rapa cell infusion with a follow up ranging from 6 weeks to 8 months with no toxicity. The last two subjects are scheduled to receive their Th1.rapa infusion in the near future as planned in the original study design.

Given this information, we propose to use this dose of $5x10^6$ autologous T1.rapa cells administered on three occasions, three months apart, and preceded by a brief lympho-depleting regimen consisting of Pentostatin and Cyclophosphamide.

1.2.7.2 The dose adjusted Pentostatin-cyclophosphamide (PC) regimen

Pentostatin is an FDA-approved medication that is primarily used for the treatment of hairy cell leukemia[87], chronic lymphocytic leukemia[88], and T cell lymphoma[89]. Pentostatin, which has a unique mechanism of action that involves inhibition of the enzyme adenosine deaminase (ADA) that is deficient in a large proportion of patients with Severe Combined Immune Deficiency (SCID), also mediates significant immune suppression and immune depletion[90].

An initial study by Pavletic et al demonstrated the lymphodepleting effects and safety of pentostatin (a total of 12 mg/m^2 over three days) when administered in sequence with nonmyeloablative doses of TBI (200 cGy) in the context of allogeneic HCT[91] . In another study, pentostatin (a total of 8 mg/m^2 over two days) was safely administered in sequence with 600 cGy of TBI prior to allogeneic HCT[92] . As such, pentostatin can represent an effective component to host conditioning regimens for the purpose of preventing graft rejection. However, to date, the pentostatin has been used less frequently than other immune modulating conditioning, which has primarily involved the purine analogue fludarabine.

In murine transplantation models, we set out to compare the immune depleting and immune suppressive effects of pentostatin relative to fludarabine[93]. In these studies we found that pentostatin was advantageous relative to fludarabine because, at a given level of host myeloid cell depletion, there was a significantly greater magnitude of host B and T cell depletion. In addition, host T cells that remained after pentostatin-based conditioning were significantly more immune supppresed relative to host T cells remaining after fludarabine-based conditioning. Importantly, in these studies, we found that optimal host immune modulation was achieved when

intermittently dosed pentostatin was administered in combination with daily dosing of cyclophosphamide. These results indicated that, pentostatin, which can have a biological half-life (in terms of inhibiting the ADA enzyme) of up to one week, is best administered in combination with low-dose therapy with DNA alkylators to achieve maximal immune depletion while minimizing myeloid cell depletion. . Finally, in these experiments, we found that host recipients of pentostatin/cyclophosphamide (PC) conditioning were significantly less likely to reject a fully MHC-disparate hematopoietic cell transplant relative to recipients of fludarabine/cyclophosphamide conditioning.

Based on these murine results, we initiated a pilot clinical trial of PC conditioning in the setting of HLA-matched sibling allogeneic HCT at the NIH Clinical Center (NCI Protocol 08-C-0088; "*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*"). In an attempt to maximize immune depletion with relative sparing of myeloid cells, we designed a 21-day pre-transplant host conditioning regimen consisting of a total of 12 mg/m^2 of pentostatin administered in equal doses on days 1, 8, and 15 of the 21-day regimen; and, in this context, patients received a relatively modest flat dose of cyclophosphamide of 200 mg per day, each day of the 21-day regimen. However, to ensure sparing of myeloid cells, we also designed a dose adjustment scheme whereby daily doses of cyclophosphamide would be held in the event that an ANC value were to be reduced. Using this regimen, in 12 consecutive patients, we achieved the protocol-defined target level of immune depletion (end of regimen ALC value of < 200) without a single case of significant neutropenia (no case of grade 3 toxicity). And, each of the 12 patients achieved prompt alloengraftment without any case of graft rejection. These results indicate that the PC regimen can be safely administered and is effective when used alone in acchieving an immune depletion sufficient for the prevention of graft rejection in the allogeneic setting.

In other murine models, we next evaluated the ability of the PC conditioning to abrogate host immune responses against foreign protein, namely the anti-cancer immunotoxin SS1P[94]. Immunogenicity of foreign protein therapies is a significant clinical obstacle, as many patients are limited to only a single course of protein therapy due to neutralizing antibody formation; such antibody formation is induced by B cells, with significant cooperativity from host T cells. As such, we hypothesized that the PC regimen might be particularly effective for preventing host immunogenicity to SS1P. Indeed, in these murine studies, we found that the PC regimen safely modulated host immunity and allowed for up to six cycles of immunotoxin therapy without induction of neutralizing antibody formation. Based on these results, we initiated a clinical translation of SS1P therapy preceded by host immune modulation with the PC regimen (NCI Protocol 11-C-0160; *"A Pilot Study of Pentostatin Plus Cyclophosphamide Immune Depletion to Decrease Immunogenicity of SS1P in Patients with Mesothelioma"*). To improve the feasibility of the regimen, this protocol is evaluating a somewhat truncated version of the PC regimen, with pentostatin currently being administered at a dose of 4 mg/m^2 (on days 1, 5, 9, and 13) with daily, dose-adjusted cyclophosphamide being administered on days 1 through 14 of the regimen. Initial results from the first 10 patients treated on this protocol indicate that the PC regimen indeed is safe and is effective in preventing or delaying host immune responses against the foreign protein therapy. In combination, the results of our studies in the allogeneic and autologous setting indicate that the dose-adjusted PC regimen can be safely administered and yield effective immune modulation.

1.2.7.3 The two PC Regimens to be used on this study (7-day and 14-day regimens)

The stated goal of the PC regimen is to receive the Th1.rapa cell infusion with substantial immune depletion (ALC value < 100) and without grade 3 neutrophil toxicity (ANC value < 1000).

- A "short" and a "long" regimen will be sequentially evaluated in each individual on cohort 5B (intra-patient escalation of the immune-depleting regimen intensity).
    - "Short regimen" ("7 day regimen"):

    Pentostatin 4 mg/m^2 iv on days 1 and 4
    Cyclophosphamide 200 mg po daily on days 1 through 5
    (days 6 and 7 are rest days; no therapy given prior to T cell infusion on day 8)

    - "Long regimen"

    Pentostatin 4 mg/m^2 iv on days 1, 4, 8 and 11
    Cyclophosphamide 200 mg po daily on days 1 to 5 and days 8 to 12.
    (days 6/7 and 13/14 are rest days; T cell infusion on day 15)

The cyclophosphamide dose will be either reduced or omitted depending on whether the lymphocyte depletion target (reduction in ALC value) has been achieved and whether there is any myeloid cell toxicity (reduction in ANC value).

1.2.8 Protocol Progress and Amendment K Rationale

With Amendment K (submitted February 2015), the protocol will evaluate two aspects of Th1/Tc1.Rapa cell therapy of multiple myeloma: cohort A, which will further define the safety and potential efficacy of Th1/Tc1.Rapa cells for prevention of relapse after AHCT; and cohort B, which will evaluate Th1/Tc1.Rapa cell therapy in patients with multiply relapsed MM. To date, n=20 patients have been treated with autologous Th1/Tc1.Rapa cells in the post-AHCT setting; one patient was not evaluable for progression-free survival because he came off-study due to logistical reasons prior to progression. There have been no adverse events attributable to the cell therapy, including five patients who received the highest dose level tested (Cohort 6: 15 x 10^6 cells/kg) and four patients on Cohort 5B who received two T cell infusions at a dose of 5 x 10^6 cells/kg (the second of which was administered after a 7-day course of pentostatin plus cyclophosphamide chemotherapy). The majority of the initial patients on the protocol received single infusions that were comprised of relatively low doses of Th1/Tc1.Rapa cells that may therefore have been sub-optimal; only 2 out of 10 of these patients remain progression-free after AHCT and subsequent Th1/Tc1.Rapa cell therapy. However, in patients treated on Cohort 6 or Cohort 5B, 6 out of 9 patients remain progression-free. Although these results are relatively preliminary, the progression-free survival in these protocol patients that received higher Th1/Tc1.Rapa cell doses or intermediate doses of Th1/Tc1.Rapa cells in combination with a 7-day immune-depleting PC regimen are potentially promising in terms of preventing relapse post-AHCT. Figure 3 below shows the progression-free survival curve of these nine protocol patients; results are informally compared to recent published data from MD Anderson Cancer Center. Although these results are compelling in terms of an anti-tumor effect of the Th1/Tc1.Rapa cells, further investigations will be required to confirm and extend these results. Towards this end, with Amendment K, the protocol will now evaluate Th1/Tc1.Rapa cell therapy on a new Cohort A

(continued evaluation of prevention of MM relapse) and a new Cohort B (new evaluation of treatment of MM relapse). With Cohort A, we will evaluate a uniform treatment regimen in an expanded cohort to provide more definitive support for a role of Th1/Tc1.Rapa cell therapy in the prevention of MM relapse after AHCT; specifically, patients will receive two infusions of Th1/Tc1.Rapa cell therapy (at the intermediate dose of 5 x 10^6 cells/kg) with each dose preceded by the 7-day PC regimen. Because of the safety observed to date with Th1/Tc1.Rapa cell therapy and the certainty of MM relapse even in patients with normal risk MM, the amended protocol will evaluate post-AHCT T cell therapy in all risk groups of MM. And, with Cohort B, we will evaluate the same intermediate T cell dose of 5 x 10^6 cells/kg combined with the PC regimen for therapy of advanced-stage, multiply relapsed patients with measurable disease; this effort seeks to provide further and more direct evidence for an anti-myeloma effect of the Th1/Tc1.Rapa cell therapy.

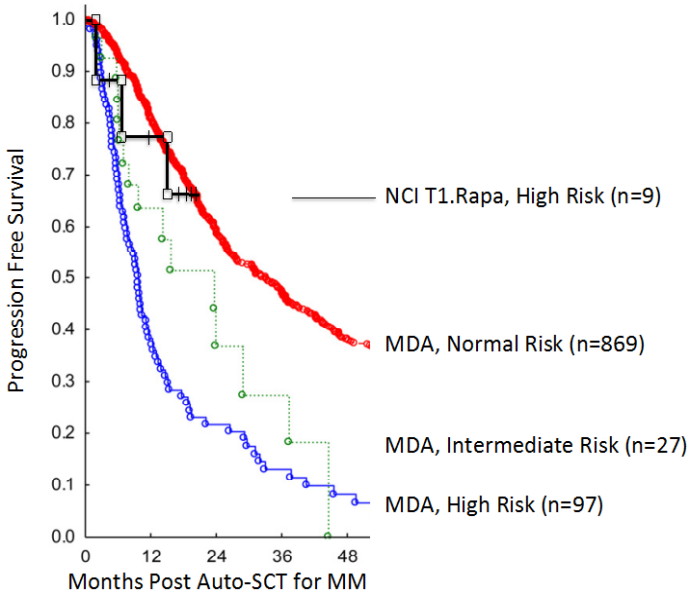


Figure 3. Patients with multiple myeloma have an ongoing risk of relapse after AHCT, the rate of which depends on whether a patient is considered normal-, intermediate-, or high-risk (progression free survival [PFS] data shown are from MD Anderson [MDA], recently published by Sasaki et al; BBMT 2013 Aug;19(8):1227-32). PFS data from patients treated on Cohort 5B or Cohort 6 of protocol 11-C-0016 are shown (NCI T1.Rapa, High Risk; n=9 patients).

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Multiple Myeloma criteria

2.1.1.1.1 Criteria for Cohort A (recently diagnosed subjects; to receive AHCT)

- Must have presence of clonal plasma cells in the bone marrow greater or equal to 10% or biopsy proven plasmacytoma
- Must have either (a) presence of an M-component (IgG or IgA) in serum greater or equal to 1g/dl or in urine greater or equal to 200 mg/24 h; or (b) presence of an abnormal serum free light chain (FLC) ratio on the serum FLC assay.

2.1.1.1.2 Criteria for Cohort B (multiply relapsed multiple myeloma)

- Must have measurable MM, as defined by: serum M-protein ≥ 1 g/dL, urine M-protein ≥ 200 mg/24 hours, involved serum free light chain (FLC) level ≥ 10 mg/dL, biopsy proven plasmacytoma, or more than 30% bone marrow plasma cells.
- Must have received at least 2 different treatment regimens for MM.

2.1.1.2 Other eligibility criteria (applies to both Cohort A and Cohort B, unless specified)

- Age ≥18 years and less than or equal to 75 years. In subjects between 65 and 75 years of age, physiologic age and co-morbidity will be thoroughly evaluated before enrolling. Specifically, any history of cardio-vascular pathology or symptoms, not clearly fitting the exclusion criteria of section **2.1.2** will prompt an evaluation by a Clinical Center Cardiologist and eligibility will be considered on a case-by-case basis.
- For Cohort A only, high-dose chemotherapy and AHCT must be planned; with amendment K, post-transplant maintenance therapy will not be permitted.
- Karnofsky performance status of 70% or greater. Lower KPS down to 50% may be acceptable if the restriction of activity is solely due to intractable pain from myeloma lesions.
- Ejection fraction (EF) by MUGA or 2-D echocardiogram within institution normal limits. In case of low EF, the subject may remain eligible after a stress echocardiogram is performed if the EF is more than 35% and if the increase in EF with stress is estimated at 10% or more.
- Serum creatinine less than or equal to 2.5 mg/dl,
- AST and ALT less than or equal to 3 x upper limit of normal,
- Bilirubin less than or equal to1.5 (except if due to Gilbert's disease).
- Corrected DLCO greater than or equal to 50% on Pulmonary Function Tests
- No history of abnormal bleeding tendency or predisposition to repeated infections.
- Patients must be able to give informed consent

2.1.2 Exclusion criteria

- Prior allogeneic stem cell transplantation
- Hypertension not adequately controlled by 3 or less medications.
- History of cerebro-vascular accident within 6 months of enrollment.
- History of documented pulmonary embolus within 6 months of enrollment
- Clinically significant cardiac pathology: myocardial infarction within 6 months prior to enrollment, Class III or IV heart failure according to NYHY, uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities.
- 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.
- HIV seropositive
- Patients known or found to be pregnant or who is unwilling to stop breast-feeding.
- Patients of childbearing age who are unwilling to practice contraception or other means of avoiding pregnancy.

Patients may be excluded at the discretion of the PI or if it is deemed that allowing participation would represent an unacceptable medical or psychiatric risk.

2.2 RESEARCH ELIGIBILITY EVALUATION

Before receiving any therapy on protocol, patients must have their eligibility confirmed and must have signed an informed consent (section**6.7**). The following evaluations will serve both purposes of eligibility determination and pre-treatment evaluation. Only the evaluations necessary for eligibility determination defined by the inclusion and exclusion criteria need to be performed before the subjects sign informed consent. The rest of the evaluations may be obtained after consent is signed.

2.2.1 History and Physical examination

All patients must have a complete history, review of systems and physical examination within 2 weeks of study entry date. Appropriate consultations (in particular with the Infectious Diseases, Radiation Oncology and Dental Services) may also be obtained as indicated for best clinical care.

2.2.2 Radiological evaluation

All patients must have had initial radiological evaluation of their disease at the time of their diagnosis (for cohort A patients) or within 8 weeks of study entry (for cohort B relapsed patients). Radiologic studies vary depending on patient presentation and institutional practices, with some combination of the below listed tests typically being performed. Tests that are informative in terms of disease monitoring will be repeated at the time of formal disease re-staging.

- Skeletal survey
- CXR
- Head CT scan or MRI
- CT scan of chest/abdomen/pelvis
- PET/CT scan, Body MRI
- Bone density by Dexa scan

2.2.3 Laboratory evaluation

The following laboratory studies must be performed within 4 weeks prior to study entry date (unless otherwise indicated):

- Bone marrow biopsy and aspirate with immunostains, amyloid stain, and BM cytogenetics must be performed in newly diagnosed subjects.
- If BM studies performed at diagnosis or at any point during the course of the disease will be used for eligibility determination, they must be available for review at the NCI in subjects who have already initiated induction therapy.
- In case the diagnosis of myeloma was initially not made on the basis of bone marrow findings (e.g. in cases of plasmacytomas), a repeat bone marrow biopsy is not mandatory (PI's discretion).
- CBC with differential and platelet count
- Electrolytes, Bun, Creatinine, creatinine clearance
- Liver profile, mineral panel
- PT/PTT
- Serum Quantitative Immunoglobulins
- Serum M protein determination; Immuno-electrophoresis **and** immuno-fixation
- β2-microglobulin, serum free light chain assay
- CRP
- 24 hour urine for protein, creatinine, protein electrophoresis immune fixation
- Peripheral blood TBNK flow cytometry
- Viral serology: HSV 1 & 2, VZV, CMV, EBV, hepatitis (A, B and C), T. Cruzi, HIVNAT testing
- Toxoplasma titers
- RPR
- PPD placed and read (per ID recommendation only)
- ABO typing
- Urinalysis
- β-HCG or urine pregnancy test in pre or peri- menopausal women only
- Pulmonary function tests with DLCO determination (within 8 weeks)
- MUGA or 2D-echocardiogram (within 8 weeks)
- ECG
- Venous assessment (any time prior to collection)
- Vitamin D

2.3 PATIENT REGISTRATION

2.3.1 NCI patients

Authorized staff must register an eligible candidate 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 sent via encrypted email to: NCI Central Registration Officencicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, 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. Please note, all registrars must acquire encrypted e-mail from NIH Help Desk, because the verification of registration includes patient information. For questions regarding registration, authorized individuals should call the Central Registration Office between the hours of 8:30AM and 5:00PM, Monday – Friday: phone (301) 402-1732. A recorder is available during non-working hours.

Patients who initially participated in cohort A aspect of the study may benefit from cohort B therapy if they subsequently have relapsed/refractory disease. As such, a patient may be registered on the cohort A, taken off study, and then later registered on the protocol for cohort B therapy. These patients will be considerd in the total sample size for cohort B as well as on cohort A.

2.3.2 For Participating Site Registration

Regardless of which institution the subject enroll at, all participating subjects will be registered at the NIH Clinical Center in order to receive an NIH ID number (for sample handling and tracking). This does not requires the subject's presence at NIH.

All patients must be registered through the NCI Central Registration Office (CRO). The CRO is open from 8:30am to 5:30pm EST Monday through Friday, excluding federal holidays. A protocol registration form will be supplied by the study coordinator and updates will be provided as needed. Subject eligibility and demographic information is required for registration. To initially register a subject, after the participant has signed consent, complete the top portion of the form and send to study coordinator, Ellen Carroll, R.N., ecarroll@mail.nih.gov; phone number: 301 496-5853.

Once eligibility is confirmed after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the patient is being registered for treatment and send to Ellen Carroll, R.N. In addition, source documents supporting the eligibility criteria must be sent to the study coordinator. The CRO will notify you either by e-mail or fax that the protocol registration form has been received. The CRO will assign a unique patient/subject ID number for each subject that will be used to enter data into the C3D data base. Questions about eligibility should be directed to the study coordinator, Ellen Carroll, R.N., ecarroll@mail.nih.gov; phone number: 301 496-5853. Technical questions about the form should be directed to the Central Registration Office (301-402-1732).

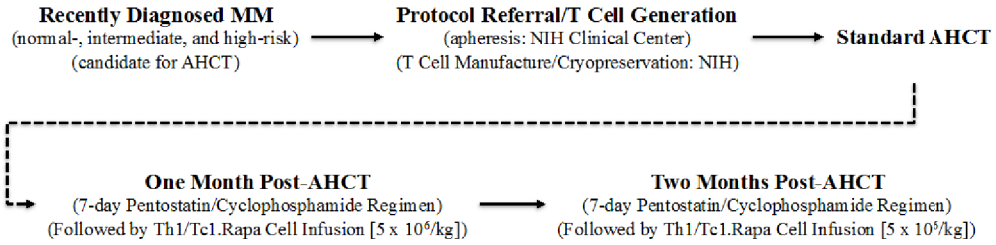
Subjects that do not meet screening criteria should be removed from the study following the procedure in section **3.5.3**.

3 STUDY IMPLEMENTATION

3.1 OVERVIEW OF COHORT A AND COHORT B THERAPY (AMENDMENT K)

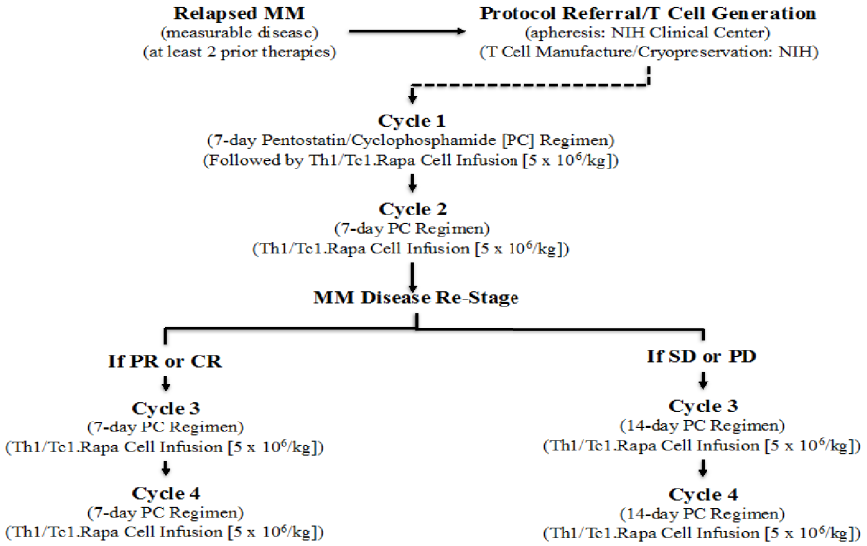
3.1.1 Current Schema for Cohort A Therapy (Th1/Tc1.Rapa Prevention of Relapse)

Cohort A Schema, Th1/Tc1.Rapa Cell Therapy for MM Relapse Prevention after AHCT



3.1.2 Current Schema for Cohort B Therapy (Th1/Tc1.Rapa Treatment of Relapse)

Cohort B Schema, Th1/Tc1.Rapa Cell Therapy for Relapsed MM



3.2 TREATMENT IMPLEMENTATION

3.2.1 Specifics of Implementation Relating to Cohort A (Amendment K)

- Lymphapheresis will be performed for all subjects at the NIH Clinical Center (DTM). This procedure can be performed either prior to or during induction chemotherapy but must be performed prior to the chemotherapy/stem cell mobilization procedure. Apheresis will consist of a 10 to 15 liter collection on CS-3000 or equivalent machine.

The experimental T cells will be generated centrally in the NIH DTM under the supervision of Dr. Fowler.

- After apheresis, subjects will return to the referring team for standard care of myeloma, including: induction therapy; chemotherapy/stem cell mobilization; single or tandem AHCT (high-dose chemotherapy not protocol-driven and will be determined by the referring team). Post-AHCT maintenance therapy will not be allowed.
- The protocol-defined PC regimen will be administered by the NIH protocol team at the Clinical Center. And, the protocol-defined Th1/Tc1.Rapa cell infusion will be administered at the NIH Clinical Center. These therapies may be administered on an outpatient basis.
- The PC Regimen will be administered over 7-days, as further detailed below in Section **3.4** The Th1/Tc1.Rapa cell infusion will occur on the day following the PC regimen (up to a 3-day delay in T cell infusion will be allowed if required for logistic reasons or any toxicity concern); the Th1/Tc1.Rapa cell dose will be 5 x 10^6 cells/kg (however, in the event that sub-optimal cell yield occurs during manufacturing, doses of 1 x 10^6 cells/kg will be allowed).
- The PC regimen followed by Th1/Tc1.Rapa cell infusion will be performed twice: once at one month post-AHCT and then again at two months post-AHCT. The first cycle will not be initiated until at least 30 days post-AHCT; up to a four week delay in cycle one initiation is permitted to account for various factors (cell manufacturing, travel logistics, recovery from toxicities related to AHCT). The second cycle of therapy will not be initiated until at least four weeks after initiation of the first cycle; however, up to a four week further delay in the initiation of cycle two will be permitted (can be up to 8 weeks between cycle one and cycle two).
- With respect to treatment administration on cohort A: all elibility criteria detailed in section **2.1** must be met prior to cycle one initiation; prior to administration of the cryopreserved T cells, premedication with diphenhydramine 25 to 50 mg IV or p.o. and acetaminophen 650 mg p.o. will be given; the cryopreserved T cells will be thawed and immediately and rapidly administered intravenously by gravity following the appropriate institutional SOP for blood product administration; no steroids will be allowed in the management of DMSO-related toxicities (chills, muscle aches) that may occur immediately after cellular infusion unless the toxicity is deemed life threatening and vital signs (including pulse oxymetry) will be taken every 20 minutes for the first hour and then every hour for the next 2 hours. In the event that vital signs are altered, more frequent monitoring will be performed.
- Within 14 days prior to initiation of cycle 1, patients must have: Karnofsky performance status of 70% or greater. Ejection fraction (EF) by MUGA or 2-D echocardiogram within institution normal limits (in case of low EF, the subject may remain eligible after a stress echocardiogram is performed if the EF is more than 35 % and if the increase in EF with stress is estimated at 10% or more); Serum creatinine less or equal to 2.5 mg/dl; AST and ALT less or equal to 3 x upper limit of normal; Bilirubin less than or equal to1.5 (except if due to Gilbert's disease); Still satisfy the exclusion criteria of section **2.1.2** above (HIV will not be repeated); must not have initiated maintenance therapy following AHCT; and

have Absolute Neutrophil Count of 1000 or greater and Platelet count of 75K or greater (these latter two lab values should be obtained within 4 days of starting cycle 1).

3.2.2 Specifics of Implementation Relating to Cohort B

- Lymphapheresis will be performed at the NIH Clinical Center (DTM) for all subjects. Apheresis will consist of a 10 to 15 liter collection on CS-3000 or equivalent machine. The experimental T cells will be generated in the NIH DTM (supervised by Dr. Fowler).
- Prior to initiation of therapy on cohort B (within one week of therapy), patients will have repeat CBC with differential in addition to repeat blood chemistry tests.

- With respect to treatment administration on cohort B: all elibility criteria detailed in section **2.1** must be met prior to cycle one initiation; prior to administration of the cryopreserved T cells, premedication with diphenhydramine 25 to 50 mg IV or p.o. and acetaminophen 650 mg p.o. will be given; the cryopreserved T cells will be thawed and immediately and rapidly administered intravenously by gravity following the appropriate institutional SOP for blood product administration; no steroids will be allowed in the management of DMSO-related toxicities (chills, muscle aches) that may occur immediately after cellular infusion unless the toxicity is deemed life threatening and vital signs (including pulse oximetry) will be taken every 20 minutes for the first hour and then every hour for the next 2 hours. In the event that vital signs are altered, more frequent monitoring will be performed.
- The protocol-defined PC regimen will be administered at the Clinical Center by the NIH protocol team. And, the protocol-defined Th1/Tc1.Rapa cell infusion will be administered at the NIH Clinical Center. These therapies may be administered on an outpatient basis.
- The PC Regimen for Cohort B will be administered over either 7-days or 14-days, as further detailed below in Section**3.4**. The Th1/Tc1.Rapa cell infusion will occur on the day following the PC regimen (up to a 3-day delay in T cell infusion will be allowed if required for logistic reasons or any toxicity concern); the Th1/Tc1.Rapa cell dose will be 5×10^6 cells/kg (however, in the event that sub-optimal cell yield occurs during manufacturing, doses of 1×10^6 cells/kg will be allowed).
- The PC regimen followed by Th1/Tc1.Rapa cell infusion will be performed on four occasions (four cycles). Cycles one and two will be performed using the 7-day PC regimen. After cycle 2, the patient will be re-staged for status of the myeloma. If a PR or CR has been obtained, cycles 3 and 4 will be performed using the same 7-day PC regimen; however, if restaging demonstrates SD or PD, then cycles 3 and 4 will consist of the 14-day PC regimen.
- Cycles 1 and 2 will be of 28 days duration (7 days of PC chemotherapy + 21 days to observe after T cell infusion); in contrast, cycles 3 and 4 will be of 35 days duration (14 days of PC chemotherapy + 21 days to observe after T cell infusion). Each cycle may be delayed up to two weeks to allow for resolution of any toxicities).

3.2.3 Specifics of the PC Regimen

- For Cohort A, the 7-day PC regimen will be administered prior to each of the two separate infusions of Th1/Tc1.Rapa cells at one and two months post-AHCT. For Cohort B, the 7-day PC regimen will be administered during cycles 1 and 2; for cycles 3 and 4, the PC regimen will remain as a 7-day regimen (if in PR or CR) or be changed to a 14-day regimen (if in SD or PD).
- The 7-day regimen will consist of pentostatin (4 mg/m^2 i.v. on days 1 and 4) in combination with cyclophosphamide (200 mg p.o. daily on days 1 through 5). Typically, for convenience and standardization, pentostatin will be administered on Monday and Thursday, with cyclophosphamide administered Monday through Friday. No chemotherapy is administered on day 6 and 7 of the 7-day regimen (typically, Saturday and Sunday). The T cell infusion occurs the following day (day 8).
- The 14-day regimen will consist of pentostatin (4 mg/m2 i.v. on days 1, 4, 8, and 11) in combination with cyclophosphamide (200 mg p.o. daily on days 1 through 5 and then again on days 8 through 12). Again, no chemotherapy is administered on days 6/7 and 13/14 of the regimen; T cell infusion occurs the following day (day 15).
- Specifics relating to pentostatin administration: (a) Preparation: pentostatin will be reconstituted by CC Pharmacy Department to a concentration of 2 mg/ml as per vial instructions. The appropriate patient specific dose will then be added to 0.9% sodium chloride to make up a total volume of 50 mL; (b) Dose and Administration: pentostatin dosing will be adjusted for renal dysfunction (see below); each dose of pentostatin will be administered intravenously over 30-60 minutes; (c) Premedication and Anti-emetic therapy: prior to infusion, infuse 1 liter of 0.9% sodium chloride over 30-60 minutes (may substitute other intravenous fluids such as 1 liter of 5% Dextrose 0.45% sodium chloride). Pentostatin can be emetogenic. Anti-emetic regimen guidelines are as follows (variations are allowed at the discretion of the PI): (1) Dexamethasone 12 mg by IV infusion 60 minutes prior to each dose of pentostatin (Days 1, 4, 8, and 11); (2) In addition, oral dexamethasone may be administered on other days at a dose of 4 mg per day; (3) Ondansetron will be administered at a dose of 8 mg by IV infusion 60 minutes prior to each dose of pentostatin (Days 1, 4, 8, and 11); (4) For the remainder of treatment, ondansetron may be administered at an oral dose of 8 mg (tablets) every 12 hours on Days 1 through 14; and (5) Aprepitant may be added as needed to the anti-emetic regimen in patients with uncontrolled nausea and vomiting.
- Specifics relating to pentostatin dose reductions: serum creatinine levels will be obtained prior to each scheduled dose of pentostatin and CrCl calculated. The CrCl will be obtained either by 24 hour urine or calculated by the Cockcroft-Gault formula. If a subject experiences an increase in creatinine level during the pentostatin and cyclophosphamide therapy, subsequent dosing will be modified as follows:
    - CrCl $\geq$ 60 mL/min/1.73 m^2: 4 mg/m^2 of pentostatin.
    - CrCl < 60 mL but $\geq$ 30 /min/1.73 m^2: 2 mg/m^2 of pentostatin.
    - CrCl < 30 mL: hold pentostatin

- 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.
- Specific aspects of cyclophosphamide administration: hydration. 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.
- Cyclophosphamide: Dose and Schedule. Oral cyclophosphamide will be given from day 1 through day 5 in the short regimen and from day 1 to day 5 and day 8 to day 12 in the long regimen. The dose of cyclophosphamide will be 200 mg each day (PO), with some provision for dose reduction as detailed below. IV infusion of this same dose may be allowed if a patient is unable to tolerate oral therapy. For IV infusion, cyclophosphamide will be reconstituted by CC Pharmacy Department to a concentration of 20 mg/ml as per vial instructions. A 200 mg dose will then be diluted in 100 ml of D5W or 0.9% sodium chloride and infused intravenously over 30 minutes.
- In some cases (for example, patient with grade 3 fatigue from myeloma or from chemotherapy), it may be appropriate to be more conservative with the immune-depleting therapy. As such, in some cases as determined by the PI, the following chemotherapy reductions will be allowed: reduction in pentostatin from 4 mg/m2/dose to 2 mg/m2/dose and reduction in cyclophosphamide from 200 mg per day to 100 mg per day.
- Cyclophosphamide: in addition, there will be monitoring and potential dose reduction based on ANC. CBC will be obtained at a minimum on days 4, 8, and 11 of the immune depleting regimen; cyclophosphamide dose will be adjusted as follows according to the values of Absolute Neutrophil Count (ANC).

Cyclophosphamide Dose Adjustment Based on ALC and ANC Values						
Day of Cycle[1]		ALC Value at time of Evaluation		ANC Value at time of Evaluation[2]		Cyclophosphamide Dose[3]
1		Any		> 1000		200
				500-999		100
4		Any		> 1000		200
				500-999		100
				< 500		0
8		Any		> 1000		200

				500-999		100
				< 500		0
11		Any		> 1000		200
				500-999		100
				< 500		0

[1] Pentostatin will not be dose-adjusted based on ALC/ANC values.

[2] For ANC values < 500, in addition to decrease in cyclophosphamide dosing, patients will receive G-CSF therapy until next ANC measurement.

[3] Cyclophosphamide dose indicated will be continued daily until the next ANC measurements (performed on days 5, 9, and 13).

3.3 PROTOCOL EVALUATION

- **Appendix 2**: Protocol For Autologous HPCS Collection By Apheresis Of Filgrastim-Stimulated Patients With Multiple Myeloma summarizes recommended evaluations and their schedule.
- At a minimum, clinical evaluation will include: (1) at least weekly (+/- 3 days) for the first two weeks after any Th1/Tc1.Rapa cell infusion; (2) then, every 2 to 4 weeks for 3 months; then, for the first year, every 1 to 3 months. After the first year, T cell infusion recipients should be seen approximately every three to six months.
- For Amendment K, cohort A, it will be important to have complete restaging performed at six months (+/- one month) after autologous transplantation to be able to determine PFS.
- Clinical evaluation, tests (not MM related): (1) interim history and physical examination; (2) CBC with differential and platelet count; (3) Electrolytes, Bun, Creatinine; (4) Liver profile, mineral panel, PT/PTT.
- Clinical evaluation, tests (MM related): (1) patients with "measurable disease" restricted to the SPEP or UPEP need to be followed only by serum immunofixation electrophoresis urine immunofixation electrophoresis, respectively; (2) unless specifically indicated clinically, skeletal survey is recommended only on a yearly basis for routine clinical evaluation; and (3) patients may be screened yearly for light chain escape with UPEP or serum FLC assay.

- Clinical evaluation of MM, schedule: (1) within 28 days preceding the first Th1/Tc1.rapa cell infusion; (2) for patients on cohort A, only serum immunofixation electrophoresisand/or urine immunofixation electrophoresis need to be measured between the first and second T cell infusions; in contrast, for patients on cohort B, who are to receive 4 cycles of therapy, all informative re-staging tests (potentially including bone marrow exam) will be repeated after cycle 2; (3) for patients on cohorts A and B, all informative re-staging tests including bone marrow exam will be repeated 4 to 6 weeks after the final T cell infusion; any PR or CR observed on cohort B should be confirmed after approximately one month of further observation; (4) thereafter, re-staging will be performed using only clinically indicated tests and will occur at a frequency of approximately every three months for the first year post-therapy, and then, as clinically indicated; and (5) FLC response criteria are only applicable to patients without measurable disease in the serum or urine and/or to fulfill the requirements of the category of sCR.
- Immune Characterization Studies: (1) Evaluation of immune status and lymphocyte recovery post Th1.rapa cell infusion. This will consist of flow cytometry experiments; the following time points represent the maximal number of collections. A reduced frequency of sampling may be appropriate depending on the frequency of patient follow-up and laboratory staffing; the decision whether to collect the sample will be made at our weekly protocol meeting. (2) Blood samples will consist of one 3 ml lavender tube to NIH Clinical Pathology or other clinical laboratory (TBNK), two red/green top CPT tubes to Dr. Fran Hakim's, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C316 contact phone: 301-594 5339. (3) Time points will be: immediately prior to the Th1/Tc1.rapa cell infusion, and at approximately 6 and 12 weeks after T cell infusion (sampling to coincide with clinic visit).
- Evaluation of type I vs. type II cytokine effects following all Th1.rapa cell infusion: (1) The following time points represent the maximal number of collections. A reduced frequency of sampling may be appropriate depending on the frequency of patient follow-up and laboratory staffing; the decision whether to collect the sample will be made at our weekly protocol meeting; (2) Blood will be drawn for cytokine evaluation into two red/green top CPT tubes and one 8 ml serum separator tube (3 SST at the baseline time point); (3) samples will be delivered to Dr. Fran Hakim's Pre-Clinical Core Lab; Attention Jeremy Rose, Bldg 10, room 12C216: contact phone: 301-594 5339; (4) time points: at the time of lymphopheresis for Th1.rapa cell generation; on the day of, but before, any Th1.rapa cell infusion; then at approximately 2 weeks, 1, 2 and 3 months post the final T cell therapy (sampling to coincide with clinic appointments).
- Evaluation of immune reconstitution following Th1.rapa cell infusion: (1) the following time points represent the maximal number of collections. A reduced frequency of sampling may be appropriate depending on the frequency of patient follow-up and laboratory staffing; the decision whether to collect the sample will be made at our weekly protocol meeting; (2) peripheral blood (30 cc; 1 green top heparinized tube, two CPT tubes) will be sent to Fran Hakim (Bldg 10, 12th floor Pre-clinical Core) for evaluation of immune reconstitution post-transplant; (3) time points: at baseline; just prior to PC chemotherapy; and then at approximately 2 weeks and 1, 2, and 3 months after the final T cell infusion (sampling to coincide with clinic visits).

- Other studies: (1) Immune studies may also be performed on bone marrow aspirate samples that are performed according to the protocol and as clinically indicated. One aspirate will be sent for clinical tests; one additional aspirate may be sent to Dr. Hakim's lab for research analysis of marrow infiltrating lymphocyte populations. (2) If a tissue biopsy is clinically indicated (e.g. to confirm a relapse), a portion of the biopsy sample may be sent to Dr. Hakim's lab for research analysis of tumor infiltrating lymphocytes.
- In Vitro Studies of Collected Cellular Products: For all subjects, in vitro studies will fall under the general category of "Immune Characterization Studies". They will focus on separation of distinct cell subsets by multi-parameter FACS analysis or separation by magnetic beads with subsequent characterization. Specifically, peripheral blood mononuclear cells (PBMC) will be analyzed by flow cytometry for expression of markers indicative of hematopoietic lineage, immune functional subsets, cytokine production, and activation state. Cell subsets may be analyzed for T cell receptor rearrangement circles (TREC) and T cell receptor repertoire diversity. Cells may be activated in vitro with a number of different stimuli including specific antigens and mitogens which are known to activate distinct pathways of T lymphocyte function. Assays may include T cell proliferation, cytokine production and gene expression. The specific assays to be used for the on-going data analyses are subject to be modified, deleted or replaced as technology and knowledge in the field evolve during the course of the study without constituting a change in research aims. These assays apply also to those samples received from outside institutions. If significant departure from these "Immune Characterization Studies" is contemplated based on accumulated data, then the protocol and consent will be amended accordingly to cover the new line of investigation and its potential risks to subjects.
- Sample management and storage: (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. The sample management is detailed in **Appendix 3**: Experimental Transplantation and Immunology Branch Preclinical Service Policy for Sample Handling.

3.4 CONCURRENT THERAPIES

For subjects treated at the NIH Clinical Center and not concurrently enrolled on a NCI myeloma therapy protocol, Concurrent Therapy consisting of Standard Care for Multiple Myeloma is included in the protocol. **Appendix 1**: Induction, HPCS mobilization and AHCT procedure guidelines for subjects enrolled at NCI describes the State of Art therapy that NCI patients will receive at the NIH Clinical Center. There is at this point no consensus in the field regarding the timing of the initiation of maintenance therapy or the treatment modality. If and when maintenance therapy is initiated on NCI patients, it will reflect the prevailing guideline and the plan will be detailed to the patient to obtain verbal consent and it will be noted in the patient's chart. For non-NCI subjects, myeloma specific Concurrent Therapy will be left at the discretion of the PI of other participating institutions.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.5.1 Criteria for removal from protocol therapy

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

- Completion of protocol therapy
- Progressive disease
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

3.5.2 Off-Study Criteria

- Voluntary withdrawal.
- Inability to generate a sufficient Th1.rapa cell number *ex vivo* to meet the minimum required cell number.
- Unauthorized initiation of maintenance therapy prior to Th1.rapa cell infusion.
- Not meeting the requirements defined in sections **3.2.2** prior to proceeding with Th1.rapa cell infusion
- Documentation, after Th1.rapa cell infusion, of disease progression requiring immediate alternative salvage therapy (i.e. subjects may remain on study for continued evaluation until a new therapy is instituted).
- Any patient may be removed from the study at any point at the discretion of the Principal Investigator if it is deemed that continued participation constitutes an unacceptable medical or psychiatric risk.
- Completion of scheduled follow-up (5 years).
- Lost to follow-up.
- Death.
- PI decision to end this study

3.5.3 Off Protocol Therapy and Off-study procedure

Authorized staff must notify NCI Central Registration Office (CRO) when a patient is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

For participating sites:

All subjects must be registered through the NCI Central Registration Office (CRO). The CRO is open from 8:30am to 5:30pm EST Monday through Friday, excluding federal holidays. An off-study form will be supplied by the study coordinator. Send the completed off-study form to the study coordinator; Ellen Carroll, R.N., ecarroll@mail.nih.gov; phone number: 301 496-5853.

3.6 FOLLOW UP EVALUATION

Annual follow up between 5 and 10 years of follow-up will occur by phone to determine survival status. In addition, we will obtain outside clinical records related to multiple myeloma re-staging to document disease status.

4 SUPPORTIVE CARE

4.1 ANTIBIOTIC PROPHYLAXIS

Patients will not be on systematic antibiotic prophylaxis if neutropenic. However, the use of antibiotics in a prophylactic or pre-emptive manner is of paramount importance in the management of patients during periods of pancytopenia. The specific guidelines for prophylaxis are periodically reviewed by the ETIB Infectious Disease physician and are susceptible to modifications according to prevailing State of the Art and institutional guidelines (NIH Clinical Center guidelines: http://intranet.cc.nih.gov/bmt/)

4.2 ANTI-VIRAL PROPHYLAXIS

All patients will be placed on oral prophylaxis for HSV or VZV with Acyclovir (or its pro-drug Valacyclovir) at the time of initiation of the immunotherapy which will be continued for a minimum of three months after the Th1.rapa cell infusion.

All NCI patients will initiate anti-viral prophylaxis at the time of the high-dose Melphalan which will be continued for a minimum of three months after the Th1.rapa cell infusion.

The specific guidelines for viral prophylaxis are periodically reviewed by the ETIB Infectious Disease physician and are susceptible to modifications according to prevailing State of the Art and NIH Clinical Center guidelines.

4.3 ANTI-FUNGAL PROPHYLAXIS

All patients will be placed on oral anti-fungal prophylaxis (first line: fluconazole) at the time of initiation of the stem cell mobilization therapy and continued for a minimum of three months from the Th1.rapa cell infusion. Drug substitution may be indicated at the discretion of the PI and the ETIB Infectious Disease physician.

4.4 PNEUMOCYSTIS PROPHYLAXIS

- All patients will initiate PJP prophylaxis
- All NCI patients will initiate pneumocystis prophylaxis at the time of the high-dose Melphalan which will be continued for a minimum of three months after the Th1.rapa cell infusion.
- Following Th1.rapa cell infusion, all patients will be placed on oral Cotrimoxazole (Trimethoprim 160 mg / Sulfamethoxazole 800 mg): one tablet po on Mondays, Wednesday and Fridays to be continued for a minimum of three months from the Th1.rapa cell infusion.
- Cotrimoxazole IV should be substituted to the oral treatment at a dose of 160 mg (based on Trimethoprim component) given on Mondays, Wednesday and Fridays at any time the patient is unable to take oral medications.
- For patients unable to tolerate or allergic to Cotrimoxazole or at the discretion of the PI, an alternative prophylactic regimen will be used according to prevailing guidelines at the

time (Dapsone, nebulized Pentamidine, etc). The specific guidelines for Pneumocystis prophylaxis are periodically reviewed by the ETIB Infectious Disease physician and are susceptible to modifications according to the prevailing State of the Art and NIH Clinical Center guidelines

4.5 ANTI-EMETIC REGIMEN

An anti-emetic regimen will be used for the high-dose Melphalan or the DCEP mobilization regimen or the lympho depleting regimens. The prescribed regimen will be consistent with the NIH Clinical Center guidelines for prophylaxis against moderately to highly emetogenic chemotherapy

The Th1.rapa cell infusion is not expected to be emetogenic. However, if initial experience with the first patients disproves this, anti-emetic therapy will be initiated in subsequent subjects according to NIH Clinical Center prevailing guidelines. However, steroids including dexamethasone must not be used at that time.

4.6 BISPHOSPHONATES

Bisphosphonates will be used in all patients with documented bone disease including osteopenia with dose adjustment for renal function according to manufacturer's recommendations.

4.7 DEEP VENOUS THROMBOSIS PROPHYLAXIS

DVT prophylaxis may be used during the induction therapy according to recommendations of the International Myeloma Working Group.

- Low Molecular Weight Heparin: Enoxiparin 40mg subcutaneously daily.
- Equivalent prophylactic regimens are acceptable.

Erythropoiesis Stimulating Agents (ESA) will not be used during the induction therapy due to the potential increased incidence in thrombo-embolic events when associated with Thalidomide, Lenalidomide or Dexamethasone.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

- All patients' data must be recorded in the progress notes and flow sheets of the patient chart maintained by the Medical Records Department of participating institutions, (or available electronically on the CRIS system of the NIH Clinical Center). Duplicate of key data may be kept in a research folder maintained by the Experimental Transplantation & Immunology Branch or the research office of participating institutions.
- Patient's demographics, disease characteristics as well as treatment and complication history during the Concurrent Therapy will be collected for research purposes.
- However, no specific toxicity data or adverse event related to the therapy prior to the first PC regimen and Th1.rapa cell infusion will be collected for study purposes.
- Data on Adverse Events requiring reporting to participating institution's IRB or FDA (see sections **7.3.2** and **7.5**) following Th1.rapa cell infusion will be collected.
- Data on baseline disease assessment and disease re-evaluation following Th1.rapa cell infusion will be collected.
- Data pertaining to a subject's death while on protocol will be collected.
- Data at both NCI and participating institutions will be prospectively collected and entered in real time into the Cancer Central Clinical Data System database (NCI C3D; information at http://ccrtrials.nci.nih.gov). It is expected that required 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.
- All patient records will be kept confidential according to individual institution policies and procedures concerning patient information.

5.1.1 Adverse event recording

- All grade 3 , 4, and 5 adverse events will be recorded regardless of attribution, including lab values for liver toxicity.
- However, grade 3 or grade 4 lymphopenia is anticipated and will not be recorded; furthermore, electrolyte abnormalities will not be recorded.
- For patients with progressive disease that receive standard of care therapy, expected adverse events due to multiple myeloma disease therapy will not be recorded.
- The following grade 1-2 adverse events will be recorded:
  - Unexpected events that are possibly, probably, or definitely related to the Th1.rapa cells during 30 days following Th1.rapa cell infusion
  - Toxicities potentially related to auto-immunity
- Any serious events that are deemed clinically significant by the PI

5.2 RESPONSE CRITERIA

Response criteria will use the consensus of the International Myeloma Working Group[97].

5.2.1 Important Considerations on response criteria

- Response criteria for all categories and subcategories of response except CR are applicable only to patients who have 'measurable' disease by at least one of the three measurements as defined below in section **5.2.1.1**.
- Patients who do not meet any of the criteria for measurable disease as listed in section **5.2.1.1** can only be assessed for stringent CR (sCR) and cannot be assessed for any of the other response categories.
- All responses must be confirmed to be stable in two evaluations, a minimum of six weeks apart.

5.2.1.1 Definition of measurable disease

Any one or combination of these abnormalities defines measurable disease:

- Serum M-protein greater or equal to 1 g/dl (10 g/l).
- Urine M-protein greater or equal to 200 mg/24 h.
- Serum FLC assay: involved FLC level greater or equal to10 mg/dl (100 mg/l) provided serum FLC ratio is abnormal.

5.2.1.2 Laboratory tests for measurement of M-protein

- Serum M-protein level is quantitated using densitometry on serum immnofixation electrophoresis (SPEP) except in cases where the SPEP is felt to be unreliable such as in patients with IgA monoclonal proteins migrating in the beta region. If SPEP is not available or felt to be unreliable (e.g., in some cases of IgA myeloma) for routine M-protein quantitation during therapy, then quantitative immunoglobulin levels on nephelometry or urbidometry can be accepted. However, this must be explicitly reported, and only nephelometry can be used for that patient to assess response and SPEP and nephelometric values cannot be used interchangeably.
- Urine M-protein measurement is estimated using 24-h UPEP only. (Random or 24 h urine tests measuring kappa and lambda light chain levels are not reliable and are not recommended)

5.2.1.3 Suggested follow-up to meet response criteria

It is recommended that patients undergoing therapy be tracked monthly for the first year of new therapy and every other month thereafter.

- Patients with "measurable disease" as defined above need to be followed by both SPEP and UPEP for response assessment and categorization;
- Except for assessment of CR, patients with "measurable disease" restricted to the SPEP will need to be followed routinely only by SPEP;
- Patients with "measurable disease" restricted to the UPEP will need to be followed routinely only by UPEP;
- Patients with "measurable disease" in either SPEP or UPEP or both will be assessed for response only based on these two tests and not by the FLC assay;
- FLC response criteria are only applicable to patients without measurable disease in the serum or urine, and only to fulfill the requirements of the category of sCR;
- Skeletal survey is not required for assessment of response unless clinically indicated, but is recommended once a year in clinical practice;

- Bone marrow is required only for categorization of CR and for patients with non-secretory disease;
- For good clinical practice patients should be periodically screened for light chain escape with UPEP or serum FLC assay.

5.2.2 International Myeloma Working Group uniform response criteria:

5.2.2.1 Complete Remission (CR)

- Negative immunofixation on the serum and urine **and**
- Disappearance of any soft tissue plasmacytomas **and**
- 5% or less plasma cells in bone marrow (a repeat bone marrow is not needed)
- No evidence of progressive or new bone lesions if radiographic studies were performed (X-Rays not required in absence of clinical indication)

Note:To be considered a CR,

- *both serum and urine immunofixation must be carried out and be negative regardless of the size of baseline M-protein in the serum or urine;*
- *patients with negative UPEP values pretreatment still require UPEP testing to confirm CR and exclude light chain or Bence–Jones escape*

5.2.2.2 Stringent Complete Remission (sCR)

- CR as defined above plus
  - Normal FLC ratio and
  - Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence (a repeat bone marrow is not needed).

5.2.2.3 Very Good Partial Remission (VGPR)

- Serum and urine M-protein detectable by immunofixation but not on electrophoresis **or**
- 90% or greater reduction in serum M-protein plus urine M-protein level <100mg per 24 h
- No evidence of progressive or new bone lesions if radiographic studies were performed (X-Rays not required in absence of clinical indication)

5.2.2.4 Partial Remission (PR)

- 50% or greater reduction of serum M-protein **and**
- 90% or greater reduction in 24-h urinary M-protein (or to less than 200mg per 24 h)
- 50% or greater reduction in the size of soft tissue plasmacytomas, if present at baseline
- No evidence of progressive or new bone lesions if radiographic studies were performed (X-Rays not required in absence of clinical indication)

If the serum and urine M-protein are not measurable (as per definition in section **5.2.1.1**),

- 50% or greater decrease in the difference between involved and uninvolved FLC levels is required (in lieu of the serum and urine M-protein criteria).

If serum and urine M-protein are not measurable and serum FLC assay is also not measurable,

- 50% or greater reduction in plasma cells is required in lieu of M-protein evaluation (provided baseline bone marrow plasma cell percentage was 30% or greater)

5.2.2.5 Stable Disease (SD)

- Not meeting criteria for CR, VGPR, PR or progressive disease

(Not recommended for use as an indicator of response; stability of disease is best described by providing the time to progression estimates)

Note:

a) *All response categories require two consecutive assessments made at anytime before the institution of any new therapy;*

b) *Confirmation with repeat bone marrow biopsy not needed.*

c) *Presence/absence of clonal cells is based upon the κ/λ ratio. An abnormal κ/λ ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is κ/λ ration of greater than 4:1 or less than 1:2.*

5.2.2.6 Progressive Disease (PD)

Requires one or more of the following:

- Increases of greater or equal to 25% in
  - Serum M-component (minimum absolute increase of 0.5g/dl) or
  - Urine M-component (minimum absolute increase of 200mg/24h) or
  - Percentage of bone marrow plasma cells (minimum absolute percentage of 10%)
  - Size of bone lesions or soft tissue plasmacytoma
- Definite development of new bone lesions or new plasmacytoma
- Development of hypercalcemia solely attributable to the disease

5.3 TOXICITY CRITERIA

5.3.1 Toxicity criteria

Toxicity will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) of the NCI available at: http://ctep.info.nih.gov/. A copy of the CTCAE version 4.0 can be downloaded from the CTEP home page. All treatment areas and personnel involved in the study should have access to a copy of the CTCAE version 4.0.

5.3.2 Definition of Dose Limiting Toxicity (DLT)

- Any grade 3, 4 or 5 toxicity (CTCAE version 4.0) with the attributions of possibly, probably or definitely related to the Th1.rapa cell administration that occurs in the first 30 days following infusion.
- The following toxicities will not be considered DLTs:
- Anticipated grade 3 or 4 toxicities that are due to the pentostatin/cyclophosphamide chemotherapy regimen (ie, fatigue, nausea, lymphopenia).

- Biochemical grade 3 or 4 toxicity for: cholesterol, triglyceride, uric acid.
- Grade 3 or 4 emesis unless it occurs after adequate pre-medication with at least 5-H2 antagonists (see section **4.5**).
- Grade 3 fever unless it occurs after adequate pre-medication with at least acetaminophen 1000 mg po and diphenhydramine 50mg IV.
- Grade 3 infections
- The occurrence of some immune mediated toxicity is not unlikely in this study, and in fact may turn out to correlate with clinical response as demonstrated by the well know graft versus tumor effect in the context of Graft versus Host disease in allogeneic Stem Cell transplantation or seen in other immunotherapy trials[98]. Grade 3 immune mediated toxicity that is clearly transient and reversible either spontaneously or with moderate therapeutic interventions, may ultimately be deemed acceptable in cost-benefit analysis. These cases will be discussed individually with NCI IRB and FDA.

5.3.3 Cytokine release (Cytokine Storm) syndrome

The cytokine release syndrome is defined clinically by nausea, headache, tachycardia, hypotension, rash and shortness of breath (CTCAE 4.0 definition). It is believed to be caused by the release of cytokines from cells under certain conditions of immune activation. A rapid rise in serum cytokine levels (cytokine storm) has been associated with Systemic Inflammatory Response Syndrome (SIRS) potentially leading to multi-organ dysfunction (MOD)[99,100]. This syndrome has been described following various forms of immunotherapy, mostly following antibody infusion; OKT3[101,102], Rituximab[103], Campath[104], anti-CD28[105] but also following cellular immunotherapy[106]. Based on these reports[101-106], the following cytokines are considered to be potential mediators of the cytokine storm: IFN-γ, GM-CSF and IL-6. Therefore, in the event that cytokine-storm toxicities are observed clinically, serum will be tested for content of these inflammatory cytokines.

5.3.4 Delayed Immune Mediated Toxicity

The possibility of Th1.rapa cell delayed toxicity, in particular Immune Mediated Toxicity as described in some immunotherapy trials[98] will be systematically explored and described in section **5.3.1**.

The following have been reported 10 days to 2 months following immune intervention (anti-CTLA4 antibody, ipilimumab: MDX-010) and were believed to constitute auto-immune mediated toxicity related to the immune intervention[98]: enterocolitis, dermatitis, hypophysitis (with pan-hypopituitarism), uveitis, hepatitis and Immune meningitis. Although the immune intervention probably related to these toxicities was very different than what subjects will undergo on this study, particular attention will be paid, within the first 3 months of the Th1.rapa cell infusion, for any toxicity occurring in these organ systems.

If Immune Mediated Toxicity were to occur following Th1/Tc1.rapa cell infusion, a reasonable assumption is for it to be of the form of known clinical manifestations encountered the allogeneic HSCT setting, such as engraftment syndrome with capillary leak syndrome and acute or chronic Graft vs Host disease. The study investigators have extensive expertise in the recognition and medical management of such syndromes in the setting of allogeneic transplantation.

Guidelines for the treatment of grade 3 or 4 auto-immune toxicity would be initiation of systemic immuno-suppressive therapy with corticosteroids (dose ranging from 1 to 2 mg/kg/d). Because

the Th1.rapa cells have been shown in the pre-clinical model to remain sensitive to rapamycin treatment in vivo, this drug may be used early in the treatment with the overall goal to allow a more rapid taper of the steroid therapy.

5.4 STATISTICAL CONSIDERATIONS

5.4.1 Previous Statistical Considerations (prior to amendment I)

This phase I dose escalation study enrolled 20 subjects, with seven cell doses evaluated; (2) cohort definitions included:

(1) Cohort 1 [1 x 10^5 Th1 cells/kg of body weight]; Cohort 2 [5 x 10^5 Th1 cells/kg]; Cohort 3 [1 x 10^6 Th1 cells/kg]; Cohort 4 [3 x 10^6 Th1 cells/kg]; Cohort 5 [5 x 10^6 Th1 cells/kg]; Cohort 5B [3 repeated doses of 5 x 10^6 Th1 cells/kg, with doses #2 and #3 being infused after immune-depleting chemotherapy]; and Cohort 6 [15 x 10^6 Th1 cells/kg]; (3) the following represented escalation requirements for cohorts 1 through 4: cohort escalation for cohorts 1 through 4 may proceed after 1 subject has been treated, provided that: the subject has encountered no DLT (as defined in section **5.3.2**) in the 30 days following Th1 cell infusion; the cytokine assays for IFN-γ, GM-CSF and IL-6 performed in the first 72 hours following Th1 cell infusion show no significant elevation over pre-treatment values (as defined in section **5.3.3**); if either of these conditions is not fulfilled, cohort accrual will be increased to 3 subjects; Th1 cell infusions in the subsequent subjects will be staggered by a minimum of 1 month; after 3 subjects have been treated, cohort escalation may proceed provided that: no subject experienced DLT (defined in section **5.3.2**) in that cohort; all 3 subjects have been followed for a minimum of 3 months; cytokine assays abnormalities in absence of any DLT will not preclude dose escalation; if 1 of 3 subjects experienced DLT, accrual to a maximum of 6 subjects will continue in that cohort; if 1 of 6 subjects experienced DLT, dose escalation may proceed to the next cohort; if 2 or more of the 6 subjects experienced DLT, accrual will stop and the MTD will have been exceeded; (4) the following represented escalation requirements for cohorts 5, 5B, and 6: each of cohorts 5, 5B, and 6 will consist of 6 subjects unless MTD is exceeded with smaller accrual; Th1 cell infusions in the first 3 subjects in each cohort will be staggered by a minimum of one month; toxicity encountered in all 6 subjects in each cohort will be considered in the determination of MTD and cohort escalation; cohort escalation will be based on the documentation of DLT (defined in section **5.3.2**); cytokine assays abnormalities in absence of any DLT will not preclude dose escalation; cohort escalation for cohorts 5 and 6 may proceed provided that: 6 subjects have been enrolled and followed for a minimum of 30 days; 3 of the 6 subjects have been followed for a minimum of 3 months; no more than 1 subject experiences DLT (defined in section **5.3.2)** in that cohort. If 2 or more of the 6 subjects experienced DLT, accrual will stop and the MTD will have been exceeded. The MTD is defined as the dose level immediately below the cohort at which 2 or more subjects experienced DLT. In cohort 1-4, if a cohort is expanded to 3 subjects, the estimated probabilities of cohort escalation based on the true probability of a patient having a DLT are similar to those of a standard phase I dose escalation trial. In cohort 5, 5B, and 6, the estimated probabilities of cohort escalation based on the true probability of a patient having a DLT are as follows:

Probability of DLT at a given dose	Probability of escalation
.1	.89
.2	.66
.3	.42
.4	.23
.5	.11

Enrollment to Cohorts 5B and 6 were simultaneous based on the following decision rules:

- A subject was enrolled on the cohort that has the smallest number of accrued subjects of these two cohorts.
- In case of a tie, the subject was enrolled on the cohort that did not enroll the previous subject.

Following cohort escalation and determination of maximum safe cell dose, the median total circulating $CD4^+$ count for each 6-subject cohort will be evaluated at 6 weeks and 12 weeks post Th1.rapa cell infusion. Data analysis from all Immune Characterization studies will determine which cell dose will be explored further by expanding the cohort to evaluate clinical outcome in a preliminary fashion. Because it will not be known until this portion of the study is concluded which cohorts to expand, the detail of a possible cohort expansion will be provided in a study amendment at a later date.

5.4.2 Th1/Tc1.Rapa Cell Prevention of Relapse Statistical Considerations (with amendment K)

1. With amendment K, Cohort A will evaluate a defined regimen of Th1/Tc1.rapa cell therapy after AHCT. Specifically, patients will receive an infusion of Th1/Tc1.rapa cells at approximately one and two months post-transplant (each infusion at a set dose of 5 million/kg; each infusion preceded by the 7-day PC chemotherapy regimen). Therefore, cohort A represents somewhat of a phase II extension of the results obtained in the initial phase I aspect of the study.
2. Accrual to Cohort A will be set at n=33 patients. This number of patients will allow a determination of the safety of this defined therapy (as detailed below); and, this number of evaluable patients will provide important information regarding the potential anti-tumor effect of this defined therapy, which will be critical for the design of subsequent, definitive clinical trials in this setting. With accrual of 1 to 2 patients per month, anticipated time to accrual completion will be approximately 24 months.
3. One primary goal of cohort A therapy is to evaluate the safety of this defined regimen. At this point in protocol implementation, we have not observed any Dose Limiting Toxicity (DLT) relating to the Th1/Tc1.rapa cell therapy or the 7-day PC regimen.

Going forward, with cohort A, we will continue to observe for serious adverse events and use any such events as stopping rule criteria.

4. For cohort A safety evaluation, the same criteria as used in the phase I portion of the trial will be utilized, as detailed in Section. **5.3.2** Specifically, a DLT will consist of any grade 3, 4 or 5 toxicity (CTCAE version 4.0) with the attributions of possibly, probably or definitely related to the Th1.rapa cell administration that occurs in the first 30 days following infusion.

5. Because this is a prevention study, any DLT of grade 5 toxicity will prompt a halt in further accrual to cohortA until full review by the IRB and FDA, with subsequent consideration of protocol amendment.

6. For DLT of grade 3 or 4 severity, to help ensure safety, the frequency of DLT will not exceed 1 out of 6, 2 out of 12, 3 out of 18, 4 out of 24, or 5 out of 30. If the DLT frequency exceeds these values, then there will be a halt in further accrual to cohort A until full review by the IRB and FDA, with subsequent consideration of protocol amendment.

7. In addition to safety evaluation, another primary goal of cohort A therapy is to determine the progression-free survival in patients who receive Th1/Tc1.rapa cells after AHCT.

8. As shown in protocol Figure 3, by 6 months post-AHCT, approximately 10 to 30% of patients will have disease progression depending upon whether the multiple myeloma is normal-risk or high-risk, respectively. Because the 25 patients that we will accrue are expected to be approximately a 1:1 mix of normal and high risk, it can be estimated that in the standard setting, approximately 20% of patients will relapse by 6 months post-AHCT. In the current study, with Th1/Tc1.rapa cell therapy, we predict that this relapse rate will be reduced. Using the method of Brookmeyer and Crowley, (Brookmeyer R and Crowley, JJ. A confidence interval for the median survival time. *Biometrics,* 38, 29-41, 1982), an estimated sample size of 33 patients will provide 80% power to identify an improvement from 80% 6 month relapse free survival to 92% 6 month relapse free survival, assuming a one-sided 0.10 alpha level significance test will be used, and that patients will be accrued for 24 months and followed for 6 months or longer after the last patient has been enrolled. As the comparative values are estimates based on a mixed population of patients, and the current trial will also have a mix of normal and high risk patients, this will just be a guideline for the sample size. In practice, the 33 patients will have Kaplan-Meier curves constructed which can be compared overall and separately by risk category to those of the historical data. The patients will be enrolled in a single stage in this cohort, however, we will determine the relapse free survival probability at six months post-AHCT after approximately 15 patients have been enrolled in this cohort and potentially followed for at least 6 months, and if RFS curve is estimated to be below that of the corresponding historical control groups during the first 6 months, then protocol accrual will be halted until further review of data by the investigative team, the IRB, and the FDA. In practice, this will mean that if the hazard ratio is below 1.0, favoring the historical results, accrual will end to this cohort.

5.4.3 Th1/Tc1.Rapa Cell Treatment of Relapse Statistical Considerations (with amendment K)

1. With amendment K, Cohort B will evaluate a defined regimen of Th1/Tc1.rapa cell therapy in advanced stage multiple myeloma patients who have progressive disease after receiving at least two prior treatment regimens. Patients will receive four infusions of Th1/Tc1.rapa cells over an interval of approximately four months (each infusion at a set dose of 5 million/kg; each infusion preceded by either the 7-day or 14-day PC chemotherapy regimen). The primary objective of cohort B therapy is to determine whether this Th1/Tc1.rapa cell therapy can mediate significant anti-tumor effects in this patient population, as defined by the rate of partial or complete remission (PR/CR).
2. Accrual to Cohort B will be accomplished using a Simon two-stage optimal design. With alpha=0.10 (probability of accepting a poor treatment) and beta=0.80 (probability of rejecting a good treatment) , the trial will seek to rule out an unacceptably low rate of response of 15% (PR+CR; p0=0.15) in favor of an acceptable rate of response of 35% (p1=0.35). Initial accrual to the first stage will be set at n=9 patients. Attainment of only 0 or 1 responses out of 9 patients will end accrual to Cohort B. If in the first stage, 2 or more patients with a PR or CR out of 9 patients will permit expansion of accrual to the second stage for a total of 23 evaluable patients. If at least six out of 23 patients achieve a PR/CR, then this would indicate that the Th1/Tc1.rapa cell therapy might be associated with a rate of response consistent with the desired 35% level, while 2 to 5 of 23 would be inadequate for further investigation in this population
3. With accrual of 1 to 2 patients per month to Cohort B, the anticipated time to accrual completion for Cohort B will be approximately 18 months.
4. To help ensure safety during cohort B implementation, the same criteria as used in the phase I portion of the trial will be utilized, as detailed in Section. **5.3.2**. Specifically, a DLT will consist of any grade 3, 4 or 5 toxicity (CTCAE version 4.0) with the attributions of possibly, probably or definitely related to the Th1.rapa cell administration that occurs in the first 30 days following the completion of the four cycles of therapy.
5. Any DLT of grade 5 toxicity will prompt a halt in further accrual to cohort B until full review by the IRB and FDA, with subsequent consideration of protocol amendment.
6. And, if the frequency of grade 3/grade 4 DLT exceeds 2 out of 9 cases during the first stage of protocol implementation, then advancement to the second stage of accrual will not occur.

5.4.4 Accrual

Accrual statement prior to amendment K:
The maximum accrual was set at 41 subjects. Enrolled subjects who are unable or unwilling to receive their Th1.rapa cell infusion will be replaced. Cohort 5B will accrue a maximum of 6 subjects. Any subject who does not complete the 3 infusions of Th1.rapa for any reason other than occurence of DLT will be replaced so that 6 subjects will be fully evaluated in cohort 5B. As for the other cohorts, evidence of cytokine elevation on the assays in absence of clinical

toxicity will not contribute to DLT determination.If the Th1.rapa cell number generated is insufficient for the assigned cohort but sufficient for a lower cohort, the subject may receive the cells and be evaluated with the lower cohort. A single subject added to a cohort of 6 will not contribute to the MTD determination. However, if more than one subject is added to a cohort, the study accrual will temporarily stop and the study will be re-evaluated in conjunction with the IRB in case more than one third of the total subjects who actually received the given cell dose experience DLT. Subjects treated in a lower cohort will be replaced in the higher cohort.

Accrual statement after amendment K:

Prior to amendment K, n=20 subjects were accrued to the study. In the new study design to address prevention of MM relapse (Cohort A), a maximum of n=33 evaluable subjects will be required. And, in the new study design to address treatment of MM (cohort B), a maximum of n=23 evaluable patients will be required. Therefore, total maximal protocol accrual will be 20 (initial phase I study) + 33 (cohort A prevention) + 23 (cohort B treatment) = 76 patients. A patient may be registered on the cohort A, taken off study, and then later registered on the protocol for cohort B therapy. These patients will be considered in the total sample size for cohort B as well as on cohort A. Some accrued patients may not be evaluable for various reasons, and as such, accrual of up to an additional ten patients may be required to complete the study.

Therefore, maximal accrual will be set at 86 subjects.

5.5 MULTI-INSTITUTIONAL GUIDELINES

5.5.1 IRB Approvals

This is a multi-institutional trial with John Theurer at Hackensack University Medical Center, Georgetown University School of Medicine and National Institutes of Health. Hackensack University Medical Center has obtained local IRB approval and Georgetown University School of Medicine will use the NCI IRB as the IRB of record.

The Coordinating PI of the NCI will ensure that no patient is entered into the trial at participating institutions without full IRB approval of the study. Thus, the NCI IRB must approve the addition of each participating institution to the protocol and a copy of the local IRB approval from each participating institution will be required before NCI IRB approval is granted. The PI will provide the NCI IRB with a copy of the participating institution's approved annual continuing review. Registration will be halted at any participating institution in which a current continuing approval is not on file at the NCI IRB.

5.5.2 Amendments and Consents

The PI will provide NCI IRB with any and all documentation of amendments, consents, and approvals from IRB reviews at all participating institutions. All amendments to the protocol or the consent are to be approved by the NCI PI and the NCI IRB and then submitted to the participating institutions IRB for approval.

5.5.3 Data Collection and Toxicity Reporting

The NCI C3D data base will be utilized; data will only be collected at the time points specified in this trial. Participating sites will be trained in the use of C3D and directly enter data at these sites into C3D. Any questions or concerns should elicit immediate contact of the NCI PI or LAI.

Guidelines for Adverse Event reporting are detailed in Section 7. All SAEs from each participating institution are reported simultaneously to IRB or delegate and to the NCI PI. At the discretion of the NCI PI, an event may be reviewed in a conference call to generate a final report that will go to the NCI IRB, sent by the NCI PI.

5.5.4 Quality Assurance/Monitoring

A site qualification visit and/or a site initiation visit will be done by the NCI PI per CCR guidance prior to the start of the trial in another institution. The coordinating center and participating institutions will undergo routine data monitoring by an NCI/CCR contractor. At each site monitoring visit, the monitor will review the source documents and data submitted into the NCI C3D. Accuracy will be checked by performing source data verification that is a direct comparison of the entries made in the database against the appropriate source documentation. Any resulting discrepancies will be reviewed with the investigator and the investigator's staff. The investigator(s) or a designated member of the investigator's staff must be available at some time during the monitoring visits to review data and resolve any queries and to allow access to the patient's records (medical records, office charts, hospital charts, and study related charts) and to provide the regulatory binder for source verification. Monitoring reports will be reviewed by the NCI PI and OCD, NCI. The investigator may be subject to a field audit by FDA inspectors to validate the participation of study subjects, adherence to the study protocol, and to verify the data reported in NCI C3D. This audit could occur while the study is in progress or several years after the study is completed. All of the patients' records and other study documentation must be filed and accessible on short notice (within 3 to 5 days) during the study and subsequent retention period. The NCI PI should be notified immediately if the participating center has been contacted by the FDA for an audit. In addition, quality assurance audits may be performed byNCI contractor. at various points throughout the study.

6 HUMAN SUBJECTS PROTECTIONS

6.1 RATIONALE FOR SUBJECT SELECTION

Median PFS of 10 months in the newly diagnosed highest risk patients vs. 32 months in the low risk subjects[32] justified the initial enrollment in the phase I aspect of the study in an attempt to improve the clinical outcome while feasibility and safety are being evaluated. With amendment K, with safety data available on n=20 Th1/Tc1.Rapa cell recipients, patient selection will be expanded to include both high-risk and normal-risk multiple myeloma patients.

6.2 PARTICIPATING OF PREGNANT OR BREASTFEEDING WOMEN

A woman who is unwilling to stop breast-feeding or pregnant, may not take part in the study since the risk of this experimental procedure is unknown to baby or unborn child.

6.3 PARTICIPATION OF CHILDREN

Multiple Myeloma is not a disease of children. The exceptional cases described in the pediatric population are unlikely to be informative for adults.

6.4 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 6.5), all NIH Clinical Center subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

6.5 EVALUATION OF BENEFITS AND RISKS / DISCOMFORTS

6.5.1 Estimated survival

6.5.1.1 In cytogenetics high-risk groups

Based on 104 MM cases that had informative FISH results for all four genetic markers, three distinct prognostic categories were proposed with genetic-based risk stratification[32]:

- Low-risk patients with none of the genetic abnormalities tested or only t(11;14) (52% of cases);
- intermediate risk and high-risk with any one or more of the genetic abnormalities other than t(11;14) (48% of cases);

The median PFS was 10 to 20 months for the higher risk groups and 32.1 months for the low risk group. The median OS was 18 months to 46 months for the higher risk group and was not reached for the low-risk group.

Abnormality	OS	PFS
- p53 del	14.7	7.9
- t(4:14):	18.3	9.9
- t(11:14)	37.2	25.2
- 13q del	34.4	22.1
- none of above:	Not reached	32.1

6.5.1.2 In other high-risk groups

Time to progression in the high-risk group defined by positivity of Circulating Myeloma Cells assay immediately before the transplant procedure has been estimated at about 14 months (versus 22 months for CMC negative patients)[36].

6.5.2 Estimated Risks and Benefits

Therefore, based on published patient distribution between High/intermediate risk and low risk group[32], it is estimated that about 50% of subjects with newly diagnosed MM will present with high risk features justifying entry on a phase I protocol.

The potentially added risks for participating in the study include:

- potential morbidity of the apheresis procedure,
- the unknown risk of the Th1 / Tc1 cell re-infusion.

The potential benefits of the study are the acquisition of general scientific knowledge and a reasonable prospect of clinical benefit to the participants.

6.6 RISK / BENEFIT ANALYSIS

The risk of apheresis is acceptable as normal volunteers, such as platelets donors, routinely undergo the procedure with minimal morbidity in the vast majority of cases. The risks of the immune depletion are equivalent to the risks of other chemotherapy regimens utilized in the treatment of MM. While the risks specific to the Th1 / Tc1 infusion are unknown in humans, the phase I design and the vast experience in lymphocyte re-infusion make this risk acceptable. With amendment K, given the safety experience obtained from the first n=20 patients treated on the protocol, the risk/benefit analysis favors accrual of both high-risk and normal-risk patients.

6.7 CONSENT PROCESS AND DOCUMENTATION

- Prior to receiving any therapy, patients must have their eligibility confirmed and must have signed an informed consent. The principal investigator or study chair will review and sign re-infusion orders for the Th1.rapa cell administration.
- The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the subjects during the Informed Consent process. A signed Informed Consent document will be obtained prior to entry onto the study by the Principal Investigator or an Associate Investigator at each participating institution. Each participating institution will be responsible for its own Informed Consent document, and will obtain IRB approval of the Informed Consent document at their institution.
- All patients must have a copy of their signed Informed Consent document maintained in the chart during therapy.
- Results of the study or of individual patients may be reported at meetings or in scientific publications with removal of specific patient identifiers.

6.7.1 Telephone re-consent procedure

Reconsent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witnessto the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process

will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

6.7.2 Short form consent process for non-English speaking patients

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OSHRP SOP 12, 45 CFR 46.117 (b) (2) and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation. Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

6.8 SUBJECT INFORMATION AND CONFIDENTIALITY

As information is gathered from this trial, clinical results will be shared with patients. Laboratory and clinical data will be frequently gathered and any new significant observation(s) found during the course of the research, which may affect a patient's willingness to participate further would be explained.

Confidentiality of information concerning participants will be maintained including in all publications and presentations resulting from this study. Names of participants or material identifying participants will not be released without permission, except as such release is required by law. Records at the National Cancer Institute are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

See section **12** for specifics of sample management and storage.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DATA REPORTING & RECORDS TO BE KEPT

All clinical data will be recorded in the patient's chart as per institutions' practices (maintained by the NIH Clinical Center Department of Medical Records and on the electronic chart CRIS system). The patient records will be maintained by the Clinical Associate and other protocol personnel. Each patient's NIH Clinical Center medical record must reflect all of the following information:

- The patient met all eligibility criteria.
- Signed Informed Consent document was obtained before treatment.
- Specific dates and times of all treatments specified in the protocol, doses administered, and documentation for the reason for any dose modification.
- Documentation of all toxicities with grading according to NCI CTCAE version 4.0 as specified in the protocol.
- Documentation of all follow-ups as specified in the protocol.

7.1.1 Adverse Events reporting

As subjects will be enrolled on protocol at the time they are receiving concurrent, off-protocol, standard of care therapy with expected toxicity, the toxicity of any concurrent therapy will not be reported for this study. Adverse Event reporting will be limited to events occurring during or immediately following the lymphapheresis procedure and events occurring during or within 6 months following the Th1.rapa cell infusion.

7.2 DEFINITIONS

7.2.1 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 **5.1**.

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 **7.4** and **7.5.**

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.

7.2.2 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.

7.2.3 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.

7.2.4 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.

7.2.5 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
- A persistent or significant disability/incapacity
- 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.

7.2.6 Disability

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

7.2.7 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.

7.2.8 Protocol Deviation (NIH Definition)

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

7.2.9 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 subjects.

7.2.10 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 *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.3 NCI-IRB AND CLINICAL DIRECTOR REPORTING

7.3.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

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

Reports must be received within 7 days of PI awareness via iRIS.

7.3.2 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 collected or reported.

7.3.2.1 Continuing Reviews at participating institutions

Continuing Reviews at participating institutions must comply with individual institutional guidelines for reporting but at a minimum must contain items outlined in **7.3.2**.

A copy of each Continuing Review submission with institutional approval must be forwarded to the NCI PI within 30 days of approval for continuing enrollment to be allowed.

7.3.3 NCI Guidance for Reporting Expedited Adverse Events for Multi-Center Trials

The site PI must immediately report to the coordinating center PI 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 within 24 hours of PI awareness of the event. The Site PI must also report any protocol deviations to the coordinating center PI within 7 days of PI awareness. Participating centers must also submit the report to their IRB in accordance with their institutional policies. As the CCR is the IND Sponsor of the study, then in addition to reporting to the PI the site PI must submit the report to the CCR as per section **7.4.**

A participating site problem form (available in section **14**) is to be filled out and sent to the Coordinating Center's PI.

7.4 IND SPONSOR REPORTING CRITERIA

An investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, 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.

- All Grade 5 (fatal) events (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- All other serious adverse events including deaths due to progressive disease must be reported within one business day

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). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to the IND sponsor at : brian@rapatherapeutics.com

7.4.1 Reporting Pregnancy

7.4.1.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as **Grade 3** "***Pregnancy, puerperium and perinatal conditions - Other (pregnancy)***" under the ***Pregnancy, puerperium and perinatal conditions*** SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The

outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.4.1.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study experimental procedure, and for 6 months after finishing the study experimental procedure.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until (180 days*)* after the last dose should, if possible, be followed up and documented.

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

In order to assure optimal concordance between the protocol requirements and the best possible clinical care of the patients, the PI or designate will co-sign a patient registration check list, review all chemotherapy orders and Th1 / Tc1 infusion orders.

PI, research nurses, will hold weekly to bi-weekly meetings to review accrued data according to primary and secondary endpoints.. The principal investigator will review adverse events, attributions, and response data on each patient to ensure safety and data accuracy. All data will be entered per protocol and reviewed by the principal investigator or designate.

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

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

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an NCI contractor. 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.

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 PHARMACEUTICAL INFORMATION

8.1 TH1/TC1 PRODUCT

Autologous Th1/Tc1.Rapa Cells

The autologous Th1/Tc1.Rapa cells will be manufactured in the NIH Department of Transfusion Medicine according to methods specified in the IND submitted to the FDA.

a) Patients on the study will undergo a 10 to 15 liter apheresis procedure (CS-3000 or an equivalent machine). The experimental Th1/Tc1.Rapa cells will be manufactured centrally in the NIH DTM according to the Investigational New Drug Application submitted to the FDA for this cell product.

b) According to the IND specifications, Th1/Tc1.Rapa cells will be cryopreserved in the NIH Department of Transfusion Medicine. As such, as long as the Th1/Tc1.Rapa cells meet the certificate of analysis as defined in the IND, the cells may be administered on protocol either at the NIH Clinical Center, or in later stages of protocol implementation, at the Hackensack University Medical Center, at the Georgetown University School of Medicine.

c) Although the specific aspects of the manufacturing method will be defined in the IND and not in this protocol, the following procedure will be utilized: (1) total T cells will be isolated after apheresis; (b) T cells will be placed into sterile tissue culture bags in incubators in the NIH-DTM; (c) T cells will be co-stimulated using anti-CD3, anti-CD28 coated magnetic beads; and (d) T cells will be expanded for a period of six days in culture (+/- 1 day) in media that contains rapamycin, IL-2, and IFN-alpha.

d) The following will be the minimal phenotypic requirements of any particular Th1/Tc1.Rapa cell culture to qualify for cryopreservation with subsequent administration. For Th1/Tc1.Rapa cell products to be infused at Hackensack and Georgetown University School of Medicine, the

cryopreserved product will be shipped by an accredited courier to Hackensack and Georgetown University School of Medicine with a certificate of analysis identifying the cell product and attainment of the specific release criteria. Cells not meeting these requirements will be discarded.

1. Flow cytometry: final release must be % CD3 of viable: ≥ 70%; CD138<1% as per the COA
2. Absence of bacterial and fungal growth.
3. Absence of endotoxin content by the limulus assay.
4. Negative mycoplasma test.
5. After magnetic bead removal, < 100 beads per 3 x 10^6 cells.

In addition, the functional characteristics of the expanded Th1/Tc1.Rapa cells will be measured (specifically, cytokine secretion pattern) and retrospectively correlated to any observed post-transplant effect, such as toxicity or pattern of immune reconstitution. However, in this pilot study, this information will not be utilized as formal release criteria for the cell product.

8.2 SUPPORTIVE CARE MEDICATIONS

The following drugs will be used along the protocol at various times: Diphenhydramine, Ondansetron, Granisetron, Prochlorperazine, Fluconazole, Cotrimoxazole. Package inserts are available from the Pharmacy Dept. for specific information.

8.3 BORTEZOMIB (VELCADE®)

8.3.1 Source:

Bortezomib is commercially available and its use is approved for treatment of multiple myeloma. FDA approved package insert is available for further information.

8.3.2 Administration

Intravenous push; reconstituted in 0.9% Sodium Chloride, USP at a final concentration of 1mg/ml

8.3.3 Adverse effects:

Most common: hematological toxicities (especially transient thrombocytopenia), decreased appetite, gastrointestinal disturbances, peripheral neuropathy, fatigue, fever, dyspnea, rash, and myalgia.

Other common: hyperglycemia, hypokalemia, insomnia, anxiety, confusion, depression, blurred vision, eye pain, dizziness, dysgeusia, tremor, epistaxis, cough, rhinorrhea, pruritus, arthralgia, edema, and orthostatic hypotension.

Uncommon: tumor lysis syndrome, hypersensitivity, rash with small vessel necrotizing vasculitis and seizures have been reported. Tachycardia, arrhythmias, palpitations, angina pectoris, and myocardial infarction have occurred. Congestive heart failure may be exacerbated and pulmonary edema has been reported. There have been rare reports of acute respiratory distress syndrome, some of them fatal.
Renal impairment is common in patients with multiple myeloma and acute renal failure has developed in patients on bortezomib. Patients with compromised renal function should be monitored, and dose reductions considered if needed. Hepato-toxicity, which may be reversible, has included

increases in liver enzyme values, hyperbilirubinemia, and acute liver failure; bortezomib should be used with caution in hepatic impairment.

8.4 LENALIDOMIDE (REVLIMID®)

8.4.1 Source

Lenalidomide is commercially available and its use is approved for treatment of multiple myeloma. FDA approved package insert is available for further information.

8.4.2 Administration

Oral; 25 mg tablet q day

8.4.3 Adverse effects

FDA approved package insert is available for further information.

Most common: Myelosuppression (neutropenia, anemia thrombocytopenia), constipation, diarrhea, fatigue

Less common: peripheral neuropathy, blurry vision, thrombo-embolism anorexia, nausea vomiting, allergic reactions, skin rash,

8.5 DEXAMETHASONE

8.5.1 Source

Dexamethasone is commercially available and its use is approved for treatment of multiple myeloma. FDA approved package insert is available for further information.

8.5.2 Administration

Oral or IV

8.5.3 Adverse effects

Hyperglycemia, hypertension, electrolytes abnormalities, increased appetite, sleep disturbance, immunosuppression.

8.6 ETOPOSIDE

8.6.1 Source

Etoposide for intravenous infusion is commercially available from the manufacturer.

8.6.2 Formulation and Preparation

Supplied in 5 ml vials containing 100 mg (20 mg/ml) in a polyethylene vehicle.

8.6.3 Stability and Storage

The drug will be freshly prepared each day by dilution in commercially prefilled bags of 0.9% sodium chloride injection to a final concentration of < 0.4 mg/ml.

8.6.4 Administration

Intravenous Etoposide is given via a central venous catheter by slow intravenous infusion.

8.6.5 Adverse effects

Etoposide may cause myelosuppression, nausea, vomiting, allergic reactions, alopecia, aftertaste, pigmentation changes, abdominal pain, constipation, dysphagia, transient cortical blindness, and optic neuritis. In addition, intravenous Etoposide can cause bradycardia or hypotension when infused too quickly.

8.7 CISPLATIN

8.7.1 Source

Cisplatin for intravenous infusion is commercially available from the manufacturer.

8.7.2 Formulation and Preparation

Cisplatin is supplied in a white lyophilized powder in 10 and 50 mg vials with mannitol and sodium chloride.

8.7.3 Stability and Storage

Reconstitution with 10 and 50 ml results in a 1mg/ml solution which is stable for 20 hours at room temperature. Further dilution to 0.05 or 0.5 mg/ml with sodium chloride yields a solution that is stable for at least 24 hours at room temperature

8.7.4 Administration

Intravenous Cisplatin is given, combined with etoposide and cyclophosphamide in a single solution, via a central venous catheter by slow intravenous infusion over 4-day continuous infusion.

8.7.5 Adverse effects

Cisplatin may cause myelosuppression mostly on red cells and platelets), moderate to severe nausea, vomiting, allergic reactions, alopecia, renal tubular toxicity (persistent hypokalemia and hypomagnesemia), renal failure, hearing loss, vestibular abnormalities, sensory motor peripheral neuropathy, rarely secondary leukemia.

8.8 INTRAVENOUS CYCLOPHOSPHAMIDE

Cyclophosphamide is not an investigational drug. FDA approved package insert is available for further information. Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

8.8.1 Pharmaceutical Information:

Cyclophosphamide is commercially available in vials containing 2000 mg of lyophilized Cyclophosphamide. The drug is reconstituted with 100 ml of sterile water for injection for a final concentration of 20 mg/ml. Intact vials should be stored at room temperature and bear an expiration date. Constituted solutions (20 mg/ml) should be used within 6 days when stored in the refrigerator (2-8 degrees Celsius). Further dilution with either 5% dextrose injection or 0.9% NaCl injection to concentrations of 0.1, 3.1 or 6.6 mg/ml results in a solution that is stable for at least 8 hours at room temperature and 24 hours when stored in the refrigerator (2-8 degrees Celsius).

8.8.2 Administration

Cyclophosphamide will be diluted in 250 ml of Normal Saline and infused as a continuous infusion.

8.8.3 Adverse effects

Myelosuppression, nausea, vomiting, diarrhea, hemorrhagic cystitis and bladder fibrosis, alopecia, occasionally hyperpigmentation, sterility, syndrome of inappropriate ADH secretion (SIADH), secondary malignancies (leukemia, and bladder cancer) and rarely interstitial pneumonitis, pulmonary fibrosis.

8.9 ORAL CYCLOPHOSPHAMIDE (CYTOXAN, NSC-26271)

(Refer to FDA-approved package insert for complete product information)

8.9.1 Supply

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a tablet form.

8.9.2 Preparation

Tablets.

8.9.3 Storage and Stability

The vials of cyclophosphamide containing the treatment doses are stable when stored at room temperature.

8.9.4 Route of Administration

The cyclophosphamide used during the immune depletion regimens will be given by oral administration on a daily basis. Tablets are not scored and should not be cut or crushed. To minimize the risk of bladder irritation, do not administer tablets at bedtime. The dose of cyclophosphamide will initially be 200 mg per day. Refer to section **3.2.3** for dose modifications during administration.

8.9.5 Mechanism of action

Cyclophosphamide is an alkylating agent that prevents cell division by cross-linking DNA strands and decreasing DNA synthesis. It is a cell cycle phase nonspecific agent. Cyclophosphamide also possesses potent immunosuppressive activity. Cyclophosphamide is a prodrug that must be metabolized to active metabolites in the liver.

8.9.6 Toxicities

a) Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].

b) Cytopenias

c) Hemorrhagic cystitis or urinary fibrosis.

d) Mucositis.

e) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

8.10 MELPHALAN

8.10.1 Source

Intravenous Melphalan will be obtained commercially from the manufacturer. FDA approved package insert is available for further information.

8.10.2 Formulation and Preparation

It is supplied in sterile vials containing 50 mg of lyophilized drug and 20 mg of povidone. Each vial comes with 10 ml of sterile diluent containing sodium citrate, propylene glycol, ethanol, and water. Vials should be stored between 15˚ C and 30˚ C. Each vial of drug should be reconstituted with 10 ml of the supplied diluent, which will produce a 5 mg/ml solution. The reconstituted solution should be filtered through a 0.45 micron filter prior to further dilution. The desired number of milligrams of Melphalan should be further diluted in 0.9% NS to a final concentration of < 2 mg / ml, and run intravenously over 15-30 minutes.

8.10.3 Stability and Storage

Administration should be complete within one hour of reconstitution.

8.10.4 Administration

Melphalan is given by slow intravenous infusion over 15-30 minutes.

8.10.5 Adverse effects

High-dose Melphalan can cause myelosuppression, nausea, vomiting, diarrhea, mucositis, pulmonary fibrosis, interstitial pneumonitis, skin hypersensitivity, vasculitis, alopecia, hemolytic anemia, and allergic reactions. Melphalan is known to be mutagenic and leukemogenic in humans.

8.11 NEUPOGEN® (FILGRASTIM, G-CSF)

Neupogen® (filgrastim, G-CSF) is a human granulocyte colony-stimulating factor (G-CSF) produced by recombinant DNA technology. Neupogen ® is indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a significant incidence of severe neutropenia with fever. Neupogen ® will be obtained from the commercial supply in the Clinical Center Pharmacy.

8.11.1 Formulation, Storage, and Stability

Recombinant granulocyte-colony stimulating factor (G-CSF) manufactured by Amgen (Thousand Oaks, CA) is supplied as a clear sterile solution of 300 mcg/mL packaged into either 1-mL (300 mcg) or 1.6-mL (480 mcg) vials. Neupogen ® should be stored in the refrigerator at 2° to 8°C (36° to 46°F). Avoid shaking. Prior to injection, Neupogen ® may be allowed to reach room temperature for a maximum of 24 hours. Any vial or prefilled syringe left at room temperature for greater than 24 hours should be discarded. Do not freeze. G-CSF is stable for at least 1 year when refrigerated.

8.11.2 Administration

G-CSF will be administered as a subcutaneous injection to mobilize peripheral blood stem cells for collection by apheresis. See Section **11.1.1** for sliding scale G-CSF dosing algorithm for mobilization and section **10.7.1** for use after AHCT

Patients may be instructed on the self-administration of G-CSF.

8.11.3 Adverse effects

Bone pain, which can sometimes be severe. Other adverse reactions include fatigue, muscle cramps, back/leg pain, splenomegaly and thinning hair.

8.12 PLERIXAFOR (MOZOBIL®)

Plerixafor is a CXCR4 chemokine receptor antagonist that blocks the binding of stromal cell-derived factor 1α (SDF-1 α). It inhibits the retention of hematopoietic stem cells in bone marrow, and increases their number in peripheral blood. It is used with granulocyte colony-stimulating factor (G-CSF) to mobilize stem cells for collection and subsequent autologous transplantation Mozobil® will be obtained from the commercial supply in the Clinical Center Pharmacy.

8.12.1 Formulation, Storage, and Stability

Subcutaneous Solution: 20 mg/ml

Inspect vial for particulate matter and discoloration prior to administration; do not use if particulate matter present or solution is discolored. (Prod Info MOZOBIL(R) subcutaneous injection, 2008).

Store at controlled room temperature, 25 degrees C (77 degrees F), with excursions permitted between 15 and 30 degrees C (59 and 86 degrees F)

8.12.2 Administration

Plerixafor 240µg/kg will be administered for one to three consecutive days as a subcutaneous injection to mobilize peripheral blood stem cells for collection by apheresis. See **Appendix 1**: Induction, HPCS mobilization and AHCT procedure guidelines for subjects enrolled at NCI for dosing algorithm.

Plerixafor will be administered on the 5th day of filgrastim administration, 6 to 8 hours before the apheresis. In case stem cell collection is insufficient with one apheresis, up to 2 additional doses of Plerixafor may be administered at the same dose, each time 6 to 8 hours before the apheresis procedures.

8.12.3 Pharmacokinetics

Peak plasma concentrations of plerixafor occur about 30 to 60 minutes after a subcutaneous dose. It is about 58% bound to plasma proteins and largely confined to the extravascular fluid space. About 70% of a dose is eliminated in the urine within 24 hours after a dose, and the terminal half-life is about 3 to 5 hours.

Plerixafor is not metabolized using human liver microsomes or human primary hepatocytes. Additionally, plerixafor does not exhibit inhibitory activity towards the major drug metabolizing cytochrome P450 enzymes nor did it induce CYP1A2, CYP2B6, or CYP3A4 enzymes.

8.12.4 Adverse effects

Common adverse effects include diarrhea, nausea, vomiting, flatulence, fatigue, arthralgia, headache and dizziness, mild injection site reactions.

Less commonly, insomnia or systemic reactions occurring about 30 minutes after injection (urticaria, periorbital swelling, dyspnea, and hypoxia).

Some cases of vasovagal reactions, orthostatic hypotension, and syncope, within 1 hour of injection, have also been reported.

8.13 PENTOSTATIN (NIPENT®; 2'-DEOXYCOFORMYCIN)

(Refer to FDA-approved package insert for complete product information)

8.13.1 Supply

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

8.13.2 Preparation

The pentostatin dose will be determined by the creatinine clearance value (as initially determined by the 24 hour urine collection method). The appropriate dose of the reconstituted pentostatin solution will be further diluted with 50 ml of 0.9% sodium chloride for IV administration

8.13.3 Storage and Stability

Pentostatin vials are stable at refrigerated storage temperature 2° to 8°C (36° to 46°F) for the period stated on the package. Vials reconstituted or reconstituted and further diluted as directed may be stored at room temperature and ambient light but should be used within 8 hours because pentostatin contains no preservatives. Once diluted for administration, pentostatin will be provided an expiration date of 8 hours.

8.13.4 Administration

Intravenous infusion over 30 to 60 minutes. Subjects will receive one liter of 0.9% sodium chloride by intravenous infusion prior to the pentostatin delivery.

8.13.5 Mechanism of action

Inhibition of adenosine deaminase, thereby increasing lymphocyte susceptibility to apoptosis.

8.13.6 Toxicities

Pentostatin is cleared by a renal mechanism (90%). As such, the pentostatin dose must be reduced for renal insufficiency (see Section **3.2.3**).

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.

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10 APPENDIX 1: INDUCTION, HPCS MOBILIZATION AND AHCT PROCEDURE GUIDELINES FOR SUBJECTS ENROLLED AT NCI

10.1 INDUCTION THERAPY

- Bortezomib (Velcade): 1.5 mg/m^2 IV on days 1, 8 & 15
- Lenalidomide (Revlimid): 25 mg po q day on days 1through 14
- Dexamethasone (Decadron): 20 mg po on days 1, 2, 8, 9, 15 & 16 q 21 days x 4 cycles.

10.2 HPCS MOBILIZATION THERAPY

The target dose for CD34$^+$cell collection is 5 x 10^6 CD34$^+$ cells/kg of body weight. This dose is intended to be sufficient for two transplant procedures of at least 2.5 x 10^6 CD34 $^+$ cells /kg of body weight each, in the event a second transplant would be clinically indicated. The minimum cell dose for any single transplant procedure is 2 x 10^6 CD34 $^+$ cells /kg of body weight. The apheresis products should be cryo-preserved accordingly.

Mobilization with Filgrastim and Plerixafor alone is the preferred method for mobilization, however, at the discretion of the NCI PI, either one of the two following mobilization strategies may be used in function of the estimate of residual disease burden following induction therapy.

Subjects enrolled on study at the time of recurrent disease, may already have collected and cryopreserved HPCs after their initial therapy at the Clinical Center or at other institutions. In the latter case, previously cryopreserved HPCs may be obtained from other institutions provided the collection, cryopreservation and shipment are in compliance with DTM's requirements.

10.2.1 Mobilization with Filgrastim and Plerixafor

- Day 1 through 4 of mobilization
    - G-CSF 10-16µg/kg/dose (according to DTM algorithm in**Appendix 1**: Induction, HPCS mobilization and AHCT procedure guidelines for subjects enrolled at NCI) subcutaneously

for 5 days if the CD34$^+$cell target dose is reached after the first apheresis,

- Day 5 of mobilization:
    - To be given eight to ten hours prior to the start of apheresis

Plerixafor 240 µg/kg (according to DTM algorithm in **Appendix 1**: Induction, HPCS mobilization and AHCT procedure guidelines for subjects enrolled at NCI) is given subcutaneously
along with G-CSF at the same dose as on days 1-4.

- Subsequent days of mobilization (if necessary):

G-CSF and Plerixafor are continued until the CD34$^+$cell target dose is reached (5 x 10^6 CD34$^+$ cells/kg)
or until the PI or alternate elects to terminate the collection in collaboration with the DTM Attending

10.2.2 Mobilization with chemotherapy

- The DCEP regimen will be used[107] and in most cases, may be delivered as outpatient. More than a single cycle may be administer prior to HPCs collection, if clinically indicated at the PI's discretion.
    - Dexamethasone: 40 mg IV over 15 min daily for 4 days,
    - Cyclophosphamide 500 mg/m^2 daily for 4-day continuous infusion
    - Etoposide 40 mg/m^2 daily (maximum dose 75 mg) for 4-day continuous infusion
    - Cisplatin 15 mg/m^2 daily (maximum dose 25 mg) for 4-day continuous infusion

The patients must be hydrated with a minimum of 1000ml of IV Normal Saline before cyclophosphamide. Adequate hydration and urine output must be maintained throughout the 4 days of continuous infusion. This may be achieved by oral hydration, if feasible. Anti emetics will be administered to all patients according to prevailing NIH guidelines (Section **4.5**).

- G-CSF daily 10-16µg/kg/dose (according to DTM algorithm in **Appendix 1**: Induction, HPCS mobilization and AHCT procedure guidelines for subjects enrolled at NCI)
    - starting 36-48 h after the end of chemotherapy
    - continued until the stem cell collection is completed.
- Starting on day 10 of the initiation of chemotherapy, a CBC will be performed daily. Peripheral CD34 count will be performed when WBC is greater than 5000/mm^3
- Apheresis will be initiated when the peripheral CD34 cell count is greater or equal to 10/µl. (Apheresis may be started at a lower CD34 count based on clinical grounds reviewed in collaboration with the DTM Attending).
- If the CD34 target dose is not reached after one apheresis procedure, one dose Plerixafor will be given eight to ten hours prior to the second apheresis procedure along with G-CSF according to DTM guidelines in**Appendix 1**: Induction, HPCS mobilization and AHCT procedure guidelines for subjects enrolled at NCI.

10.3 HPCS APHERESIS

- To start 8 to 10 hours following the last dose of G-CSF

If the target dose is reached after a single apheresis procedure:

- the patient may proceed to the preparative regimen (as soon as clinically and logistically appropriate) as outlined in section **10.4** below starting on day -5.

If the target dose is not reached:

- Plerixafor and G-CSF, given 8 to 10 hours prior to apheresis are repeated on the night of the first collection and a second collection is performed the next day.
- then the patient proceeds to the preparative regimen (as soon as clinically and logistically appropriate) as outlined in the section **10.4** below on what will become day -5 regardless of the number of days of collection.

- If after 2 days of collection, the target cell dose of 5 x 10^6 cells / kg is not reached:

- If total cell dose is greater or equal to 4 x 10^6 cells / kg, the collection will be deemed sufficient for the performance of potentially 2 AHCT procedure and the patient may proceed with the preparative regimen.
- If the total cell dose is between 2 and 4 x 10^6 cells / Kg, the collection will be deemed sufficient for the performance of a single AHCT procedure and the patient may proceed with the preparative regimen.
- If the total cell dose is less than 2 x 10^6 cells / Kg, the collection will be deemed insufficient for the performance of a single AHCT and a second cycle of mobilization will be planned at a minimum of 3 weeks after the completion of the first mobilization cycle.

- The apheresis product will be cryopreserved without manipulation according to SOPs of Dept of Transfusion Medicine

10.4 PREPARATIVE REGIMEN FOR TRANSPLANT PROCEDURES

- Anti emetics will be administered to all patients according to prevailing NIH guidelines or at PI's discretion (Section **4.1**) starting day -5.
- High-dose Melphalan will be given in preparation for
  - The 1st AHCT, as soon as clinically and logistically appropriate following HPCS collection
  - The 2nd AHCT, a minimum of 3 months after the first transplant.
- Day -2 Melphalan 200 mg/m^2 via slow intravenous infusion over 30 minutes.
- Day 0 HPCS reinfusion
  - At most, 50% of the total cell dose collected should be re-infused since the collection should provide for two transplant procedures (if clinically indicated).
  - The minimum cell dose for a transplant procedure is 2 x 10^6 CD34 + cells /kg of body weight.
- Day + 5: Begin G-CSF 5 mcg/kg/day s.q. the dose maybe rounded to accommodate vial sizes at the PI's discretion) until ANC is greater than 1000 /mm^3 for two consecutive days. (

10.5 ELIGIBILITY IMMEDIATELY PRIOR TO HIGH-DOSE THERAPIES

Within two weeks prior to each of the two high-dose chemotherapy regimens, subjects must meet the following eligibility criteria and none of the exclusion criteria of section **2.1.2**below (HIV testing will not be repeated).

- Karnofsky Performance Status ≥70% (ECOG performance status of 0 or 1)
- Ejection fraction (EF) by MUGA or 2-D echocardiogram within institution normal limits. In case of low EF, the subject may remain eligible after a stress echocardiogram is performed if the EF is more than 35 % and if the increase in EF with stress is estimated at 10% or more.
- Serum creatinine less or equal to 2.5 mg/dl,
- AST and ALT less or equal to 3 x upper limit of normal,
- Bilirubin less or equal to1.5 (except if due Gilbert's disease).

- Corrected DLCO greater or equal to 50% on Pulmonary Function Tests

10.6 DOSE MODIFICATIONS

1.1.1 DCEP

In case of abnormal renal function, the dose of cisplatin will be reduced to 10mg/m^2 daily.

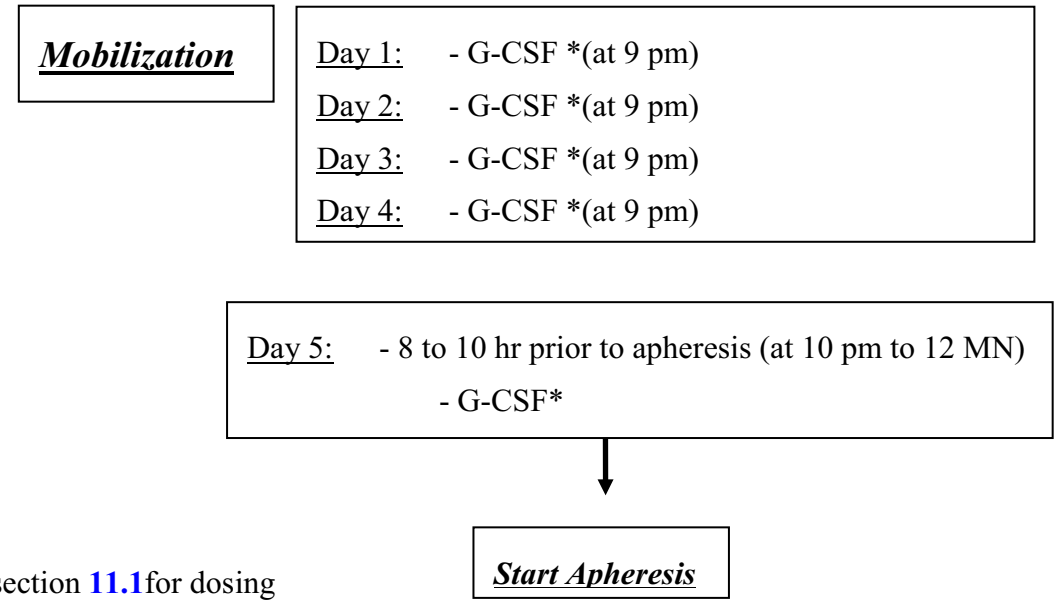
1.1.2 Melphalan

There are conflicting data about possible increased transplant related mortality with the preparative regimen of Melphalan 200mg/m^2 in individuals older than 65 years (increase in toxicity[39] ; no increase in toxicity[108]) while an increase in gastro-intestinal toxicity (mucositis and diarrhea) seems better established in the same publications.

Therefore, individuals older than 65 years will receive a reduced dose of Melphalan of 140 mg/m^2 and will receive a single transplant procedure.

10.7 TREATMENT SCHEMA

10.7.1 Mobilization with G-CSF and Plerixafor alone



* See section **11.1** for dosing

10.7.2 Mobilization with chemotherapy

Mobilization

Days 1 though 4:
- Dex: 40 mg IV over 15 min
- Continuous IV infusion over 24 hours:
  Cyclophosphamide 500 mg/m^2
  Etoposide 40 mg/m^2 (max dose 75 mg)
  Cisplatin 15 mg/m^2 (max dose 25 mg)

Day 5: finish the continuous infusion

Day 6: - G-CSF* (at 9 pm)

Day 7: - G-CSF *(at 9 pm)

↓

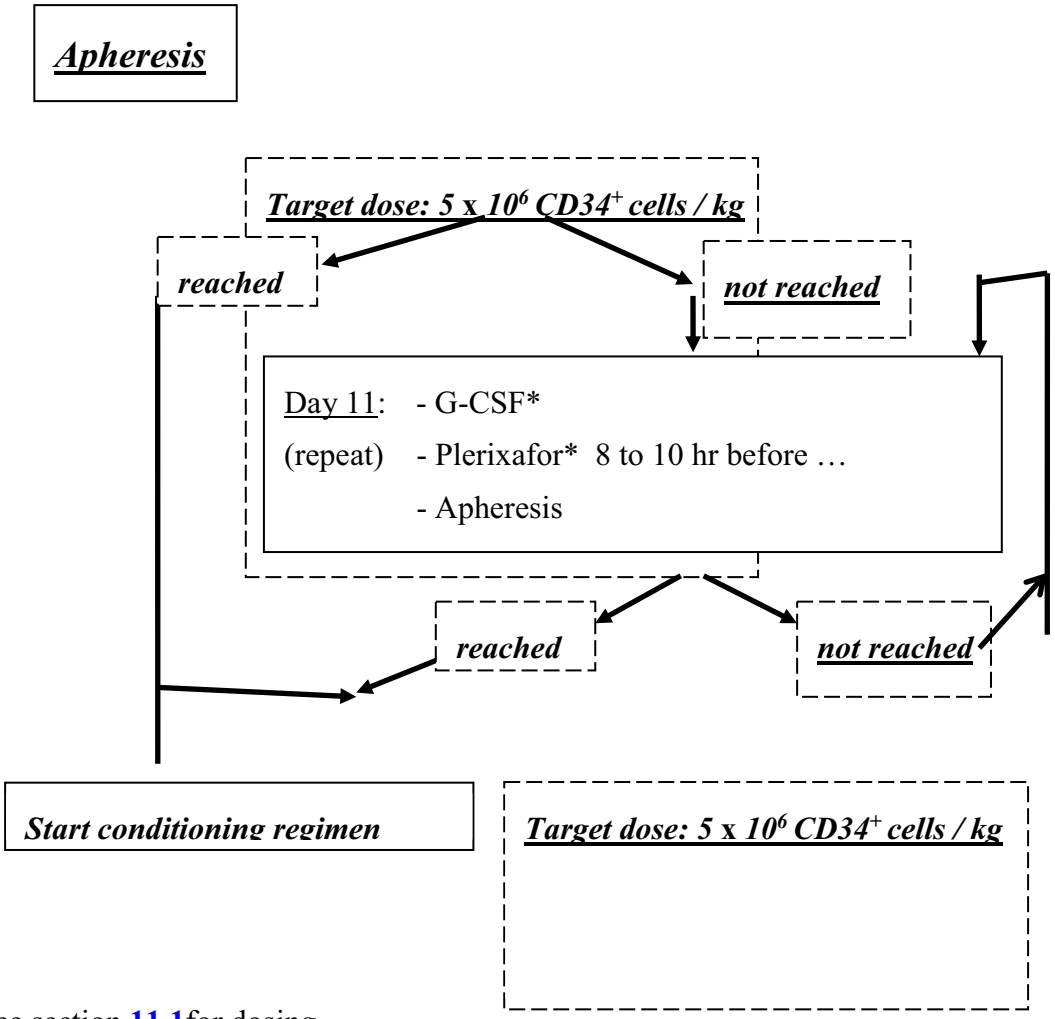
Day 10: - CBC starts daily
- peripheral CD 34 count if WBC > 5k
- if CD 34 >10 µl: start apheresis next day
- 8 to 10 hr prior to apheresis (at 10 pm to 12 MN)

↓

Start Apheresis

* See section **11.1** for dosing

10.7.3 Apheresis



* See section **11.1** for dosing

10.7.4 Conditioning and transplant

Conditioning regimen	- Day -2 - Melphalan 200 mg/m^2 - Dexamethasone 20 mg Day -1: - Dexamethasone 20mg
AHCT	Day 0: - AHCT: minimum 2 x 10^6 $CD34^+$cells/kg
	Day +1: - Dexamethasone 20mg Day +5: - G-CSF 5µg/kg q day until ANC > 1000/mm^3 for 2 days

10.8 EVALUATION FOLLOWING AHCT:

Day/ Week / Month	**Apheresis Blood Draw**	Study	**Where ?**	**What?**	**Handling**
At registration: *2 liter research apheresis*					
	Ap.	Th1.rapa culture	*DTM Cell Proc. Sect.*	apheresis bag storage	
	Ap.	Th1.rapa culture	*DTM Cell Proc. Sect.*	autologous serum per DTM	
	Bl.		Hakim Lab: 12 C216	3 red top tubes	
	Bl.	Cytokine evaluation (long-term)	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 7 ml SS Tube	Immediate centrifugation and freezing
Baseline pre 1st AHCT	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
1st AHCT					
Day +14:	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
Day +21:	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
Day +28:	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
2 months:	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
3 months:	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	

Day/ Week / Month	**Apheresis Blood Draw**	Study	**Where ?**	**What?**	**Handling**
Baseline pre 2nd AHCT	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
pre 2nd AHCT					
Day +14 (2nd)	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
Day +21 (2nd)	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
Day +28 (2nd)	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
2 months (2nd)	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	
3 months (2nd)	Bl.	Immune reconstitution	Hakim Lab: 12 C216	2 CPT 8ml tubes 1 10ml Green Top	

11 APPENDIX 2: PROTOCOL FOR AUTOLOGOUS HPCS COLLECTION BY APHERESIS OF FILGRASTIM-STIMULATED PATIENTS WITH MULTIPLE MYELOMA

11.1 MOBILIZATION OF CD34 CELLS WITH G-CSF AND PLERIXAFOR

Patients with myeloma will undergo mobilization with filgrastim (Neupogen, Amgen) and plerixafor (Mozobil, Genzyme). The filgrastim will be administered as a single daily dose in a dose range of 10-15.9 ug/kg/day subcutaneously for 5-6 days (see Table below for sliding-scale filgrastim dose schedule). The filgrastim doses for days 1-4 may be given at any time of day. Filgrastim administered on day 5, and if necessary day 6, will be combined with a concurrent dose of plerixafor 240 mcg/kg subcutaneously.

On days 5 and 6, both drugs must be given at 2:00 AM, approximately six hours prior to starting apheresis.

Predictable side effects of filgrastim, including headache, bone pain, and myalgia, will be treated with acetaminophen or ibuprofen.

11.1.1 Filgrastim administration

Filgrastim will be administered according to a vial-based algorithm to reduce wastage and increase the total filgrastim dose given to lighter weight donors in order to improve CD34 yields.[1]

Subject Weight	Total Filgrastim Dose	Dose /kg (range)
38 - 48 kg	600 mcg	(12.5 to 15.8 mcg/kg)
49 - 56 kg	780 mcg	(13.9 to 15.9 mcg/kg)
57 - 60 kg	900 mcg	(15.0 to 15.8 mcg/kg)
61 - 67 kg	960 mcg	(14.3 to 15.7 mcg/kg)
68 - 108 kg	1080 mcg	(10.0 to 15.9 mcg/kg)
≥ 109 kg	1200 mcg	(11.0 or less)

11.1.2 Plerixafor Administration

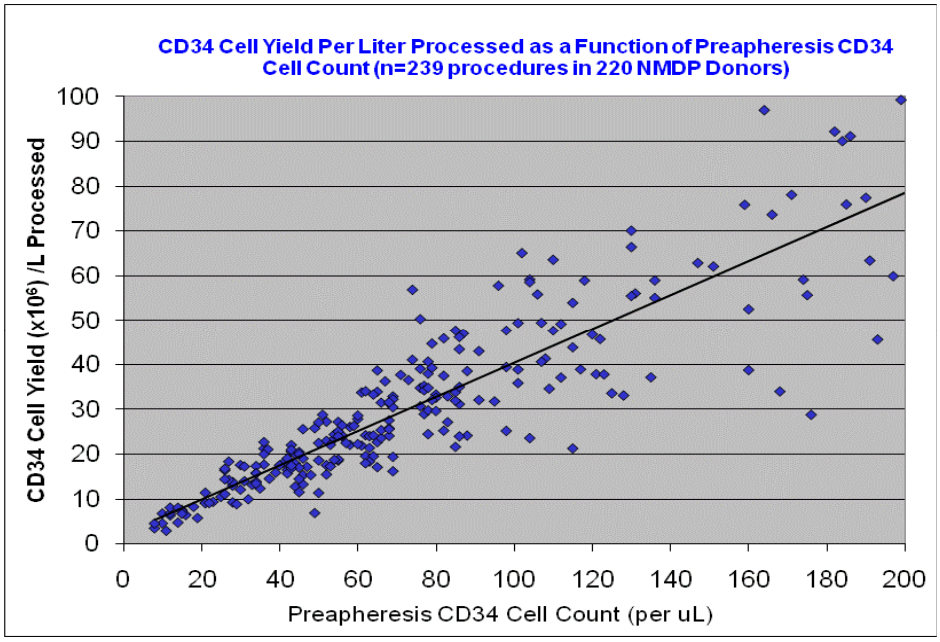
Subject Weight	Total Plerixafor	Dose (range)
≤ 90 kg	calculate dose	240 mcg/kg
> 90 kg	24,000 mcg (flat dose, equal to one full 1.2-mL vial)	

11.2 HPCS COLLECTION BY APHERESIS

Peripheral blood stem cell (HPCS) collection will be performed in the Dowling Apheresis Clinic of the Department of Transfusion Medicine (DTM). A DTM physician is within the immediate vicinity of the procedure or available within one minute by pager. The minimum CD34 dose that must be collected in order to proceed with a single autologous transplantation is 2 x 10^6/kg. When feasible, based on the mobilization response of the donor, a higher dose of 5 x10^6 CD34 cells/kg will be targeted, to permit more than one cycle of high dose chemotherapy with autologous stem cell rescue.

The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis, based on peak CD34 cell mobilization response to filgrastim and optimum and minimum CD34 cell dose needed (see graph below). Volume processed will range from 12 to 25 liters per procedure for 1 to 3 consecutive daily procedures, not to exceed a total of 75 liters over 3 days.

Collections will be performed with use of a dual-access continuous-flow apheresis device (Spectra Apheresis System, Caridian). Most donors will require a central double lumen apheresis catheter. Donors will receive continuous intravenous calcium prophylaxis to prevent citrate toxicity during apheresis, in accordance with standard DTM policies.



CD34 cell yield per liter processed as a function of pre-apheresis CD34 cell count in 220 NMDP donors.

[1]Vasu S, Leitman SF, Tisdale JF, et al. Donor demographic and laboratory predictors of allogeneic peripheral blood stem cell mobilization in an ethnically diverse population. Blood 2008;112:2092-100.

12 APPENDIX 3: EXPERIMENTAL TRANSPLANTATION AND IMMUNOLOGY BRANCH PRECLINICAL SERVICE POLICY FOR SAMPLE HANDLING

12.1.1 Storage/Tracking in the Preclinical Development and Clinical Monitoring Facility (PDCMF)

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

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

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

Samples are stored in locked freezers. All samples will be labeled solely with a bar code (which includes the date, and serially determined individual sample identifier). The key will be available to a restricted number of ETIB investigators and associate investigators on the protocol. Coded samples will be stored frozen at -20 to --80° or liquid nitrogen vapor phase according to the stability requirements under the restricted control of the PDCM Facility of ETIB. These freezers are located onsite at the Preclinical Service laboratory (12C216) or in ETIB common equipment space (CRC/3-3273).

Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol in order to be used (1) for research purposes associated with protocol objectives for which the samples were collected, or (2) for a new research activity following submission and IRB approval of a new protocol and consent, or (3) for use only as unlinked or coded samples under the OHSRP Exemption Form guidelines stipulating that the activity is exempt from IRB review. Unused samples must be returned to the PDCMF laboratory.

Samples, and associated data, will be stored permanently unless the patient withdraws consent. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the PDCMF laboratory.

These freezers are located onsite at the PDCMFPreclinical Service laboratory (12C216) or in ETIB common equipment space (CRC/3-3273).

12.2 PROTOCOL COMPLETION/SAMPLE DESTRUCTION

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

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

The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

13 APPENDIX 4: DATA COLLECTION ELEMENTS REQUIRED BY PROTOCOL

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

13.1 PATIENT ENROLLMENT

- Date of birth, age, gender, race, ethnicity
- Height
- Weight
- Performance Status
- Date of original diagnosis (only need year of diagnosis)
- Stage at diagnosis ISS or Durie-Salmon
- Risk category
- Baseline disease assessment
- Sites of disease at diagnosis and study entry
- Tumor Histology and date of confirmation
- Cohort
- Date of Informed Consent signature, consent version and date of registration
- Baseline History/Physical
- Baseline Symptoms (at the time of T cell infusion)
- Prior therapy (only need regimen and year of prior therapy)
- Prior surgery (only need year of surgery; bone marrow procedures not needed)

13.2 PRIOR AND CONCURRENT THERAPY WITH AUTOLOGOUS TRANSPLANT DOCUMENTATION

- For the following, need only name of regimen or transplant (not doses; year as date)
    - Dates, regimen and number of cycles of induction therapy
    - Date, regimen, collection data of stem cell mobilization / collection
    - Dates, regimen, engraftment data, for each AHCT

13.3 STUDY DRUG ADMINISTRATION AND RESPONSE TO THERAPY

- Th1.rapa generation data: apheresis yield, yield
- Date Th1.rapa infusion
- Dose level, actual dose, schedule and route given

- Separate listing for all infusion reactions (within 48 hrs of the Th1.rapa cell infusion)
- Response assessment for each restaging performed

13.4 LABORATORY AND DIAGNOSTIC TEST DATA

- All Clinical laboratory results done at screening and until day 100 post Th1.rapa infusion with the following exceptions:
  - All clinical laboratory and diagnostic tests done after day 100 that support a possible, probable or definite diagnosis of autoimmunity, infection or secondary malignancy and those done to document a change in grade and the end of these adverse events.
  - HLA, KIR and other NK receptors data.
  - Serologies-CMV, HSV, EBV, toxoplasmosis, adenovirus (patient and donor)
  - TTV data

13.5 ADVERSE EVENTS

Please see section **5.1.1** Adverse Event Reporting Recording

13.6 CONCOMITANT MEASURES

- List of any immunosuppressive medications or anti-cancer drugs
- Other therapy for recorded adverse events

13.7 CLINICAL EVALUATION

- For the first 3 months following Th1.rapa cell infusion
  - Weekly visit for the first 2 weeks, then every 2 to 4 weeks (record only yes or no to visit performed)
  - Then twice monthly visits with weekly interim history by phone or e-mail
- For the rest of the first year, visits every one to two months

13.8 TUMOR RESPONSE AND MEASUREMENTS

- Restaging studies performed at protocol specified time points and as clinically indicated (M-spike are not required to be manually entered).
- Determination of Persistent/Progressive Disease
- Schedule:
  - Prior to 1st AHCT (suggested only)
  - At 3 months following 1st AHCT (suggested only)
  - The required schedule is as follows:
  - Within 14 days preceding the Th1.rapa cell infusion
  - Then, following Th1.rapa cell infusion:
  - At 1 month (optional if research team indicates) and at 3 months (+ / - 7 days),
  - Then every 3 to 6 months for 3 years, unless earlier clinical suspicion of disease progression,

- Then every 4 to 8 months thereafter for 2 years, unless clinical suspicion of disease progression

13.9 OFF STUDY

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

14 APPENDIX 5: PROBLEM REPORT FORM

<table>
<tr><td>NCI Protocol #:</td><td>Protocol Title:</td></tr>
<tr><td></td><td>Report version: (select one)
____Initial Report
____Revised Report
____Follow-up</td></tr>
<tr><td>Site Principal Investigator:</td><td></td></tr>
<tr><td>Date of problem:</td><td>Location of problem: (e.g., patient’s home, doctor’s office)</td></tr>
<tr><td colspan="2">Who identified the problem? (provide role (not name of person): nurse, investigator, monitor, etc…)</td></tr>
<tr><td>Brief Description of Subject (if applicable)
(Do NOT include personal identifiers)</td><td>Sex: ___ Male ___ Female Age:
___ Not applicable (more than subject is involved)</td></tr>
<tr><td colspan="2">Diagnosis under study:</td></tr>
<tr><td colspan="2">Name the problem: (select all that apply)
[ ] Adverse drug reaction
[ ] Abnormal lab value
[ ] Death
[ ] Cardiac Arrest/ code
[ ] Anaphylaxis
[ ] Sepsis/Infection
[ ] Blood product reaction
[ ] Unanticipated surgery/procedure
[ ] Change in status (e.g. increased level of care required)
[ ] Allergy (non-medication)
[ ] Fall
[ ] Injury/Accident (not fall)
[ ] Specimen collection issue
[ ] Informed consent issue
[ ] Ineligible for enrollment
[ ] Breach of PII
[ ] Tests/procedures not performed on schedule
[ ] Other, brief 1-2 word description: _____</td></tr>
</table>

Detailed Description of the problem: (*Include any relevant treatment, outcomes or pertinent history*):
***Is this problem unexpected?** *(see the definition of unexpected in the protocol))* __YES __NO **Please explain:**
***Is this problem related or possibly related to participation in the research?** __YES __NO **Please explain:**
***Does the problem <u>suggest</u> the research places subjects or others at a greater risk of harm than was previously known or recognized?** __YES __NO **Please explain:**
Is this problem? (*select all that apply*) [ ] An Unanticipated Problem* that is: [ ] Serious [ ] Not Serious [ ] A Protocol Deviation that is: [ ] Serious [ ] Not Serious [ ] Non-compliance **Note if the 3 criteria starred above are answered, "YES", then this event is also a UP.*
Is the problem also *(select one)* [ ] AE [ ] Non-AE
Have similar problems occurred on this protocol at your site? __YES __NO **If "Yes", how many? ____ Please describe:**
Describe what steps you have already taken as a result of this problem:
In addition to the NCI IRB, this problem is also being reported to: (*select all that apply*) [ ] Local IRB [ ] Study Sponsor [ ] Manufacturer : _____ [ ] Institutional Biosafety Committee [ ] Data Safety Monitoring Board [ ] Other: _____ [ ] None of the above, not applicable

INVESTIGATOR'S SIGNATURE:	**DATE:**