TITLE: A Randomized Phase II Study of Autologous Stem Cell Transplantation with Tadalafil and Lenalidomide Maintenance with or without Activated Marrow Infiltrating Lymphocytes (MILs) in High Risk Myeloma

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This study will be conducted in compliance with the protocol and all other applicable regulatory requirements.

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PROTOCOL APPROVAL PAGE

Signature of Principal Investigator

A Randomized Phase II Study of Autologous Stem Cell Transplantation with Tadalafil and Lenalidomide Maintenance with or without Activated Marrow Infiltrating Lymphocytes (MILs) in High Risk Myeloma

I, the undersigned, have reviewed this protocol and I agree to conduct this protocol in accordance with all applicable laws and requirements, and any conditions required by a regulatory authority and/or Institutional Review Board (IRB).

I understand that the protocol may not be modified without written approval of the sponsor. All changes to the protocol must be submitted to the applicable regulatory authority and IRB, and must be approved by the IRB prior to implementation except when necessary to eliminate immediate hazards to the subjects or when the change(s), as deemed by the sponsor, involve(s) only logistical or administrative changes. Documentation of IRB approval must be sent to the sponsor immediately upon receipt.

Signature and date: _____

Name and Title: _____

Address: _____

_____

Telephone number: _____

Site/Center Number (if available): _____

TRIAL SYNOPSIS

Patient Population:

Patients with active myeloma (Durie-Salmon Stage II/III) that have high risk features, have completed induction therapy and are eligible for an autologous peripheral stem cell transplant.

High risk features are defined by any **one** of the following criteria:

- High risk chromosomal translocations by FISH: t(4;14), t(14;16), t(14;20), del(17p), 1p del, 1q amplification;
- MyPRS GEP-70 high risk signature;
- LDH at baseline >300U/L;
- Relapse within 12 months from prior therapy.

Number of Patients:

Will treat a total of 90 evaluable patients. More patients may be consented such that the target of 90 evaluable patients can be met. There will be a 2:1 randomization of patients to MILs vs no MILs.

Study Objectives:

Primary Objective

- Progression free survival.

Secondary Objective

- Toxicity;
- Overall survival;
- Subset analysis of immune responsiveness:
  - Quantification of anti-myeloma immunity;
  - Validation of immune responsive phenotype.

Eligibility Criteria:

Inclusion

- Patient age 18 - 80 years old;
- Patients with active myeloma requiring systemic treatment;
- Newly diagnosed patients. Relapsed myeloma patients that have not previously had a transplant;
- Patients who meet the criteria for high-risk disease;
- Measurable serum and/or urine M-protein from *prior to induction therapy* documented and available. A positive serum free lite assay is acceptable; ECOG performance status of 0 – 2 (see Appendix C).
- Meet all institutional requirements for autologous stem cell transplantation;
- The patient must be able to comprehend and have signed the informed consent;
- Patients must have had > than PR after last therapy in order to proceed to transplant (MILs can be harvested prior to treatment)

Exclusion

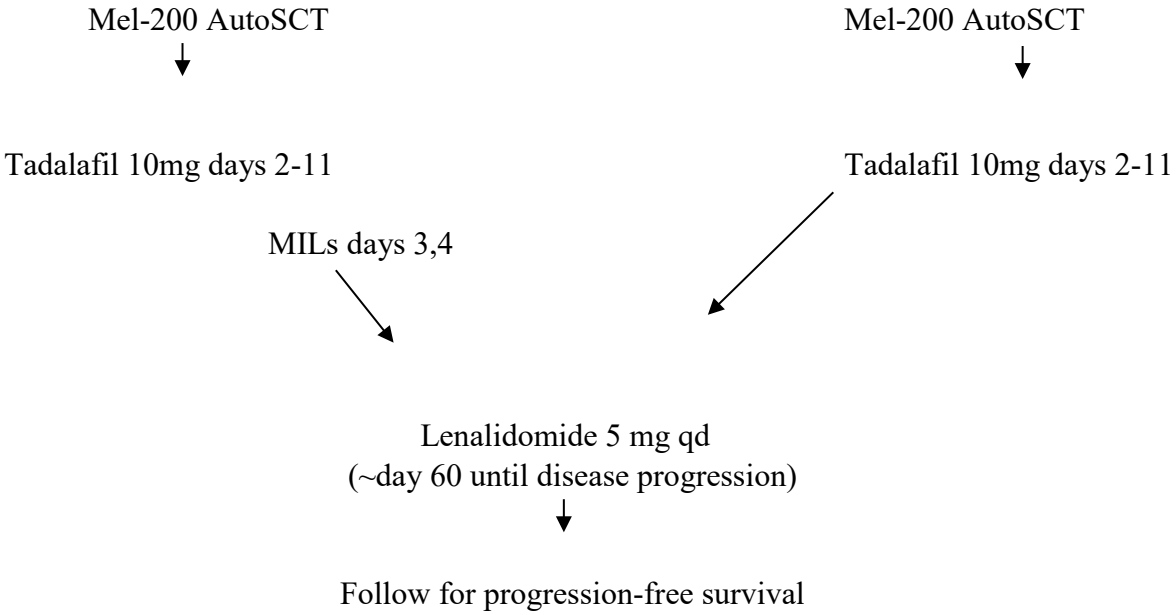
- Diagnosis of any of the following cancers:
  - POEMS syndrome (plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, monoclonal protein [M-protein] and skin changes);
  - Non-secretory myeloma (no measurable protein on Serum Free Lite Assay);
- Diagnosis of amyloidosis;
- Previous hematopoietic stem cell transplantation;
- Use of corticosteroids (glucocorticoids) within 21 days of bone marrow collection;
- Use of any myeloma-specific therapy within 21 days of bone marrow collection;
- Infection requiring treatment with antibiotics, antifungal, or antiviral agents within seven days of registration;

- Participation in any clinical trial within 21 days of bone marrow collection involving an investigational drug or device;
- History of malignancy other than multiple myeloma within five years of registration, except adequately treated basal or squamous cell skin cancer;
- History of an autoimmune disease (e.g., rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosis) requiring active systemic treatment. Hypothyroidism without evidence of Grave's disease or Hashimoto's thyroiditis is permitted.
- HTLV 1 or 2 positive;
- Contraindication to phosphodiesterase-5 inhibitors (e.g. currently on nitrates).

SCHEMA

MILs Bone Marrow Harvest

Randomization (2:1)



STUDY OVERVIEW

This randomized Phase II clinical study is designed to examine the clinical efficacy of antiCD3/CD28 activated marrow infiltrating lymphocytes (aMILs), an activated, autologous bone marrow derived T cell product, in the study of myeloma patients with high risk disease undergoing their first autologous bone marrow transplant. Additional screening will be performed on those eligible patients to determine whether they meet the criteria for exclusion based on immune unresponsiveness. This will be accomplished by analysis of blood that will be sent to the Borrello Lab and run for a panel of parameters. The PI or patient will be informed of these results.

A bone marrow aspiration will be performed to collect ~200ml of marrow. During the *in vitro* expansion process, T cells will be activated and expanded *ex vivo* by co-stimulation with anti-CD3 and anti-CD28 monoclonal antibodies covalently attached to super-paramagnetic microbeads. Patients will be treated with standard high-dose chemotherapy regimen for multiple myeloma consisting of single agent melphalan (200mg/m^2). Patients will then receive peripheral blood stem cells. For patients randomized to MILs arm, activated MILs will be infused on two consecutive days between days 2 and 5 following the stem cell infusion. Because of the potential negative impact of G-CSF on T cell trafficking to the bone marrow, patients will not receive post-transplant G-CSF. Tadalafil will be administered to all patients from day 2- 11post-transplant. The rationale is to both reduce the immune-suppressive myeloid-derived suppressor cells (MDSCs) as well as to increase the bone marrow infiltration of MILs which will hopefully increase the anti-tumor efficacy of the adoptive T cell therapy approach. Lenalidomide will be started as per standard of care around day 60.

The primary objective of the study is progression free survival. Additional secondary endpoints in the study include: overall survival and toxicity of MILs.

1. STUDY OBJECTIVES

1.1 Primary Objective

Determine the progression free survival (PFS) of autologous stem cell transplant (ASCT) alone vs ASCT plus MILs.

1.2 Secondary Objectives

1.2.1 Evaluate Toxicity

Toxicities according to the NCI Common Toxicity Criteria will be tabulated by body system, grade, and attribution of causality only for those felt to be related to MILs. Expected transplant related toxicities will not be recorded.

1.2.2 Evaluate Overall Survival

Patients will be monitored for progression/relapse on Days 60, 180, and 360, and as clinically indicated. Following one year follow-up, patients will be followed quarterly for the next five years.

1.2.3 Determine Immune Responses

Immune responses will be determined only in a subset of patients in certain sites for whom samples can be obtained.

1.2.4 Anti-Tumor Immune Responses

Lymphocytes from patients will be collected and banked at several time points during the study. Tumor specific anti-tumor immunity will be determined by analysis of samples obtained throughout the study.

2. BACKGROUND

2.1 Multiple Myeloma

Multiple myeloma is a plasma cell dyscrasia that is the most common cancer of the bone marrow.[1, 2] The American Cancer Society estimates that over 14,000 new cases of multiple myeloma will occur and that over 10,000 people will die of this disease in the United States in 2002.[3]

Multiple myeloma is most often diagnosed in middle aged and elderly individuals. The most common sites of disease are the bone and bone marrow. Malignant plasma cells arise from clonal

expansion and accumulate in the bone marrow in masses known as plasmacytomas. These plasma cells produce large amounts of monoclonal immunoglobulins, most commonly IgG (50-60%) and IgA (20-25%) and occasionally IgD, IgM and IgE.[4] Patients often suffer from bone pain and skeletal fragility.[5] Plasmacytomas are osteolytic in nature and often confined to the central skeleton, skull, and femur. Bone destruction is usually localized but can be present throughout the skeleton.[6]

The etiology remains unknown, but risk factors are thought to include chronic immune stimulation, autoimmune disorders, exposure to ionizing radiation, occupational exposure to pesticides or herbicides, occupational exposure to dioxin, and perhaps prolonged use of certain hair coloring products. [7,8]

The diagnosis is made using several criteria including results of radiographic skeletal survey, bone marrow examination, and measurement of serum and/or urine monoclonal protein (M-protein).[9,10] Patients are also classified by stage (Stage I, II, III) according to the status of bone lesions, hemoglobin, serum calcium, □-2 microglobulin, C-reactive protein and M-protein.[11] The M-protein is often used to monitor response to treatment via measurement of serum and/or urine analysis of Bence-Jones protein, the light chain component of the M-protein.

Multiple myeloma is a largely incurable disorder and most patients will die of their disease. A variety of chemotherapy agents have been used to treat the disease but few patients experience long-term disease-free survival with current therapeutic approaches. There is an urgent need for more effective therapies to treat this challenging disease.

2.2 Current Therapies for Multiple Myeloma

Treatment for multiple myeloma is dependent on the stage of the disease. Patients with Stage I disease are often monitored without treatment. Patients with Stage II and III disease are usually treated with chemotherapy until a response is achieved. A variety of different combination chemotherapy regimens have been developed in an attempt to improve on these results. Unfortunately, regardless of the type of initial treatment, the disease will recur and the 5-year survival is less than 30%.[12,13]

Dose intensification using high-dose chemotherapy followed by autologous stem cell transplantation has recently been used to increase the response rate and improve the outcome of patients with multiple myeloma.[14-25] In 1996, the French Myeloma Intergroup reported the results of a randomized clinical trial, which compared high-dose chemotherapy supported by autologous bone marrow support to conventional chemotherapy in patients with previously untreated multiple myeloma.[21] The study demonstrated the superiority of high-dose therapy in terms of response rate, event-free survival and overall patient survival. This pivotal study has led to the widespread use of high-dose chemotherapy with autologous stem cell support as standard of care in multiple myeloma patients with good performance status.

More recently, the addition of lenalidomide maintenance in two multi-center randomized trials has resulted in a significant improvement in progression free survival from 23 months with no maintenance to 45 months with maintenance[26, 27]. These data confirm the difficulty of eradicating the disease. Interestingly, the benefits observed with chronic lenalidomide are likely related to two attributes, continuous suppression of the malignant clone and immunomodulation resulting in priming of myeloma-specific immunity.

In the allogeneic transplant setting, long-term responses have been demonstrated. However, this treatment is associated with severe graft versus host disease (GVHD) and substantial mortality, which has limited its use.[28] However, the presence of long-term remissions in patients developing GVHD points to an immune-mediated response.

A major goal of newer studies has been to increase the overall efficacy of autologous stem cell transplantation without added toxicity. Clearly, the ability to impart a myeloma-specific immune response without the toxicity seen with allogeneic transplants offers significant appeal. Recent studies attempting to utilize vaccine approaches alone or in combination with adoptive immunotherapy have shed significant light into the potential efficacy of these approaches. More importantly, these studies underscore the profound limitations of the current interventions and enabled the development of novel strategies with greater anti-tumor specificity.

2.3 Overview of Cell-mediated Immunity

The human immune system is made up of many kinds of cells responsible for eliminating harmful invaders such as viruses or cancer from the body. One type of lymphocyte, the T cell, plays a central role in orchestrating most immune responses. T cells become activated when they recognize antigens, specific elements of microbes or tumor cells, as foreign to the body. This occurs when antigens are taken up, processed and presented by an antigen-presenting cell (APC) to a molecular complex on the T cell. This molecular complex contains the T cell receptor (TCR) associated with the CD3 signaling complex. [28]
The primary signal for activating a T cell takes place when the TCR expressed on its surface binds to a processed antigen present on the surface of an APC. Each individual T cell only expresses a single TCR capable of recognizing a specific antigen. However, the many billions of T cells found in the human body express millions of different TCRs, thereby enabling recognition of millions of distinct antigens. Only a specific T cell that recognizes a particular antigen will become activated during a normal immune response.

APCs must deliver a second signal in order to activate T cells. This co-stimulatory signal occurs when receptors on APCs bind to CD28 receptors on T cells. Activation of T cells takes place when APCs bind to the TCR/CD3 complex and CD28 receptor. These activated T cells are exquisitely sensitive to further stimulation and also secrete a variety of chemical messengers called cytokines. This process further augments the immune response both by driving continued activation and proliferation of T cells and recruiting and stimulating other cells of the immune system. This cascade of events ultimately leads to destruction of pathogens such as tumor cells and viruses.

2.4 Immune Defects in Patients with Cancer Including Multiple Myeloma

The inability of a patient's own immune system to respond to and control cancer may be due to a number of problems. Defects in both the afferent (responding) as well as efferent (acting) arms of the immune system are well documented in cancer patients. Deficits in the afferent arm of immunity include zeta chain defects in the TCR, which contribute to signaling problems in T cells. In addition, patients with hematological malignancies including chronic lymphocytic leukemia and multiple myeloma demonstrate significant narrowing of the broad spectrum of T cell receptors present in healthy individuals.[29-31] This narrow T cell receptor repertoire may limit the patient's ability to recognize and respond to tumor cells as well as other pathogens. This may contribute not only to cancer progression, but also to the infections that are often observed in patients with hematological malignancies. These defects are both a result of the malignancy itself as well as cytotoxic therapy that can damage T cells. Additionally, ineffective induction of CD40L (CD154) on T cells has been demonstrated in cancer patients.[32] Without CD40L signaling, APCs are not capable of being activated or presenting antigen to T cells. Poor co-stimulation by APCs due to non-responsive elements or defects in the co-stimulatory pathway has been observed in cancer patients.[28] Problems with the effector arm of the immune system in cancer patients include the presence of relatively low numbers of cytotoxic T lymphocytes (CTL), which are required to kill the tumor cells. Additionally, some cancers including multiple myeloma produce cytokines that inhibit the function of normal T cells or APCs.[33] The deficits in immunity may limit the ability of the patients' own T cells to mount an effective immune response to their own cancers.

2.5 Rationale for Immunotherapy of Multiple Myeloma

Immunotherapy is one approach to improving the outcome of patients with multiple myeloma. As noted above, defects in the host's immune system are present in patients with cancer including multiple myeloma. These deficits are thought to play a role in the patient's inability to generate an anti-tumor response and control the disease. A variety of therapeutic approaches are now being developed that stimulate the patient's immune system. Several groups are using idiotype vaccines to stimulate T cell-mediated responses to the patient's tumor cells.[34,35] Using this approach, patients are typically treated with an autologous transplant followed by vaccination with their own idiotype, which is derived from their unique M-protein. Promising clinical results have been observed in some of these clinical trials. However, many patients have weakened immune systems after the transplant and have been unable to respond to the vaccine[36]

We have recently completed a clinical study utilizing autologous tumor vaccines in the autologous transplant setting. In this study, newly diagnosed patients undergo a bone marrow harvest to collect autologous tumor that will be combined with the K562/GM-CSF producing bystander cell line in the final vaccine formulation [37]. Patients are administered the vaccine pre-transplant in an effort to prime tumor-specific T cell responses *in vivo.* The lymphocytes are then collected and infused at the time of transplantation in an attempt to impart an early anti-tumor effect. The posttransplant vaccinations start 6 weeks post-transplant and are administered every 3 weeks for a total of 8 post-transplant vaccines. The rationale for starting vaccinations this early post-transplant is based on murine data demonstrating the existence of an early endogenous tumor-specific

lymphocyte expansion that can then be maintained with vaccinations in the post-transplant setting [38]. While this study shows evidence of the generation of tumor-specific T cell responses, the degree of lymphopenia post-transplantation is greater than initially predicted with absolute CD4 numbers considerably below normal up to one year post-transplant. One attempt to increase vaccine efficacy is the ability to enhance T cell reconstitution and utilize vaccines at a time when maximal T cell responsiveness can be guaranteed.

Recently, several investigators have documented the potent anti-tumor effects of donor lymphocyte infusions (DLI) administered to patients who relapse after allogeneic stem cell transplantation.[39-43] Unfortunately, the limited availability of suitable donors coupled to the high incidence of GVHD observed with DLI has significantly limited its therapeutic application.

Investigators have documented that T cells with anti-tumor activity are present in the blood of patients with multiple myeloma.[44] If sufficient numbers of the patient's own T cells could be activated and expanded, they could be used in combination with an autologous stem cell transplant. This would provide a potentially safer therapeutic alternative to DLI. Patients would avoid the risks of GVHD as well as the substantial morbidity and mortality (up to 40%) that has been documented in multiple myeloma patients undergoing allogeneic stem cell transplantation. [45]

Additionally, recent clinical data provide further rationale for the administration of autologous T cells in patients undergoing autologous bone marrow transplantation. Several clinical studies have documented improved therapeutic outcome in patients with multiple myeloma (as well as nonHodgkin's lymphoma, breast cancer, and ovarian cancer), who experience more rapid and/or complete recovery of their peripheral blood lymphocytes after autologous stem cell transplantation.[46-48]

2.6 Rationale for the Use of CD3xCD28 Bead-Activated T Cells

Carl June and colleagues developed technology to activate T cells of the immune system outside of the body (*ex vivo*)[49]. This procedure is based on the roles of the CD3 signaling complex and CD28 receptor in the activation of T cells. In the manufacturing process, T cells are stimulated *ex vivo* using monoclonal antibodies that bind to the CD3 and CD28 molecules expressed on the surface of T cells. The antibodies are attached to microscopic beads, thereby creating artificial APCs. As a result, a universal reagent can be developed to activate all T cells. Single beads, which coordinate CD3 and CD28 signals, optimize T cell activation and allow rapid expansion of T cells. Preclinical studies have shown that T cells can be generated *ex vivo* using these beads from patients with human immunodeficiency virus (HIV) or cancer. [50,51]

Studies have demonstrated that T cells can be activated and expanded more than one hundred fold in less than 10-12 days with anti-CD3/CD28 bead activation with a predominant expansion of CD4 over CD8 T cells. Further studies have shown that the patients' activated T Cells express high levels of CD154 (CD40L), CD137 (4-1BB) and other key effector molecules such as CD134 (OX40), CD54 (ICAM) as well as important receptors such as CD25 (IL-2 receptor). Finally, this process generates T cells that display a Th1 phenotype secreting high levels of IL-2 and

interferongamma that are known to play essential roles in activating both helper T cells as well as cytolytic T cells. These features demonstrate the ability to reverse tolerance in cancer patients and restore T cell responsiveness that may enable these T cells to restore anti-tumor immunity.

A number of independent clinical trials have been conducted in which patients have been treated with T cells activated *ex vivo* using a CD3/CD28 bead-based technology. T cells activated in this manner have previously been tested in patients undergoing a peripheral blood stem cell transplant for relapsed or refractory Non-Hodgkin's lymphoma[52]. T cells were collected prior to high-dose chemotherapy. Fourteen days following the peripheral blood stem cell infusion, activated and expanded T cells were administered. Three patients were treated at a median cell dose of 0.4×10^9, twelve patients were treated at a median cell dose of 1.6×10^9, and two patients were treated with a median cell dose of 9.8×10^9. Infusion related toxicities experienced by the two patients at the highest dose level included transient fever, dyspnea, rigors and pulmonary edema. Maximal responses included five patients with complete responses, seven patients with partial responses, and five patients with stable disease.

We recently completed a Phase I/II study in multiple myeloma in which 32 patients were administered anti-CD3/CD28 activated T cells. T cells were effectively expanded in all patients with an average fold-increase of 268 (± 101). T cells were then infused on day + 3 following an autologous stem cell transplant. Interestingly, in addition to the in vitro expansion, patients experienced an additional in vivo expansion reaching maximal expansion on day +21 that far exceeded the T cell numbers seen in a non-transplanted healthy individual. However, despite the feasibility, safety and evidence of both in vitro and in vivo expansion, the overall clinical response showed CR 6%, PR 34% with an overall response rate of 40% which is no better than standard autologous stem cell transplants. There were no significant toxicities related to the infusion of activated T cells and the majority of adverse events related to T-cells were mild in severity and included fever (19%), chills (17%), asthenia (10%), headache (10%), and nausea (10%).

2.7 Rationale for the Use of Activated MILs in Myeloma

From the above-mentioned clinical studies, we have shown the ability of this technology to effectively overcome the inherent unresponsiveness seen in T cell from tumor-bearing hosts and to expand upon anti-CD3/CD28 stimulation. This data also underscores a significant limitation of the polyclonal expansion - the lack of antigen specificity. To this effect, we have developed a strategy aimed at increasing the tumor specificity of this approach. Specifically, we discovered that marrow infiltrating lymphocytes (MILs) can be effectively activated and expanded with properties suggestive of an effector/memory population. More importantly, they possess several critical

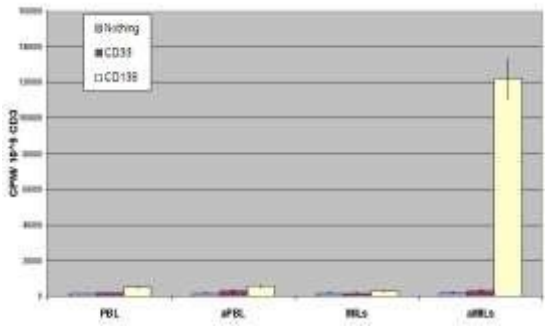


Figure 1: aMILs exhibit marked anti-myeloma activity. T cells from blood or marrow were either activated with anti-CD3/CD28 beads or left unstimulated. Tumor specificity was determined by incubating the cells either with autologous plasma cells (CD138), autologous non-malignant myeloid cells (CD33) or nothing and determining H-thymidine incorporation.

features required for effective anti-tumor adoptive immunotherapy:
1) they can be activated and expanded to reasonable numbers; 2) they demonstrate significant specificity against mature plasma cells; 3) the possess surface unique surface markers that increase their likelihood of trafficking to the marrow upon re-infusion; and 4) that possess a memory phenotype that increases their likelihood of persisting over time. As shown in **Figure 1**, whereas activated PBLs (aPBLs) failed to show measurable tumor specificity, activated MILs (aMILs) exhibited marked tumor reactivity. Interestingly, no reactivity was appreciated against normal hematopoietic elements. In addition to their significant activity against mature plasma cells, aMILs were also capable of significantly limiting the outgrowth of clonogenic myeloma precursors suggesting a broad range of tumor antigen recognition.

Another critical aspect of effective adoptive immunotherapy is the ability of T cells to traffic to the tumor microenvironment. SDF-1 (stromal derived factor -1) and its cognate chemokine receptor, CXCR4 are critical factors in cell trafficking in the marrow. We have shown that a significantly higher percentage of MILs express CXCR4 as compared to PBLs thus increasing the likelihood of trafficking of these cells to the appropriate compartment. [53]. To confirm this, we performed an experiment utilizing NOD/SCID mice. Mice were irradiated and challenged with the H929 myeloma cell line and then 18 days later either given activated MILs, activated PBLs or no T cells. The T cell doses used corresponded to doses ranging from 3 – 20 x 10^7 CD3/kg (doses easily achievable in humans). As shown below in **Figure 2**, activated PBLs imparted no measurable anti-tumor effect compared to no T cells whereas the mice receiving activated MILs demonstrated 100% survival with no evidence of detectable tumor. Furthermore, T cells were detectable in the marrows of mice having received aMILs whereas no T cells but CD138+ plasma cells were seen in the bone marrows of mice treated with HBSS or aPBLs. These findings confirm our in vitro data and suggest the overall efficacy and durability of this approach.

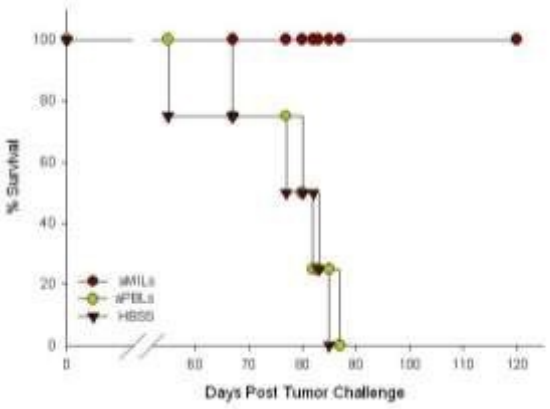


Fig 2: aMILs confer significant tumor-free survival advantage. NOD/SCID mice were challenged with the human myeloma cell line, H929. 18 days later they either received HBSS, aPBLs, or aMILs at doses ranging from 1-5 x 10^6 /mouse and were followed for tumor-free survival.

The first trial was conducted in patients that had never undergone an autologous stem cell transplant (ASCT) and that were not in a complete remission at the time of transplant. Patients underwent a standard Mel-200 ASCT with the infusion of aMILs on day +3. To monitor the ability of T cells to adoptively transfer vaccine-primed immunity, patients were administered the polyvalent pneumococcal vaccine conjugate, Prevnar (PCV) prior to MILs harvest and on day +21 post-transplant. We were able to grow MILs on all patients, the median T cell dose infused was 1.96 x10e9 total cells. We observed a 38% incidence of graft vs host disease which was mostly limited to the skin

2.8 Results from the First MILs Trial

and resolved spontaneously. No grade 3 or 4 toxicities were appreciated that could be related to the infusion of MILs. Interestingly, the median number of prior therapies for patients on this study was 2.3 (range 1-6). Considering that the greatest predictor of CR post-transplant is entering transplant in a CR[54] and that patients in CR were excluded from the trial, we feel the overall response rate seen of 72% with a CR rate of 27% was possibly better than anticipated in this pretreated relatively high risk patient population.

One of the major aspects of this study was the demonstration of persistence of MILs and evidence of induction of measurable myeloma-specific immunity. To accomplish this, we performed immune monitoring only within the tumor microenvironment utilizing MILs obtained at harvest and at various time points post-transplant. PCV immunity and tumor specificity is shown below in **Fig 3**. Patients showed evidence of PCV immunity with the harvest T cells (collected 2 weeks after the pre-transplant vaccine). This immunity was transferred post-transplant, peaked at 6 months but was still measurable one –year post-transplant. With regards to myeloma-specific immunity, there was no evidence of tumor specific immunity at harvest (as expected). However, measurable anti-tumor immunity was detectable in the post-transplant setting that peaked at 6 months but was still present at the 1-year

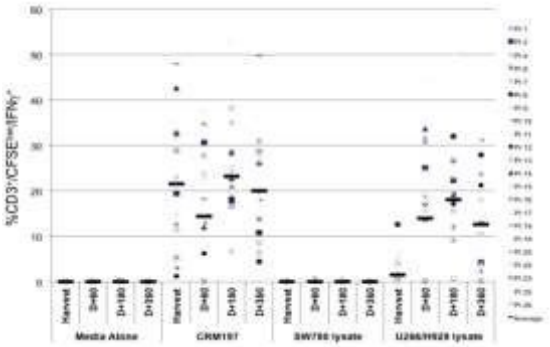
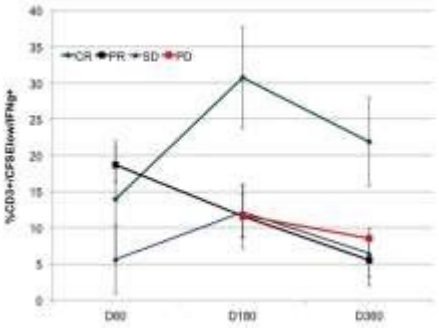


Fig 3: PCV and tumor specificity. BM was analysed at harvest and post-SCT. CRM-197 antigen specificity was appreciable early and preserved. Tumor specificity was generated and maintained over time.

Fig 4: Tumor specific immunity correlates with clinical response.

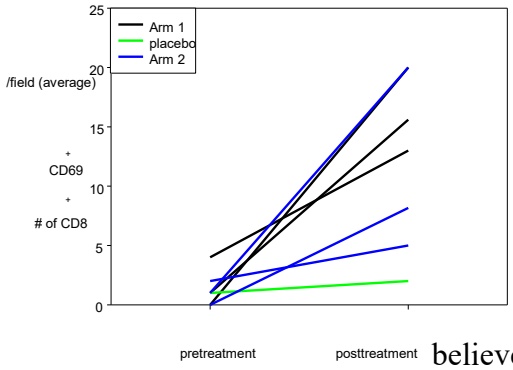
Tumor specificity of MILs was time-point. Taken together atients showed evidence of PCV determined at the indicated time immunity with the harvest T cells (collected 2 weeks after the pre-transplant vaccine). This immunity was transferred post-transplant, peaked at 6 months but was still measurable one –year post-transplant. With regards to myeloma-specific immunity, there was no evidence of tumor specific immunity at harvest (as expected). However, measurable antitumor immunity was detectable in the post-transplant setting that peaked at 6 months but was still present at the 1-year time-point. Taken together these data prove that MILs were able to impart a MM-specific immune response and that these cells persisted over time. We also observed a range in tumor-specific immunity and upon further investigation were able to show a direct correlation between the ability to achieve a significant response with the ability to enter into complete remission (**Fig 4**). This correlation was subsequently lost with patients that achieved a PR or less. Taken together, these data suggest that, at least in this study, achieving a CR was in part mediated by the ability to induce and maintain of a vigorous anti-myeloma immune response.



2.9 Phosphodiesterase-5 (PDE-5) Inhibition

A major obstacle to the achieving clinically meaningful results with immunotherapy is largely due to the multitude of immunosuppressive mechanisms in place in a tumor bearing-host. Myeloid derived suppressor cells (MDSCs) are one population associated with tumor-induced immunosuppression that has been implicated. It exerts its immunosuppressive function through the production of nitric oxide (NO) and up-regulation of arginase-1 (Arg-1). We have previously shown that the use of PDE-5 inhibitors such as tadalafil can significantly downregulate production of NO and Arg-1 and restore tumor-specific T cell immunity.[55]

There is also extensive data to suggest that improved clinical outcomes are associated with a higher degree of tumor infiltrating lymphocytes as shown in both colon and ovarian cancers. [56, 57] As such, strategies that increase the ability of T cells to infiltrate the tumor potentially increase the ability to augment anti-tumor efficacy. In our original murine studies, we observed an increased in anti-tumor efficacy when T cells were adoptively transferred in the conjunction with PDE5 inhibition. [55] This data was subsequently confirmed in a recent clinical trial in head and neck squamous cell carcinoma in which tadalafil (Cialis) or placebo was administered in the neo-adjuvant setting. We observed a significant increase in activated tumor infiltrating lymphocytes as defined as CD8+ CD69+ compared to the placebo treated group (**Fig 5).** We already believe that a major therapeutic benefit of MILs compared to peripheral T cells rests with their increased tumor specificity and increased tropism for the bone



marrow. However, we have also shown that granulocytic MDSCs in our earlier study correlated with worse clinical outcomes**.** As such, any strategy that could increase the ability of these tumor specific T cells to traffic to the tumor site immediately following T cell transfer would likely increase our therapeutic benefit of the study.

2.10 Rationale for Study Design

Current treatments for high-risk multiple myeloma are unsatisfactory. Data obtained from the Arkansas group utilizing a 70-gene expression panel has demonstrated that this subgroup fails to achieve a meaningful response despite high dose chemotherapy regimens such as Total Therapies 2 and 3 as manifest by depth of response, progression free survival or overall survival. [58, 59]

Fig 5. Tadalafil increases the percentage of activated TILs. Patients with head and neck cancer were treated with either tadalafil 10 or 20mg or placebo for 21 days. Tumor specimens obtained pre- and post-treatment were stained for CD8 and CD69.

Similar poor outcomes were also observed in the ECOG Lenalidomide high vs low-dose dexamethasone trial as well as in IFM studies. [60] Taken together, these results underscore the lack of effective therapies for this poor risk group. Interestingly, This GEP-70 is now commercially available as the MyPRS that can be run as a send-out test by Signal Genetics. Interestingly, it represents approximately 18% of newly diagnosed, untreated patients and this percentage has been shown to increase with subsequent relapses.

Other parameters associated with high risk disease and worse overall prognosis include poor risk chromosomal rearrangements identified by FISH such as t(4;14), t(14;16), t(14;20), del(17p) as well as ISS stage III and an elevated LDH at diagnosis. [61] In this protocol, we will extend the inclusion criterion to these other high-risk features. The rationale for this is based on the fact that we are attempting to demonstrate the clinical efficacy of this approach in a high risk disease setting, would also like to assure that the protocol can be enrolled in a relatively short period of time without requiring an extensive number of screen failures, and that with the randomized nature of the study we will be able to still determine the clinical efficacy of this approach. From a practical stand point, tertiary referral centers usually see patients at the time of consultation for transplant and most will not have had the GEP-70 profile done. This is further complicated by the fact that the induction regimens have increased their overall efficacy to the point that an increasingly larger percentage of patients are able to achieve a complete remission prior to transplant – a factor that makes obtaining GEP-70 even more difficult. However, FISH and ISS from diagnosis is generally known which will enable us to determine eligibility criteria for the patients.

The first MILs trials has shown a significantly high response rate for patients with several high risk features including ISS III and poor risk chromosomal translocations by FISH. Immunotherapy offers the possibility to kill tumors with mechanisms very different from those utilized by conventional cytotoxic chemotherapy. [62] Taken together, there is both significant biologic as well as clinical rationale for the proposed study.

Lenalidomide is included in the study for two major reasons: 1) two studies examining its role as maintenance in the post-transplant setting have demonstrated its significant clinical efficacy in terms of its ability to significantly increase progression free survival (PFS) in patients undergoing an autologous SCT; and 2) it has significant immunomodulatory effects including its ability to enhance T cell co-stimulation, [63] increase NK function, [64] and overall enhance tumor specific immunity. [65]

The PDE-5 inhibitor, tadalafil, is being used early post-transplant to increase the possibility of trafficking of the aMILs to the tumor site. We have previously shown that this drug was able to increase the therapeutic efficacy of adoptive T cell therapy in a murine model and also to increase the percentage of tumor infiltrating lymphocytes in a recently conducted head and neck cancer trial. As such, administration of tadalafil in association with the infusion of MILs should enhance their therapeutic efficacy.

3. PATIENT SELECTION

3.1 Eligibility Criteria for Enrollment

Patient eligibility for the study will be determined when patients are deemed eligible to proceed to transplant. To determine patient eligibility, evaluations for inclusion and exclusion criteria must be performed prior to patient registration. Patients who meet these criteria can then be registered on the trial. All evaluations for inclusion and exclusion criteria must be performed within 30 days prior to registration unless otherwise noted. Please refer to Patient Study Calendars, Appendix E.

At the time of study registration, patients will be **eligible** if they meet <u>all</u> of the following inclusion criteria:

- Previous diagnosis of multiple myeloma based on standard criteria as defined in Appendix A (*Diagnostic Criteria for Multiple Myeloma*). Tests need not be performed within 30 days of registration;
- Active myeloma as defined as the presence of CRAB criteria: hypercalcemia, renal insufficiency, anemia and/or bone disease;
- Measurable serum and/or urine M-protein from *prior to induction therapy* documented and available. A positive serum free lite assay is acceptable;
- Presence of at least <u>one</u> of the features defining high risk. These include:
  - High risk chromosomal translocations by FISH: t(4;14), t(14;16), t(14;20), del(17p), del(1p), amplification 1q.;
  - MyPRS GEP-70 high risk signature either from diagnosis or at time of registration for the study;
  - LDH > 300 U/L <u>at diagnosis;</u> ○ Relapse from prior therapy within 12 months.
- Patient age 18 - 80 years old;
- ECOG performance status of 0 - 2
- Life expectancy 6 months;

- Corrected serum calcium < 11 mg/dL, and no evidence of symptomatic hypercalcemia. (Corrected serum calcium is calculated by adding 0.8 mg/dL to the measured serum calcium for every 1 g/dL that the serum albumin falls below 4.0 g/dL.);
- Serum total bilirubin and SGPT (ALT) ≤ 2.0 times the upper limit of normal;
- Serum creatinine < 2.0 mg/dL;
- The patient must be able to comprehend and have signed the informed consent.

At the time of study registration, patients will be **ineligible** if any of the following exclusion criteria apply:

- Diagnosis of any of the following cancers:
  - POEMS syndrome (plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, monoclonal protein [M-protein] and skin changes); ○ Non-secretory myeloma (no measurable protein on Serum Free Lite Assay); ○ Plasma cell leukemia; ○ HTLV1 / HTLV2 positive.
- Diagnosis of amyloidosis;
- Previous hematopoietic stem cell transplantation. Patients can have had prior relapsed disease as long as they have never been previously transplanted;
- Known history of HIV infection;
- Use of corticosteroids (glucocorticoids) within 21 days of the MILs collection;
- Use of any myeloma-specific therapy within 21 days of the MILs collection;
- Systemic infection requiring treatment with antibiotics, antifungal, or antiviral agents within seven days of registration;
- Patients should be excluded if they are known to be positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection.
- Participation in any clinical trial, within four weeks prior to registration on this trial, which involved an investigational drug or device;
- History of malignancy other than multiple myeloma within five years of registration, except adequately treated basal or squamous cell skin cancer;
- Active autoimmune disease (e.g., rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosis) requiring active systemic treatment. Hypothyroidism without evidence of Grave's Disease or Hashimoto's thyroiditis is permitted;
- Known contraindication to phosphodiesterase-5 inhibitors (e.g. currently on nitrates).

3.2 Eligibility Criteria for Bone Marrow Transplant

Prior to transplant, patients will be **eligible** if they meet all of the following inclusion criteria:

- Myeloma specific therapy with a minimum of 3 cycles;
- Achieved at least a partial response (PR) to therapy
- Institutional criteria for and have institutional approval to undergo autologous peripheral blood stem cell transplantation;
- Females of child-bearing potential must have a negative serum HCG test and be willing to use effective contraception (i.e. a hormonal contraceptive, intra-uterine

device, diaphragm with spermicide, or condom with spermicide, or abstinence) up to Day 180.

Prior to transplant, patients will be **ineligible** if any of the following exclusion criteria apply:

 Evidence of spinal cord compression;

- Major organ system dysfunction including (but not limited to): New York Heart Association Class III or IV (Appendix D), pulmonary disease requiring the use of inhaled steroids or bronchodilators, renal, hepatic, gastrointestinal, neurologic, or psychiatric dysfunction which would impair patient's ability to participate in the trial; HIV infection.

3.3 Confirmation of Eligibility

Data collected from time of diagnosis, prior to induction therapy must be available for eligibility:

- M-protein results from the time of diagnosis, prior to induction therapy;
- ISS which includes -2 microglobulin and serum albumin;
- Myeloma FISH bone marrow results (if available);
- Baseline LDH (if available);
- Durie-Salmon Stage;
- Date of diagnosis;
- Duration of induction;
- Type of prior therapy(s); GEP-70 MyPRS (if available).

4. REGISTRATION ON STUDY AND RANDOMIZATION

At registration, patients will have the baseline evaluations listed in Section 7. Once eligibility requirements are met, the patient will proceed to bone marrow collection to obtain MILs.

Randomization will occur after the bone marrow has been collected for MILs expansion. The following criteria will be utilized:

- Newly diagnosed vs. relapsed;
- Presence of high risk GEP-70 signature. For patients with an indeterminate or unavailable GEP-70 profile, they will be assigned to the "low-risk" group.

5. MULTICENTER GUIDELINES

5.1 Registration Guidelines

Eligible patients will be entered on study centrally at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University by the Lead Study Coordinator.

To verify eligibility and coordinate MILs collection all sites should contact:

- lead study coordinator Dmitry Kololo (dkololo1@jhmi.edu)
- lead research nurse Laura Cucci, RN (lcucci1@jhmi.edu)
- Coordinating Center mailbox (onc-coordcnt@jhmi.edu)

The Registration Form and Eligibility Worksheet will be supplied to each participating site.

Following registration, patients are eligible to begin protocol treatment. Issues that would cause treatment delays should be discussed with Dr.Imus . If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5.2 Registration Process

To register a patient, the following documents should be completed by the site's Register Nurse or Study Coordinator and ***emailed to all the addresses provided above***:

- Registration Form;
- Signed patient consent form;
- HIPAA authorization form; Eligibility screening checklist;
- Copy of required screening tests.

5.3 Responsibilities

Protocol Chair

The Protocol Chair is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE) Reviewing data from all sites.

Coordinating Center

The Coordinating Center is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.

- Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

Participating Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP). Submitting data to the Coordinating Center.
- Registering all patients with the Coordinating Center by submitting patient registration form, and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
- Collecting and submitting data according to the schedule specified by the protocol.

6. BASELINE EVALUATIONS

Harvest Consent:

After the patient has been registered in the study at the time of initial consent, the following evaluations will be performed to determine the patient's baseline status. All tests must be completed within 30 (±_7 days) of the MILs bone marrow harvest:

- Complete clinical exam, including complete medical history, review of systems, ECOG performance status, vital signs, and physical examination.
- Current medications;
- Serum chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, ALT, AST, total bilirubin, alkaline phosphatase, albumin;
- CBC with differential and platelet count;
- HTLV-1, HTLV-2 (tests must all be negative).

The following myeloma labs should be obtained on the day of the MILs harvest:

- M-protein, serum:
  - Protein electrophoresis; ○ Immunofixation;
  - Freelite (sensitive assay for kappa and lambda light chains) only if most recent immunofixation result showed no evidence of M-protein.
- M-protein, urine (24 hour collection) for:

- Total protein; ○ Protein electrophoresis; ○ Immunofixation.
- Quantitative immunoglobulins (IgG, IgM, IgA);
- □-2 microglobulin;
- MyPRS GEP-70 from Signal Genetics (if not previously obtained)

Transplant Consent:
Prior to proceeding to autologous stem cell transplantation, patients should undergo the routine testing to meet the institution requirements for autologous stem cell transplantation for myeloma. This should include myeloma labs (SPEP, UPEP, quantitative immunoglobulins, serum free light chains)

7. MARROW INFILTRATING LYMPHOCYTES (MILs) COLLECTION

The bone marrow harvest will be performed to obtain the marrow infiltrating lymphocytes (MILs) required for the in vitro activation. The patient's treatment schedule is summarized in Appendix E, *Patient Study Calendar*. Patient should undergo MILs collection within 30 of consent. If outside the window, the infectious serologies need to be repeated.

7.1 MILs Collection Procedure

Each eligible patient will have approximately 200cc of bone marrow collected in syringes containing 2ml of 10,000 units/ml heparin under steady state conditions. The marrow will be collected in 60cc syringes and transferred into a 300ml transfer pack. Sedation and analgesia will be administered per standard institutional practices.

- Patients should also have 20ml of blood collected in heparinized tubes.
- 15ml of bone marrow should be collected in green top (heparinized tubes) For patients on the immune sub-study (Hopkins only), 100ml of blood will be collected in heparinized syringes instead of the 20ml and 20 ml of aspirate will be collected in a heparinized syringe instead of 15 ml in a green top tube.

The marrow and blood will be shipped overnight at room temperature between Monday and Thursday (NO PRODUCTS SHOULD BE SHIPPED ON FRIDAY) to:

Johns Hopkins Cell Therapy Lab
401 N Broadway
Weinberg Building, Rm 2450
Baltimore, MD 21287
Tel: 410 955 2354
Fax: 410 502 6749

8. MILs ACTIVATION PROCESS, CELL DOSE & PRODUCTION FORMULATION

8.1 MILs Activation Process

The MILs bone marrow product will undergo *ex vivo* activation and expansion of T cells in the Johns Hopkins Cell Therapy Laboratory (CTL). In the activation process, the T cells are activated and expanded by co-stimulation of T cells with anti-CD3 and anti-CD28 antibodies conjugated to super-paramagnetic microbeads (Dynabeads M-450 CD3/CD28 T). The super-paramagnetic microbeads are removed at the completion of the process. The product will not be released, unless acceptance criteria have been met.

8.2 Formulation Storage and Dose of Activated MILs T Cells

The activated MILs will be formulated in cryopreservation bags in a final volume of up to 50 ml. Each bag will contain aMILs formulated in a cryoprotectant consisting of Hetastarch [6% Hetastarch in 0.9% sodium chloride injection] supplemented with 2% Human Serum Albumin (HSA) and 5% dimethylsulfoxide (DMSO), The aMILs T Cell product will be frozen in a controlled-rate freezer until it reaches –80ºC. The product will then be stored in the vapor phase of a liquid nitrogen freezer at less than -135ºC.

8.3 Shipping and Storage of Activated MILs

Shipment of the aMILs will be coordinated between the Johns Hopkins CTL and the receiving site. Upon receipt, the product will be stored in the vapor phase of a liquid nitrogen freezer at less than 135^0 C.

9. MOBILIZATION AND COLLECTION OF PERIPHERAL BLOOD STEM CELLS

Following MILs collection, the patient may begin the mobilization regimen, which should be performed according to institutional standards.

9.1 Mobilization Regimen

The mobilization regimen will occur as per institutional standards. The procedure used for mobilization will be recorded.

9.2 Peripheral Blood Stem Cell Collection

Peripheral blood stem cell leukapheresis will be performed per standard institutional practice. Leukapheresis will be performed to obtain a minimum of 2.0 x 10^6 $CD34^+$ cells/kg. Standard leukapheresis will be performed according to institutional guidelines for the collection of peripheral blood stem cells.

Should patients fail to mobilize, they will be able to proceed as per institutional guidelines which could include: bone marrow harvest or mobilization with other agents including but not limited to plerixafor.

9.3 Nomenclature for Numbering of Days

The day of infusion of the peripheral blood stem cells is called Day 0. Days following peripheral blood stem cell transplant are numbered accordingly, e.g., the first day after transplant is designated as Day 1. Days before the transplant are designated in the negative, e.g., the day prior to transplant is referred to as Day –1.

9.4 Day -2 and Day -1: Melphalan

Melphalan 200mg/m^2 will be administered per institutional standard of care: either on two consecutive days (Day –2 and Day –1) at a dose of 100 mg/m^2/day intravenously in sterile water (5mg/ml) over 20-30 minutes or all in one day. Current medications will be documented.

CBC with differential will be obtained on Day –2 and Day –1 prior to Melphalan infusion.

9.5 Peripheral Blood Stem Cell Transplant (Day 0)

A CBC with differential and platelets will be obtained prior to stem cell infusion.

A minimum of 2.0 x 10^6 $CD34^+$ cells/kg stem cells will be infused. The infusion will occur the day after the last dose of melphalan. **Corticosteroids may have an adverse effect on the activity of T cells contained in the stem cell product and should, therefore, be avoided.**
See Section 12.7, *Contraindicated Medications*.

9.6 Supportive Care

All patients will receive supportive care according to institutional clinical guidelines for autologous peripheral blood stem cell transplantation. This may include but not be limited to allopurinol, menstrual suppression, prophylactic antibiotics, empiric antibiotics, intravenous immunoglobulin (IVIG), transfusion of blood products, hyperalimentation, and erythropoietin.

9.7 Post-Transplant Filgrastim

Post-transplant filgrastim (Neupogen; G-CSF) **will not be permitted** on this trial because of the known ability of this agent to down-regulate CXCR4 which may negatively impact on the ability of aMILs to traffic to the bone marrow following infusion.

9.8 Tadalafil

Tadalafil 10mg po will be administered to all patients starting on day +2 and given through day +11 to both cohorts of patients. Should patients demonstrate unacceptable hypotension, the tadalafil will be discontinued and restarted once the blood pressure has normalized.

10. ADMINISTRATION OF ACTIVATED MILs (~DAY 3 & 4)

10.1 Evaluations Prior to Activated MILs T Cell Infusion

The following evaluations will be made prior to infusion of activated MILs:

- Clinical assessment: review of systems, vital signs;
- CBC with differential and platelet count;
- Serum chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, ALT, AST, total bilirubin, alkaline phosphatase, albumin;
- Serum separator for cytokine measurement (only for immune monitoring substudy) Concurrent medication documentation;
- Adverse event documentation.

10.2 Timing of Infusion of Activated MILs (aMILs)

Activated MILs T Cell infusions will be infused on two consecutive days. One third of the total product will be infused on Day 3 -/+ 1 day and the remainder of the product on the next day. Infusion may be delayed if required by cell processing issues or if warranted by the patient's clinical condition.

Date and time of each infusion must be documented.

Infusion should be given on <u>two</u> consecutive days.

NOTE: If medically unstable, the MILs infusion(s) will be delayed until those issues have been resolved.

aMILs will not be infused if:

- Temperature **>38.5ºC;**
- Oxygen **saturation < 90% on room** air**;**
- Patient **is felt to be clinically unstable by the medical team.**

Infusion can be given once these symptoms have resolved and the patient is felt to be medically fit to receive it after discussion with protocol chair.

10.3 Pre-Medication Prior to Infusion of aMILs

The patient will be pre-medicated with acetaminophen 650 mg by mouth and diphenhydramine hydrochloride 25-50 mg by mouth or IV, no greater than 30 minutes prior to the infusion of activated MILs. These medications may be repeated every six hours as needed. A course of nonsteroidal anti-inflammatory medication may be prescribed if the patient continues to have a high fever not relieved by acetaminophen.

Intravenous diphenhydramine hydrochloride, epinephrine, hydrocortisone and supplemental oxygen will be available at the patient's bedside in the event of anaphylaxis or an infusion reaction.

Other medications may also be prescribed to treat additional side effects during the course of treatment. However, there are some medications and therapies that may have adverse effects on the activity of activated MILs T Cell therapy and therefore are contraindicated (see Section 12.7, *Contraindicated Medications*). **In particular, patients should not receive systemic corticosteroids** such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on activated MILs. If steroids are required for an acute infusional reaction, an initial dose of hydrocortisone 100 mg is recommended.

10.4 Procedure for Administering aMILs

For the administrating of the activated MILs, the patient will be hydrated with D5½NS at approximately 200 ml per hour for at least one hour. If the activated MILs product appears to have a damaged or leaking bag, or otherwise to be compromised, it will not be infused. Otherwise the activated MILs will be thawed and infused through standard blood tubing containing a 170-260 micron filter **without an additional leukoreduction filter** into a central IV site. Each of the bags will be infused at a rate of approximately 10 ml per minute. Following aMILs infusion, the patient will be hydrated with D5W½NS at approximately 200 ml per hour for two hours.

10.5 Safety Monitoring During Administration of aMILs

During the infusion of activated MILs, the patient's blood pressure, heart rate, respiratory rate, temperature, and peripheral oxygen saturation will be monitored at the following time intervals: Immediately prior to infusion of first bag of activated MILs;

- 15 minutes after initiation of first bag of activated MILs;
- 30 minutes after initiation of first bag of activated MILs;
- 1 hour after initiation of first bag of activated MILs;
- 2 hours after initiation of first bag of activated MILs;

In the event of a severe allergic reaction (e.g., anaphylaxis, bronchospasm, hypotension) or other serious adverse reaction (i.e., cardiac failure, respiratory failure, severe nausea and vomiting) to the, activated MILs the infusion of the activated MILs will be stopped immediately and the patient will be provided with the supportive care deemed necessary by the medical staff at the clinical site.

The reaction should be noted as a serious adverse event, and the IRB, the Cell Therapy Lab as well as the Protocol chair,Philip Imus, , should be notified within 24 hours.

11. OBSERVATION, EVALUATION, AND MANAGEMENT AFTER TREATMENT WITH aMILs

Follow-up evaluation of the patient will be carried out according to standard institutional guidelines for autologous peripheral blood stem cell transplantation. In addition, the patient will have a limited number of tests performed specifically for this protocol. Any toxicity felt to be related to the infusion of aMILs will be reported as adverse events. **See Appendix E, *Patient Study Calendar***

11.1 Evaluations from Day 5 Through Day 28 after Peripheral Blood Stem Cell Transplant

The following tests will be performed from Day 4 until the day of confirmed engraftment for both neutrophils and platelets as clinically indicated (See section 15.8 for definition of engraftment):

- CBC with differential and platelet count (differential will only be performed if the total WBC > 100/µl);
- Adverse event documentation related to the infusion of aMILs will continue until resolution of those events.

The following tests will be performed on Day 7, 11, 14 and 28 (± 2 days):

- Serum chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, ALT, AST, total bilirubin, alkaline phosphatase, albumin;
- Serum separator for measurement of cytokines (only for immune monitoring substudy)
- Adverse event documentation – in any way related to the infusion of aMILs or tadalafil.

11.2 Evaluations from Day 28-360 after Peripheral Blood Stem Cell Transplant

The patients will be evaluated post transplant on Day 28 □ 2 days, 60 ± 7 days, Day 180 ± 30 days, and Day 360 ± 30 days for:

- Clinical assessment: review of systems, ECOG performance status, vital signs; □ CBC with differential and platelet count;
- Serum chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, ALT, AST, total bilirubin, alkaline phosphatase, albumin; □ M-protein, serum and urine:
  - Protein electrophoresis;
  - Immunofixation (if no measurable M-spike in serum or urine);
  - Freelite (sensitive assay for kappa and lambda light chains) only if most recent immunofixation result showed no evidence of M-protein. ○ Urine eletropheresis

can be done with a spot urine if the patient has no measurable disease in the urine at diagnosis

- Quantitative immunoglobulins (IgG, IgM, IgA);
- Disease response/progression/relapse assessment;
- Adverse event assessment in any way related to either the administration of tadalafil or administration of MILs as appropriate
- Concurrent medication documentation;
- Bone marrow aspirate and biopsy. The bone marrow examination will be performed on days 28± 2 days, 60 ± 7 days, 180 ± 30 days and 360 ±30 days only for patients enrolled on the immune sub-study. In addition to aspirate and biopsy, additional bone marrow (at least 20ml) will be obtained in a heparinized syringe only for the immune monitoring sub-study;
- Study Blood 100ml in heparinized syringe only for the immune monitoring sub-study.

11.3 Initiation of Lenalidomide Maintenance Therapy

- 5mg to start around day 60 once the following parameters are met:
  - Absolute neutrophil count >1500/µl;
  - Platelets > 50,000/µl; Hemoglobin > 10.5g/dL.
- Up to 10mg from approximately 6 months to be continued until disease progression or as long as medically tolerated;
- Dose can be adjusted up or down as medically indicated; but cannot be increased above 10mg.
- Dose of lenalidomide will be recorded
- Those subjects with revlimid dose higher than 10mg will be considered off study.

If lenalidomide needs to be discontinued because of toxicity, it should be restarted once the toxicities have been resolved. The interval off drug and dose upon reinstitution of the drug should be recorded.

11.4 Discontinuation of Evaluations after Treatment

There are several reasons that a patient may be discontinued from the post-treatment protocol evaluations. These include, but are not limited to:

- Patient decision to withdraw from the study;
- Protocol non-compliance;
- Patient lost to follow-up;
- Activated MILs product failed to meet specifications; Activated MILs product was not infused.
- Revlimid dose exceeds 10mg

The reason for discontinuation of the protocol evaluations will be documented. The patient should continue to be followed annually for survival when possible.

11.5 Contraindicated Medications

There are some medications that may have adverse effects on the activity of activated MILs. These medications and/or therapies should **NOT** be given to patients at any time during the entire study unless indicated in the protocol or to treat life-threatening conditions. Patients should not be placed on any maintenance therapy following transplant. Contraindicated medications and therapies include:

- Corticosteroids (e.g., hydrocortisone, prednisone, prednisolone [66], dexamethasone (Decadron), etc.). Inhaled steroids for the use of allergic rhinitis or pulmonary disease is allowed and not contraindicated;
- Thalidomide;
- Interferon;
- Nitrates;
- Growth factors, interleukins, or other cytokines (except filgrastim as outlined in the protocol, or erythropoietin);
- Cytotoxic agents (except cyclophosphamide for stem cell mobilization, fludarabine and high-dose melphalan as outlined in the protocol); □ Other immunosuppressive drugs; □ Other experimental therapies.

After disease progression, use of corticosteroids and other medications are not dictated by the protocol.

NOTE: Corticosteroids treatment may have an adverse effect on the activity of activated MILS. Each patient's hospital and clinic chart will be labeled "NO STEROID MEDICATIONS EXCEPT FOR EMERGENCIES" on the cover.

12. TADALAFIL

Tadalafil is currently FDA approved for the use of erectile dysfunction and pulmonary hypertension with an extensive clinical track record. In this study we propose to utilize this agent in the early post-transplant period in an effort to alter the tumor microenvironment and thereby increase the likelihood of maximizing trafficking of T cells. In an effort to maximize our ability to determine the clinical efficacy of MILs, tadalafil will be administered to both groups. Tadalafil administration will be recorded by patient on drug diary (Appendix I) and/or by the nurse in medical records.

13. MANAGEMENT OF PROGRESSIVE DISEASE

13.1 Documentation of Progressive Disease

Patients with disease progression/relapse shall have this documented.

Disease relapse for patients needs to be documented with:

- SPEP
- Quantitative immunoglobulins
- UPEP (if the patients have light chain disease)
- Serum free light chain
- CBC with differential
- Chemistries: Na, K, Cl, CO2, BUN, creatinine, total protein, albumin, calcium, alkaline phosphatase, AST, ALT
- Bone marrow biopsy and aspirate to include the MyPRS (if available). Hopkins patients should also have 20ml of marrow collected in a heparinized syringe for research purposes.

At relapse, patients are eligible to proceed with an alternative acceptable myeloma regimen.

14. RISK AND TOXICITY ASSESSMENT

If the Protocol Chair or Site Principal Investigator determines that an adverse event is sufficiently severe to remove the patient from the study, an assessment should be performed and the patient should be given appropriate treatment under medical supervision.

Patients will be subject to the risks associated with high-dose chemotherapy and peripheral blood stem cell transplant. Because autologous stem cell transplantation is considered a standard treatment option in multiple myeloma, this section will focus on the risks associated with the infusion of activated MILs, as well as unforeseen adverse events that could result from the interaction of activated MILs with the stem cell transplantation therapy.

14.1 Risks of Venous Access

Complications associated with venous access for leukapheresis of stem cells, infusion of stem cells and activated MILs include discomfort, bruising, and/or bleeding at the catheter insertion site, thrombosis of the accessed vessel, and infection. Complications from placement of a central line required for the high-dose chemotherapy and stem cell transplantation procedure and activated MILs include increased risk and severity of the above complications, as well as pneumothorax.

14.2 Risks of MILs Bone Marrow Harvest

The patients will undergo a standard bedside bone marrow aspiration with conscious sedation and sterile technique to collect the marrow infiltrating lymphocytes for subsequent T cell activation. There is a potential risk of excessive pain that will be handled with analgesia as needed. There is also the possibility of local and/or systemic infection.

14.3 Microbial Contamination of Activated MILs

There is the potential that microorganisms inadvertently introduced during cell collection and processing could cause an infection in the patient following infusion of the activated MILs. All precautions to maintain sterility will be taken. Cultures will be obtained prior to and after completing the T cell activation process.

14.4 Potential Toxicity of Storage Solutions

14.4.1 Dimethyl Sulfoxide (DMSO)

The activated MILs product is cryopreserved in a solution containing approximately 5% DMSO. DMSO is a cryoprotectant used to store a variety of infused cell products including peripheral blood stem cells and bone marrow. After cells are thawed, DMSO contained in the freezing solution is infused along with the cells. Toxicity associated with DMSO infusion is usually limited to minor changes in heart rate, blood pressure, fever, chills, sweats, nausea, vomiting and headaches. These are usually self-limited, lasting no more than a few hours. The most notable effect is a garlicky odor due to exhalation of a DMSO metabolite that may last up to one day. More severe adverse effects include severe acute allergic reactions including anaphylactoid reactions, pulmonary embolism, respiratory failure, renal failure, cardiac arrhythmias, seizures, and death. [68,69]These side effects are rarely observed and occur primarily in patients receiving much higher volumes of cryopreserved cell products than being infused in the present study. Nevertheless, patients will be carefully monitored for any adverse effects as outlined in Section 15, *Adverse Events*.

14.4.2 Human Serum Albumin (HSA)

The aMILs are formulated in a cryopreservative supplemented by 2% human serum albumin. Albumin solutions are FDA approved for volume resuscitation (treatment of hypovolemia, shock, and burns). Although hypersensitivity reactions are possible, reactions are not anticipated to the small amount of albumin administered.

14.4.3 Hetastarch

The cryopreservative for the aMILs will also contain 6% hetastarch. Hetastarch is FDA approved for hypovolemia and as a volume expander administered during leukapheresis. Hypersensitivity reactions have occurred but are not common. Considering the small amount of hetastarch to be administered to the patient, adverse reactions are not anticipated.

14.5 Potential Adverse Effects of Tadalafil

Tadalafil has been evaluated extensively for safety. Initial safety was evaluated in doses up to 20 mg daily for 12 weeks (n=258), with a 3% discontinuation rate[70] and did not demonstrate any gender related differences in pharmacokinetics or adverse effects. A recent meta-analysis did not demonstrate any higher cardiovascular risk compared to placebo.[71] Non-arterial anterior ischemic optic retinopathy has been proposed as a rare side effect of PDE-5 inhibitors, but definitive data have not been provided due to the rarity of this phenomenon in both the population and subject using PDE-5 inhibitors, as well as the widespread use of PDE-5 inhibitors.[72]

The most common side effects of tadalafil at a dose of 20mg are headache (15%), dyspepsia (10%), back pain (6%), myalgia (3%), flushing (3%), limb pain (3%) and nasal congestion (3%). These side effects usually go away after a few hours. Men who get back pain and muscle aches usually get it 12-24 hours after taking tadalafil. Back pain and muscle aches usually go away within 2 days. Some uncommon side effects include priapism, color vision changes, sudden decrease or loss of vision in one or both eyes, sudden loss or decrease in hearing, sometimes with tinnitus and dizziness.

Drug interactions:

- Nitrates: Administration of tadalafil to patients who are using any form of nitrate is contraindicated. Tadalafil has been shown to potentiate the hypotensive effect of nitrates;
- Alpha blockers: Tadalafil and alpha adrenergic blockers are vasodilators with blood pressure lowering effects and when used in combination an additive effect on blood pressure may be anticipated;
- Cytochrome P450 inhibitors: Tadalafil is a substrate of and predominantly metabolized by CYP3A4. Drugs that inhibit CYP3A4 such as ketoconazole and ritonavir can increase tadalafil exposure;
- Cytochrome P450 inducers: Drugs that induce CYP3A4 such as rifampin can decrease tadalafil exposure.

Contraindications:

- Known hypersensitivity to tadalafil;
- Concurrent use of organic nitrates in any form.

14.6 Potential Adverse Effects Associated with Activated MILs

Previous clinical trials using CD3xCD28 bead-activated and expanded peripheral blood T cells were described above. Eighty-nine HIV patients have been treated, with the most common toxicities being fevers, chills, fatigue, rash, sinusitis, asthenia, headaches, and nausea. [73-76]Grade 3 and 4 toxicities were predominantly associated with IL-2, which was infused in some of the patients in these trials. T cells activated with CD3xCD28 beads have also been administered to patients undergoing peripheral blood stem cell transplantation for relapsed or refractory NonHodgkin's lymphoma.[52] T cells were collected prior to high-dose chemotherapy. Fourteen

days following peripheral blood stem cell infusion, activated and expanded T cells were administered. Three patients were treated at a median cell dose of 0.4 x 10^9, twelve patients were treated at a median cell dose of 1.6 x 10^9, and two patients were treated with a median cell dose of 9.8 x 10^9. The latter two patients experienced infusion related toxicities including transient fever, dyspnea, rigors and pulmonary edema (one patient).

It is possible that activated T cells could attack the patient's own tissues, resulting in autoimmune disorders. However, this was thought to be due to high-dose IL-2, a known cause of this disorder, which was given with the cell therapy. Autoimmune disease has not been observed in previous studies using CD3xCD28 bead-activated T cells. Considering that in this case, the lymphocytes are obtained from the marrow microenvironment, it is possible that an immune response could develop towards normal hematopoietic elements. For this reason, hematologic engraftment will be closely monitored throughout the trial.

To date, over 50 patients have been treated with activated MILs. No Grade 2 or greater toxicity was observed in any of the patients. Patients did not have any infusion related toxicities. We have, however, noticed a 35% incidence of acute Grade 1 or 2 skin graft vs host disease (GVHD) that was self-limiting and required no local or systemic treatment.

14.7 Potential Adverse Events Associated with Peripheral Blood Stem Cell Transplant

Patients will be at risk for all of the complications associated with the peripheral blood stem cell transplant procedure. The Investigator is responsible for discussing these risks in detail with the patient. The following is a brief overview of the major risks associated with peripheral blood stem cell transplantation, and is not intended to be comprehensive. An extensive list of the expected complications associated with an autologous peripheral stem cell transplant is listed in Appendix G.

14.7.1 Peripheral Blood Stem Cell Mobilization Chemotherapy Regimen

Patients receive cyclophosphamide chemotherapy and filgrastim to mobilize peripheral blood stem cells for collection. Cyclophosphamide can cause alopecia, nausea, vomiting, bladder irritation and hemorrhage, and myelosuppression including anemia, leukopenia, and thrombocytopenia. Patients are at risk of bleeding and infection.

14.7.2 Administration of Filgrastim (G-CSF)

Filgrastim is given in the peripheral blood stem cell mobilization regimen and can cause significant bone pain in many patients.

14.7.3 Melphalan

High doses of melphalan can cause nausea, vomiting, fatigue, anemia, and alopecia. Patients are at significant risk of serious and life-threatening infections during the neutropenic period, which usually lasts for approximately two weeks. Patients will likely require red blood cell and platelet transfusions, both of which are associated with infectious risks such as hepatitis and HIV.

14.7.4 Leukapheresis

Patients will require leukapheresis to collect the peripheral blood stem cells.

14.7.5 Peripheral Blood Stem Cell Infusion

The peripheral blood stem cells may become contaminated with microbes during their collection or processing. Infusion of peripheral blood stem cells contaminated with microbes such as bacteria could cause an infection. The peripheral blood stem cells are stored in DMSO. Similar to the administration of activated MILs, the DMSO is infused along with the cells.

14.8 Lenalidomide

Lenalidomide can commonly cause myelosuppression, skin rashes, and fatigue. The drug will not be initiated until the patients meet the required hematologic criteria as specified by the protocol. However, because of our desire to increase the immunostimulatory effects of lenalidomide on the MILs, we will attempt to start treatment around 60 days post-transplant and to start with the low dose of 5mg.

Lenalidomide has been associated with a significant improvement in progression free survival and possibly also overall survival. It has also been associated with a 3-5% incidence of secondary malignancies when administered either in association with melphalan or following high dose melphalan in the post-transplant setting.

15. ADVERSE EVENTS

15.1 Definitions

15.1.1 Adverse Event (AE)

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product (refer to ICH E6 GCP Guidance – section 1.2).

15.1.2 Serious Adverse Event (SAE)

A Serious AE (SAE) is any untoward medical occurrence that at any dose produces any of the following outcomes (refer to ICH E6 GCP Guidance – section 1.50): Results in death;

- Is life threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see NOTE below for exceptions);
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect (note: reports of congenital anomalies/birth defects must also be reported on the Pregnancy Supplemental Form);
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization).
- Transplant-related complications that are expected but exceed the severity and are felt to be related to either tadalafil or the MILs need to be reported as SAEs. Examples include but are not limited to: prolonged aplasia, excessive fevers, excessive diarrhea
- Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

NOTES:

1. The following hospitalizations are not considered SAEs:

- Admissions as per protocol for a planned medical/surgical procedure;
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy);
- Medical/surgical admission for purpose other than remedying ill health state and was planned prior to entry into the study. Appropriate documentation is required in these cases;
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).

15.1.3 Non-serious Adverse Event

A non-serious adverse event is any adverse events not classified as serious (as described in previous section).

15.2 Adverse Event Assessment and Recording

15.2.1 Collection and Assessment of AEs/SAEs

The process of reviewing of *all AEs* by the PI will be documented on the "Adverse event assessment chart" (Appendices J). Source documentation, including all available clinic notes, will be reviewed from the time of bone marrow collections of MILs through Day 360/disease progression post-transplant. AEs/SAEs that occur after Day 360 or disease progression/relapse would likely reflect the disease process, and not the activated MILs or tadalafil.

All toxicities, abnormal laboratory results and/or potential adverse events experienced by accrued subjects during this time will be assessed by the PI. These assessments are to occur regularly for the time periods specified in Appendices K and confirmed by the PI based on attribution to study intervention(s).

- Only Adverse Events considered in any way related to the either infusion of aMILs or Tadalafil are recorded on the AE Log in database.
- Only clinically significant laboratory results considered in any way related to either aMILs or Tadalafil and not at all related to bone marrow transplant are recorded on the AE Log.

For purposes of this study, clinical significance is defined as any laboratory result that is outside the range of normal, as defined by each laboratory's recorded reference range and is deemed clinically significant by the Principal Investigator.

This assessment chart does not pertain to SAEs. Each SAE is individually assessed by the PI at the time of the event. All SAEs will be recorded on the AE Log in database.

15.3 Grading of Adverse Events and Toxicities

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all AE reporting.

Attribution of AE's:

- Mild (Grade 1) – near the lowest intensity (or within the lower one-third) typically seen with the observed sign or symptom;
- Moderate (Grade 2) – average intensity typically seen with the observed sign or symptom;
- Severe (Grade 3) – near the highest intensity (or within the top third) typically seen with the observed sign or symptom;
- Life-threatening or disabling (Grade 4) – any AE that places the patient, in the view of the investigator, at immediate risk of death from the reaction as it occurred. It does not refer to an event which hypothetically might have caused death if it were more severe;
  Fatal (Grade 5).

Note: These terms are used to describe intensity of a specific event; the event itself may be of relatively minor medical significance (such as severe headache).

15.4 Attribution of Causality

The association or relationship of the AEs to activated MILs or tadalafil will be defined according to the NCI Common Toxicity Criteria guidelines as follows:

- Definite – The AE is *clearly related* to the study product, e.g., an event that follows a reasonable temporal sequence from administration of the drug or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of drug;
- Probable – The AE is *likely related* to the study product, e.g., an event that follows a reasonable temporal sequence from administration of the drug, that follows a known or expected response pattern to the suspected drug, that is confirmed by stopping or reducing the dosage of the drug, and that could be reasonably explained by the known characteristics of the subject's clinical state;
- Possible – The AE *may be related* to the study product, e.g., an event that follows a reasonable temporal sequence from administration of the drug, that follows a known or expected response pattern to the suspected drug, but that could readily have been produced by a number of other factors;
- Unlikely – The AE is *doubtfully related* to the study product;
- Unrelated – The AE is *clearly not related* to the study product;
- Not applicable – The AE occurred prior to administration of the study product; Unknown – No evaluation for causality can be made.

15.5 Serious Adverse Event Capture and Reporting

The Sponsor/Investigator is obligated to report to the FDA suspected adverse reaction that is both serious and unexpected. (see 21 CRF, part 312.32):

- Serious Adverse Event - any adverse experience which is fatal or life-threatening, permanently disabling, requires inpatient hospitalization, or is a congenital anomaly, cancer, or overdose;
- Unexpected Adverse Event - any adverse experience that is not identified in nature, severity or frequency in the current Investigator Brochure.

ALL serious adverse events, regardless of causality must be reported to the following entities:

- IND Sponsor – Dr.Cooke ;
- Study PI – Dr Imus;
- IRB (per the IRB's reporting requirements);

15.5.1 Investigator Reporting Responsibilities

The conduct of the study will comply with all FDA safety reporting requirements. All adverse experience reports must include the patient number, age, sex, weight, severity of reaction (mild, moderate, severe), relationship to study drug (probably related, unknown relationship, definitely not related), date and time of administration of test medications and all concomitant medications, and medical treatment provided. The investigator is responsible for evaluating all adverse events to determine whether criteria for "serious" and "unexpected" as defined above are present.
This SKCCC clinical trial requires all study sites to maintain a study-specific master adverse event log and a protocol deviation log in Excel format. Templates will be provided by the Coordinating Center.

15.5.2 SAE Reporting to the IND Sponsor

SAEs that meet the definition of an unanticipated event must be reported to the IND Sponsor, Dr. Cooke within 24 hours of the event. Subsequently each participating site must document such an SAE using MedWatch form (FDA 3500A) and send it to the coordinating center's study research nurse Laura Cucci at Johns Hopkins and to onccoordcnt@jhmi.edu within 7 calendar days of the event.

15.5.3 Sponsor SAE Reporting to the FDA

All SAEs are reported to the FDA via the IND annual report per 21 CFR 312.33. SAEs deemed unexpected and related to the investigational product qualify for expedited reporting and must be submitted by the IND Sponsor to the FDA per 21 CFR 312.32 as shown immediately below.

15.5.3.1 7 Calendar-Day Telephone or Fax IND Safety Report to FDA

The Sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the Sponsor Investigator to be possibly related to the use of activated MILs or tadalafil within 7 calendar-days of first learning of the event. An unexpected adverse event deemed possibly related to the use of an investigational study drug is defined as any adverse drug experience of which the specificity or severity is not consistent with the current investigator brochure, the general investigational plan, or elsewhere in the current application, as amended.

Such reports are to be telephoned or faxed to the FDA and Laura Cucci, R.N. at Johns Hopkins (tel: 410 614 5407 or fax 410 502 9690) within 7 calendar-days of first learning of the event. Each telephone call or fax transmission should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever department is responsible for the review of the IND.

15.5.3.2 15 Calendar-Day Written IND Safety Report to FDA

The IND Sponsor is required to notify the FDA, and all participating investigators, in a written IND Safety Report, of any serious, unexpected adverse event considered by the

IND Sponsor to be possibly related to the use of activated MILs and tadalafil within 15 calendar-days of first learning of the event. If applicable, the IND Sponsor must also notify the FDA, and all participating investigators of any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity within 15 calendar-days of first learning of the event.

A serious, unexpected adverse event deemed possibly related to the use of an investigational study drug is any adverse drug experience of which the specificity or severity is not consistent with the current investigator brochure, the general investigational plan, or elsewhere in the current application, as amended, and results in any of the following outcomes:

- Death (reported first as a 7-day telephone/fax report);
- Life-threatening adverse drug experience (reported first as a 7-day phone/fax report);
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant disability/incapacity, or a congenital anomaly/birth defect;

Or is an important medical event that may not result in death, be life-threatening, or require hospitalization but is considered a serious adverse drug experience when, based upon appropriate medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Note: Aggregate reporting of hospitalizations due to neutropenic fever

Stem cell transplant is the background regimen in the study. Admissions during the period of aplasia associated with the transplant are expected complications of the transplant. An occurrence of such an event is not an adequate basis to conclude that the event is a suspected adverse reaction to the investigational products tadalafil and MILs. Therefore, neutropenic fevers *will not be reported individually as they occur* because they are uninformative as single cases. These SAEs will be monitored and assessed for causality to investigational products, and the numbers of events in each arm will be compared. An IND safety report based on data in the aggregate will be submitted as a part of annual report.

All written IND Safety Reports should include an Analysis of Similar Events in accordance with 21 CFR 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports and be submitted to the FDA, and all participating investigators within 15 calendardays of first learning of the event. The FDA prefers these reports be documented on a MedWatch 3500A Form, but alternative formats are acceptable (e.g., summary letter).

The address of the Food and Drug Administration is:

Center for Biologics Evaluation and Research
Food and Drug Administration
HFM-99, Room 200
1401 Rockville Pike
Rockville, MD 20852-1448

15.5.3.3 Follow-up Reports to FDA

All follow-up information concerning IND Safety Reports should be submitted to the FDA as soon as possible (and no later than 15 calendar days after receiving an FDA request for more information).

15.6 Definitions of Response (IMWG Criteria)

Definitions of response are adapted from the combined criteria developed for patients with multiple myeloma treated by high-dose chemotherapy and hematopoietic stem cell transplantation (Blade's Criteria) and are updated based upon the consensus report of the International Myeloma Working Group (IMWG) criteria.[77] If confirmation of a measurement is required, the date of response/progression/relapse will be the date at which the M-protein measurement first meets the required criterion. M-protein measurements by serum protein electrophoresis (SPEP) will take precedence over quantitative immunoglobulins for determining response. The date of response/progression/relapse will be determined by the date when the relevant tests were obtained, and not by the date when the investigator reviews the test results.

Criteria for Evaluation and Endpoint Definitions:

- The serum and urine M-protein levels obtained at baseline will be used as the reference baseline for calculation of increases or decreases in the M-protein level. The M-protein levels from the time of diagnosis will also be obtained for the patients that proceed directly from induction chemotherapy to transplant. For patients with relapsed myeloma, the M-protein level baseline will be considered the highest value prior to the last chemotherapy given leading up to the transplant. The response calculations using those respective values as baseline will also be performed in secondary analyses.

15.6.1 Complete Response (CR) Requires

all of the following:

- Absence of the original M-protein in serum by immunofixation, maintained for a minimum of 6 weeks. Presence of M-protein by Freelite assay does not exclude CR. The presence of oligoclonal bands consistent with oligoclonal immune reconstitution does not exclude CR. Normalization of serum concentrations of normal immunoglobulins is not required;
- If a urine M-protein is present at baseline or other prior evaluation, absence of this Mprotein in urine by immunofixation, maintained for a minimum of 6 weeks;
- < 5% plasma cells in a bone marrow aspirate and on bone biopsy (must be performed)

- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response;

 Disappearance of any soft tissue plasmacytomas.

15.6.2 Stringent Complete Response (sCR)

Requires all of the criteria for CR to be met, plus all of the following:

- Normal FLC ratio (0.26 to 1.65); and
- Absence of clonal plasma cells in the bone marrow, defined by 2-4 color flow cytometry OR immunohistochemistry

15.6.3 Very Good Partial Response (VGPR) Requires all of the following:

- A decrease in the serum M-protein by 90%, maintained for a minimum of 6 weeks;
- If a urine M-protein is present at baseline or other prior evaluation, the urine M component must be less that 100mg per 24 hours;
- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response;

 Disappearance of any soft tissue plasmacytomas.

15.6.4 Partial Response (PR) Requires all of the following:

- A decrease in the serum M-protein by 50%, maintained for a minimum of 6 weeks;
- If a urine M-protein is present at baseline or other prior evaluation, a $\geq$ 90% reduction in this protein, or a decrease to less than 200 mg in a 24 hour urine collection. This response must be maintained for a minimum of 6 weeks;
- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response;
- Decrease of 50% in the size of any soft tissue plasmacytomas (by radiography or clinical examination)

15.6.5 Minimal Response (MR) Requires all of the following:

- A decrease in the serum M-protein by 25%, maintained for a minimum of 6 weeks;
- If a urine M-protein is present at baseline or other prior evaluation, a $\geq$ 50% reduction in this protein in a 24 hour urine collection. This response must be maintained for a minimum of 6 weeks;
- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response;
- Decrease of 25% in the size of any soft tissue plasmacytomas (by radiography or clinical examination).

15.6.6 Stable Disease

Not meeting the criteria of CR, VGPR, PR, MR, progression, or relapse from CR

15.6.7 Relapse from CR (NB this is not distinguished in new IMWG criteria) Requires one or more of the following:

- Reappearance of serum or urinary M-protein on immunofixation or routine electrophoresis, confirmed by at least one further investigation, and excluding oligoclonal immune reconstitution;
- ≥ 5% plasma cells in a bone marrow aspirate or on bone biopsy;
- Development of new lytic bone lesions or soft tissue plasmacytomas or definite increase in the size of residual bone lesions. Development of a compression fracture does not exclude continued response and may not indicate progression;
- Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL or 2.8 mmol/L) not attributable to any other cause.

15.6.8 Progression

Requires one or more of the following:

- A > 25% increase in the level of serum M-protein, which must also be an absolute increase of at least 5 g/L and confirmed by at least one repeat investigation;
- A > 25% increase in the 24 hour urinary light chain excretion, which must also be an absolute increase of at least 200 mg/24 hours and confirmed by at least one repeated investigation;
- Only in subjects with no measurable serum or urine M spike: a >25% increase in the difference between involved and uninvolved FLC levels (absolute increase must be > 10 mg / dL)
- Only in subjects without meausurable serum and urine M protein levels AND without measurable disease by FLC levels: a > 25% increase in plasma cells in a bone marrow aspirate or on biopsy, which must also be an absolute increase of at least 10%;
- Definite increase in the size of existing bone lesions or soft tissue plasmacytomas;
- Development of new bone lesions or soft tissue plasmacytomas at any time since baseline. Development of a compression fracture does not exclude continued response and may not indicate progression;
- Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL or 2.8 mmol/L) at any time since baseline, which is not attributable to any other cause. (Corrected serum calcium is calculated by adding 0.8 mg/dL to the measured serum calcium for every 1 g/dL that the serum albumin falls below 4.0 g/dL.).

Reference level for increase in M-protein, plasma cells, or lesion sizes is the lowest level documented since (and including) baseline evaluation.

15.6.9 Response Criteria for serum free light chain responses

	Minimum deemed measurable	PR	CR	sCR	Progression
MM without measurable serum or urine M- protein	iFLC >/= 100mg/l and rFLC abnormal	50% reduction of dFLC	ND	Normal rFLC & CR by IFE and bone marrow	50% increase of iFLC to > 100 mg/l
MM with measurable disease	Use of FLC not recommended	Use of FLC not recommended	Use of FLC not recommended	Normal rFLC & CR by IFE and bone marrow	Use of FLC not recommended

Abbreviations: iFLC, inv-restricted disease; dFLC, difference between iFLC and uninvolved FLC; rFLC, free light chain ratio; ND, not defined. Measurable M protein includes serum M protein of at least 1 g per 100 ml or a urine Mprotein of at least 200 mg/24 h for myeloma patients (100 mg/24 h for AL patients).

15.7 Survival Endpoints

15.7.1 Overall Survival

Measured as the time from Day 0 to death from any cause.

15.7.2 Progression-Free Survival

Measured as the time from initial registration Day 0 to progression/relapse of disease or death from any cause, whichever occurs first.

15.8 Definitions of Engraftment and Graft Failure

15.8.1 Engraftment

Neutrophil engraftment is defined as the day post-transplant when the absolute neutrophil count is > 500/mm^3 for two consecutive days post-transplant (note: bands as well as neutrophils are included in calculations). The first day on which this criterion is met is considered the day of neutrophil engraftment.

Platelet engraftment is defined as the first of three consecutive counts post-transplant when the platelet count is > 20,000/mm^3 without platelet transfusion support. The first day on which this criterion is met is considered the day of platelet engraftment.

15.8.2 Failure of Engraftment

Primary graft failure is defined as failure to recover an absolute neutrophil count > 500/mm^3 or platelet count > 20,000/mm^3 by Day 28 post-transplant. Secondary graft failure is defined as a fall to less than these levels for three or more consecutive days after Day 28 without another cause.

16. STATISTICAL CONSIDERATIONS

NOTE: The original statistical plan is noted here in Section 16. Due to protocol violations that were discovered in the conduct of the study, this statistical plan is no longer valid. The current statistical plan is noted in Appendix K. The original statistical plan is left in the protocol for historical reference only, and will not be followed.

16.1 Sample Size

Ninety patients will be treated in this clinical trial. If patients are registered but do not proceed to transplant for any reason, then additional patients will be registered such that the target of 90 patients treated can be achieved.

16.2 Statistical Analysis

This is a prospective, randomized clinical trial in multiple myeloma (MM) patients with high risk disease undergoing their first autologous bone marrow transplant. In this study patients randomized to the MILs arm will receive a post-transplant infusion of anti-CD3/CD28 activated marrow infiltrating lymphocytes (aMILs) in an attempt to induce durable myeloma-specific immune responses. The primary endpoint will assess the impact of infusing aMILs on progression free survival (PFS). Patients will be random ized in a 2:1 ratio to receive aMILs (Arm A) or not (Arm B) following ASCT. All patients will be given tadalafil from days 2-21. Lenalidomide will be started on day +60 post ASCT. Randomization will be stratified by relapse status prior to transplant and high-risk phenotype based on the GEP-70 profile – if available.[78]

Primary Objective: Determine the efficacy of aMILs in prolonging PFS in patients with high risk multiple myeloma followed by ASCT. Relapse for patients in CR prior to transplant is defined in section 16.6.6. Progression for patients not in CR prior to transplant is defined in section 16.6.7. PFS will be defined as the time from ASCT to documented progression/relapse, death from any cause or last follow-up. Assessment of progression will be made by the treating physician and confirmed through laboratory biochemical analyses.

The time-to-event in both arms of this study will be assumed to follow an exponential distribution. We hypothesize that the median PFS is 15 months for the control (Arm B) and 33.3 months for the aMILs treated group (Arm A). The stratification factors for randomization will consist of relapse status prior to transplant and the presence or absence of high risk GEP-70 profile. A randomized block procedure will be used.

Secondary Objectives:

1. Evaluate hematopoietic and infusional toxicities associated with this treatment;
2. Evaluate progression-free survival at 2 years;
3. Evaluate overall survival;
4. Quantify anti-myeloma immunity in the subset participating on the immune study;
5. Validate immune response phenotype identified in previous MILs trial.

Sample Size: The median PFS in high-risk MM patients on the study arm not receiving aMILs (Arm B) is expected to be 15 months. Hazard rates over four time intervals were estimated based on the TT2 PFS curve for MyPRS-defined high-risk patients[1]. Simple linear regressions were used to fit the natural logarithm of the Kaplan-Meier product limit estimate of the PFS function as a function of time in four intervals: from 1 to 7 weeks, 7 to 15, 15 to 21, and 21 to 23 weeks. These estimated hazard rates: 0.0072, 0.0136, 0.0058, and 0.0009 per person week were used in simulations to evaluate power for this study. We hypothesize that the addition of aMILs will translate into a relative improvement of 46% on the 15 month PFS (15 month PFS improved from 50% to 73%). Patients will be randomized to the aMILs (Arm A) and no aMILs arms (Arm B) in a 2:1 ratio. Using a one sided 5% type I error allowance, accrual of 36 patients per year for 2.5 years (Arm A and B target sample sizes of 60 and 30 respectively) with an additional 2 years of follow up, this study will provide sufficient events to have 83% power to detect an improvement of the median PFS from 15 months to 33.3 months, i.e. a hazard ratio of 0.45.

Futility monitoring plan: We will monitor the study for futility using a non-parametric Bayesian predictive probability approach[79,80,81]. This monitoring plan is based on calculations of the posterior distribution of the survival distribution and simulations to determine the probability that the PFS at two years on the treatment arm will be greater than that on the control arm at the end of the study. When the trial is monitored, Gibbs sampling is used to generate samples from the posterior distribution of the survival distribution via Monte Carlo simulation, given the data and a Dirichlet process prior. Censored observations are treated as random quantities and PFS event times are simulated for each censored observation. This is repeated many times and ultimately converges to a realization of the parameters drawn from the posterior distribution. The algorithm is that reported in Kuo and Smith.[80] If the probability that the treatment 2 year PFS is greater than the control PFS at 2 years is very low, less than 33.3%, the study will be stopped for futility.

The reference hazard rates were estimated based on the TT2 PFS curve for MyPRS-defined high risk patients[78]. Simple linear regressions were used to fit the natural logarithm of the KaplanMeier product limit estimate of the survival function as a function of time in four intervals: from 1 to 7 weeks, 7 to 15, 15 to 21, and 21 to 23 weeks. Survival times were then generated using a piecewise exponential distribution with these estimated hazard rates: 0.0072, 0.0136, 0.0058, and 0.0009 per person week. For simulation purposes, the effect of treatment was assumed to multiply these hazard rates by factors of 1.0 (no difference), 0.805 0.75, 0.65, 0.55, 0.50, and 0.45. The randomization ratio for these simulated studies was 2:1 and a single interim analysis, evaluating 2 year PFS, was performed after 50% of the patients were enrolled, i.e. n=30 in Arm A (aMils) and n=15 in Arm B (no aMils). A one-sided 0.05 alpha level log-rank test was used for the final analysis. Plots of the reference curve (black) and six scenarios of treatment effects (red) are shown in figure 1. In scenario one, the reference and treatment hazard rates are equal. Table 1 gives operating characteristics of this monitoring plan for these scenarios. Column 4 in table 1 is the proportion of studies rejecting the null hypothesis out of the number of studies that did not stop for futility. Column 5 is the overall proportion of studies with a positive result.

Table 1. Operating characteristics of monitoring rule from 500 simulated studies with interim analysis on 4 month PFS and final analysis based on the log-rank test.

Scenario	Control hazard rate (λ) multiplier	Stop for Futility %	Rejected at final analysis %*	Overall studies rejecting Ho %
1	1.00	10.4%	6.5%	5.8%
2	0.85	3.2%	9.7%	9.4%
3	0.75	2.0%	20.4%	20.0%
4	0.65	1.0%	40.6%	40.2%
5	0.55	0.0%	62.6%	62.6%
6	0.50	0.0%	77.4%	77.4%
7	0.45	0.0%	83.4%	83.4%

*Denominator is the number of simulations that do not stop early for futility.

Primary analysis: The primary analysis will be intent to treat analysis of all patients treated on protocol, regardless of treatment modification or discontinuation. The PFS event time distributions for patients receiving aMILs (Arm A) and patients not receiving aMILs (Arm B) will be estimated with the method of Kaplan and Meier and compared using the log-rank statistic. The primary analysis will use a two sided 0.05 alpha-level test.

Secondary analyses:

1. The time to neutrophil and platelet engraftment (absolute neutrophil count >500/mm^3 and platelet >20,000/mm^3) will be summarized using cumulative incidence curves with death as the competing risk. The rate of primary graft failure will be estimated and reported with 95% exact binomial confidence intervals. Any adverse events appreciated during the infusion of aMILs or felt to be related to the infusion will be summarized and reported.
2. Overall survival event time distributions for the two arms of the study will be estimated with the method of Kaplan and Meier and compared using the log-rank statistic.
3. Lymphocytes from the peripheral blood and marrow will be collected and banked at the indicated time points during the study: blood on days +7, +14, +28, +60, +180 and +360; and bone marrow, on days +28, +60, +180 and +360 for the patients participating in this substudy (Hopkins only). Tumor-specific assays will be performed in these patients. We expect tumor specific responses post-transplant. Results will be summarized and correlated with clinical responses. For patients receiving the aMILs following disease relapse, blood will be collected and banked on days +7, +14, +28, +60, +180 and +360; and bone marrow, on days +28, +60, +180 and +360 following the administration of cyclophosphamide for the patients participating in this sub-study (Hopkins only). For patients with ongoing responses, bone marrow samples (and possibly blood) will be obtained and stored whenever the patient is getting a bone marrow as part of the standard clinical care. It is routine at Hopkins to have patients undergo annual bone marrow biopsies for up to 5 years post-transplant assuming the patients are still in remission.

4. Data from the first MILs trial suggests that high endogenous CD8/IFN and an increased percentage of effector as well as effector memory CD8 cells at baseline was associated with progressive disease. These parameters will be correlated with progression free and overall survival.

16.3 Safety Data Monitoring

16.3.1 Review of Safety Data

The Investigators will review safety data on an ongoing basis. Clinical research meetings are held at least monthly via teleconference with participating sites in which all the patient data is reviewed including adverse events and safety. The trial may be prematurely terminated should the occurrence of adverse events warrant such action.

16.3.2 Premature Termination of the Study

If at any time four patients die of treatment related causes or experience Grade 4 pulmonary or hepatic toxicity, the study will be terminated. The study will also be terminated if there are two or more deaths, which are definitely or probably attributable specifically to the activated MILs or vaccination.

16.4 Plan for Statistical Summaries and Analyses

16.4.1 Subject Accountability

The following **data** will be summarized:

- Number (%) of subjects registered;
- Number (%) of subjects undergoing autologous stem cell transplant;
- Number (%) of subjects who receive activated MILs.

16.4.2 Demographic and Baseline Analysis

Demographic, disease, and baseline characteristics will be summarized for each of the study groups specified above.

For continuous variables, the analysis will include mean, median, standard deviation, and range. For categorical variables, number and percentages of subjects possessing a characteristic will be provided. The data will include, but will not be limited to:

- Subject age;
- Subject gender;
- Subject disease history and relevant concomitant medical conditions.

16.4.3 Protocol Compliance

The registered study subject population will be described in reference to the study criteria.

At a minimum, the following tabulations will be provided:

- Number of subjects with violations of inclusion/exclusion criteria; Number of subjects with major on-study deviations or violations.

16.4.4 Primary Endpoint

The primary endpoint of the study is progression free survival.

Evaluate Response Rates

Inclusion of patients into the efficacy analysis data sets is secondary to meeting entrance criteria and receiving activated MILs and vaccination. Overall event free survival and relapsed rates will be determined.
Exact methods will be used to compute a 95% confidence interval for the estimated proportion of CR and overall response (CR, VGPR, PR, MR). Additional analyses may be carried out to examine the significance of baseline prognostic variables.

The Kaplan-Meier method will be used to estimate duration of response. This is defined as the date of response to the first date when the patient meets the criteria for progression/relapse as defined in Section 13.5, *Definitions of Response*.

16.4.5 Secondary Endpoint

Evaluate Toxicity

Hematopoietic Toxicity:
The mean and median time to neutrophil and platelet engraftment (absolute neutrophil count >500/mm^3 and platelet >20,000/mm^3) will be summarized among those with successful engraftment, along with the rate of primary graft failure. It should be remembered that in the absence of post-transplant G-CSF, we would expect neutrophil engraftment to be slightly delayed. However, should red cell, platelet and neutrophil engraftment all show evidence of significant delay in more than 2 patients, the study will be placed on hold until a more formal evaluation can be performed.

Infusional Toxicity:
This will include any adverse appreciated during the infusion of aMILs or felt to be related to the infusion.

Evaluate Overall Survival

Patients will be monitored for overall survival during and following the study. The KaplanMeier method will be used to estimate the overall survival as defined in Section 16.7, *Survival Endpoints*.

Evaluate Anti-Tumor Immune Responses

Lymphocytes from the peripheral blood and marrow will be collected and banked at the indicated time points during the study for the patients participating in this substudy.

Tumor-specific assays will be performed in these patients. Results will be summarized and correlated with clinical responses.

Correlate Baseline Immune Function with Clinical Outcomes

Data from the first MILs trial suggests that high endogenous CD8/IFN and an increased percentage of effector as well as effector memory CD8 cells at baseline was associated with progressive disease. These parameters will be examined and will be correlated with clinical outcomes.

16.4.6 Results of Activated MILs Manufacturing Process

T cell activation and expansion will be summarized using descriptive statistics. A table will be generated to summarize the activated MILs product profiles for all the patient samples processed. The variables may include: Total cell count, %$CD3^+$, Total $CD3^+$ Count, %$CD3^+CD4^+$, %$CD3^+CD8^+$, CD4:CD8 Ratio, %Viability, Sterility, Mycoplasma, Endotoxin.

17. ETHICAL, REGULATORY, AND ADMINISTRATIVE CONSIFERATIONS

17.1 Informed Consent

The principles of informed consent are described in the Code of Federal Regulations 21 CFR, part 50.

Patients or their legal guardians must be made aware of the investigational nature of this treatment protocol, and have the possible risks, hazards and benefits of the protocol explained to them. The information that is given shall be in a language understandable to the patient. No informed consent, whether oral or written, may include any exculpatory language through which the patient is made to waive or appear to waive any of their legal rights, or releases or appears to release the Principal Investigator, the institution, or its agents from liability for negligence.

The patient, or legal guardian, must be able to comprehend the informed consent and must sign the document prior to registration on study. The patient will receive a copy of the respective signed consent form.

17.2 Institutional Review

The principles of Institutional Review Board (IRB) are described in the Code of Federal Regulations 21 CFR, part 56.

The Principal Investigator will obtain approval for the study from the IRB. The Principal Investigator must notify the IRB within 5 days of protocol deviations in emergency situations regarding patient safety. The Principal Investigator will be responsible for obtaining annual IRB

renewal through the duration of the study, or more frequently if required by the IRB. Copies of the Principal Investigator's report and copies of the IRB's continuance of approval must be maintained in the regulatory binder located at the clinical site.

17.3 Tissue Use for Research Purposes

A portion of the MILs bone marrow product and/or final activated MILs product may remain unused at the conclusion of the study. Patients will have the option on the study consent form to allow this tissue to be used for further research purposes by the Principal Investigator, or to be destroyed one year after collection.

In addition, various patient tissues, including peripheral blood and bone marrow will be collected from patients during the course of the study expressly for research assays. Baseline bone marrow specimens with adequate tumor involvement may be used to develop primers required for a PCR based assay for minimal residual tumor in the final activated MILs product. Quantification of minimal residual disease will also be performed in bone marrow from patients in complete remission for whom primers can be developed. Peripheral blood and tumor containing marrow specimens may also be used in immune assays.

Results from assays performed for research purposes will not be provided to patients. At the conclusion of the study and follow-up period (some of this tissue may remain unused. Patients will have the option in the study consent form to allow this tissue to be used for further research purposes or to be destroyed.

18. STUDY MONITORING AND DATA COLLECTION

18.1 Study Monitoring

This is a DSMP Level II study under the SKCCC Data Safety Monitoring Plan (12/6/2012). Data Monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. The protocol will be monitored internally at SKCCC by the Principal Investigator and the SKCCC CRO, as well as, by a CRO contracted by the Leukemia Lymphoma Society (sponsor of this study) in accordance with SKCCC guidelines. The SKCCC Safety Monitoring Committee requires all DSMP Level I-IV study teams to maintain a study-specific master adverse event log and a protocol deviation log in Excel format. Templates available here: http://cro.onc.jhmi.edu/. External participating sites are required to maintain updated versions of these logs, and submit them to the CC team upon request Trial monitoring at Johns Hopkins site will be done through the Safety Monitoring Committee (SMC) at SKCCC. Additionally, Leukemia and Lymphoma Society will monitor the study at all sites.

Authorized representatives of the Coordinating Center may visit participating sites to perform audits or inspections, including source data verification. The purpose of these audits or

inspections is to systematically and independently examine all trial related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements.

18.1.1 Completion of electronic Case Report Forms (eCRFs)

The Principal Investigator/sub-investigator or his designee will be responsible for completing, in a timely manner, a eCRF for each patient who is registered to participate in this study. eCRFs should be completed within 10 days after the date of study visit, or as information becomes available.

Master Adverse Event Log located in the study database will be utilized for AEs/SAEs collection. Master Adverse Event Log should be sign in database by the PI at least monthly. Master Adverse Event Log can be converted into Excel file if needed.

18.1.2 Cell Therapy Lab (CTL) Cell-Processing Facility

The Cell Therapy Lab (CTL) is the processing lab for all cellular products (bone marrow, vaccines, and adoptive T cell products) administered in the Cancer Center at Johns Hopkins. It operates according to Good Tissue and Manufacturing Practices and is fully accredited by FACT.

The results of cell processing using the activated T cell process will be recorded on CRFs or in the Batch Production Records at the Johns Hopkins CTL facility. CRFs and other study records will be reviewed in detail by the CRA, or designee, who will have access to all patient medical records, laboratory data, and other source documentation to verify the accuracy of the data recorded on these documents.

18.2 Maintenance of Study Documentation

It is the responsibility of the Principal Investigator and study staff to maintain a comprehensive and centralized filing system of all study-related documentation. This filing system must be suitable for inspection at any time by the CRO or Quality Assurance (QA) designee of Johns Hopkins and the FDA. Elements should include:

- Subject Files - containing the completed patient CRFs, supporting source documentation, and a signed and dated Informed Consent;
- Regulatory Binder - containing the protocol with all amendments, current Consent Form, the Investigator Brochure, clinical site logs, accountability records and laboratory documents (e.g., certification, norms/ranges, etc.).

18.2.1 Retention of Records

The FDA requires that each Principal Investigator retain records for a period of two (2) years from the date of FDA approval to market the product for this indication, or two (2) years from the date that the Sponsor withdraws the application for approval.

18.3 Final Study Report

Upon completion of the study, the Principal Investigator is required to submit a final study summary report to the Institution. This report must also be submitted to the IRB.

18.4 Investigational Product Labeling and Accountability

18.4.1 Investigational Product Labeling

The activated MILs product will be labeled with the patient name, history number, unique product number and the volume within the bag.

18.4.2 Investigational Product Accountability

The Investigator should take adequate precautions, including storage of the investigational products, to prevent theft or diversion of the products into unauthorized channels of distribution. The processing of each product will be documented on a Batch Production Record. Release test results will be summarized on the Product Specification Report.

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20. APPENDICES

20.1 APPENDIX A DIAGNOSTIC CRITERIA FOR MULTIPLE MYELOMA

Major criteria:

1. Plasmacytomas on tissue biopsy;
2. Bone marrow plasmacytosis (>30% plasma cells);
3. Monoclonal immunoglobulin spike on serum electrophoresis IgG >3.5 g/dL or IgA >2.0 g/dL; kappa or lambda light chain excretion > 1 g/day on 24 hour urine protein electrophoresis.

Minor criteria:

1. Bone marrow plasmacytosis (10 to 30% plasma cells);
2. Monoclonal immunoglobulin present but of lesser magnitude than given under major criteria;
   3. Lytic bone lesions;
4. Normal IgM <50 mg/dL, IgA <100 mg/dL or IgG <600 mg/dL.

Any of the following sets of criteria will confirm the diagnosis of multiple myeloma:

1. Any two of the major criteria;
2. Major criterion 1 plus minor criterion b, c, or d; 3. Major criterion 3 plus minor criterion a or c; 4. Minor criterion a, b and c or a, b and d.

Reference: Durie, B. G. 1986. Staging and kinetics of multiple myeloma. Semin.Oncol. 13[3], 300-
309.

20.2 APPENDIX B DURIE-SALMON STAGING OF MULTIPLE MYELOMA

Stage I

All of the following must be present:

1. Hemoglobin > 10.5 g/dL or hematocrit > 32%;
2. Serum calcium level normal;
3. Low serum myeloma protein production rates as evidenced by all of the following:
    - IgG peak < 5g/dL;
    - IgA peak < 3g/dL;
    - Bence Jones protein < 4g/24 h.
4. No bone lesions.

Stage II

All patients who do not meet criteria for Stage I or III are considered to be Stage II.

Stage III

One of the following abnormalities must be present:

1. Hemoglobin < 8.5 g/dL, hematocrit < 25%;
2. Serum calcium > 12 mg/dL;
3. Very high serum or urine myeloma protein production rates as evidenced by one or more of the following:
    - IgG peak > 7g/dL;
    - IgA peak > 5g/dL;
    - Bence Jones protein > 12 g/24 h;
4. 3 lytic bone lesion on bone survey (bone scan not acceptable).

Sub-classification

A: Serum creatinine < 2.0 mg/dL
B: Serum creatinine ≥ 2.0 mg/dL

Adapted from: Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 1975;36:842-54.

20.3 APPENDIX C ECOG PERFORMANCE STATUS

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self care. Totally confined to bed or chair
5	Dead

AS PUBLISHED IN AM. J. CLIN. ONCOL. (CCT) 5:649-655, 1982

20.4 APPENDIX D. NEW YORK HEART ASSOCIATION CLASSIFICATION OF PATIENTS WITH DISEASES OF THE HEART

Functional Classification:

Class l	Patient with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
Class ll	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
Class lll	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
Class lV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

(New York Heart Association Criteria Committee: Diseases of the Heart and Blood Vessels: Nomenclature and Criteria for Diagnosis, 6th ed. Boston, Little, Brown, & Co. 1964)

APPENDIX E PATIENT STUDY CALENDAR FOR TRANSPLANT PATIENTS

	Study Registration Eligibility	Marrow Harvest	Baseline	Stem Cell Mobilization	Stem Cell Collection	Day -2 (Melphalan)	Day -1 (Melphalan)	Day 0 (Stem Cell Infusion)	Therapy: Day 1	Day 2	Day 3 aMILs Infusion[10]	Day 4 aMILs Infusion[10]	Day 5	Day 7	Day 11	Day 14	Follow-up: Day 28[15]	Day 60[15]	Day 180	Day 360	Quarterly until disease progression[16]	Off Study[14]
Informed Consent	X																					
Inclusion/Exclusion criteria	X																					
Clinical Assessment[1]	X																X	X	X	X		
Bone marrow examination[2]		X															X	X	X	X		X
PRS GEP-70		X																		X		X
Skeletal survey/PET[3]		X																		X		
Beta2-microglobulin		X																		X		
CBC w/diff, platelets	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Serum Chemistries[4]	X										X			X	X	X	X	X	X	X		X
HTLV-1, 2, HIV-1, 2	X																					
M-protein, serum[5]		X	X															X	X	X	X	X
M-protein, urine[6]		X	X															X	X	X	X	X
Quantitative immunoglobulins		X	X															X	X	X	X	X
Tadalafil[7]										X	X	X	X	X	X							

Lenalidomide[8]																		X	X	X	X	
Study Blood [9]		X									X_a			X		X	X	X	X	X		
Disease Response/Progression[11]			X															X	X	X	X	X
Survival Status[12]																		X	X	X	X	X
Current meds	X			X	X				X						X	X	X	X	X	X	X	
Adverse events[13]		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

APPENDIX E PATIENT STUDY CALENDAR FOR TRANSPLANT PATIENTS (CONTINUED)

1 Clinical assessment: history, review of systems, weight, vital signs, physical exam

2 Collect 20ml of marrow in a heparinized 60 ml syringe For Hopkins patients on the immune sub-study: 100ml of blood will be collected in the heparinized syringes (Hopkins only) instead of the 20ml and 20 ml of aspirate will be collected in a heparinized syringe instead of 15 ml in a green top tube..

3 Skeletal survey/PET scan is optional but recommended for patients with extensive bone involvement before transplantation and on Day 360

4 Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, AST, ALT, alk. phosph., total bili, albumin

5 M-protein, serum: serum protein electrophoresis & immunofixation, Freelite (sensitive assay for kappa and lambda light chains)

6 M-protein, urine: 24 hour urine collection for total protein, protein electrophoresis & immunofixation should be done at marrow harvest. For patients that do not have Bence-Jones proteinuria, 24-hour urine will only be collected to document complete remission.

7 Tadalafil will be given from days 2-11

8 Lenalidomide: start at 60 days as clinically indicated at 5mg 21 out of 28 days. Other dose adjustments per clinical practice a

9 100ml of blood will be collected in heparinized syringes, and an additional tiger top tube. Days1, 7, 11 and 14 should only be a tiger top tube and 2 EDTA (lavender tubes).. Only for patients participating on the substudy at Hopkins o

10 For aMILs infusion, patients must have T<38.5 C and room air O2 saturation of >90%. If T cell infusion is delayed, perform Day 7 evaluations on second day following T cell infusion

11 Disease progression will be captured at every visit for the first year and quarterly thereafter

12 Survival and disease status will be follow-up for 5 years from Day 0 of transplant

13 Captured adverse events are those felt to be in any way related to the infusion of aMILs and/or tadalafil. Standard stem cell transplant toxicities will not be captured

14 Attempts should be made for annual bone marrow biopsies. Biopsy done at relapse needs to have the MyPRS repe as well as Myeloma labs, CBC w/diff, Platelets, Serum Chemistries

15 For Clinical Assessment, Disease Response/Progression and Survival Status Assessment, Current meds and AEs collection allowed window is ±14 days

16 Upon relapse, the visit documenting relapse can serve as the end of study visit

20.5 APPENDIX F SKCCC DATA AND SAFETY MONITORING PLAN

http://cro.onc.jhmi.edu/pageData/dsmp.pdf

20.6 APPENDIX G: STANDARD EXPECTED TRANSPLANT RELATED TOXICITIES

Toxicity	Up to Grade
Investigations	
White blood cell decreased	4
Anemia	3
Platelet count decreased	4
Alanine aminotransferase increased	3
Aspartate aminotransferase increased	3
Blood and lymphatic system disorders	
Febrile neutropenia	3
Metabolism and nutrition disorders	
Anorexia	3
Hypo- or hypercalcemia	4
Hypo- or hyperkalemia	4
Hypo- or hyperphosphatemia	4
Hypo- or hypermagnesemia	4
Hypo- or hypernatremia	4
Skin and subcutaneous tissue disorders	
Rash maculo-papular	2
Alopecia	2
Gastrointestinal disorders	
Nausea	3
Vomiting	3
Oral mucositis	3
Diarrhea	3
Renal and urinary disorders	
Acute kidney injury	3
Infections and infestations	
Lung infection	3
Other infections and infestations	3
Respiratory, thoracic and mediastinal disorders	

Pulmonary edema	3
General disorders	
Fatigue	3
Musculoskeletal and connective tissue disorders	
Muscle weakness	3
Nervous system disorders	
Seizure	2
Reproductive system disorders	
Irregular menstruation	3

20.7 APPENDIX H: NCI CTCAE V4.0

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

20.8 APPENDIX I. A Randomized Phase II Study of Autologous Stem Cell Transplantation with Tadalafil and Lenalidomide Maintenance with or without Activated Marrow Infiltrating Lymphocytes (MILs) in High Risk Myeloma

Tadalafil Diary

Site Number		Patient's Initials	
Patient's Number		Patient's MRN	

Instructions for patient:

Please indicate the date and time you took Tadalafil 10 mg PO. If dose is missed please add comments. Please sign, date and return at the end of the treatment.

Study Day	Date	Time	Comments
Day +2		:	
Day +3		:	
Day +4		:	

Day +5		:	
Day +6		:	
Day +7		:	
Day +8		:	
Day +9		:	
Day +10		:	
Day +11		:	

Research Nurse's Comments (if Tadalafil doses were missed, treatment was interrupted or discontinued, please provide all related information):

_____

_____

_

Research Nurse Signature: _____ Date: _____

Patient Signature: _____ Date: _____

20.9 Appendix J. Adverse Event assessment Chart

A Randomized Phase II Study of Autologous Stem Cell Transplantation with Tadalafil and Lenalidomide Maintenance with or without Activated Marrow Infiltrating Lymphocytes (MILs) in High Risk Myeloma.

Adverse Event Assessment Chart

Site Number		Patient's Initials	
Patient's Number		Patient's MRN	

Source documentation, including all available clinic notes, is reviewed from the time of bone marrow collections of MILs through Day 360/disease progression. All toxicities, abnormal laboratory results and/or potential adverse events experienced by accrued subjects during this time are assessed by the PI. These assessments are to occur for each of the following time periods and confirmed by the PI based on attribution to study intervention(s).

- Only Adverse Events considered in any way related to the either infusion of aMILs or Tadalafil are recorded on the AE Log.
- Only clinically significant laboratory results considered in any way related to either aMILs or Tadalafil, and not at all related to bone marrow transplant, were also recorded on the AE Log.
- Serious Adverse Events are defined per protocol section 15.1.2 and this assessment chart does not pertain to those events (SAEs are individually assessed by PI on the AE Log).

Study Visits	PI signature	Assessment Date	Comments
BM Harvest – Day 1			
Day 2 – Day 14			
Day 14 – Day 28			

Day 28 – Day 60			
Day 60 – Day 180			
Day 180 – Day 360			

20.10 Appendix K: Revised Statistical Plan

Overview

The following describes a plan to analyze the data from study J1343 ("A Randomized Phase II Study of Autologous Stem Cell Transplantation with Tadalafil and Lenalidomide Maintenance with or without Activated Marrow Infiltrating Lymphocytes (MILs) in High Risk Myeloma") in light of reported lenalidomide dosing deviations. Briefly, these deviations occurred when on-study patients received doses of lenalidomide greater than 10 mg per day prior to disease progression during the time such higher doses were not allowed by the protocol.

This study's original design sought to evaluate the possible ability of activated marrow infiltrating lymphocytes (aMILs) to delay disease progression after autologous stem-cell transplant (ASCT). The study's primary endpoint is progression-free survival, defined in the protocol as "the time from ASCT to documented progression/relapse, death from any cause or last follow-up." The higher dose of lenalidomide prior to progression could serve to delay disease progression. Because these deviations occurred predominantly within one treatment arm (i.e., those receiving aMILs), the two treatment groups in J1343 did not receive the same care in all respects except for aMILs. A higher percentage of one group than the other appears to have received higher doses of lenalidomide treatment than the protocol prescribed, and these higher doses could affect the study's primary endpoint.

These dosing violations have compromised the randomization; therefore, we will not perform comparative analyses of efficacy. There is no simple way to salvage the analysis of a randomized clinical trial to reduce bias completely once the randomization has been compromised in a manner that affects the primary endpoint as in this case. Any straightforward comparison of the outcomes in the two treatment groups in our study will provide a biased estimate of the effect of adding aMILs to ASCT, keeping all other aspects of patient care the same.

Therefore, our primary analysis will focus on safety and feasibility of using MILs to treat patients with high-risk myeloma (as defined in the protocol) in the context of a multi-center study. There will not be a comparison of efficacy (progression-free survival) between the two treatment groups. Instead, the primary endpoint will be feasibility, defined below. A key secondary endpoint will be safety, which will be evaluated in the interval between transplant and 60 days after transplant.

Analysis Populations

The last subject on trial reached 60 days on May 3, 2019 and thus the data collection for the primary and key secondary endpoint was complete on that date. No new data were collected after that date. We will consider May 3, 2019 as the last possible date for follow-up times relating to time-to-event data (e.g., overall survival and progression-free survival).

Safety population

The safety population consists of all registered subjects who underwent MILs harvest. Any patient in the MILs arm who does not receive MILs will be so identified but will remain in that arm for analysis in order to provide an unbiased comparison to the control arm for safety.

Feasibility

Feasibility is defined as the ability to harvest, expand, and infuse the MILs product within 120 days, which is a typical timeframe between BMT referral and stem cell infusion. Based on our experience using MILs, we expect that MILs will be feasible for 90% of patients. We will declare the use of MILs not to be feasible if there is high probability that no more than 75% of patients can feasibly receive MILs. *A priori* our best guess is that MILs will be feasible for 90% of patients, and we think there is only 5% probability that the proportion for whom it is feasible is 75% or less. This assessment corresponds to a prior beta (23, 3.4) distribution for the proportion of patients for whom MILs is feasible. After treating 60 patients with MILs, we will declare MILs not feasible if we can only harvest, expand, and deliver MILs to 40 or fewer patients. If we are only successful with 40 of 60 patients, the probability that MILs is feasible for at most 75% of patients is 0.46 (i.e., less than even odds in favor of feasibility).

Safety Analyses

Safety and tolerability will be assessed, where applicable, by incidence and grade for adverse events (AEs) and deaths.

Adverse events (AEs)

The number and percentage of patients experiencing AEs of grade three or higher will be summarized. The incidence of AEs will be presented by treatment arm for those occurring from MILs harvest through 60 days after transplant. A patient who experiences repeated episodes of the same AE will be counted only once, with the highest severity grade used in tabulations of incidence.

A summary of the number of deaths, cause of death (progressive disease vs. other), and time of death (from the date of the MILs harvest) will be provided.

Efficacy Analysis

Efficacy analyses will be conducted on the population of all patients randomized to the MILs arm.

Progression-Free Survival (PFS) and Overall Survival (OS)

PFS time will be calculated from the time of randomization (in months) until disease progression, death due to any cause, or protocol deviation with respect to lenalidomide dosing (above 10mg), whichever occurs first. Subjects still at risk for one of the PFS events at the time of the analysis will be censored at the date last known to be at risk for one of these events. OS time will be calculated from time of randomization until death due to any cause. The last possible follow-up date for the study is May 3, 2019. Patients whose follow-up data indicate that they were alive without progressive disease (PFS) or known to be alive after having progressed (OS) at the time of last contact will be censored at the date of last contact in the analyses. If the patient's last contact occurs after May 3, 2019, then the patient's follow-up time will be censored on May 3, 2019.

Any presentation of the data will include a description of the protocol violations (dosing above 10mg of lenalidomide per day), the number of patients affected, and our assigning the timing of each violation as the occurrence of an event in the analysis of progression-free survival.

ORR

Best response on study will be reported according to IMWG criteria. The associated 95% CI will be estimated using the Clopper Pearson method (Clopper and Pearson, 1934).