Title: **A Pilot Study of Enhancing Anti-Tetanus Vaccine Response After Autologous Stem Cell Transplantation**

Principal Investigator: **Christopher D'Angelo, MD**

986840 Nebraska Medical Center

Omaha, Ne 68198-6840

Co-Principal Investigator: **Muhamed Baljevic, MD**

986840 Nebraska Medical Center
Omaha, Ne 68198-6840

Co-Principal Investigator: **Zaid Al-Kadhimi, MD**

986840 Nebraska Medical Center
Omaha, Ne 68198-6840

UNMC Secondary Investigators: **Sarah A. Holstein MD, PhD**

Julie M. Vose, MD

Matthew A. Lunning, DO

James Talmadge, PhD

Alison G. Freifeld, MD, FIDSA

BMT Immunotherapy
University of Nebraska Medical Center

Biostatistician: Lynette Smith, PhD

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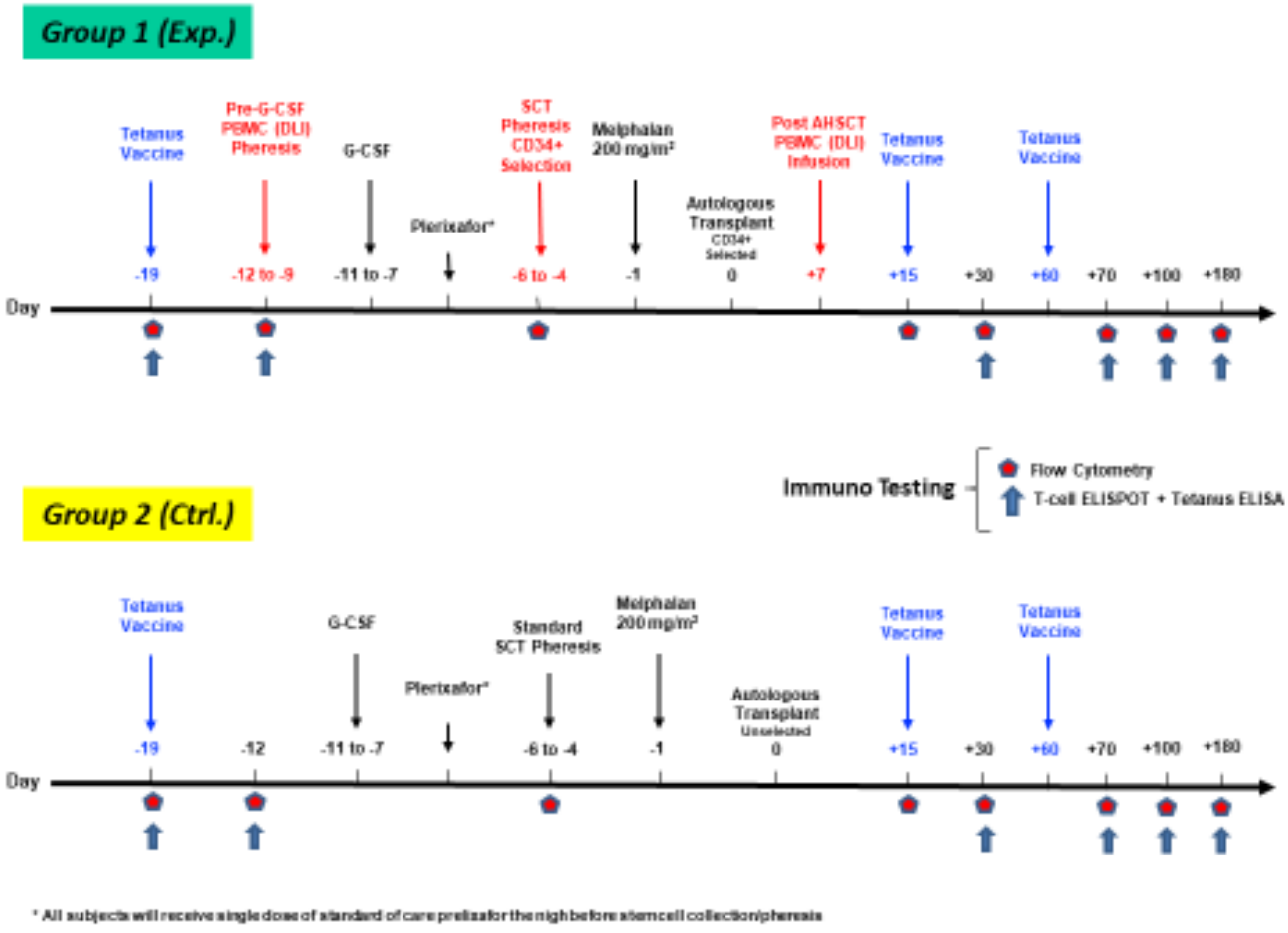
Abstract:

Because curative therapy for multiple myeloma is not yet available, the treatment goal is to produce lengthy asymptomatic disease remissions. High dose chemotherapy with autologous hematopoietic stem cell transplant (AHSCT) provides optimal remission outcomes, and is the current standard of care for transplant eligible patients. However, some patients experience early relapse. One possible explanation for early relapse is poor immune recovery after high dose chemotherapy given before AHSCT. Harvesting an autologous transplant graft product from circulating blood is facilitated by administration of granulocyte colony stimulating factor (G-CSF) to mobilize large numbers of hematopoietic stem cells into the circulation. The cells are collected from the blood with apheresis and stored until the transplant. Our preliminary studies show that immune suppressor cells are also mobilized and collected in the graft product. Depletion of these suppressor cells in the graft before transplant provided enhanced T-cell reactivity to allogeneic antigens and lymphocyte homeostatic expansion. The hypothesis is that depleting majority of immune suppressive cells from G-PBSC graft in vitro in the setting of AHSCT will tip the balance towards accelerated lymphocyte expansion in-vivo, which will be reflected in high ALC numbers on day 15 (>500/µl). The goal is to achieve this depletion via selection and infusion of CD34+ cells, thereby eliminating MDSC's and T-regs which have an immunosuppressing effect on immune reconstitution. This pilot randomized Phase II trial (10 subjects per arm) will compare immune reconstitution following transplantation of an autologous mobilized graft product to reconstitution following transplantation of a mobilized graft product followed by an autologous lymphocyte infusion collected prior to G-CSF mobilization. All subjects will receive tetanus vaccines pre and post-transplant. The primary end point will be tetanus vaccine immune responses post-transplant.

Schema:



As shown in the schema above:

1- Subjects in all groups will receive tetanus vaccine 7-10 days prior to mobilization.

2- Subjects in group 1 will have mononuclear cells collected and frozen 7-10 days after tetanus vaccination pre-transplant. These subjects will then receive a CD34+ selected graft on day 0 and autologous donor lymphocyte infusion (DLI) on day +7.

3- Subjects in group 2 will receive a standard G-CSF mobilized graft on day 0 without any DLI post-transplant.

4- Subjects in all groups will receive G-CSF as subcutaneous injections daily (10 µg/kg) x 5 days starting on day -11 (following the DLI pheresis in the experimental group). Subjects will receive one dose of plerixafor, a stem cell mobilizer, followed by pheresis on the next day to collect G-PBSC graft, with the goal of > 6.0 × 10^6 CD34+ cells/kg.

5- On day of G-PBSC pheresis, the graft in group 2 will be analyzed for CD34, lymphocyte content, and T-regs and MDSCs (cell differential and flow cytometry). Then it will be processed and frozen as per local institutional standards.

6- The stem cell product will start to be washed while waiting for the CD34+ content to be known. CD34+ stem cells will then be selected out of the G-PBSC mobilized graft the same day using clinical grade CD34+ magnetic beads and Clinimacs device from Miltenyi as per manufacturer's instructions and as described below. The CD34 content will be analyzed before freezing in liquid nitrogen.

7- The subjects in all groups will receive high dose chemotherapy Melphalan at 200 mg/m^2 on day -1.

8- On day 0, subjects in group 1 will receive the thawed CD34+ stem cell graft, while subjects in the group 2 will receive the standard thawed G-PBSC graft. Transplanted CD34+ stem cell dose both groups will be in the range of 2-10 X 10^6/Kg body weight.

9- On day +7 post-transplant, the stored autologous donor lymphocyte product in group 1 will be thawed and infused back to the subjects.

10- **No planned G-CSF post-transplant** (unless medically deemed necessary due to slow engraftment by day 15)

11- Subjects will be followed closely in the hospital until engraftment and resolution of all transplant related toxicities.

12- Subjects will be closely evaluated for any trial related adverse events, specifically: delayed engraftment, auto-immune side effects (autologous graft versus host disease), and infections.

13- At day +15(+/-3) and day +60 (+/-3) post-transplant, all subjects will receive tetanus vaccines.

14- At days + 30(+14), +70(+14), +100(+/-10), and 180(+/-10) post-transplant subjects will undergo immune evaluation for tetanus vaccine responses.

Section 1.0 Objectives:

Hypotheses

1. The abundance of immune suppressive cells in G-PBSC grafts results in poor immune reconstitution post AHSCT, leading to higher risk for infection and myeloma relapse.
2. Depletion of immune suppressor cells from G-PBSC graft will allow a robust *in vivo* expansion of the adoptively transferred lymphocytes as well as a robust recovery of antigen specific tetanus immune response post-transplant compared with the control group.
3. It is safe and feasible to deplete all immune suppressive cells from G-PBSC grafts by positively selecting CD34+ cells from G-PBSC grafts and combining that with an autologous donor lymphocyte infusion product.

1.1 Primary:

1- To compare the **cellular and humoral** vaccine response post-transplant between the two arms by performing Elisa, and T-cell enzyme-linked immunospot (ELISPOT) assays

2- To determine the feasibility and safety of this approach

1.2 Secondary:

1- To compare post-transplant recovery of innate and adaptive immune cells (CD8, CD4, CD19, NK, γδ T-cells), in addition to T-cell phenotype markers between the two arms.

2- To compare post-transplant recovery of T-regs and MDSCs between the two arms.

3- To compare progression free survival (PFS) at 2 years post-transplant

1.3 Exploratory objective:

To compare the depth of multiple myeloma (MM) response by international myeloma working group (IMWG) criteria, including analyzing minimal residual disease (MRD) status at 3 and 6 months post-transplant for those subjects who are close to achieving CR (defined as monoclonal serum protein of ≤ 0.1 g/dL, with negative urine protein electrophoresis) between the two arms.

Section 2.0 Introduction:

2.1 Myeloma relapse is the main challenge post autologous hematopoietic Stem cell transplant (AHSCT)

Multiple Myeloma (MM) is a hematologic malignancy with a rising incidence in the US (about 30,000 cases newly diagnosed per year and 12,000 related myeloma deaths per year). Unfortunately, myeloma is considered incurable despite widening available standard therapies. New and innovative approaches are needed to improve the outcomes of this growing disease[1]. AHSCT has significantly improved disease free and overall survival (OS) for patients with MM. However, relapses continue to limit long term survival of these patients.[2] The efficacy of AHSCT as a treatment modality has relied mainly on the hope that high dose chemotherapy will eradicate the resistant tumor clones that survived standard chemotherapy. This paradigm has not changed in more than 30 years. Unfortunately, the same intensive preparative transplant regimen that overcomes resistance to standard dose chemotherapy, depletes various immune cells as well as compromises their function, increasing the risk for serious infections from various agents as well as potentially increasing the risk of myeloma relapse post-transplant.[3] Advances in modifying the chemotherapy preparative regimen and supportive care have resulted in less transplant related morbidity and mortality, but no improvement in relapse rates. Attempts at improving AHSCT efficacy by tumor cell purging, CD34 selection or switching the preparative regimen have not been successful.[4,5] Therefore, new interventions are needed to improve autologous transplant outcomes.

2.2 High dose chemotherapy and autologous peripheral blood stem cell transplant can be a favorable vaccine platform for multiple myeloma.

The state of minimal residual disease and abundance of homeostatic cytokines (IL-7 & IL-15) can make the early phase post AHSCT a favorable platform for vaccine response.[6] The recovery phase of lymphopenia enhances tumor vaccine responses in multiple animal models. [7-14] Mitchell et al. has demonstrated a favorable immune response to PP65 pulsed dendritic cell vaccine given during chemo induced lymphopenia in patients with glioblastoma.[15] Sampson et al. showed that greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma.[15] However, studies that tested early vaccine responses post AHSCT showed poor immune responses, which reflects the immune suppression state in the first year post AHSCT.[17,18]

2.3 Immune reconstitution after AHSCT.

Today's AHSCTs are almost all done with G-CSF mobilized PBSC (G-PBSC), rather than bone marrow grafts based on faster engraftment, decreased risk of infections and days of hospitalization.[19] Early hematopoietic reconstitution of white blood cells, hemoglobin and platelets occur within the first two weeks post AHSCT. However, there is a substantial delay in reconstitution of the non-granulocytic immune components. B-lymphocytes (B-cell) recover to normal numbers within 2-4 months.[20] Natural killer cells (CD56+3- or CD16+3- NK) reached normal values within one month post-transplant.[20] Total T-lymphocyte (T-cell) CD3+ reconstitution occurs within 2-4 months post AHSCT. All patients showed a low CD4/CD8 ratio during the first year post transplant, due both to low numbers of CD4+ cells and elevated numbers of CD8+ cells. The low number of CD4+ cells was due to a persistently low level of naive CD4+CD45RA+ cells. While a high proportion of the CD8+ cells displayed a phenotype compatible with activated T-cells (CD8+DR+) up to 10 months after AHSCT. This seems to be secondary to the intrinsic difference between CD4 versus CD8 regeneration pathways post intensive chemotherapy. CD4 regeneration post intensive chemotherapy is more dependent on thymic function, which starts to regress even in young adults.[21-23] Therefore CD4 reconstitution post AHSCT is mainly dependent on expansion of mature T-cells in the graft.[24] A clear global

suppression of T-cell proliferative, and cytokine release in response to mitogenic agents *in vitro* has been observed in the first 6-12 months post AHSCT.[23-26]

2.4 Antigen specific immune reconstitution

Riemer et al. evaluated the antigen specific humoral immune response post AHSCT in 38 patients. He found that IgG antibody titers against measles, mumps, rubella, and polio were present in almost all patients pre-transplant and during 12 months post-transplant, indicating persistent humoral immunity.[27] Svane et. al. evaluated the antigen specific cellular immune responses post AHSCT in breast cancer patients.[28] Peripheral blood was collected from five breast cancer patients at serial time points in connection with chemotherapy treatment and in a follow-up period of 1 year. The frequencies of CD8 and CD4 T-cells responsive to cytomegalovirus (CMV), varicella zoster virus (VZV), and tetanus in antigen-activated whole blood were determined by flow cytometric analysis of CD69, (intra-cellular) TNFα, and IFNγ expression. Mononuclear cells were labeled with PKH26 dye and the CMV, VZV, and tetanus toxoid-specific proliferation of T-cell subpopulations was analyzed by flow cytometry. In none of the patients did the treatment result in loss of overall T-cell reactivity for any of the antigens. Prior to chemotherapy 5/5 patients possessed TNF expressing T-cells specific for CMV, 4/5 for VZV, and 3/5 for tetanus. One year after stem cell transplantation all patients possessed TNF expressing T-cells specific for CMV, VZV and tetanus. The highest percentages of cytokine responding T-cells were seen after stimulation with CMV antigen. **In general, the lowest antigen specific immune reactivity (close to zero) was measured in G-CSF-mobilized blood at the time of leukapheresis.** Despite a continuously reduced CD4 to CD8 ratio after transplantation, recovery of antigen specific CD4 T-cells usually occurred prior to CD8 recovery and often to a higher level. This study demonstrated that natural as well as vaccine-induced specific immunity established prior to AHSCT can be regained after stem cell transplantation.

2.5 The impact of post AHSCT immune suppression on risk of infections:

The risk of viral infections is higher in the first year post AHSCT. Varicella zoster virus VZV seems to be the main one, with a risk of 23-50%.[29,30] The risk of VZV infections was higher when CD4<200/µl, and CD8<800/µl. Patients who met both of these predictors had a VZV risk of about 48% at one year.[31] Other less common infections include CMV. Wingard et. al. in 1988 reported a 45% rate of CMV infections in 165 patients post auto transplant (based on a rise in CMV antibody titer). However, the rate of CMV pneumonia in that study was 2%.[32] Kadakia et. al. reported on human herpes virus 6 (HHV6) viral infections in the first year post autologous transplant. By using direct viral isolations, HHV6 DNA, and HHV6 antibody titer testing, they could detect active HHV6 infection in 10/11 autologous transplant patients. Furthermore there was a 55% association between HHV6 isolation and sinusitis (P=.002).[33, 19] The risk of CMV, VZV, parainfluenza and bacterial infections were all increased in CD34 selected AHSCT compared to standard AHSCT, based on the excessive immune suppression post-transplant caused by T-cell depletion.[34]

2.6 The impact of post AHSCT immune suppression on the risk of cancer relapse:

There is no clear direct evidence to link the post AHSCT immune suppression, slow numerical and functional T-cell recovery to rates of relapse. However, animal models and clinical data strongly suggest a link between immunosuppression and occurrence/progression of cancer. Furthermore, retrospective studies and one prospective study in AHSCT suggest that such link exists.

Animal studies of gene-targeted and lymphocyte subset-depleted mice were used to establish the relative importance of NK and NK1.1+ T (natural killer T, NKT) cells in protecting against tumor initiation and metastasis. In these models, CD3+ NK cells were responsible for rejection and

protection from metastasis of chemically induced (methylcholanthrene MCA) tumor.[35,36] Similarly Rag2-/- mice (no T-cells, and B cells) showed increased susceptibility to chemically MCA induced tumors.[37] Studies of T-cell receptor deficient mice demonstrated a non-overlapping role of γδ T-cells and αβ T-cells in blocking the formation and progression of chemically induced tumors.[38,39] These studies seem to suggest that γδ T-cells act to inhibit initial tumor formation, while αβ T-cells directly inhibit tumor progression by using their cytotoxic mechanisms to kill tumor cells.

Clinical studies have shown that the high-grade density of CD8+ T-cells in cancer cell nests correlated with prognosis. The presence of tumor infiltrating T-cells (TILs) was able to predict a better survival as an independent prognostic factor in various types of cancers including colon cancer,[40,41] esophageal cancer,[42] oral squamous cell carcinoma,[43] breast cancer,[44] ovarian cancer,[45,31] and malignant melanoma.[46]Other studies have shown a similar positive correlation between NK cell infiltration and the survival for gastric cancer,[47] colorectal cancer,[48] and squamous cell lung cancer.[49]

Increased relative risk for various types of cancers has been observed in immunosuppressed solid organ transplant recipients that have no apparent viral origin. Information on 5692 Nordic recipients of renal transplants in 1964–82 was linked to the national cancer registries in 1964–86 and to population registries. Significant overall excess risks of two-to-five-fold were seen in both sexes for cancers of the colon, larynx, lung and bladder, and in men for cancers of the prostate and testis. Notable high risks ranging from 10-30 fold above expectations, were associated with cancers of the lip, skin (non-melanoma), kidney, endocrine glands, non-Hodgkin's lymphoma, and in women with cancers of the cervix and vulva–vagina.[50] Additionally, there have been case reports of growth and flare up of epithelial cancers in chronic lymphocytic leukemia (CLL) patients after the use of nucleoside analogs fludarabine or 2-chlorodeoxy adenosine (2CdA).[51,52] Allogeneic bone marrow transplants have been associated with increased risk for squamous cell carcinoma of the buccal cavity and skin.[53]

In AHSCT patients, the group from Mayo clinic has retrospectively evaluated the role of early lymphocyte count recovery post-transplant. In multiple reports, they found that absolute lymphocyte count ALC of 500/µl on day 15 post AHSCT correlated with improved overall and disease free survival in patients with Hodgkin's lymphoma, NHL, acute myeloid leukemia, multiple myeloma, amyloidosis, and breast cancer.[54-58] Porrata et al reported one prospective study on 50 NHL patients undergoing AHSCT, confirming the same above observation.[59]
More recently peripheral blood absolute monocyte count (AMC) has been used as a surrogate marker of tumor microenvironment immune suppression.[60] Porrata et al. showed a correlation between AMC excess at day 15 post-transplant with risk of relapse and low PFS. He showed that day+ 15 ALC/AMC ratio> 1.0 correlated with better survival. Furthermore, he demonstrated that the recovery of ALC and AMC at day 15 post AHSCT correlated with the numbers of these cells in the autologous graft.

The value of immune recovery was further highlighted in a recent report that looked at immune signatures associated with improved PFS and OS for myeloma patients treated with AHSCT.[61] In this study, MM patients underwent comprehensive immune profiling (IP) in peripheral blood and had the marrow tested for MRD before and approximately 100 days after AHSCT. It was found that higher $\gamma\delta$ T-cell counts post-AHSCT correlated with improved 2-year PFS and OS[61]. Furthermore, the higher $CD4^+$ central memory (CM) cell counts post-AHSCT were associated with improved 2-year OS but not PFS. The higher $\gamma\delta$ T-cell and $CD4^+$ CM-cell count associations were primarily observed in MRD-negative patients post-AHSCT and in patients not receiving maintenance therapy.[61]

Collectively these studies suggest that high ALC and low AMC on day 15 post AHSCT are markers of accelerated immune recovery, and lower immune suppression, resulting in lower risk of relapse and better long-term survival.

2.7 The autologous graft is enriched with immune suppressive cells:

One of the immune suppressive cellular elements enriched in the G-PBSC grafts are regulatory T cells (T-reg). These $CD4^+CD25^+$hi T-reg is a specialized subset of T-cells that act to suppress immune activation, and thereby maintain immune tolerance to self. FOXP3 is thought to be a characteristic marker for T-regs (a master transcriptional regulator in the development and function of regulatory T-cells).[62] Seddiki et al. showed that expression of $CD4^+CD25^+$CD127low phenotype gave an accurate estimation of T-reg cell numbers and correlated well with FOXP3 expression. [63,47] The bone marrow is an important reservoir for functional $CD4^+CD25^+$hi T-regs. G-CSF mobilizes T regs from the bone marrow to the peripheral blood through down regulation of bone marrow CXCL12, a ligand for CXCR4 receptor expressed on the surface of T-reg.[64] Ruttella et al. showed that *in vivo* exposure to G-CSF induced suppressor T-cells with a peculiar cytokine profile (IL-10 high, TGF-β1 high, IL-2 low, IL-4 low), and suppression of antigen-driven proliferation.[65] A clinical trial evaluating the role of AHSCT in juvenile idiopathic arthritis demonstrated an accelerated recovery of $CD4^+CD25^+$hi T-regs in peripheral blood post-transplant.[66] This phenomenon correlated with the clinical quiescence of the autoimmune process. *In vitro* depletion of these T-regs from peripheral blood samples post-transplant partially corrected the T-cell dysfunctional state. Early after AHSCT, T-reg expansion occurs through homeostatic proliferation from the G-PBSC graft rather than through thymic differentiation. [65]

Another immune suppressive population abundant in the G-PBSC grafts is immature monocytes. Fraser et al. showed an increased proportion of CD14+ monocytes in G-PBSC grafts. Majority of these monocytes were immature, expressed low CD14, had surface bound IL-10, and induced significant T-cells suppression *in vitro*.[67] Activation of STAT3 pathway and expression of FAS ligand are possible mechanisms of their immunosuppressive phenotype.[68,69] G-CSF mobilized immature monocytes are recruited to the tumor microenvironment, where their phenotype is further modified to become myeloid suppressor cells (MDSC).[70] These MDSCs are usually CD33+, CD11b+,HLA-DR low and CD15+/-.[71]

As mentioned above Saven et al. noticed the lowest level of antigen specific T-cell immune responses in G-PBSC grafts.[28] The abundance of immune suppressor cells in G-PBSC grafts raises concern about their *in vivo* effect on the immune recovery post-transplant.

2.8 The effect of T-regs on T-cell homeostatic expansion *in vivo*

In an autoimmune disease animal model, Shen et al. demonstrated that T-regs inhibited homeostatic proliferation of adoptively transferred T-cells, reduced their survival, and reduced their functional differentiation into cytokine producing effector/memory T-cells. Eventually these T-regs prevented the development of clinical autoimmune disease.[72] A similar observation of enhanced T-reg recovery was made by Alexander et al. in patients with refractory systemic lupus erythematosus (SLE) who underwent AHSCT.[73] Collectively the above data offer a possible explanation for the lack of robust T-cell homeostatic expansion post AHSCT. Therefore, in the absence of immune suppressor cells (no G-CSF), chemotherapy induced lymphopenia can stimulate significant *in vivo* expansion and activation of donor derived lymphocyte infusions both in animal models and human clinical trials. [74,75]

2.9 *In-vivo* depletion/modulation of immune suppressive cells improves anti-tumor immune response:

Kline et al. demonstrated that depletion of T-regs enhanced *in vivo* expansion of adoptively transferred tumor specific T-cells, increased their cytokine secretion and their ability to eradicate pre-injected subcutaneous tumors in RAG2-/- (lymphocytes deficient) B16 melanoma animal model. To simulate a transplant model, similar results were observed when C57BL/6 (immune intact) mice irradiated and received adoptive transfer of total or T-reg depleted splenocytes.[76] Collectively the above data showed that T-reg depletion not only enhanced T-cell homeostatic expansion, but also improved their ability to eradicate tumors. Jennifer Ko et al. demonstrated that sunitinib dropped the proportion of MDSC and T regs in the peripheral blood of renal cancer patients, as well as improved T-cell immune function.[71] The use of monoclonal antibodies (CD33 or CD15) for *in vitro* purging of myeloid leukemic cells from PBSC grafts have been attempted before in clinical trials.[77,78] However, significant delays in neutrophil and platelet engraftment post-transplant have prevented further development of this approach.

2.10 Feasibility of CD34+ selection for clinical applications

The feasibility of CD34+ hematopoietic stem cell selection in AHSCT was demonstrated in the late 90s as a strategy of purging the graft from cancer cells.[79 – 83] This approach was tested in a large randomized phase III MM trial and shown to be safe, however there was no improvement in PFS or OS.[4] CD34+ stem selection using the immune-magnetic bead approach is now FDA approved allogeneic stem cell transplant in acute myeloid leukemia. Previously completed phase II trial demonstrated feasibility of engraftment with as low as 2.0 million x 10^6/Kg body weight CD34+ selected stem cell graft for patient with hematologic malignancy[93].

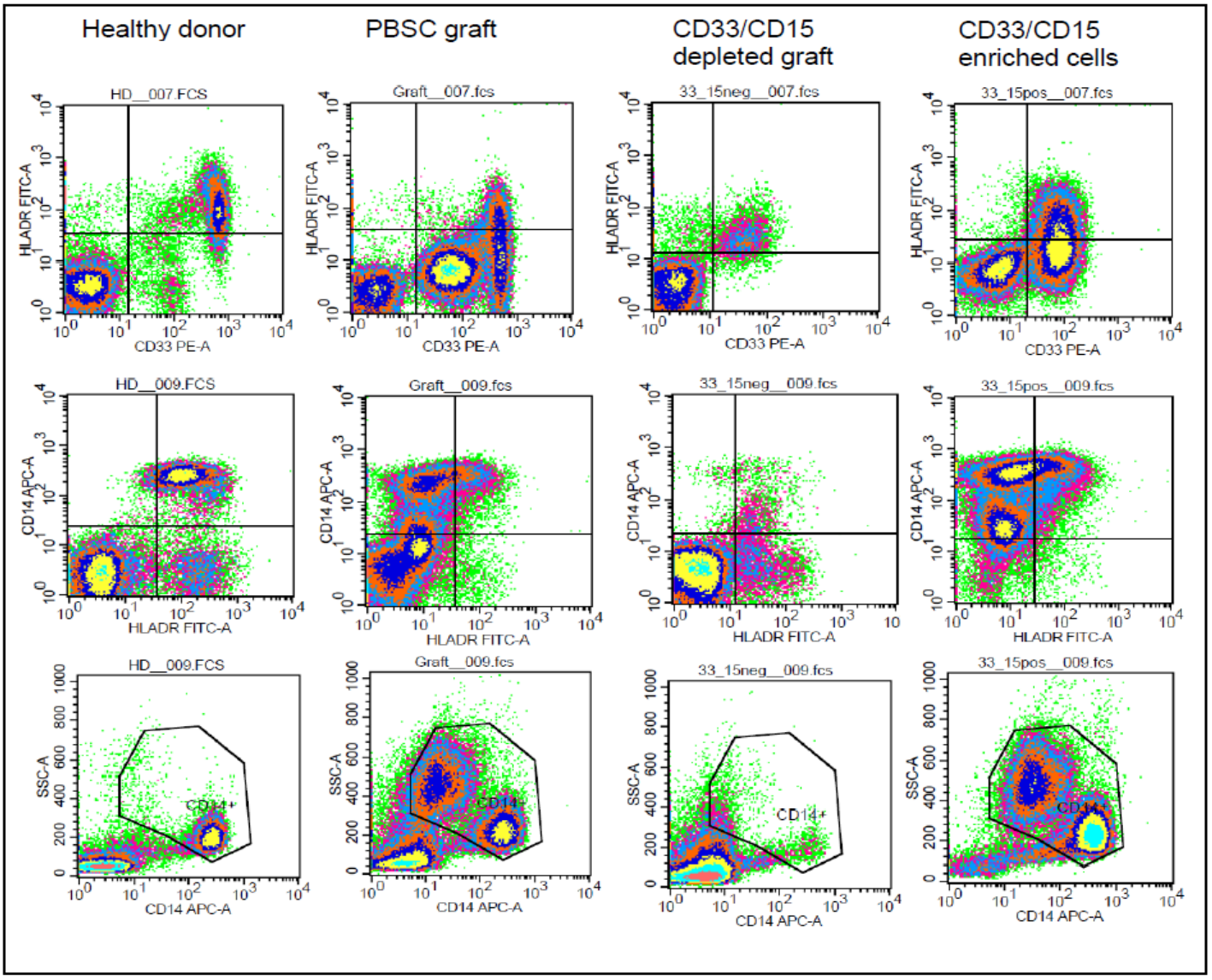
Therefore, combining a pre G-CSF peripheral blood mononuclear cells (PBMC) pheresis product (autologous DLI) with CD34+ selected stem cells may produce a graft with minimum immune suppresser cells, enhanced immune reconstitution and vaccine immune response post AHSCT.

2.11 Preliminary data:

2.11.1 - G-CSF mobilized grafts are enriched with myeloid derived suppressor cells MDSCs.

A- We have evaluated the proportion of MDSC in G-PBSC graft samples and compared them with healthy donor's peripheral blood. As shown below in **Figure 1** (first two columns on the left), the proportion of CD33+/HLA DR low, CD14+/HLA DR low, CD14 low/side scatter high population (immature monocytes), were all higher in the G-PBSC graft compared with healthy donor blood samples (six patient samples). While all the CD33+/HLADR low cells were positive for CD11b, a proportion of these MDSCs were positive for CD15 (data not shown). We demonstrated the *in vitro* feasibility of depleting MDSCs using CD33/15 Miltenyi's magnetic beads (third column from the left showed the depleted graft), while the fourth shows the positively selected population.

Figure 1

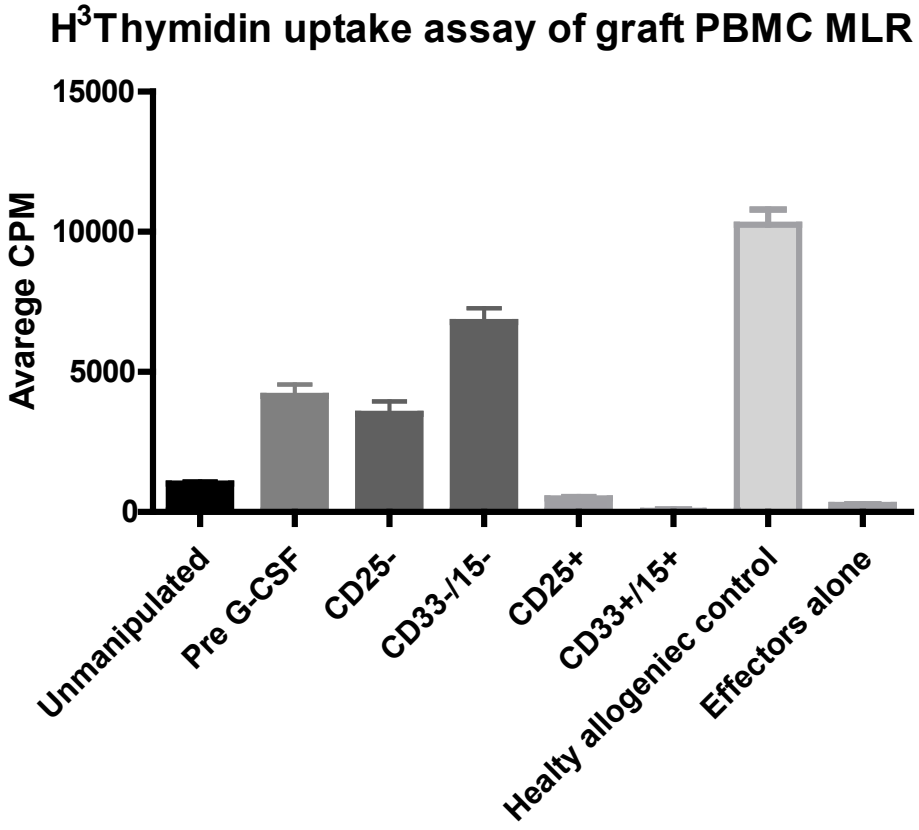


2.11.2- Depletion of T-regs and or MDSC led to improved T-cell proliferation, in response to an allogeniec mixed lymphocyte reaction.

We performed a standard one way mixed lymphocyte reaction (MLR) with responder G-PBSC graft cells (1 x 10^5 cells) co-cultured with a similar number of irradiated (2500 rads) allogeneic stimulator PBMC for 6 days in a round bottomed 96-well microtiter plates in 37° humidified 5% CO_2 incubator. The cells then were pulsed with 2 µCi of ^{3}H-thymidine for 16 hours, then harvested and counted in a beta scintillation counter. G-PBSC graft samples (six patients) were divided to 5 groups: (1) un-manipulated G-PBSC graft sample, (2) CD25 depleted, (3) CD33/CD15 depleted, (4) CD25 enriched, (5) CD33/CD15 enriched. Controls included: (1) patients' own pre G-CSF PBMC sample MLR (2) a healthy donor MLR, and (3) G-PBSC effecter cells only with no targets. We observed an increase in proliferation of CD25 or CD33/15 depleted G-PBSC graft sample over un-manipulated G-PBSC graft in 6 patients, while CD25 or CD33/15 enriched groups in all

the patients had very little reaction as shown in the **Figure 2** below. Each bar shows the average counts per minute reflecting ^{3}H-thymidine uptake for patients in that group.

Figure 2



2.11.3- Summary data on two patients treated on the experimental arm of this protocol at Emory University.

Two high risk MM patients were treated on the experimental arm of this protocol at Emory University. They received all pre- and post-transplant vaccines in addition to graft manipulation. Both patients started to show rise in WBC on days (15 and 16) with ANC engraftment on days (17 & 18). Platelets started to rise on day 2 13 & 14 with platelet engraftment on days 15 & 16. Both patients experienced transient neutropenic fever with no positive cultures. Both patients were discharged from the hospital and had no further complications post-transplant. Both patients continue to be in remission >12 months post-transplant. Day 15 post-transplant ALC/AMC ratio was >1 in both patients.

Section 3.0 Eligibility Criteria:

3.1 Inclusion Criteria

1. Age ≥ 19 years to 70 years old at time of study entry (consent)
2. Diagnosis of Multiple Myeloma (MM) as per updated International Myeloma Working Group (IMWG) criteria[84].
3. Must have received bortezomib, lenalidomide and dexamethasone (VRd) as a form of induction therapy pre-AHSCT (use of cyclophosphamide, bortezomib and dexamethasone may be allowed for up to 2 weekly doses before initiation of VRd induction, if necessary clinically for cytoreduction)
4. Able to understand and sign a consent form.
5. Creatinine clearance equal or > 60 ml/min (calculated)
6. Ejection fraction equal or > 50% before admission for transplant as per institutional standards. Patients with coronary heart disease (recent myocardial infarctions, angina, cardiac stent, or bypass surgery in the last 6 months or arrhythmia) need to be cleared by cardiology as per institutional BMT standards.
7. Total bilirubin ≤2.5 times the upper limit of the institutional normal values, and total AST (SGOT) and ALT (SGPT) ≤2.5 times the upper limit of the institutional normal values.
8. FVC, FEV1 or DLCO equal or >50% predicted before admission for transplant as per institutional standards. Patients on home oxygen are not allowed on the protocol.
9. No more than 6 months of pre-transplant MM chemotherapy is allowed (from the date of the start of the induction therapy).
10. KPS ≥ 70%or ECOG 0-2.
11. Must be eligible to receive Melphalan dose of 200mg/m^2
12. A female of child-bearing potential, must have two negative urine pregnancy test results at time of eligibility testing and prior to GCS-F pre-transplant as a way of ensuring safe transplant planning.

3.2 Exclusion Criteria

1. Participation in another clinical study with an investigational product during the last 28 days.
2. Prior stem cell transplant (either autologous or allogeneic)
3. Creatinine clearance < 60 ml/min (calculated)
4. Subjects who failed to achieve ≥ PR to induction therapy (i.e. VRd) and required salvage induction prior to AHSCT.
5. Documented central nervous system or extramedullary disease.
6. Significant organ dysfunction deemed to carry inappropriate risk for AHSCT.
7. Intention or plans for cyclophosphamide mobilization.
8. Known allergic reactions after previous tetanus diphtheria vaccination or had a condition of Guillain Barre Syndrome (GBS)
9. Known active hepatitis B, C or HIV infections on initial assessment.
10. Enrollment on any other transplant related protocols.

3.3 Minorities and Women

Both genders and from all racial/ethnic groups will be recruited equally into this trial.

Section 4.0 Registration Procedure:

The date of enrollment is the date of consent. However, all eligibility criteria do not need to be met until the date of the first study related treatment.

All subjects need to sign the consent form in order to enroll on the study. They will receive a copy of the consent form. In case of any issues, the treating physician or PI should be called for clarification. The subject will be registered as describe below in section 4.2.

4.1 Recruitment

Subjects, who are referred to the Nebraska Medical Center (NMC)/UNMC with a diagnosis of MM and considered eligible for autologous hematopoietic stem cell transplantation will be identified from the outpatient clinics or inpatient service. Potential subjects will be recruited for the study by the principal investigator (PI), co-investigators or participating clinicians.

Screening eligibility is based on standard clinical care performed by Bone Marrow Transplant (BMT) physician. Pre-transplant work up performed for determining the eligibility of the subject for high dose chemotherapy and autologous transplant, prior to signing of consent form will be acceptable. All other study related interventions and evaluation can only be performed after signing the consent form.

If the subject is screened as potentially eligible, he/she will then be offered the option to participate. An informed consent will be signed by the subject after thorough review of the study is completed with the physician and his/her designee.

4.2 Eligibility Verification/Registration

Before potential subjects are registered into the study, an eligibility checklist (Appendix A) must be completed to confirm the subject meets the eligibility criteria. The eligibility checklist will be maintained in the study file as source documentation if it has been reviewed, signed, and dated prior to registration by the treating physician.

Subjects will be registered through the sponsor site (UNMC) by contacting the UNMC Project Coordinator. Study personnel from UNMC will contact the UNMC Research Project Coordinator if a subject appears to meet the eligibility criteria. They will email the following information:

- Registration request with Demographics cover sheet (located in the Study site Manual)
- Signed, completed eligibility checklist (Appendix A)
- Copy of the signed and dated consent form

Once the UNMC Research Project Coordinator confirms that the subject meets criteria, and target accrual has not been met, approval for the subject will be given and study subject number assigned. An email confirmation of registration is sent by the UNMC Research Project Coordinator to the site. The project coordinator can assign a subject study number prior to final registration confirmation of a subject.

Each subject consented to the protocol is loaded into the UNMC Clinical Trial Management System (CTMS) within 7 days of consent. CTMS registration includes entering the required demographic information as stated in the SRC policies and procedures.

4.3 Randomization

The protocol will randomize subjects to two subsequent arms (10 subjects per arm): group 1 will get a CD34+ selected graft + autologous DLI on day+7, while group 2 will get standard G-CSF mobilized graft only.

The process of a 1:1 randomization between the experimental and control arms. During the registration procedures, study personnel are provided the randomization assignment from the the

IIT Office project coordinator. The 1:1 randomization will be conducted using blocked randomization with blocks of size 2 or 4.

4.4 Instructions for Subjects Who Do Not Start Assigned Protocol Treatment

If a subject does not receive any assigned protocol treatment after consenting, baseline data will be collected and included in the electronic data capture system.

Section 5.0 Treatment Plan of Research Design:

5.1 Outline of research plan

The protocol will randomize subjects to two subsequent arms (10 subjects per arm): group 1 (experimental) will get a CD34+ selected graft + autologous DLI on day+7, while group 2 (control) will get standard G-CSF mobilized graft only. Nebraska Medicine Biological Production Facility (BPF) personnel will perform processes outlined in Sections 5.1.4 through 5.1.9 below.

5.1.1 Pre-transplant Tetanus Vaccine

Vaccination can start whenever the subject is in reasonable physical condition to move forward to AHSCT (VRd 3-6 cycles) with a minimum of partial response by IMWG, and at least 2 weeks since the last does of bortezomib, lenalidomide or dexamethasone. Tetanus (without pertussis) is given pre-transplant as an intramuscular injection. Tetanus vaccination will be documented in the medical record per usual clinical practice. Data needed for the study data set will be recorded from this information.

5.1.2 Immune Correlative Studies

In all subjects, 70 mls of peripheral blood will be obtained (four tablespoon full) will be collected from all subjects for baseline immune correlative studies . A similar amount of blood will be collected at pre-G-CSF, graft pheresis and on days +15 (+/- 3), +30 (+14), +70 (7-14 days post last dose of tetanus vaccine), +100 (+/-10), and 180 (+/-10) post-transplant to analyze initial post-vaccine immune response. More details in section 5.7.

Test	Time point	Pre vaccine	Pre - PBMC harvest/Pre G-CSF	Graft Pheresis/PBSC graft day	Day + 15 (+/- 3)	Day + 30 (+14)	Day + 70 (+14)	Day + 100 (+/- 10)	Day + 180 (+/- 10)
T-cell Elispot ELISA		X	X			X	X	X	X
Flow		X	X	X	X	X	X	X	X
ELISA to Tetanus		X	X			X	X	X	X

5.1.3 Pre-G-CSF PBMC (DLI) Pheresis

Seven to 10 days after vaccine, subjects in group 1 will undergo mononuclear leukapheresis to collect and freeze PBMC product for DLI. Subjects will be apheresed for a target blood processed volume of 24-28 liters or as the patient tolerates and per standard UNMC BPF practice. Lymphocyte content of the apheresed product as well as subset analysis will be determined by a cell differential count and flow cytometry.

5.1.4 Stem cell lab processing of the pheresis product

Depending on the volume of the product and the total nucleated cell count (TNC) in it, additional processing may be required to minimize volume. The recommended storage cell concentration should not exceed $2x10^8$/mL. This will require centrifugation and plasma

reduction at 1000X G for approximately 15 minutes. The excess plasma is discarded then. The total cell count and volume of the concentrated product is determined. The proportion of the following components in the final product should be approximately: PBMC 75%, plasma/serum 5%, Plasma-Lyte 9.5%, DNase 0.5% and DMSO 10% per unit volume. For example for a 100 ml final product volume the PBMC should be 75ml, plasma/serum 5 ml, plasma-Lyte 9.5 ml, DNase 0.5 ml, and DMSO 10 ml. The product is then taken to the controlled rate freezer before final transfer to liquid nitrogen.

5.1.5 Stem cell mobilization

Subjects in all groups will receive G-CSF as subcutaneous injections daily (10 µg/kg/day) x 5 days starting on day -11 (after 1st leukapheresis for groups 1). Subjects will receive one dose of plerixafor followed by pheresis on the next day to collect G-PBSC graft. No Cyclophophamide mobilization is allowed on this protocol. Follow standard of care for stem cell collection.

5.1.6 Graft processing on the experimental arm

The stem cell product may start to be washed while waiting for the CD34+ content to known. Subjects who fail to mobilize a minimum of 6 million CD34/kg on day 1 of leukapheresis will be removed from the experimental arm of the study (these subjects will be treated and followed like group 2 for a separate analysis at the end).

- Subjects who mobilize 6-10 million CD34/kg on day 1:
    - will proceed to CD34 stem cell selection on the Milteny device, provided that the TNC content does not exceed the column capacity. They will proceed to day 2 collection to complete CD34 need for second stem cell transplant 2 million CD34/kg + 2 million CD34/kg reserve for potential “boost” need in case of graft failure on protocol (hence 4 total).
- Subjects who mobilize >10 million CD34/kg on day 1:
    - They can have approximately up to 10 million CD 34/kg processed on the Miltenyi column, if the TNC content does not exceed the column capacity (remainder difference for the second AHSCT and the boost will be collected on the following day 2 of collection).
- Collecting at least 14 million CD34/kg on day 1 will complete stem cell collection process(10 for Miltenyi, 2 for second AHSCT and 2 for boost).

5.1.7 Stem Cell lab processing for CD34+ selection

The PBSC pheresed graft is washed initially with PBS containing 5mM EDTA and 0.05% human albumin as per manufacture’s standard operating procedure SOP. After reducing the total volume to 95 mls, the anti CD34 antibody (directly conjugated to magnetic micro beads Miltenyi) will be added and incubated for 30 minutes. The cells then washed with Max buffer and attached to Clinimax tubing set. The enriched cells will be cryopresrved with DMSO 7.5%, PBS/HAS 4%/heparin and stored in the gas phase of liquid nitrogen. CD34 content of the final product should be no less than 2 million/Kg body weight.

All subjects in the control group will have the graft absolute lymphocytes quantified (differential count). A sample of the graft will be sent to the stat hematology lab for white cell count and differential. In addition, another sample will be sent for flow cytometry for subset analysis.

5.1.8 Conditioning Regimen

Transplant preparative regimens**:** a single intravenous dose of Melphalan 200mg/m^2 on day -1 is used as the standard preparative regimen in subjects with multiple myeloma.

5.1.9 Day zero

The CD34 stem cells will be thawed and infused back to subjects in group 1. Subjects in group 2 will receive their unmanipulated graft as per standard procedures. The CD34 graft content should be in the range of 2-10 million/kg body weight in all groups. Subjects will receive maximal standard of care premedication excluding glucocorticoids.

Glucocorticoid Use

Due to lymphodepleting effect on the immune system, no steroids will be allowed from day 0 of ASCT until day+181, unless unavoidable for urgent clinical use (such as autoimmune diagnosis or adrenal insufficiency).

5.1.10 On day +7 post-transplant

Subjects in groups 1 will receive the stored autologous donor lymphocyte product after thawing via intravenous infusion. Subjects will receive maximal standard of care premedication excluding glucocorticoid use before donor lymphocyte infusion. No dexamethasone should be given on or after the day of DLI infusion.

G-CSF post AHSCT will not be given.

Subjects who show no rise in WBC as a sign of engraftment by day +15 post-transplant are allowed to receive G-CSF at 5 µg/kg daily until absolute neutrophil count is equal or > 500/µl.

5.1.11 Other Therapy:

All standard of care therapy provided for multiple myeloma subjects undergoing autologous transplant will be allowed.

5.1.12 At day +15 (+/-3) and day +60 (+/-3) post-transplant

All subjects will receive tetanus vaccines as intramuscular injection. Platelets will be transfused if platelet count is < 30k on day +15 to allow for the intramuscular injection.

5.1.13 Immune evaluation assays (anti-tetanus & immunophenotyping)

Assays will be performed on day +15 (+/- 3 days), +30 (+14days), day+70 (+14days), day+100 (+/-10 days), and Day 180(+/-10) post-transplant on both groups.

5.2 Post transplant Myeloma Management:

5.2.1 All post-transplant myeloma staging and therapy will be based on local standard of clinical practice for myeloma subjects. Subjects will undergo *Euroflow*-based[85] MRD staging if they are deemed to be close to CR (serum protein electrophoresis < 0.1 gdL, but serum immunefixation positive) based on IMWG criteria.

5.2.2 For all subjects, post-transplant myeloma specific therapy may not be started before the day 100 (+/-10) visit on this protocol. The only exception would be disease progression that occurs before day+100, in which case the subject will be taken off study.

5.2.3 Subjects on this protocol may not enroll on other treatment/interventional protocols for the first 100 (+/-10) days post-transplant. The only exception would be disease progression before day+100, in which case, the subject is off study.

5.3 Possible Complications:

Subjects in the experimental arm (group 1) will be closely monitored after day zero for any possible treatment related toxicity. These toxicities may include but are not limited to:

1- *Engraftment failure*:
Although quite unlikely based on previous studies with CD34+ selected grafts in AHSCT[3], in the event of slow engraftment or failure, the subject can receive part or all of his back up CD34+ grafts. No engraftment failure was observed in the two subjects treated on the experimental arm at Emory University.

2- *Autoimmunity (including autologous graft versus host disease autoGVHD)*:
Subjects will be closely monitored for any clinical evidence of auto-immunity secondary to excessive immune suppressor cells depletion. Subjects can be treated according to the severity and organ involved based on physician discretion after consulting with the study PI.

3- *Severe lymphopenia*:
Has been described in CD34+ selected AHSCT[34,86]. In this trial, the chance of lymphopenia is small, since subjects will receive a DLI product on day +7, and these lymphocytes have shown evidence of accelerated *in vivo* expansion in the past.[74] Nevertheless, we will closely watch subjects for lymphopenia beyond the general expected range at 4 weeks post AHSCT. If three consecutive subject's had ALC equal or <1000 cells/µl at 4 weeks post AHSCT, further accrual will be held until their ALC >1000 with no infectious complications (including: CMV, EBV, Adeno- virus, mold). These subjects will be closely monitored for infections (CMV, EBV, adeno virus screening on lymphopenic subjects is optional).

5.4 Duration of the study.

The study is expected to last approximately 24 months.

5.5 Number of Subjects:

In this pilot phase II study, 10 subjects per arm will be enrolled for a total of 20 subjects. To account for screen failures up to 29 subjects may be screened for the whole trial.

5.6 Removal from the study

Subjects who for whatever reason do not meet the cardiac ejection fraction, pulmonary function test requirement or develop significant organ dysfunction before the transplant admission will be removed from the study.

The investigator will withdraw a subject from the study treatment whenever:

1. Continued participation is no longer in the subject's best interests. Reasons for discontinuing treatment may include the occurrence of a SAE or an inter-current illness. These subjects will be taken off treatment and followed according to the study calendar.
2. The subject requests to end treatment.
3. Subjects who fail to collect a minimum of 6 million CD 34 cells/kg body weight at the end of 1st day of Leukapheresis will be either removed or transferred to arm 2 of the protocol after discussion with the PI.

5.7 Immuno-Correlative Laboratory Studies

5.7.1 Laboratory studies to determine the general immunologic benefit of depleting immune suppressive cells from G-PBSC grafts.

The recovery of ALC and AMC will be recorded at days 15(+/-3) and 30 (+14) post-transplant, as surrogates for immune recovery and predictors of relapse and survival. These values will be obtained as part of the standard clinical care of these subjects post AHSCT. ALC/AMC ratio represents a surrogate for the balance between the host adaptive immune response versus tumor microenvironment immune suppression, as has been shown in the background section. The hypothesis is that depleting majority of immune suppressive cells from G-PBSC graft in the setting of AHSCT will tip the balance towards accelerated lymphocyte expansion *in-vivo*, which will be reflected in high ALC numbers on day 15 (>500/µl). The above intervention is hypothesized to lead to minimum AMC expansion by day 15 post AHSCT, leading to a high ALC/AMC ratio (>1). Such effect may jeopardize self-immune tolerance and trigger an auto-immune reaction or auto-GVHD. The development of autoimmune side effects in the context of cancer immunotherapy has been associated with augmented anti-tumor immune response and survival advantage. This was demonstrated in the use of CTLA4 (Cytotoxic T-Lymphocyte Antigen) blockade in renal cell carcinoma.[90]

5.7.2 Laboratory studies to determine phenotypic recovery of immune cells post AHSCT.

The phenotypic recovery can play a role in cancer immune surveillance and tolerance, specifically T-cells (CD8, CD4), B cells, NK cells, γδ T-cells, regulatory T-cells (CD4/CD25+/FOXP3+/CD26-) and MDSCs (CD33+/CD11b+/HLADR low/CD14 low/CD15+/-).These recovery patterns of immune phenotype data collected between the experimental group and the control group in the trial will be compared. PBMCs will be collected for these subjects on day of graft pheresis, +15 (+/-3), +30 (+14), +70(+14, 7-10 days post last tetanus vaccine), +100 (+/-10), and day 180(+30/-7) post-transplant. The number/proportion of MDSCs and T-regs are hypothesized to be much lower in the experimental group compared with the control group.

Subject's blood Samples (70 mls) will be collected on days: Pre-G-CSF, PBSC graft day, days +15 (+/- 3), +30 (+14), +70 (7-14 days post last tetanus vaccine), +100 (+/- 10), and day 180(+/-10) post-transplant.

5.7.3 Studies to determine antigen specific immune response post AHSCT.

To determine whether depletion of immune suppressive cells from G-PBSC graft will improve post AHSCT antigen specific (Ag) immune reconstitution, tetanus vaccine immune response post-transplant in the graft manipulation group will be studied and compared with the control groups and pre-transplant levels. The tetanus specific immune response will be determined by performing both ELISPOT assay to anti-IFNγ and ELISA to anti-tetanus IgG antibody.

Section 6.0 Measurement of Effect

Statistical analysis of both T-cell Elispot, ELISA and flow cytometric assays will be conducted among the subjects in the two groups of subjects, to determine if meaningful and/or statistically significant response has occurred after study intervention in experimental group compared to the control group.

Section 7.0 Study Parameters

Examinations and Assessments	Baseline (Screening)	Pre-Transplant (SCT)				Transplant	Post Transplant Period										Follow up Period
	Pre vaccine	**Vaccine (day -19)**	**Day -12 to -9**	**Day -11 to -7**	**Day -6 to -4**	**AHSCT Hospitalization**	**Day +7**	**Day +15**	**Day +30**	**Day +60**	**Day +70**	**Day +100 (mo. 3)**	**Day +130 (mo. 4)**	**Day +150 (mo. 5)**	**Day +180 (mo. 6)**	**Day +365 (1 yr)**	**Survival status (2 years)**
Window Days for Study Visit[4]	-30 days (+/-10 days)					Day 0		+/-3	+14	+/-3	+14	+/-10	+/-7	+/-7	+/-10	+/-30	+/-30 days
Informed Consent	X																
Inclusion/Exclusion Criteria	X																
Pregnancy testing	X[5]																
Medical History	X																
Clinical Assessment	X[2]																
Data collection to assess organ function (labs)[6]	X																
Disease response[3]	X											X			X	X	X
Data collection for Disease Assessment												X					

Section 7.0 Study Parameters

Examinations and Assessments	Baseline (Screening)	Pre-Transplant (SCT)				Transplant	Post Transplant Period										Follow up Period
	Pre vaccine	**Vaccine (day -19)**	**Day -12 to -9**	**Day -11 to -7**	**Day -6 to -4**	**AHSCT Hospitalization**	**Day + 7**	**Day +15**	**Day +30**	**Day +60**	**Day +70**	**Day + 100 (mo. 3)**	**Day + 130 (mo. 4)**	**Day + 150 (mo. 5)**	**Day + 180 (mo. 6)**	**Day + 365 (1 yr)**	**Survival status (2 years)**
(imaging and bone marrow)																	
Data collection for Disease assessment (blood and urine)	X											X			X	X	X
Adverse Events						X	X	X	X	X	X	X	X	X	X	X	X
Assessment for Subject/Graft Survival								X	X		X	X			X		X
Hematology (complete blood count (CBC) with differential ALC platelets)	X					X	X	X	X	X	X	X			X	X	X
Randomization	X																

Section 7.0 Study Parameters

Examinations and Assessments	Baseline (Screening)	Pre-Transplant (SCT)				Transplant	Post Transplant Period										Follow up Period
	Pre vaccine	**Vaccine (day -19)**	**Day -12 to -9**	**Day -11 to -7**	**Day -6 to -4**	**AHSCT Hospitalization**	**Day + 7**	**Day +15**	**Day +30**	**Day +60**	**Day +70**	**Day + 100 (mo. 3)**	**Day + 130 (mo. 4)**	**Day + 150 (mo. 5)**	**Day + 180 (mo. 6)**	**Day + 365 (1 yr)**	**Survival status (2 years)**
Tetanus Vaccine		X						X		X							
Group 1[1]/ Pre G-CSF PBMC pheresis			X														
Group 1 – Post-AHSCT PBMC infusion							X										
G-CSF (both groups)				X													
Pre Stem Cell harvest / Graft Pheresis (both groups)[7]					X												
Immune correlative study blood draw		X	X[1]		X			X	X		X	X			X		

[1] Pre-PBMC harvest in group 1 7-10 day post vaccine only
[2]Clinical Assessment is billed as physical exam with performance score. Data will be collected for MM disease specifics. Height and weight are also collected only at screening.
[3] Disease response to be done at discretion of physician in keeping with standard of care.
[4] Transplant day is day 0.

[5] Female of child-bearing potential must have two negative urine pregnancy test results within at time of eligibility testing and prior to GCS-F pre-transplant as a way of ensuring safe transplant planning.

[6]Organ function measured by standard clinical care labs collected from CBC, complete metabolic panel to assess the kidney, liver function.

[7] Subjects will receive one dose of plerixafor, a stem cell mobilizer, followed by pheresis on the next day to collect G-PBSC graft

7.1 SCREENING AND BASELINE EVALUATION

A flow chart of study procedures and assessments is provided above.

The transplant patients that meet the study eligibility criteria and are consented for the study will undergo baseline evaluations. Much of the information listed below will be obtained at the time of determining eligibility/screening prior to enrollment on the protocol.

The following baseline evaluations should represent those assessments/labs performed **prior to the start** of the conditioning regimen according to institutional standards.

- History, physical examination, height and weight.
- CBC with differential, platelet count
- Blood for immunocorrelative studies at pre-transplant pre-vaccine (day -30 to day -20)

7.2 PRE-TRANSPLANT PERIOD

The following procedures will be performed pre-transplantation:

- Blood for immunocorrelative studies on days: 7-10 days post last vaccine (pre-PBMC harvest and Pre-G-CSF), stem cell harvest day

7.3 STUDY PROCEDURES: POST-TRANSPLANT PERIOD

The following procedures will be performed post-transplantation

- History and physical examination to assess morbidity daily during the in-patient phase, then after transplant at designated timepoint on study parameters table
- Record specific adverse events and opportunistic infections.
- CBC (hemoglobin, hematocrit, WBC with differential and platelet count).
- Blood for immunocorrelative studies all time points noted on the study parameter table.

Section 8.0 Investigational Agents

8.1 CliniMacs CD34 beads and CliniMacs Devise by Miltenyi

The CliMacs devise and accompanying beads is approved for CD34 stem cell selection in allogeneic stem cell transplant from sibling donors. CD34+ stem cell selection in autologous stem cell transplant has been shown to be feasible and safe by Imai et al. BMT 2005 & Stewart et al. JCO 2005.

8.2 Tetanus Vaccine

The Tetanus vaccine that will be administered to subjects for this study is Tetanus/Diphtheria Toxoid (Td). Td will be provided as 5-2FLU/0.5mL ready to inject prefilled syringes. Active ingredients include *Clostridium Tetani*, Toxoid antigen (Formaldehyde inactivated) 5Lf/0.5mL, *Corynebacterium Diphtheriae* Toxoid antigen (Formaldehyde inactivated) 2Lf/0.5mL. Inactive ingredients present are Aluminum phosphate, Formaldehyde, Latex, Sodium Chloride and Sterile Water for injection.

Td is commercially available and is manufactured by Sanofi under the name Tenivac. Td will be obtained from Cardinal Health by the Investigational Drug Service (IDS) Pharmacy.

If patients experience any grade ≥ 3 adverse event/vaccine related toxicity following the first vaccine administration, they will be excluded from subsequent vaccine administration and taken off trial.

8.2.1 Contrainidications[92][Tenivac-FDA Approved Sanofi Pasteur Limited. Full Prescribing Package Insert]:

Hypersensitivity: A severe allergic reaction (e.g., anaphylaxis) after a previous dose of TENIVAC vaccine or any other tetanus toxoid or diphtheria toxoid-containing vaccine or any other component of this vaccine is a contraindication to administration of TENIVAC vaccine. Because of uncertainty as to which component of the vaccine may be responsible, none of the components should be administered.

8.2.2 Warnings/ Precautions[92] [Tenivac-FDA Approved Sanofi Pasteur Limited. Full Prescribing Package Insert]:

A. Acute allergic anaphylactic reactions could occur. Epinephrine hydrochloride solution (1:1,000) and other appropriate agents and equipment must be available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.
B. Latex is present in this TD preparation. The tip caps of the TENIVAC prefilled syringes may contain natural rubber latex, which may cause allergic reactions in latex sensitive individuals.
C. Arthus reactions can occur. Persons who experienced an Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine usually have high serum tetanus antitoxin levels and should not receive TENIVAC vaccine more frequently than every 10 years, even for tetanus prophylaxis as part of wound management.

8.2.3 Adverse Reactions[92] [Tenivac-FDA Approved Sanofi Pasteur Limited. Full Prescribing Package Insert]:

A. Injection site reactions could occur such as pain, redness and swelling.
B. Systemic reactions consist of fever, headache, muscle weakness, general malaise, pain in joints, dizziness, syncope, vomiting and lymphadenopathy.

Section 9.0 Toxicity and Adverse Event Reporting Guidelines:

This protocol will comply with monitoring and adverse event reporting requirements of the UNMC Fred & Pamela Buffett Cancer Center Data Monitoring plan. The protocol will adhere to both institutional IRB and FDA guidelines for toxicity reporting.

All subjects will be closely followed for toxicity from between the start of any protocol intervention and day 100 during the study and all grade 3 or 4 adverse events (or highly unusual grade 2 adverse events) will be recorded on the CRF.

Per NCI guidelines, Serious Adverse Events (SAEs) and Adverse Events (AEs) will be assessed and graded by reports from subjects to their physician/Investigator and by physical examinations. SAEs and AEs will be graded and toxicity assessed using the revised NCI CTCAE version 5.0. AEs will be followed until resolution, return to baseline or ≤ grade 1 levels. The outcome of all SAEs will be graded as resolved; recovering; ongoing; resolved with sequela; fatal.

SAEs will be followed until resolution, return to baseline or ≤ grade 1 levels, death, or until no further improvement is reasonably expected. Deaths occurring within 30 days of study treatment regardless of relationship will be reported to the UNMC DSMC.

9.1 Definitions:

Adverse Event

An AE is defined as any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not the event is considered causally related to the use of the product.

Any worsening of a pre-existing condition or illness is considered an AE. Laboratory abnormalities and changes in vital signs are considered to be AEs if they result in discontinuation from the study, necessitate therapeutic medical intervention, meet protocol specific criteria (see Section 5.0, Treatment Plan) and/or if the investigator considers them to be AEs.

Expected Adverse Event

Any event in which the severity or specificity is consistent with the risk information described in the protocol, or is anticipated based on the subject's medical history.

Unexpected Adverse Event

An AE in which the specificity, severity, or frequency is not consistent with (a) the IRB application and detailed protocol; (b) Risk information in the ICF; (c) and the event is not anticipated from the subject's disease history or status.

Serious Adverse Event

An SAE is one which results in any of the following outcomes:

- Death
- Is a serious injury, or otherwise seriously impacts the health, safety or welfare-fare of subject[1]
- Inpatient hospitalization or prolongation of existing hospitalization
- Required intervention to prevent permanent impairment or damage
- Persistent or significant disability or incapacity[2]
- Is a congenital anomaly or birth defect
- Other serious important medical event[3]
- Any event that requires treatment to prevent one of the outcomes listed above

[1] means that the subject was at immediate risk of death at the time of the SAE.

[2] "Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[3] Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

9.2 Adverse Event Reporting Requirements Per University of Nebraska Medical Center, IRB and Fred & Pamela Buffett Cancer Center Data and Safety Monitoring Committee (DSMC)

9.2.1 IRB Reporting

All internal adverse events (AEs) will be reported to the local IRB promptly per institutional human research protection program policy.

9.2.2 Fred & Pamela Buffett Cancer Center Data and Safety Monitoring Committee (DSMC) Reporting

In its initial review, the DSMC will make a recommendation for the frequency of monitoring based on an assessment of risk associated with study-associated therapy, per the DSMC policy. Reporting of the following will be done in accordance with DSMC guidelines:

- All SAEs and toxicity reporting will be reported to the DSMC.

- All AE grade 3 or higher (expected or unexpected, regardless of attribution) will be reported to the DSMC. In certain investigator-initiated institutional trials where virtually 100% of subjects are guaranteed to experience very specific known grade 3 or higher toxicities (e.g., hematologic toxicity in stem cell transplant studies) , the requirement for reporting of those toxicities may be waived at the discretion of the DSMC based on initial protocol review and in conjunction with investigator input if required.

Attribution of AE: The likelihood of relationship of the AE to the study drugs will be determined by the investigator based on the following definitions:

Not related: The subject was not exposed to the study treatment or another cause that is obvious.
Probably not related: The AE is most likely explained by another cause, and the time of occurrence of the AE is not reasonably related to the study treatment.
Possibly related: Study treatment administration and AE occurrence reasonably related in time, and the AE is explained equally well by causes other than study treatment, or treatment administration and AE occurrence are not reasonably related in time, but the AE is not obviously a result of other causes.
Probably related: Study treatment administration and AE occurrence are reasonably related in time, and the AE is more likely explained by study treatment than by other mechanisms.
Definitely related: The occurrence and timing of the AE are clearly attributable to the study treatment.

AEs will be collected from the time the start of any protocol intervention and day 100 post transplant. All AEs will be followed until resolution or a cause is identified. AEs judged by the investigator as not related or probably not related to the treatment will NOT be followed beyond the 100 days post transplant.

As a part of this study subjects will receive an autologous hematopoietic stem cell transplant (AHSCT). As part of a routine AHSCT process there are many adverse events expected to potentially occur. Below are known or frequent grade 3 or above Adverse Events that occur post AHSCT.

Hematologic Toxicities
Electrolyte Abnormalities
Mucositis
Fatigue
Fevers, including Neutropenic Fevers

Per DSMC approval, for this protocol these AEs will not be reported to the PRMS office during the trial. All other grade 3 and above AEs and any Serious Adverse Event that is unexpected will still be reported using the DSMC approved forms.

All SAEs and AE reporting will be completed using DSMC approved forms. Detailed policy and procedures for this section may be reviewed at:
http://www.unmc.edu/cancercenter/clinical/prms.html

9.3 Monitoring

Various methods will be implemented to exchange information with study personnel:

- Site Initiation/Orientation
- Investigator meetings as feasible
- Email distributions/reports as needed

9.3.1 Data Monitoring

For this study, data monitoring is the act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirement(s). Monitoring is a Quality Control, continuous process during the entire trial.

Scheduled meetings will occur depending upon the activity of the protocol. These meetings will include the Principal Investigator, Co-PI, data managers, and anybody deemed important to attend by the PI.

During these meetings the following points will be reviewed and discussed:

1. Safety of protocol participants (AE reporting).
2. Validity and integrity of the data.
3. Enrollment rate relative to expectations and the characteristics of participants.
4. Retention of participants and adherence to the protocol (potential or real protocol violations).
5. Completeness of collected data.

9.4 Auditing

Auditing is a systematic and independent examination of trial-related activities and documents to determine:

- whether the evaluated trial-related activities were conducted
- the data were recorded, analyzed, and accurately reported, according to the protocol, to the sponsor's SOPs, GCP, and applicable regulatory requirement(s).

Auditing is a Quality Assurance, one point process during the trial.

The UNMC Fred & Pamela Buffett Cancer Center Scientific Review Committee (SRC) will review this protocol on at least an annual basis.

This study will undergo audit on at least a semi-annual basis by the UNMC Fred & Pamela Buffett Cancer Center Audit Committee.

Detailed policy and procedures for this section may be reviewed at: https://www.unmc.edu/cancercenter/clinical/prms.html .

Section 10.0 Statistical Considerations:

10.1 Primary Objectives

Given the known lack of vaccine response in the first few months post autologous stem cell transplantation, we intend to look for evidence of vaccine response in the experimental arm via increase in tetanus specific T-cell Elispots and anti-tetanus antibody (Elisa) on days 30, 70, 100 and 180 post-transplant. Given the pilot/explorative nature of this trial and the lack of good pre trial assumptions about vaccine response, we will evaluate the vaccine response by Elisa and Elispot on days 30, 70, 100 and 180 will be compared between groups using a Mann-Whitney test.

10.2 Secondary Objectives

For the secondary objectives, engraftment, development of any auto immune side effects, and infections will be treated as binary variables. Chi-square tests will be used to compare each of these 3 outcome variables between the two groups, respectively. A Wilcoxon signed-ranks test will be employed to test the change in absolute lymphocyte (ALC), absolute monocyte counts (AMC), immune cells (T cells CD8, CD4), natural killer cells, γδ T-cells, T-regs and MDSCs) at days 15 and 30 post AHSCT from the baseline among each group, respectively. Wilcoxon signed-ranks test will be further used to compare the change of change in ALC, AMC and immune cells between the two groups at days 15 and 30 post- AHSCT, respectively. Generalized linear mixed models will be employed to compare the change pattern of ALC, AMC and immune cells between the two groups over the entire follow up periods. Time to relapse and overall survival will plotted using the methods of Kaplan and Meier and the log rank test will be used to compare the time to event curves between the two treatment groups.

Lastly, as an explorative objective will also be depth of anti-myeloma response as evaluated by the standard IMWG criteria, including the rate of sCR, and subsequent MRD status for the subjects in sCR.

10.3 Study Design:

This is a pilot phase II trial with 1:1 randomization between the experimental and control arms.

10.4 Stopping Rules

If more than three subjects on the experimental arm develop a treatment related grade 3-4 toxicity, which is irreversible, protocol will be halted and the Institutional Review Board will be notified.

10.5 Sample Size and Power Consideration

This is exploratory trial with a total sample size of 20 subjects total (10 subjects in each phase II arm). There is no preliminary data on the primary endpoint of the study (baseline tetanus vaccine response early after transplant). However, it is known that vaccine response in general is muted in the first three months post-transplant. We hypothesize to see a wide difference in Elispot values between the two arms. Two-sided two sample t-test is used to for power calculation. The sample size of 20 will achieve a power of at least 80% to detect an effect size of d=1.36 for a Mann-Whitney test comparing two independent groups at the significance level of 5% assuming that the actual distribution is Norma[91]. Because of the exploratory nature of the study, corrections for multiple comparisons will not be performed. Significance for each test will be set at $P < .05$.

10.6 Expected Accrual Rate, Accrual Duration, and Study Duration
The estimated accrual rate is 20 subjects enrolled in 12 -18 months.

Section 11.0 Records to be Kept:

Information regarding the actual treatments, adverse effects, radiographic and laboratory information are to be recorded on appropriate forms. See attached Data form plan. Serious adverse events, when noted, will be recorded on site via the standard serious adverse effects form.

11.1 Quality assurance
Complete records must be maintained in a research chart on each subject treated on the protocol. Research chart can be maintained either as an electronic record, paper chart or a combination or both. These records should include primary documentation (e.g., lab. report slips, physician notes, etc.) which confirm that:

- The subject met the eligibility criteria.
- Signed informed consent was obtained prior to treatment.
- Treatment was given according to protocol (dated notes about doses given & reasons for any dose modifications).
- Toxicity was assessed according to protocol (laboratory report slips, etc.).
- Response was assessed according to protocol (dated notes on clinical assessment, lab reports as appropriate).

11.2 Responsibilities of the Principal Investigator
The PI is responsible for ensuring that the protocol and its appendices are scrupulously followed, particularly when other departments are involved in the trial. The PI oversees the quality of the data collected in the case reports. The data obtained during the trial are recorded directly, with all modifications of data signed, dated, and justified as stated above. Modifications must conform to the procedures defined for paper and electronic records.

For the entire group of persons involved in carrying out the trial (day, night and emergency personnel), the PI is responsible for the following:

1. Ensuring that the personnel are informed of the protocol used and that they understand the part they are responsible for implementing.
2. Training personnel if necessary.
3. Designating individual(s) specifically responsible for the administrative management of the trial.
4. Ensuring that other departments or services involved in this trial are informed of the trial and determining with them the specific operating procedures necessary to conduct the trial.

11.3 Records Retention
The Principal Investigator will maintain all records related to the study as required by regulations and institutional policy.

11.4 Forte Electronic Data Capturing (EDC) System
Data will be stored electronically for this study on the Forte secure server. Data forms will not differ from the paper versions with the exception of an electronic format containing the UNMC Fred & Pamela Buffett Cancer Center and Forte logo.

Forte EDC provides for remote data collection that meets FDA 21 CFR Part 11 requirements as well as HIPAA and other regulatory requirements designed to enhance data security and protect subject confidentiality. Authorized users log into Forte through a secure connection and must provide a valid username, password, and database ID. This data may be made available to the public at large.

Section 12.0 Patient Consent:

In accordance with Title 21 CFR (Part 50), the PI commits to obtaining the informed consent of the subject by signature and clearly indicates the content of the information given to the subject and the means by which consent is obtained.

The PI commits him/herself to conform to GCP, 21 CFR Parts 50, 54, 56 and 312 regulations, concerning his/her duties.

12.1 Subject Competency
Subjects will be eligible to participate in the study only if they are competent to give informed consent. A subject that the investigators judges to be incompetent will not be enrolled.

12.2 Process of Informed Consent and subject comprehension
If the patient chooses to be a participant in this study informed consent will be obtained by the investigators. The study and procedures involved including the risks will be explained in detail to each subject and that participation is entirely on a voluntary basis. The participating investigators will be available to discuss the study with them.

The subject will be asked to state in his/her own words the purpose of the study, the procedures that will be carried out, potential risk, potential benefits to the subject, the alternatives and the right to withdraw from the study. If there are any indications that a given subject's comprehension is anything less than accurate, the points of confusion will be discussed and clarified.

Section 13.0 References:

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Section 14.0 Data Collection Forms:

A data collection plan for electronic data capture forms (eCRF) is submitted as part of this protocol for the scientific review committees review. This plan includes data capture forms for the following:

- Adverse Event Log
- Clinical Laboratory Values
- Death Record
- Eligibility Checklist (including both inclusion and exclusion criteria to match the protocol)
- Medical History
- Physical Examination

In addition, the study team has the following items provided, at the time of site activation, as an accompaniment to the protocol. These items are provided electronically as a study site manual and Manual of Operations (MOP) to guide the study operations. Below are the included forms.

- Manual of Operations document
- Study site manual
    - Delegation log template
    - Training log template
    - Enrollment/Registration documents – registration request, eligibility exception request and ineligibility report and enrollment log
    - Td Vaccine Information sheet for subjects
    - Investigator's brochure, package insert
    - DSMC/ Federal AE/SAE reporting documents
    - Specimen collection and prep worksheet – Lab Manual
    - eCRF completion guidelines
    - FDA 1572, FDA 3455 Financial disclosure, as appropriate

Appendix A: Eligibility checklist

Date Completed:	Institution: UNMC	Subject ID#:
IRB # 669-19 Title: A Pilot Study of Enhancing Anti-Tetanus Vaccine Response After Autologous Stem Cell Transplantation PI: Muhamed Baljevic, MD		Waiver #:

Inclusion criteria:	Yes No N/A
1. Age ≥19 years to 70 years old at time of study entry (consent).	[ ] [ ] [ ]
2. Diagnosis of Multiple Myeloma as per updated International Myeloma Working Group (IMWG) criteria[84].	[ ] [ ] [ ]
3. Must have received bortezomib, lenalidomide and dexamethasone (VRd) as a form of induction therapy pre-AHSCT (use of cyclophosphamide, bortezomib and dexamethasone may be allowed for up to 2 weekly doses before initiation of VRd induction, if necessary clinically for cytoreduction)	[ ] [ ] [ ]
4. Able to understand and sign a consent form.	[ ] [ ] [ ]
5. Creatinine clearance equal or > 60 ml/min (calculated)	[ ] [ ] [ ]
6. Ejection fraction equal or > 50% before admission for transplant as per institutional standards. Patients with coronary heart disease (recent myocardial infarctions, angina, cardiac stent, or bypass surgery in the last 6 months or arrhythmia) need to be cleared by cardiology as per institutional BMT standards.	[ ] [ ] [ ]
7. Total bilirubin ≤2.5 times the upper limit of the institutional normal values, and total AST (SGOT) and ALT (SGPT) ≤2.5 times the upper limit of the institutional normal values.	[ ] [ ] [ ]
8. FVC, FEV1 or DLCO equal to or >50% predicted before admission for transplant as per institutional standards. Patients on home oxygen are not allowed on the protocol.	[ ] [ ] [ ]
9. Is there less than 6 months of pre-transplant MM chemotherapy (from the date of the start of the induction therapy)?	[ ] [ ] [ ]
10. KPS ≥ 70%or ECOG 0-2.	[ ] [ ] [ ]
11. Must be eligible to receive Melphalan dose of 200mg/m^2	[ ] [ ] [ ]
12. A female of child-bearing potential, must have two negative urine pregnancy test results at time of eligibility testing and prior to GCS-F pre-transplant	[ ] [ ] [ ]
*All of the above must be **yes** to be eligible.*	
Exclusion criteria	Yes No N/A

1. Participation in another clinical study with an investigational product during the last 28 days.	[ ] [ ] [ ]
2. Prior stem cell transplant (both autologous and allogeneic)	[ ] [ ] [ ]
3. Creatinine clearance < 60 ml/min (calculated)	[ ] [ ] [ ]
4. Subjects who failed to achieve ≥ PR to induction therapy (i.e. VRd) and required salvage induction prior to AHSCT.	[ ] [ ] [ ]
5. Documented central nervous system or extramedullary disease.	[ ] [ ] [ ]
6. Significant organ dysfunction deemed to carry inappropriate risk for AHSCT.	[ ] [ ] [ ]
7. Intention or plans for cyclophosphamide mobilization.	[ ] [ ] [ ]
8. Known allergic reactions after previous tetanus diphtheria vaccination or had a condition of Guillain Barre Syndrome (GBS)	[ ] [ ] [ ]
9. Known active hepatitis B, C or HIV infections on initial assessment.	[ ] [ ] [ ]
10. Enrollment on any other transplant related protocols	[ ] [ ] [ ]
*All of the above must be **no** to be eligible.*	

Eligibility: [ ] Subject satisfies all criteria.
[ ] Subject not formally eligible, but admitted to study because (state reason)

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Subject Initials: _____MR #_____DOB _____

ELIGIBILITY reviewed and confirmed

Site Investigator Signature _____Date_____

Appendix B – Correlative Lab Specimen Collection Schedule

Visit	Specimen Type	Collection amount	Tube	Handling Instructions
Pre vaccine	Blood	70 mls	6 Sodium Heparin tubes – 10 mls each 1 red top tube – 10 mls	Sodium heparin tubes must be placed in 4°C temperature if not delivers to the lab within 30 minutes. Red top tube – deliver to Al-Kadhimi lab directly.
Pre G-CSF PBMC pheresis	Blood	70 mls	6 Sodium Heparin tubes – 10 mls each 1 red top tube – 10 mls	
Graft Pheresis/PBSC graft day	Blood	10 mls	1 Sodium Heparin tube	Sodium heparin tubes must be placed in 4°C temperature if not delivers to the lab within 30 minutes.
Day + 15 (+/- 3)	Blood	70 mls	6 Sodium Heparin tubes – 10 mls each 1 red top tube – 10 mls	Sodium heparin tubes must be placed in 4°C temperature if not delivers to the lab within 30 minutes. Red top tube - deliver to Al-Kadhimi lab directly.
Day + 30 (+14)	Blood	70 mls	6 Sodium Heparin tubes – 10 mls each 1 red top tube – 10 mls	
Day + 70 (+14)	Blood	70 mls	6 Sodium Heparin tubes – 10 mls each 1 red top tube – 10 mls	
Day + 100 (+/- 10)	Blood	70 mls	6 Sodium Heparin tubes – 10 mls each 1 red top tube – 10 mls	
Day + 180 (+/- 10)	Blood	70 mls	6 Sodium Heparin tubes – 10 mls each 1 red top tube – 10 mls	