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Title: Non-steroidal anti-inflammatory drugs (meloxicam) to mobilize hematopoietic stem cells: A Phase II randomized trial

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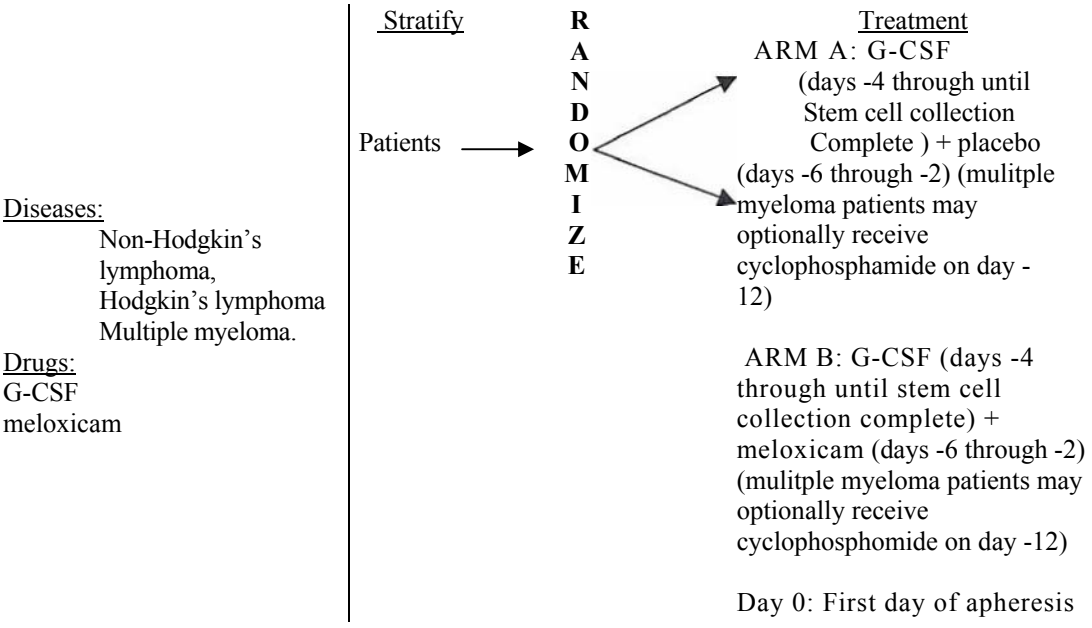

TABLE OF CONTENTS

1. OBJECTIVES

We hypothesize that patients in the meloxicam group (G-CSF + meloxicam) will have higher numbers of CD34+ cells (above 1.5 fold) in the apheresis product collected on the first day, will require fewer apheresis sessions to achieve ≥ 4 x 10^6 CD34+ cells/kg for multiple myeloma patients and ≥2 x 10^6 CD34+ cells/kg for lymphoma patients compared to patients in G-CSF + placebo group and will have faster neutrophil and platelet engraftment compared to patients transplanted with HSC graft mobilized with G-CSF + placebo.

1.1 Study Design

Patients eligible for AHSCT will be randomized to receive G-CSF + placebo (Arm A) or G-CSF + meloxicam (Arm B) for their stem cell mobilization. Patients who will have <10/μl CD34+ cells in the peripheral blood will be declared as "poor mobilizers" and be rescued with Plerixafor and G-CSF.

1.2 Primary Objectives

1. Determine if meloxicam in conjunction with G-CSF leads to increased numbers of circulating CD34+ cells on the first day of apheresis compared to G-CSF + placebo.

2. Determine how many apheresis sessions are required to collect ≥ 4 x 10^6 CD34+ cells/kg for multiple myeloma patients and ≥2 x 10^6 CD34+ cells/kg for lymphoma patients in the G-CSF + meloxicam group compared to the G-CSF group.

1.3 Secondary Objectives

1. To determine that meloxicam is safe when used in combination with G-CSF for stem cell mobilization.

2. The time to neutrophil and platelet engraftment after AHSCT.

3. To evaluate transfusion support (number of red blood cells and platelet transfusions needed prior to red blood cell and platelet engraftment).

4. Proportion of patients failing stem cell mobilization.

2. BACKGROUND

2.1 Rationale

Autologous hematopoietic stem cell (HSC) transplantation plays an important role in the treatment of many hematologic malignancies and some pediatric cancers. The utility of current mobilization regimens which use cytokines (G-CSF) after chemotherapy (chemomobilization) or cytokine(s) (e.g. G-CSF) alone, is limited by failure to mobilize adequate numbers of CD34+

cells in up to 40% of selected patient population. Plerixafor (AMD 3100, a CXCR4 antagonist) in combination with G-CSF has recently been used as a rescue with (second) successful mobilization of HSC in approximately 63% of these patients, which still leaves a large number of patients without an adequate HSC graft for their potentially curative autologous HSC transplantation. In addition, current strategies have differing HSC yields, safety considerations, resource utilization and costs. Novel agents are clearly needed that will adequately mobilize HSC with few or no toxicities, thereby improving patient outcomes. Here, we propose to demonstrate that oral meloxicam, an inexpensive, well-tolerated, and widely available non-steroidal anti-inflammatory drug (NSAID), can be a safe and effective stem cell mobilizing agent. Recently, we have shown in a mouse model that meloxicam increases egress of hematopoietic progenitor cells (HPC) and the phenotypic HSC-enriched populations Sca-1^+ c-Kit^+ $lineage^-$ (SKL) or the highly purified $CD150^+$ $CD48^-$ (SLAM) SKL populations [Nature in press]. When baboons were mobilized with a standard clinical regimen of G-CSF, or the combination of G-CSF + meloxicam, in all cases meloxicam resulted in increased numbers of circulating $CD34^+$ cells compared to G-CSF alone, and in addition, meloxicam treatment on its own also resulted in significant HSC/HPC mobilization. In healthy human volunteers, short-term meloxicam treatment resulted in significant increases in $CD34^+$ cells. The inhibition of PGE2 signaling which is an important positive regulator of HSC CXCR4 and HSC homing to the niche, appears to be one (reduced CXCR4 levels) of the main mechanisms of NSAID-induced HSC mobilization and this is a cyclooxygenase (COX) pathway specific effect. Staggered administration of meloxicam and G-CSF allowed for restored CXCR4 levels but maintaining enhanced HSC mobilization and, in transplantation models HSC grafts collected this way resulted in significant enhancement in hematopoietic engraftment. Meloxicam inhibits both COX-1 and COX-2 and when compared to other dual inhibitors it has much less inhibition of platelet aggregation. In this proposal, we will address the hypothesis that oral meloxicam - an inexpensive, well-tolerated, and widely available non-steroidal anti-inflammatory drug (NSAID), can be a safe and effective stem cell mobilizing agent. These studies have the potential to lead to the establishment of a novel drug, that is widely available, affordable, easy to administer, safe, yet effective in predictably mobilizing CD34+ cells for both autologous and allogeneic HSC transplantation.

BACKGROUND AND SIGNIFICANCE

Factors limiting clinical AHSCT. Autologous hematopoietic stem cell (HSC) transplantation (AHSCT) provides hematopoietic support after high dose chemotherapy and is the standard of care for patients with multiple myeloma, chemosensitive relapsed/refractory Hodgkin's disease, high- or intermediate-grade non-Hodgkin lymphoma (NHL) and relapsed/refractory germ cell tumor [1-3]. AHSCT is also used as a salvage treatment in follicular lymphoma and recent data suggest that more than10-year disease-free survival is possible after high-dose chemotherapy and AHSCT [4]. In addition, AHSCT may lead to long-term progression-free survival in patients with mantle cell lymphoma, when used as part of front-line treatment in first remission [5].

The numbers of CD34+ cells play an important role in the success of AHSCT. Higher HSC doses are associated with faster neutrophil (>0.5 x 10^9/l) and faster platelet engraftment (plt count >20 x 10^9/l) and reduction in the need of supportive measures such as blood product transfusions and prophylactic antibiotics [6, 7]. Other factors that influence the HSC mobilization and, therefore, the success of AHSCT include patient age, gender, degree of exposure to previous chemotherapy and irradiation, disease characteristics such as bone marrow involvement and previous mobilization attempts [8]. Unsuccessful initial HSC mobilization often leads to expensive additional mobilization attempts and may preclude ASHCT altogether [9-12].

Today, the two most common HSC mobilization regimens use cytokines (G-CSF) alone or cytokines (G-CSF) after chemotherapy (chemomobilization). While cytokine alone can mobilize adequate HSC, the addition of a myelosuppressive chemotherapy followed by cytokine mobilization increases HSC numbers by a factor of 2.5 [11] and can reduce the number of apheresis required for targeted HSC collection. The use of chemotherapy in chemomobilization is associated with variable rates of complications due to neutropenia and thrombocytopenia and, therefore leads to increased resource use, hospitalization, transfusions and the use of antibiotic therapy [13, 14]. In addition, the variation in chemotherapeutics and individual responses to chemomobilization often results in unpredictable/irregular collection schedules that increase resource utilization [15]. Current HSC mobilization regimens also have high failure rates, failing to mobilize sufficient HSC to proceed to AHSCT in 5-30% of patients, necessitating additional mobilization attempts or precluding transplantation [16, 17].

Biology of HSC Mobilization. There has been significant recent advances in specific cellular and molecular mechanisms governing the interactions between HSC and BM microenvironment, i.e., HSC/niche interactions. The HSC niche is represented by stromal cells, endothelial cells, osteoblasts and other matrix components such as collagens, fibronectins and proteoglycans. HSCs express a variety of cell surface receptors, specifically adhesion molecules, namely lymphocyte function-associated Ag-1 (LFA-1), very late Ag-4 (VLA-4) and Mac-1, the chemokine receptors CXCR4 and CXCR2, the cell surface glycoproteins CD44 and CD62L and the tyrosine kinase receptor c-kit [18, 19]. The BM stroma contains stromal cell-derived factor-1 (SDF-1), CXC chemokine GRO-β, vascular cell adhesion molecule-1 (VCAM-1), kit ligand, P-selectin glycoprotein ligand-1 (PSGL-1) and hyaluronic acid, all of which are ligands for HSC adhesion molecules/receptors [18, 19]. Inhibition of these receptor-ligand interactions results in HSC and HPC egress from the bone marrow [20, 21]. Human hematopoietic progenitor cells (HPC) and their precursors, including the majority of HSCs, express the CD34 antigen, despite the available evidence that CD34- cells may have multilineage reconstitution capacity [22, 23]. Therefore, the CD34 surface marker is commonly used as a marker for HSC/HPC collection. The target dose of CD34+ cells ranges from a minimum of 2.0 x 10^6 CD34+ cells/kg to an optimal dose of 5.0 x 10^6 CD34+ cells/kg [12, 24-26]. The numbers of CD34+ cells are positively correlated with the pace of neutrophil and platelet engraftment following AHSCT. Higher CD34+ cell doses are more beneficial for patients with myeloma and NHL, with a few studies reporting improvement in overall survival in myeloma patients [27, 28].

G-CSF. Under G-CSF stimulation, myeloid cells release active neutrophil serine proteases cathepsin G and neutrophil elastase along with stromal metalloproteinase-9. This results in cleavage/degradation of the adhesion molecules VCAM-1, c-kit, CXCR4 and SDF-1 and in release of CD34+ cells [29-32]. G-CSF is the most commonly used HSC mobilizing agent. When G-CSF alone is used for mobilization of CD34+ cells, it is given at doses varying from 10 ug/kg s.c. daily to 32 ug/kg s.c. daily, beginning at least 4 days before the first apheresis session and continued until the last apheresis session (Neupogen, filgrastim [package insert]. Amgen Inc.: Thousand Oaks, CA, 1991-2006) [32]. In many patients with myeloma and malignant lymphoma, mobilization with G-CSF alone results in suboptimal CD34+ cell yields, with “mobilization failures”, defined as CD34+ cell yields of <2 x 10^6/kg, in up to 30% of patients. Moskowitz et al reported that G-CSF mobilization alone yielded significantly fewer CD34+ cells and was inferior to mobilization with chemotherapy + G-CSF (1.5 x 10^6/kg vs. 6.7 x 10^6/kg) [33].

G-CSF with chemotherapy. It has been speculated that treatment with cyclophosphamide (CY) and G-CSF have a synergistic effect on protease release in marrow microenvironment, because when administered individually, both CY and G-CSF enhance the release of neutrophil

proteases that cleave the adhesion molecules CXCR4, SDF-1 and c-kit [34]. Besides CY, some of the other chemotherapeutics chosen from the salvage regimens, that are used in conjunction with G-CSF for HSC mobiliozation are ICE (ifosfamide, carboplatinum, etoposide), high-dose cytarabine, DHAP and ESHAP (etoposide, cytarabine, methylyprednisolone and cisplatinum), etc. Salvage chemotherapy followed by G-CSF is widely used for mobilization of CD34+ cells because the addition of suppression of the bone marrow to G-CSF results in higher HSC yields with fewer apheresis sessions needed to collect the targeted CD34+ cell dose, which may result in better patient outcomes [35, 36]. While chemotherapy has been noted to reduce the risk of tumor-contaminated cell collections, it has not been proven yet that the cytoreductive effects of chemomobilization confer any survival benefit to the patients [37, 38].

The effect of chemomobilization is highly variable and CD34+ cell egress peaks 10-18 days after administration of chemotherapy which generally correlates with rebound of white cell count after a chemotherapy-induced nadir [39]. Following chemomobilization, the length of time before the number of CD34+ cells peaks differs substantially between patients resulting in unpredictable collection schedules [11, 15, 40]. White cell and CD34+ cell counts, on occasions, must be monitored over the course of many days to determine when to initiate the collection. Besides, the gains of adding chemotherapy to a G-CSF mobilization regimen may be offset by the increased risk of complications, including higher risk of infection, hospitalizations, the need for transfusion and antibiotic therapy, greater resource utilization with considerably higher cost overall [13, 35].

Plerixafor (AMD 3100). AMD 3100 is a selective and reversible inhibitor of CXCR4 and disrupts its interaction with SDF-1, thereby releasing HSC into the peripheral blood [41]. Plerixafor in combination with G-CSF has been shown to predictably enhance the numbers of circulating CD34+ cells [compared to G-CSF alone group, median increase of 4.4 fold in lymphoma patients and 3- to 3.5 fold in myeloma patients] [42]. In a study on poor mobilizers, collections of $\geq$ 2x 10^6 CD34+ cells/kg after administration of G-CSF (10 μg/kg s.c. daily, with apheresis initiated on day 5, continued until the collection is complete or declared "failed") and plerixafor (240 μg/kg s.c. daily, beginning on the evening of day 4, continued every evening until collection is complete or declared "failed") were achieved in 60.3% of patients with NHL, 71.4% of patients with myeloma and 76.5% of patients with Hodgkin lymphoma [43]. Perixafor is establishing its recognition as a potential HSC mobilizing agent. However, this is an expensive drug, needs hospital visits for the injections and it has adverse side effects such as diarrhea, nausea, vomiting and injection-site erythema/edema.

PRELIMINARY STUDIES: Despite significant advances in specific cellular or molecular mechanisms governing HSC/niche interactions, little is understood about regulatory function within the intact mammalian hematopoietic niche. Recently, we and others described a positive regulatory role for the lipid mediator Prostaglandin E_2 (PGE_2) on HSC function *ex vivo* [44,45]. However, to date, the function of PGE_2 produced endogenously in mammalian niches in regulation of hematopoiesis is unknown. Given the known roles of PGE_2 on HSC and the abundance of clinically used compounds that alter PGE_2 signaling, particularly nonsteroidal anti-inflammatory drugs (NSAIDs), we sought to determine the

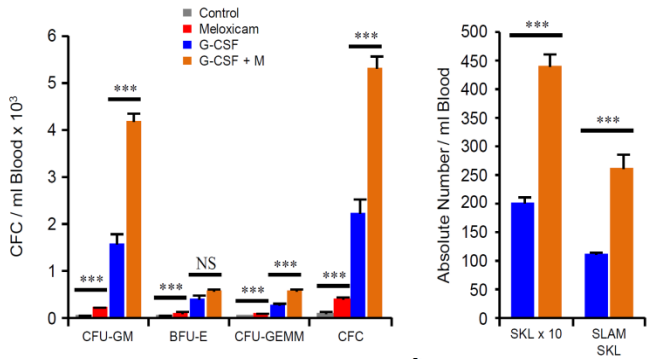

Figure 2. Meloxicam enhances HPC and HSC mobilization. **(a)** Colony forming units and **(b)** phenotypically defined HSC in the peripheral blood of mice treated with G-CSF or the combination of G-CSF and Meloxicam. Data are mean ± SEM 3 individual epxeriments, of 5 mice per group, per experiment, each assayed individually. ****P*<0.001.

role of endogenous PGE_2 on systemic niche-HSC regulation.

NSAIDs increase hematopoietic stem and progenitor cell egress

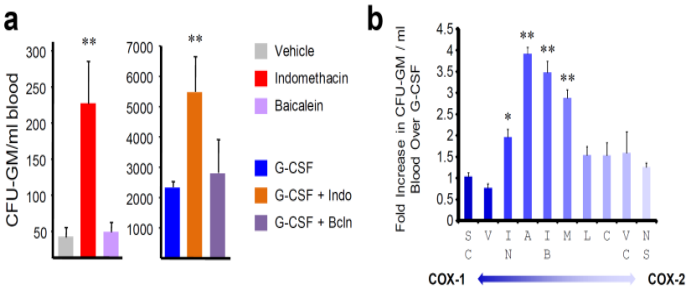

Figure 1. NSAIDs mobilize hematopoietic stem and progenitor cell (**a**) CFU-GM mobilization by indomethacin, G-CSF and combination treatment (n=5 mice per group) (**b**) Fold increase in CFU-GM over G-CSF with NSAIDs with varying COX-1 and COX-2 selectivity; SC-560 (SC), valeryl salicylate (V), indomethacin (IN), aspirin (A), ibuprofen (IB), meloxicam (M), licofelone (L), celecoxib (C), valdecoxib (VC) and NS-398 (NS). X ± SEM of 5 mice/group, each assayed individually.*P<0.05:

Mice were treated with the prototypical NSAID indomethacin (50 μg/mouse) for 4 days to reduce endogenous PGE_2 production. Treatment resulted in a significant increase in hematopoietic progenitor cells (CFU-GM) in peripheral blood [46] (Figure 1a), that was not accompanied by an increase in total white blood cell count (not shown), which likely accounts for lack of previous detection of this observation despite decades of clinical NSAID use. No increase in HPC egress was seen in mice treated with the lipoxygenase inhibitor baicalein (Figure 1a), suggesting a cyclooxygenase (COX) pathway specific effect. When indomethacin was co-administered with the clinically used mobilizing agent granulocyte-colony stimulating factor (G-CSF), significant enhancement (~2 fold) in HPC mobilization resulted (Figure 1a, right panel). NSAIDs with varying COX-1- and COX-2-selectivity demonstrated significant mobilization with indomethacin, aspirin, ibuprofen, and meloxicam (Figure 1b). Meloxicam inhibits both COX-1 and COX-2 within the bone marrow microenvironment and when compared to other dual inhibitors it has a reduced incidence of gastrointestinal discomfort [47] and inhibition of platelet aggregation [48]. Therefore, meloxicam was used in the majority of the described studies below, and is our choice for clinical study in this proposal. Meloxicam, similar to indomethacin, increased egress of HPC (Figure 2, left panel) and the phenotypic HSC-enriched populations Sca-1^+ c-kit^+ $lineage^-$ (SKL) or the highly purified $CD150^+$ $CD48^-$ (SLAM) SKL populations (Figure 2, right panel).

Enhanced hematopoietic engraftment with G-CSF + NSAID mobilized grafts We then explored the hematopoietic engraftment potential of an NSAID-mobilized graft. Despite significant increases in phenotypic HSC and functional HPC in the graft, two early attempts at transplant did not show a significant effect on HSC engraftment (data not shown). Since we had previously shown that PGE_2 signaling was an important positive regulator of HSC CXCR4 expression and HSC homing to the niche [1], we hypothesized that while there was an increase in HSC/HPC yield in NSAID grafts, CXCR4 expression might be reduced, accounting for apparent lack of enhancement in engraftment. To test this hypothesis we used a simple treatment regimen modification, staggering the administration of NSAID and G-CSF to allow for hematopoietic mobilization and restoration of normal endogenous PGE_2 signaling pathways before transplant (Figure 3a). As hypothesized, CXCR4 levels were significantly lower on G-CSF mobilized HSC/HPC after NSAID treatment, while staggered administration allowed for restored receptor levels (Figure 3b), while maintaining enhanced HSC mobilization (Figure 3c). Using these novel dosing strategies, we competitively transplanted mobilized grafts from G-CSF-mobilized mice, or non-staggered and staggered G-CSF + meloxicam treated mice. Staggered NSAID administration along with G-CSF resulted in a significant enhancement in LT-HSC engraftment as measured by enhanced chimerism and competitive repopulating unit frequency in the G-CSF + NSAID mobilized graft (Figure 3d). In addition to long-term engraftment, early recovery of blood cell counts, particularly neutrophils and platelets, is important to reduce the duration of neutropenia and thrombocytopenia associated with

myeloablative conditioning regimens and subsequent transplant related morbidity and mortality [49-51]. Increasing the number of HSC/HPC contained within a hematopoietic graft has been

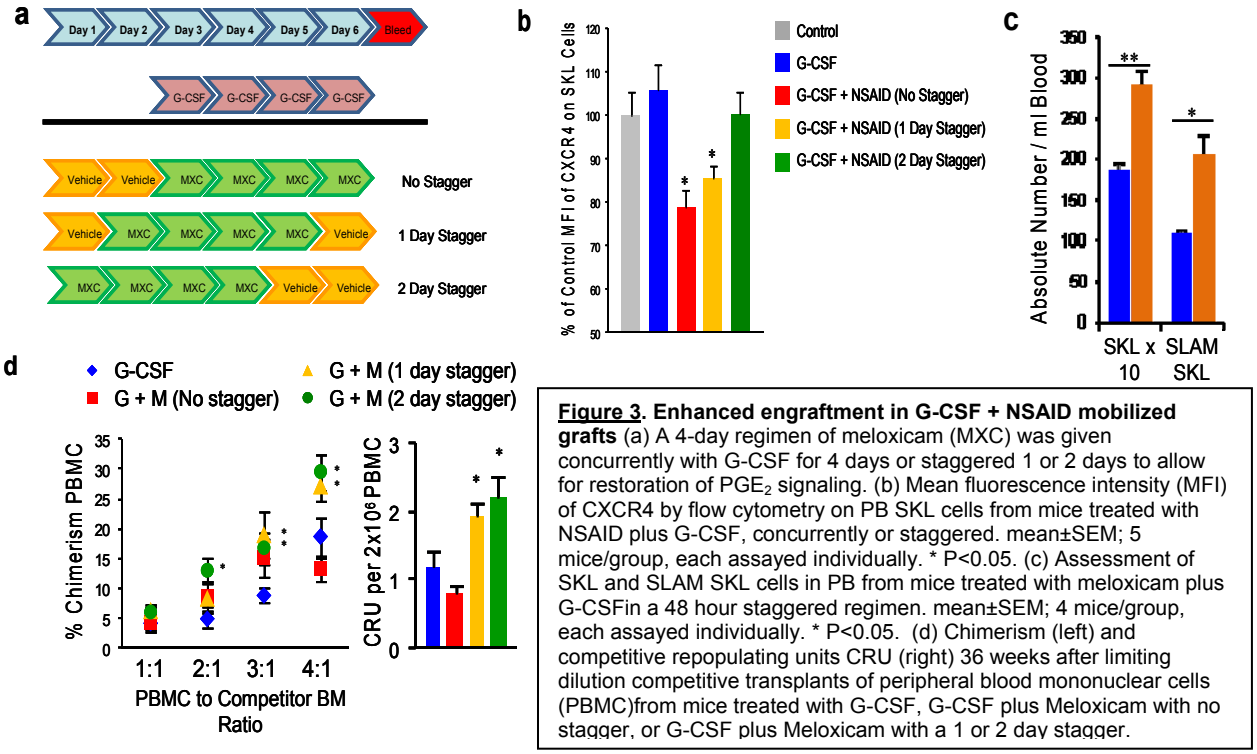

Figure 3. Enhanced engraftment in G-CSF + NSAID mobilized grafts (a) A 4-day regimen of meloxicam (MXC) was given concurrently with G-CSF for 4 days or staggered 1 or 2 days to allow for restoration of PGE_2 signaling. (b) Mean fluorescence intensity (MFI) of CXCR4 by flow cytometry on PB SKL cells from mice treated with NSAID plus G-CSF, concurrently or staggered. mean±SEM; 5 mice/group, each assayed individually. * P<0.05. (c) Assessment of SKL and SLAM SKL cells in PB from mice treated with meloxicam plus G-CSFin a 48 hour staggered regimen. mean±SEM; 4 mice/group, each assayed individually. * P<0.05. (d) Chimerism (left) and competitive repopulating units CRU (right) 36 weeks after limiting dilution competitive transplants of peripheral blood mononuclear cells (PBMC)from mice treated with G-CSF, G-CSF plus Meloxicam with no stagger, or G-CSF plus Meloxicam with a 1 or 2 day stagger.

demonstrated to reduce the time necessary for neutrophil and platelet recovery [52]. When grafts were transplanted non-competitively, G-CSF plus staggered co-administration of meloxicam resulted in a 4-day faster recovery of both neutrophils (Figure 4, left panel) and platelets (Figure 4, right panel) compared to G-CSF alone. Competitive secondary transplantation confirmed sustained LT-HSC activity in these mice with full multi-lineage reconstitution 36 weeks post-transplant (data not shown). These results clearly demonstrate that endogenous PGE_2 signaling is required for HSC niche retention and robustly define a novel therapeutic mobilization strategy with NSAIDs that significantly enhances hematopoietic transplantation efficiency and efficacy. Our proposed studies will utilize this novel NSAID dosing strategy to enhance hematopoietic mobilization.

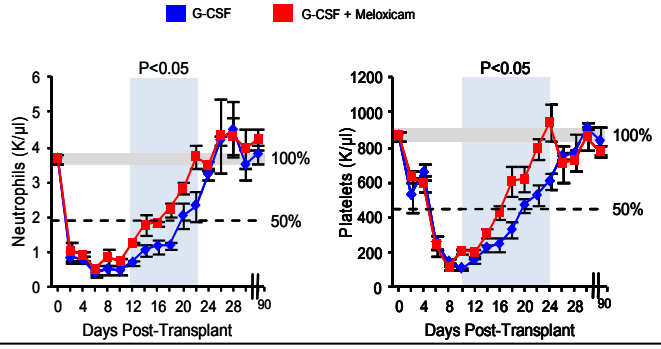

Figure 4. Recovery of ANC (left) and platelets (right) in irradiated mice after non-competitive transplant of PB cells mobilized by G-CSF or G-CSF plus Meloxicam (2 day stagger). Counts were monitored for 90 days post-transplant. Data presented as X±SEM; n=5 mice /group bled on alternate 4 day cycles.

Non-human primates and healthy volunteers mobilize after NSAID treatment To confirm the NSAID mediated hematopoietic effects in higher species, 4 baboons were mobilized with a standard clinical regimen of G-CSF, or the combination of G-CSF + meloxicam in a crossover design (Figure 5a). While individual baboon response to G-CSF varied, similar to what is seen in humans, in all cases meloxicam treatment resulted in increased numbers of $CD34^+$ cells (Figure 5b) and CFU-GM (Figure 5c) in

peripheral blood. In addition, as seen in mice, meloxicam treatment on its own resulted in significant HSC/HPC mobilization compared to baseline levels (Figures 5 d,e). In healthy human volunteers, short-term meloxicam treatment resulted in significant increases in $CD34^+$ cells (Figure 5f), determined using the ISHAGE procedure [53], and functionally defined HPC (Figures 5 g, h and i), matching hematopoietic egress seen with meloxicam treatment in baboons and mice. Thus, short-term endogenous PGE_2 inhibition, closely resembling current clinical NSAID treatment, results in a previously unappreciated increase in stem and progenitor cells in the peripheral blood.

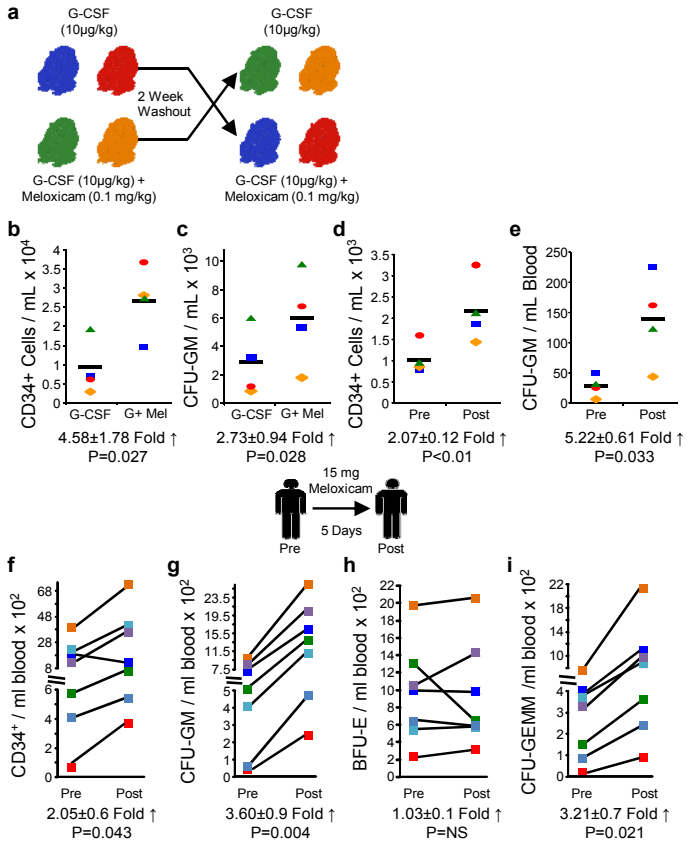

Figure 5. Non-human primates and healthy human volunteers mobilize HSC/HPC in response to NSAID treatment.
a, Four baboons were mobilized with G-CSF or G-CSF + Meloxicam in a cross-over design and **b,** $CD34^+$ cells and **c,** CFU-GM in peripheral blood determined. **d,** $CD34^+$ cells and **e,** CFU-GM in peripheral blood determined pre- and post- 5 days of meloxicam alone treatment in baboons. Seven healthy human volunteers were treated with 15 mg/day p.o. for 5 days, and were assessed for **f,** $CD34^+$ cells; **g,** CFU-GM; **h,** BFU-E, and **i,** CFU-GEMM pre- and post-treatment. Statistics represent paired, two-tailed t-test.

2.2 Correlative Studies Background

Characterize the hematopoietic stem and progenitor cells mobilized by meloxicam.
There is increasing evidence that grafts collected after different mobilization methods differ in CD34 subclasses and immune cell content, which may have important clinical impact on transplant outcome, although further studies are still required [54,55]. In preclinical studies, grafts mobilized by chemokine agonists/antagonists contain populations of more immature HSPC with enhanced engraftment potential compared to G-CSF alone [54,56,57]. Administration of myeloid progenitor cells has also shown utility as a bridging therapy in animal models. Co-administration of purified common myeloid progenitor cells (CMP) and granulocyte

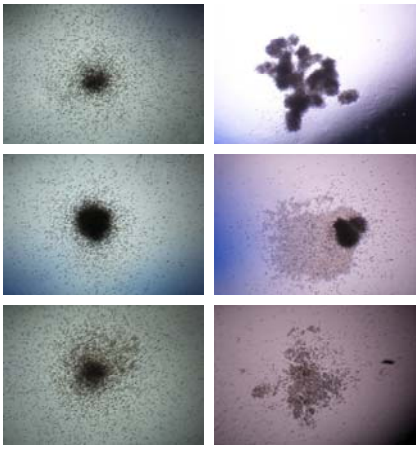

Figure 6. Representative CFU-GM colonies from G-CSF and G-CSF + NSAID mobilized mice. Mice were mobilized with a 4 day regimen of G-CSF or G-CSF + indomethacin. Shown on left are prototypical colonies found in blood mobilized by G-CSF and on the right various non-prototypical colonies found in PB of mice mobilized by G-CSF with NSAID. This representation is not inclusive of all colonies present, but highlights distinct colony types not normally seen in cultures of PB mobilized by G-CSF alone.

macrophage progenitor cells (GMP) with HSC showed enhanced reconstitution of tissue myeloid cells and protection from fungal and bacterial infections [58], and significantly improved survival in mice receiving lethal doses of radiation [59]. Therefore, not only are the absolute numbers of CD34+ cells important in determining post-transplant outcome, but the functional repertoire of stem and progenitor cells, as well as their mature counterparts, also substantially contribute. We have recently demonstrated that treatment with NSAIDs alone or in combination with G-CSF results in an increase in functionally defined myeloid progenitors both in bone marrow and in peripheral blood (preliminary data and [46]). Intriguingly, morphologic and microscopic analysis of myeloid hematopoietic colonies from mice mobilized with G-CSF vs. G-CSF plus NSAIDs showed a marked shift in multi-centric and monocytic colony formation (Figure 6), with many colonies exceeding the field of view (40X) in size (not shown). Histological analysis revealed that these colonies were primarily monocytic, and are highly reminiscent of multipotent, high proliferative potential colony forming cells (HPP-CFC) [60].

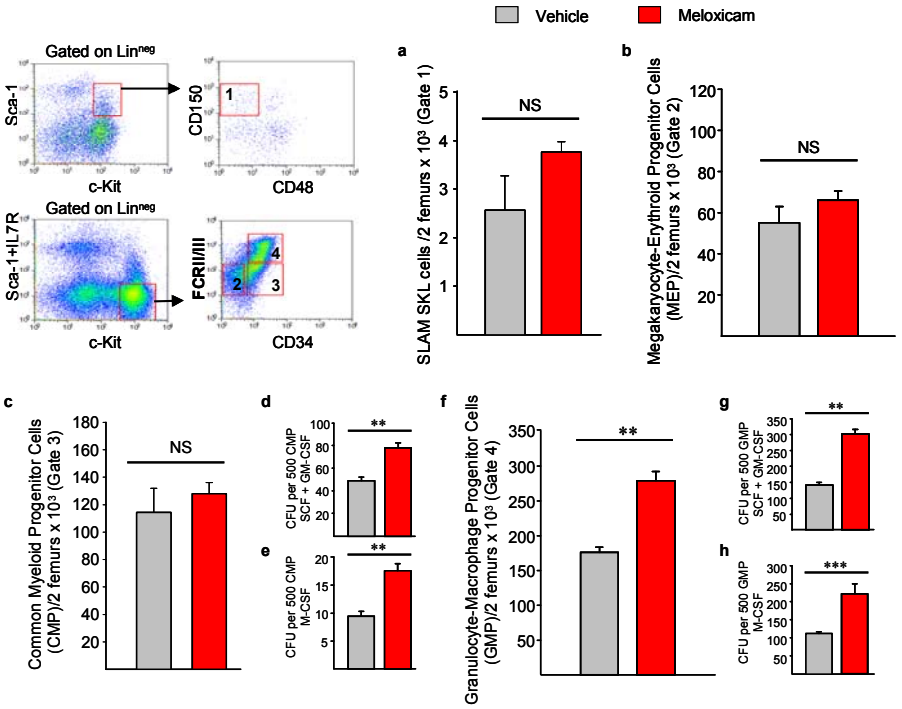

Figure 7. Representative FACS gating of bone marrow SLAM SKL cells (gate 1), MEP (gate 2), CMP (gate 3) and GMP. **a,** SLAM SKL, **b,** MEP, **c,** CMP and **f,** GMP cell number between vehicle and meloxicam treated mice (n=4 mice/group, each assayed individually). CMPs were sorted and plated in **d,** GM-CSF 10 ng/ml) + SCF (50 ng/ml) or **e,** M-CSF (2 ng/ml); GMPs were sorted and plated in **g,** GM-CSF + SCF or **h,** M-CSF. CFU per 500 cells determined (n=10 plates per treatment and condition group). *P<0.05, **P<0.01, ***P<0.001; unpaired two-tailed t-test. All error bars represent X + s.e.m.

While functional studies demonstrated an increase in CFU-GM in bone marrow following NSAID treatment, immunophenotypic studies by flow cytometry did not show an increase in HSC or other HPC populations, despite clear increases in their colony forming ability and enhanced long-term repopulating ability. These results suggest that perhaps a qualitatively more robust progenitor population was mobilized by NSAIDs. To answer this question we performed extensive functional and immunophenotypic analysis of bone marrow of control vs. NSAID treated mice [46] (Figure 7). The striking findings

were **1)** phenotypic analysis showed no increase in SLAM SKL (highly purified LT-HSC), phenotypically defined CMP, or phenotypically defined myelo-erythroid progenitors (MEP), with a ~2-fold increase in phenotypically defined GMP; **2)** flow cytometry sorted GMP from NSAID treated mice showed 2-fold enhanced colony formation on an equal cell number basis compared to GMP from control mice (Figure 7g,h), and in addition, **3)** flow cytometry sorted CMP from NSAID treated mice showed 2-fold enhanced colony formation on an equal cell number basis compared to control CMP (Figure 7d,e). These data clearly demonstrate that NSAIDs increase the proliferative potential or proliferative program of both CMP and GMP, resulting in the mobilization of a qualitatively different progenitor subset. In our patient populations mobilized with meloxicam, we hypothesize that the CD34+ cells will be qualitatively different than those mobilized with G-CSF + placebo.

3. PARTICIPANT SELECTION

Patients with Hodgkin's disease, non-Hodgkin lymphoma and multiple myeloma for whom AHSCT is an appropriate therapeutic strategy, will be the study population for this trial. Standard inclusion and exclusion criteria will be followed. In the randomized arm A, patients will receive G-CSF + placebo for the mobilization of their HSPC. Patients in arm B will receive G-CSF + meloxicam.

Eligibility tests for patients on BMT protocols must be completed within 42 days of registration, unless otherwise stated.

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Patients with hematologic malignancies for whom autologous stem cell transplantation is deemed clinically appropriate:

3.1.2 **Inclusion criteria**

3.1.2.1 . Non-Hodgkin's lymphoma, or Hodgkin's lymphoma either in a CR1 or refractory/relapsed with chemosensitive disease in a CR or PR.

3.1.2.2.. Multiple myeloma in first or second remission. Patients with CR or PR will be eligible for this protocol.

3.1.2.2 Ages 18-75 years

3.1.2.3 ECOG performance status of 0, 1, or 2.

3.1.2.4 Ability to understand and the willingness to sign a written informed consent

3.1.2.5 Patients on NSAIDs will be eligible only when they are off NSAIDs for 14 days.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Cardiac disease: symptomatic congestive heart failure or RVG, active angina pectoris, or uncontrolled hypertension. Participants may not be receiving any other study agents.

3.2.2 Pulmonary disease: severe chronic obstructive lung disease, or symptomatic restrictive lung disease.

3.2.3 Renal disease: serum creatinine > 2.0 mg/dl.

3.2.4 Hepatic disease: SGOT or SGPT > 3 x normal; serum bilirubin >2.0 mg/dl that is not due to Gilbert's syndrome or hemolysis

3.2.5 Uncontrolled infection.

3.2.6 Pregnancy or lactation.

3.2.7 Patients with NSAIDs allergies, known lactose allergy, a history of recent GI bleed (less than 2 weeks), and those who are on therapeutic dose anticoagulants will be excluded from this protocol.

3.2.8 Known CNS lymphoma.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If

a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at [redacted]

   Exception: DF/PCC Affiliate sites must fax the entire signed consent form including HIPAA Privacy Authorization and the eligibility checklist to the Network Affiliate Office. The Network Affiliate Office will register the participant with the QACT.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

5. TREATMENT PLAN

Treatment will be administered on both an outpatient and inpatient basis. Expected toxicities and potential risks as well as dose modifications for G-CSF and Meloxicam are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

All patients will sign an informed consent and register through the Dana Farber

QACT. ***The day of 1st apheresis will be regarded as day 0. Patients in whom the numbers of CD34+ cells in the peripheral blood are <10/ul, will be declared as "poor mobilizers" and they will be rescued with plerixafor.***

The patients will be randomized into two main arms as follows (A & B). There will also be further randomization according to the type of the disease, i.e., malignant lymphoma vs. multiple myeloma.

A. *G-CSF Alone (+ placebo):* Patients in this group will receive G-CSF 10 ug/kg s.c. daily, beginning 4 days prior to the 1st apheresis [days -4, -3, -2, -1] and continued on daily G-CSF for a total of 4 apheresis or until ≥ 4 x 10^6 CD34+ cells/kg for multiple myeloma patients or ≥2 x 10^6 CD34+ cells/kg for lymphoma patients are collected. They will also receive oral placebo for 5 days on days -6 through -2. Patients will undergo apheresis for 300 minutes to achieve approximately 3 to 4 whole blood volumes processed. This is a standard institutional protocol for autologous HSPC collection at the MGH. Multiple myeloma patients opting for mobilization with cyclophosphomide will have cyclophosphomide on day -12.

B. *G-CSF + meloxicam:* Patients in this group will be treated with meloxicam and G-CSF in an approximate two-day staggered dose schedule as described in our preclinical studies. Meloxicam will be given orally at a dose of 15 mg/day for 5 days (days -6 through -2). G-CSF at 10 ug/kg/day subcutaneously will be started on day -4 and continued daily for a total of 4 apheresis or until ≥ 4 x 10^6 CD34+ cells/kg for multiple myeloma or ≥2 x 10^6 CD34+ cells/kg for lymphoma patients are collected. Multiple myeloma patients opting for mobilization with cyclophosphomide will have cyclophosphomide on day -12.

Any patient in any of the above groups who has <10/ul circulating CD34+ cells on day 0 (poor mobilizer) will be started on daily (evening) plerixafor at 240 ug/kg/day s.c. as a rescue, will undergo apheresis on day +1 if circulating CD34+ cells are ≥ 10/ul and be continued on daily G-CSF + plerixafor for a total of 4 apheresis or until ≥ 4 x 10^6 CD34+ cells/kg are collected for multiple myeloma patients and > 2 x 10^6 CD34+ cells/kg for lymphoma patients. Patients who have < 10/μL $CD34^+$ cells on day +1 after the plerixafor on day 0 will come off the study.

5.1 Agent Administration

5.1.1 Meloxicam: Patients will receive meloxicam 15 mg by mouth daily for 5 days on days -6 through -2. Please see below "safety concerns of meloxicam".

5.1.2 G-CSF(Neupogen, Granix, filgrastim, tbo-filgrastim): Patients will receive G-CSF 10 ug/kg/day S.C. The dose of G-CSF is based on actual body weight. The dose of G-CSF may be rounded as follows: Patients < 65 kg, receive 600 mcg, patients ≥ 65<80 kg, receive 780 mcg, patients ≥ 80<96 kg, receive 900 mcg, patients ≥ 96<110 kg, receive 1080 mcg, patients ≥110kg receive 1200 mcg. The G-CSF is used to mobilize stem cells. G-CSF is commercially available.

5.1.3 Placebo: Patients in Arm A will receive oral placebo for 5 days on days -6 through -2.

5.1.4 Cyclophosphamide: Optional and only for multiple myeloma patients. Patients will receive cyclophosphamide 2000 mg/m^2 intravenously once a day for stem cell

mobilization on day -12. Mesna and intravenous hydration be given in conjunction for hemorrhagic cystitis prophylaxis per institutional standard.

5.2 General Concomitant Medication and Supportive Care Guidelines

A. Access to vessels. Pheresis will be obtained either via an indwelling central venous catheter (Hickman pheresis), a temporary venous catheter, or peripheral access.
B. Patients will receive standard supportive care for stem cell transplant patients according to institutional guidelines.
C. Transfusions: Transfusion practices will follow institutional guidelines. All blood products will be irradiated and leukoreduced.
D. Management of infections. Principles of infection prophylaxis and treatment will vary according to the spectrum of organisms and their antibiotic sensitivity and concurrent infection management practices and/or antibiotic clinical trial participation.

5.3 Duration of Therapy

Patients will receive meloxicam or placebo daily for 5 days, on days -6 through -2 along with daily G-CSF starting on day -4. G-CSF will be continued for a total of four sessions of apheresis or until $\geq 4 \times 10^6$ CD34+ cells/kg for multiple myeloma patients or $\geq 2 \times 10^6$ CD34+ cells/kg for lymphoma patients is collected. Multiple myeloma patients opting for mobilization with additional cyclophosphomide will start on day -12. The patients, who on day 0 are declared to be poor mobilizers, will be rescued with Plerixafor/G-CSF.

5.4 Duration of Follow Up

Participants will be followed for 6 months after autogeneic transplantation or until death whichever occurs first. If participants are removed from study, they will be followed for survival until 6 months after SCT or until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Participants who experience relapse of disease will be followed at the discretion of the treating physician.

5.5 Criteria for Removal from Study

Participants will be removed from study for death, relapsed or progressive disease, discretion of PI or treating physician, or withdrawal of consent. In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator: Bimalangshu Dey, MD, [redacted]

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

No dose delays or modifications are allowed. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through 30 days after the last apheresis session. . Additionally all serious infection or bleeding events post transplant should be collected through day 100 post transplant. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

Meloxicam:

Non-steroidal anti-inflammatory drugs (NSAIDs) have been associated with adverse effects such as peptic ulcers, gastritis, and bleeding. Meloxicam is a selective inhibitor of cyclooxygenase 2 (COX-2), inhibiting this enzyme with approximately six times the potency of COX-1. Originally approved in 2000, meloxicam has a long track record and a favorable side effect profile. Trials in healthy volunteers showed that this agent did not prolong the bleeding time or affect platelet aggregation in contrast to other NSAIDs tested such as nabumetone and indomethacin [69,70]. Data from these studies suggest that even at supratherapeutic doses, meloxicam shows relatively low inhibition of thromboxane A2, the eicosanoid that is responsible for platelet aggregation and maintenance of the bleeding time. With regard to the potential gastrointestinal side effects of NSAIDs, these almost always occur in the context prolonged use at high doses ; as we will be using the standard dose of meloxicam for a total of 5 days, GI adverse events are highly unlikely. Additionally, at least one large-scale cohort study that followed 13,000 patients on meloxicam for three months found only a 0.8% rate of GI adverse events [71]

G-CSF:

Rare anaphylactic reactions with the first dose; bone pain at sites of active marrow with continued administration. Local reactions at injection sites. Constitutional symptoms, increased alkaline phosphatase, LDH, uric acid; worsening of pre-existing inflammatory conditions. Please refer to the package insert for a complete listing of all toxicities.

Cyclophosphamide:

Alopecia, nausea, vomiting, leukopenia, amenorrhea, cardiomyopathy, Stevens-Johnson syndrome, hemorrhagic cystitis, azospermia,

oligozoospermia, interstitial pneumonia, infections, and rare cases of toxic epidermal necrolysis. Please refer to the package insert for a complete listing of all toxicities.

7. DRUG FORMULATION AND ADMINISTRATION

7.1.1 Meloxicam: in arm B, patients will receive meloxicam 15 mg by mouth daily for 5 days on days -6 through -2. Please see below "safety concerns of meloxicam".

7.1.1.1 Availability: Meloxicam tablets are commercially available and will be used in the compounding of meloxicam capsules. Pharmacy will be ensuring the blinding and distributing of both placebo and study drug (meloxicam) among the participating patients. "Blinding" will ensure accurate assessments of the toxicities of the "study drug" arm [G-CSF + meloxicam].

7.1.1.2 Preparation: Meloxicam will be compounded into a capsule formation in order to ensure the blind. Missed and/or vomited doses should not be made up. Meloxicam can be taken with or without food. Capsules should be swallowed whole and not crushed, chewed or dissolved.

7.1.1.3 Administration: oral

7.1.2 Placebo: In arm A, patients will receive placebo by mouth daily for 5 days on Days -6 through -2.

Study staff will provide pharmacy with a fund number to purchase and blind study drug. The compounding pharmacy will blind active drug and provide a matching placebo. The placebo will be composed of lactose in a gelatin capsule. The pharmacy will dispense the blinded study drug to subjects free of charge.

7.1.3 G-CSF(Neupogen, Granix, filgrastim, tbo-filgrastim): Patients will receive G-CSF 10 ug/kg/day S.C. The dose of G-CSF is based on actual body weight. The dose of G-CSF may be rounded as follows: Patients < 65 kg, receive 600 mcg, patients ≥65<80 kg, receive 780 mcg, patients ≥80<95 kg, receive 900 mcg, patients ≥96<110 kg, receive 1080 mcg, patients ≥110kg receive 1200 mcg. The G-CSF is used to mobilize stem cells. G-CSF is commercially available.

7.1.3.1 Availability

Commercially available in single use vials containing 480 mcg/vial (300 mcg/ml, 1.6 mg/vial).
Should be stored at 2-8oC (do not freeze and do not shake) and is stable for at least 1 year at this temperature.

7.1.3.2 Preparation

Refer to the FDA approved package insert for complete product information.

7.1.3.3 Administration

Filgrastim or tbo-filgrastim will be given by subcutaneous injection at 10 μg/kg/d; patient or other caregiver will be instructed on proper injection technique

7.1.4 Cyclophosphamide: Optional and only for multiple myeloma patients. Patients will receive cyclophosphamide 2000 mg/m^2 intravenously once a day for stem cell mobilization on day -12.
The chemical name is 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate. The molecular formula is C7H15Cl2N2O2P•H2O. The molecular weight is 279.1. Cyclophosphamide is a nitrogen mustard alkylating agent. Following
intravenous administration, the elimination half-life of cyclophosphamide ranges from 4.1 to 16 hours. There is extensive hepatic metabolism, as cyclophosphamide is a prodrug that is converted by hepatic microsomal enzymes (cytochrome P450 mixed-function oxidase) to the active form. Specifically, the activation pathway of cyclophosphamide involves CYP2B6, CYP2C9 and CYP3A4 isoenzymes. CYP3A4 also catalyzes the inactivation of cyclophosphamide to dechloroethylcyclophosphamide. Metabolites include 4-hydroxycyclophosphamide, aldophosphamide, phosphoramide mustard (believed to be the active antineoplastic metabolite of cyclophosphamide), acrolein (believed to cause hemorrhagic cystitis), and a number of inactive metabolites. Excretion of cyclophosphamide is exclusively by the kidney; however, due to the non-ionized nature of the intact drug, renal tubular reabsorption is avid, resulting in a large fraction of the dose being eliminated by hepatic metabolism.

7.1.4.1 Availability

Cyclophosphamide is commercially available and supplied as a lyophilized powder which is compatible in 0.9% sodium chloride or 5% dextrose in glass or polyolefin containers and is incompatible with aluminum. Reconstituted solutions of lyophilized cyclophosphamide are chemically and physically stable for 24 hours at room temperatures or for 6 days in the refrigerator. Specific temperatures are not provided by the manufacturer.

7.1.4.2 Preparation
Cyclophosphamide will be prepared per institutional standards.

7.1.4.3 Administration

Cyclophosphamide will be given intravenously once a day at 2000 mg/m$^{2.}$

8. CORRELATIVE/SPECIAL STUDIES

Characterize the hematopoietic stem and progenitor cells mobilized by meloxicam.

Our scientific studies clearly demonstrate the importance of defining and characterizing the cellular content and function of grafts mobilized following the novel regimen of G-CSF + meloxicam. 1 10 mL EDTA tube of blood will be sampled from patients at the following timepoints: baseline prior to initiating meloxicam or placebo (day -6), and on day 0 (first apheresis day), and on subsequent days of apheresis if no additional mobilization rescue is given. The following assays will be performed on blood samples: **1)** $CD34^+$ cells will be quantified by flow-cytometry using the ISHAGE protocol [53] as performed routinely in our clinic; **2)** Functionally defined hematopoietic progenitors (CFU-GM, CFU-GEMM and BFU-E). Assays will be performed using methylcellulose media with 30% HI-FCS, 1 U/ml Epo, 50 ng rhSCF and 10 ng rhGM-CSF, and CFU-GM, BFU-E and CFU-GEMM quantified after 7 days incubation at 37°C, in 5% CO_2, 5% O_2 in humidified air, as we have previously described (Figure 5 and _ENREF_40 [46]).

In addition to the above analysis, the sample collected on the first day of apheresis will also be assessed for content of **1)** Total $CD34^+$ cells and the most primitive subset $CD34^+CD38^-CD90^+CD45RA^-CD49f^+$ [64-68] **2)** CXCR4 expression on $CD34^+$ cells; ; and **3)** immune cell subsets by flow-cytometry. Immune cell subsets to be analyzed include B ($CD19^+$) cells, NK ($CD3^-CD56^+$) cells, total T cell subsets ($CD3^+CD4^+CD8^-$, $CD3^+CD4^-CD8^+$), and Treg cells ($CD45^+CD25^+FOXP3^+$). Also, for $CD4^+$ and $CD8^+$ T cells, we will determine the proportion of naïve cells ($CD45RA^+CD27^+$), CD45RA-expressing effector memory cells ($CD45RA^+CD27^-$), central memory ($CD45^-CD27^+CD62L^+$) cells, effector memory type I cells ($CD45RA^-CD27^+CD62L^-$), and effector memory type II cells ($CD45RA^-CD27^-$) cells. As dendritic cell (DC) content of the graft may also impact on outcome of allogeneic HCT, we will also evaluate the plasmacytoid ($Lin^-DR^+CD11c^-CD123^+$) and myeloid ($Lin^-DR^+CD11c^+CD123^-$) DC mobilized.

9. STUDY CALENDAR

	Pre-Study	Day -12	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 1-2[7]	Day +3[7]	Day[5] 100 post	Day 180[5]
Informed consent	X												
G-CSF					X	X	X	X	X	X	X		
Meloxicam or placebo			X	X	X	X	X						
Cyclophosphomide[8]		X											
History, Physical Exam	X		X						X	X	X	X	
Physical exam (Ht, Wt, BSA, VS)	X[1]		X						X		X	X	
CBC w/diff, plts[3]	X		X						X	X	X	X	
Chemistry panel[2]	X												
Serum Hcg	X												
Flow cytometry			X						X	X	X		
Adverse event evaluation[6]			X----------										----------X
Disease assessment as per SOC for disease	X												
Research samples[4]			X						X	X	X		

1. Height, weight and BSA required only at pre-study visit.
2. Chemistry panel must include: creatinine, bilirubin, SGOT, SGPT
3. Daily CBC after AHSCT until ANC> 500 th/cmm and PLT > 20 th/cumm (transfusion independent), for 3 consecutive days.
4. 1 10 mL EDTA tube of blood at D-6, D0, D+1, D+2, D+3
5. Day 100 and 180 assessments refer to post SCT
6. Formal toxicity assessments to be documented at days -6, 0, 3 and day 100 post stem cell infusion.
7. Days 1, 2, and 3 required if insufficient cells collected on day 0.
8. Cyclophosphomide optional for multiple myeloma patients only and not applicable for lymphoma patients.

10. ADVERSE EVENT REPORTING REQUIREMENTS

10.1 Definitions

10.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms.

10.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more sever form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical 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.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

10.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

10.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

10.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

10.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

10.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

10.3 Reporting Requirements

10.3.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Grade 4 (life threatening) events are not required to be reported as SAEs if they are expected events related to stem cell transplantation. These include neutropenia, neutropenic fever within 14 days of transplantation, oral mucositis, thrombocytopenia, minor bleeding episodes (e.g. epistaxis), infections in the first 100 days of transplantation (e.g. pneumonia, line sepsis, cellulitis).

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator as soon as possible, but not later than 10 days of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 10 days after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

10.3.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms. Grade 2 and higher adverse events will be captured.

10.4 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

Other investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to:

[*DF/HCC Overall Principal Investigator*]
[*telephone #*]
[*email*]
[*fax#*]

The DF/HCC Principal Investigator will submit SAE reports from outside institutions to the DFCI Office for Human Research Studies (OHRS) according to DFCI IRB policies and procedures in reporting adverse events.

10.5 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

10.6 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

11. DATA AND SAFETY MONITORING

11.1 Data Reporting

11.1.1 Method

The QACT will collect, manage, and monitor data for this study.

11.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 30 days of registration
Baseline Assessment Form	Within 30 days of registration
Treatment Form	Within 30 days of the last day of the cycle
Adverse Event Report Form	Within 30 days of the last day of the cycle
Response Assessment Form	Within 30 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 30 days of the protocol defined follow up visit date or call

11.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

11.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

12. REGULATORY CONSIDERATIONS

12.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

12.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and

dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

12.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
  www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

    - Title 21 Part 11 – Electronic Records; Electronic Signatures
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html

    - Title 21 Part 50 – Protection of Human Subjects
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html

    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

    - Title 21 Part 56 – Institutional Review Boards
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html

    - Title 21 Part 312 – Investigational New Drug Application
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws

- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

12.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records,

recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

12.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13. STATISTICAL CONSIDERATIONS

A sample size of 15 patients per group provides 90% power to detect a 1.5 fold increase in the number of circulating CD34+ cells on the first day of apheresis with the addition of meloxicam compared to G-CSF + placebo. The fold change is defined as the difference between group means that is expressed as a multiple of the standard deviation assumed to be common in both groups. A two-sided Mann-Whitney test is used at a 1% level, under the assumption of a normal distribution for the number of circulating CD34+ cells. The Mann-Whitney test will also be used to compare the number of apheresis sessions required to collect ˜ 5 x 10^6 CD34+ cells/kg between the patient groups. To show that meloxicam-treated patients will need 1 less apheresis session to achieve ˜5 x 10^6 CD34+ cells/kg, 50 patients in each group will be required to achieve 81% power at a one-sided 10% alpha level, assuming an average of two sessions will be required to collect ˜5 x 10^6 CD34+ cells/kg in G-CSF-treated patients. The time to neutrophil or platelet engraftment will estimated by the Kaplan-Meier method and the difference between groups compared by the logrank test. Cell population numbers and HPC in peripheral and apheresis products will be summarized respectively by the median, mean and standard deviation. In peripheral blood, the difference in cell types following meloxicam and G-CSF mobilization relative to baseline will be compared to the change following G-CSF alone using the Mann-Whitney test or two-sample t-test. The proportion of patients failing stem cell mobilization will be a secondary comparison between the two groups. Fisher's exact test will be used to explore the effect of adding meloxicam but will have limited power.

14. PUBLICATION PLAN

The outcomes of the study including the correlative science studies will be analyzed and hopefully be presented at the professional clinical and scientific meetings. The results will then be submitted to peer reviewed journals for publication.

15. REFERENCES

1. Attal, M., et al., *A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome.* N Engl J Med, 1996. **335**(2): p. 91-7.
2. Philip, T., et al., *Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma.* N Engl J Med, 1995. **333**(23): p. 1540-5.
3. Einhorn, L.H., et al., *High-dose chemotherapy and stem-cell rescue for metastatic germ-cell tumors.* N Engl J Med, 2007. **357**(4): p. 340-8.

4. Rohatiner, A.Z., et al., *Myeloablative therapy with autologous bone marrow transplantation for follicular lymphoma at the time of second or subsequent remission: long-term follow-up.* J Clin Oncol, 2007. **25**(18): p. 2554-9.
5. Geisler, C.H., et al., *Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic Lymphoma Group.* Blood, 2008. **112**(7): p. 2687-93.
6. Siena, S., et al., *Therapeutic relevance of CD34 cell dose in blood cell transplantation for cancer therapy.* J Clin Oncol, 2000. **18**(6): p. 1360-77.
7. Duggan, P.R., et al., *Predictive factors for long-term engraftment of autologous blood stem cells.* Bone Marrow Transplant, 2000. **26**(12): p. 1299-304.
8. Ikeda, K., T. Kozuka, and M. Harada, *Factors for PBPC collection efficiency and collection predictors.* Transfus Apher Sci, 2004. **31**(3): p. 245-59.
9. Sugrue, M.W., et al., *Characterization and outcome of "hard to mobilize'" lymphoma patients undergoing autologous stem cell transplantation.* Leuk Lymphoma, 2000. **39**(5-6): p. 509-19.
10. Boeve, S., et al., *Analysis of remobilization success in patients undergoing autologous stem cell transplants who fail an initial mobilization: risk factors, cytokine use and cost.* Bone Marrow Transplant, 2004. **33**(10): p. 997-1003.
11. Bensinger, W., et al., *Factors that influence collection and engraftment of autologous peripheral-blood stem cells.* J Clin Oncol, 1995. **13**(10): p. 2547-55.
12. Weaver, C.H., et al., *An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy.* Blood, 1995. **86**(10): p. 3961-9.
13. Koc, O.N., et al., *Randomized cross-over trial of progenitor-cell mobilization: high-dose cyclophosphamide plus granulocyte colony-stimulating factor (G-CSF) versus granulocyte-macrophage colony-stimulating factor plus G-CSF.* J Clin Oncol, 2000. **18**(9): p. 1824-30.
14. Fitoussi, O., et al., *A comparison of toxicity following two different doses of cyclophosphamide for mobilization of peripheral blood progenitor cells in 116 multiple myeloma patients.* Bone Marrow Transplant, 2001. **27**(8): p. 837-42.
15. Hicks, M.L., et al., *Optimizing the timing of chemotherapy for mobilizing autologous blood hematopoietic progenitor cells.* Transfusion, 2007. **47**(4): p. 629-35.
16. Dreger, P., et al., *Autologous progenitor cell transplantation: prior exposure to stem cell-toxic drugs determines yield and engraftment of peripheral blood progenitor cell but not of bone marrow grafts.* Blood, 1995. **86**(10): p. 3970-8.
17. Pusic, I., et al., *Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation.* Biol Blood Marrow Transplant, 2008. **14**(9): p. 1045-56.
18. Nervi, B., D.C. Link, and J.F. DiPersio, *Cytokines and hematopoietic stem cell mobilization.* J Cell Biochem, 2006. **99**(3): p. 690-705.
19. Winkler, I.G. and J.P. Levesque, *Mechanisms of hematopoietic stem cell mobilization: when innate immunity assails the cells that make blood and bone.* Exp Hematol, 2006. **34**(8): p. 996-1009.
20. Nakamura, Y., et al., *Soluble c-kit receptor mobilizes hematopoietic stem cells to peripheral blood in mice.* Exp Hematol, 2004. **32**(4): p. 390-6.
21. Craddock, C.F., et al., *Antibodies to VLA4 integrin mobilize long-term repopulating cells and augment cytokine-induced mobilization in primates and mice.* Blood, 1997. **90**(12): p. 4779-88.

22. Opie, T.M., L.E. Shields, and R.G. Andrews, *Cell-surface antigen expression in early and term gestation fetal hematopoietic progenitor cells.* Stem Cells, 1998. **16**(5): p. 343-8.
23. Dao, M.A., J. Arevalo, and J.A. Nolta, *Reversibility of CD34 expression on human hematopoietic stem cells that retain the capacity for secondary reconstitution.* Blood, 2003. **101**(1): p. 112-8.
24. Fu, S. and J. Liesveld, *Mobilization of hematopoietic stem cells.* Blood Rev, 2000. **14**(4): p. 205-18.
25. Pavone, V., et al., *Poor mobilization is an independent prognostic factor in patients with malignant lymphomas treated by peripheral blood stem cell transplantation.* Bone Marrow Transplant, 2006. **37**(8): p. 719-24.
26. Bolwell, B.J., et al., *Patients mobilizing large numbers of CD34+ cells ('super mobilizers') have improved survival in autologous stem cell transplantation for lymphoid malignancies.* Bone Marrow Transplant, 2007. **40**(5): p. 437-41.
27. O'Shea, D., et al., *Predictive factors for survival in myeloma patients who undergo autologous stem cell transplantation: a single-centre experience in 211 patients.* Bone Marrow Transplant, 2006. **37**(8): p. 731-7.
28. Toor, A.A., et al., *Favourable results with a single autologous stem cell transplant following conditioning with busulphan and cyclophosphamide in patients with multiple myeloma.* Br J Haematol, 2004. **124**(6): p. 769-76.
29. Vermeulen, M., et al., *Role of adhesion molecules in the homing and mobilization of murine hematopoietic stem and progenitor cells.* Blood, 1998. **92**(3): p. 894-900.
30. Heissig, B., et al., *Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand.* Cell, 2002. **109**(5): p. 625-37.
31. Levesque, J.P., et al., *Characterization of hematopoietic progenitor mobilization in protease-deficient mice.* Blood, 2004. **104**(1): p. 65-72.
32. Levesque, J.P., et al., *Mobilization by either cyclophosphamide or granulocyte colony-stimulating factor transforms the bone marrow into a highly proteolytic environment.* Exp Hematol, 2002. **30**(5): p. 440-9.
33. Moskowitz, C.H., et al., *Factors affecting mobilization of peripheral blood progenitor cells in patients with lymphoma.* Clin Cancer Res, 1998. **4**(2): p. 311-6.
34. Ford, C.D., et al., *CD34+ cell adhesion molecule profiles differ between patients mobilized with granulocyte-colony-stimulating factor alone and chemotherapy followed by granulocyte-colony-stimulating factor.* Transfusion, 2006. **46**(2): p. 193-8.
35. Desikan, K.R., et al., *Comparable engraftment kinetics following peripheral-blood stem-cell infusion mobilized with granulocyte colony-stimulating factor with or without cyclophosphamide in multiple myeloma.* J Clin Oncol, 1998. **16**(4): p. 1547-53.
36. Narayanasami, U., et al., *Randomized trial of filgrastim versus chemotherapy and filgrastim mobilization of hematopoietic progenitor cells for rescue in autologous transplantation.* Blood, 2001. **98**(7): p. 2059-64.
37. Olavarria, E. and E.J. Kanfer, *Selection and use of chemotherapy with hematopoietic growth factors for mobilization of peripheral blood progenitor cells.* Curr Opin Hematol, 2000. **7**(3): p. 191-6.
38. Dingli, D., et al., *Cyclophosphamide mobilization does not improve outcome in patients receiving stem cell transplantation for multiple myeloma.* Clin Lymphoma Myeloma, 2006. **6**(5): p. 384-8.
39. Demirer, T., C.D. Buckner, and W.I. Bensinger, *Optimization of peripheral blood stem cell mobilization.* Stem Cells, 1996. **14**(1): p. 106-16.
40. Bargetzi, M.J., et al., *Mobilization of peripheral blood progenitor cells with vinorelbine and granulocyte colony-stimulating factor in multiple myeloma patients is reliable and cost effective.* Bone Marrow Transplant, 2003. **31**(2): p. 99-103.

41. Matthys, P., et al., *AMD3100, a potent and specific antagonist of the stromal cell-derived factor-1 chemokine receptor CXCR4, inhibits autoimmune joint inflammation in IFN-gamma receptor-deficient mice.* J Immunol, 2001. **167**(8): p. 4686-92.
42. Flomenberg, N., et al., *The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone.* Blood, 2005. **106**(5): p. 1867-74.
43. Calandra, G., et al., *AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data.* Bone Marrow Transplant, 2008. **41**(4): p. 331-8.
44. J. Hoggatt, P. Singh, J. Sampath, and L. M. Pelus, Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* 113, 5444 (2009).
45. T. E. North, W. Goessling, C. R. Walkley, C. Lengerke, K. R. Kopani*, et al.*, Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* 447, 1007 (2007).
46. J. Hoggatt, K.S. Mohammad, P. Singh, A.F. Hoggatt, B.R. Chitteti, *et al.* Differential stem and progenitor cell trafficking by prostaglandin E_2. *Nature,* (2013), *in press*.
47. M. Ahmed, D. Khanna, and D. E. Furst, Meloxicam in rheumatoid arthritis. *Expert. Opin. Drug Metab Toxicol.* 1, 739 (2005).
48. H. M. Rinder, J. B. Tracey, M. Souhrada, C. Wang, R. P. Gagnier*, et al.*, Effects of meloxicam on platelet function in healthy adults: a randomized, double-blind, placebo-controlled trial. *J. Clin. Pharmacol.* 42, 881 (2002).
49. S. L. Gerson, G. H. Talbot, S. Hurwitz, B. L. Strom, E. J. Lusk*, et al.*, Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. *Ann. Intern. Med.* 100, 345 (1984).
50. K. M. Taylor, S. Jagannath, G. Spitzer, J. A. Spinolo, S. L. Tucker*, et al.*, Recombinant human granulocyte colony-stimulating factor hastens granulocyte recovery after high-dose chemotherapy and autologous bone marrow transplantation in Hodgkin's disease. *J. Clin. Oncol.* 7, 1791 (1989).
51. P. A. Pizzo, Granulocytopenia and cancer therapy. Past problems, current solutions, future challenges. *Cancer* 54, 2649 (1984).
52. H. Bittencourt, V. Rocha, S. Chevret, G. Socie, H. Esperou*, et al.*, Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 99, 2726 (2002).
53. D. R. Sutherland, L. Anderson, M. Keeney, R. Nayar, and I. Chin-Yee, The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. *J. Hematother.* 5, 213 (1996).
54. S. Fruehauf, M. R. Veldwijk, T. Seeger, M. Schubert, S. Laufs*, et al.*, A combination of granulocyte-colony-stimulating factor (G-CSF) and plerixafor mobilizes more primitive peripheral blood progenitor cells than G-CSF alone: results of a European phase II study. *Cytotherapy.* 11, 992 (2009).
55. E. Jantunen and S. Fruehauf, Importance of blood graft characteristics in auto-SCT: implications for optimizing mobilization regimens. *Bone Marrow Transplant.* 46, 627 (2011).
56. J. Hoggatt and L. M. Pelus, Hematopoietic stem cell mobilization with agents other than G-CSF. *Methods Mol. Biol.* 904, 49 (2012).
57. L. M. Pelus and S. Fukuda, Peripheral blood stem cell mobilization: the CXCR2 ligand GRObeta rapidly mobilizes hematopoietic stem cells with enhanced engraftment properties. *Exp Hematol* 34, 1010 (2006).
58. A. BitMansour, S. M. Burns, D. Traver, K. Akashi, C. H. Contag*, et al.*, Myeloid progenitors protect against invasive aspergillosis and Pseudomonas aeruginosa infection following hematopoietic stem cell transplantation. *Blood* 100, 4660 (2002).

59. V. K. Singh, J. Christensen, O. O. Fatanmi, D. Gille, E. J. Ducey*, et al.*, Myeloid progenitors: a radiation countermeasure that is effective when initiated days after irradiation. *Radiat. Res.* 177, 781 (2012).
60. T. R. Bradley and G. S. Hodgson, Detection of primitive macrophage progenitor cells in mouse bone marrow. *Blood* 54, 1446 (1979).
61. P. Schroeter, A. Sablotzki, and D. Riemann, CD13 and CD10 expression of granulocytes as markers for the functioning of the immune system: quantification of the expression of membrane molecules using 1:1 labeled monoclonal antibodies and flow cytometry. *Methods Mol. Biol.* 378, 71 (2007).
62. K. K. Pannu, E. T. Joe, and S. B. Iyer, Performance evaluation of QuantiBRITE phycoerythrin beads. *Cytometry* 45, 250 (2001).
63. L. Jiang, C. M. Yuan, J. Hubacheck, J. E. Janik, W. Wilson*, et al.*, Variable CD52 expression in mature T cell and NK cell malignancies: implications for alemtuzumab therapy. *Br. J. Haematol.* 145, 173 (2009).
64. C. M. Baum, I. L. Weissman, A. S. Tsukamoto, A. M. Buckle, and B. Peault, Isolation of a candidate human hematopoietic stem-cell population. *Proc. Natl. Acad. Sci. U. S. A* 89, 2804 (1992).
65. M. Bhatia, J. C. Wang, U. Kapp, D. Bonnet, and J. E. Dick, Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc. Natl. Acad. Sci. U. S. A* 94, 5320 (1997).
66. M. Bhatia, D. Bonnet, B. Murdoch, O. I. Gan, and J. E. Dick, A newly discovered class of human hematopoietic cells with SCID-repopulating activity. *Nat. Med.* 4, 1038 (1998).
67. R. Majeti, C. Y. Park, and I. L. Weissman, Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. *Cell Stem Cell* 1, 635 (2007).
68. F. Notta, S. Doulatov, E. Laurenti, A. Poeppl, I. Jurisica*, et al.*, Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science* 333, 218 (2011).
69. H.M.Rinder, J.B.Tracey, M. Souhrada, C.Wang, R.P.Gagnier, et al. Effects of meloxicam on platelet functionin healthy adults: a randomized, double-blind, placebo-controlled trial. J. Clin. Pharmacol. 42, 881(2002)
70. D.J.van Kraaij, A.H.Hovestad-Witterland, M. de Metz, E.J.Vollaard. A comparison of the effects of nabumetone vs. meloxicam on serum thromboxane B2 and platelet function in healthy volunteers. Br J Clin Pharmacol. 53(6):644-7 (2002)
71. H. Zeidler, J.P.Kaltwasser, J.P.Leonard, T.Kohlmann, R.Sigmund, F.Degner, M.Hettich. Prescription and tolerability of meloxicam in day-to-day practice: postmarketing observational cohort study of 13,307 patients in Germany. J Clin Rheumatol. 8(6):305-15 (2002)

Appendix A: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix B: Lab Manual

The following research samples will be collected:

10 mL EDTA tube of blood at D-6, D0, D+1, D+2, D+3

Sample Handling:

Samples should be received by the lab within 24 hours of collection and should be sent ambient.

Sample labeling:

Samples should be labeled with pt initials, pt ID number, Study number (13-195), sample time point, date and time obtained.

Samples with Sample Submission Form to be sent to:

Protocol 13-195

Non-steroidal anti-inflammatory drugs (meloxicam) to mobilize hematopoietic stem cells: A Phase II randomized trial

Sample Submission Form

Date: _____

Initials:_____ PT ID #:_____

16. MRN: _____

Sample Information

1. Sample type: _____
2. Sample time point: _____
3. Date sample obtained: _____
4. Relation to G-CSF administration (circle one): PRE/ POST
5. WBC (attach a copy of CBC report if available): _____
6. Sample sent out by: _____ Date: _____
7. FedEx tracking # or Skycom Job #: _____

OTHER MEDICATIONS TAKEN

If you take a daily medication (prescribed or otherwise), please use one line per drug and indicate the start and stop dates under the "Date(s) Taken" section (i.e., 6/2/09-6/5/09).

Drug Name	Dose	Dates Taken	Reason Taken

Study Participant Initials _____ Date _____

FOR OFFICE USE	
Staff Initials:	
Date Dispensed:	Date Returned:
# pills/caps/tabs dispensed:	# pills/caps/tabs returned:
# pills/caps/tabs that should have been taken:	
Discrepancy Notes:	

Study Participant Self-Administration Study Drug Diary

Dana-Farber/Harvard Cancer Center

Participant Identifier: _____
Protocol # 13-195
Your MD_____ Phone_____
Your RN_____ Phone_____

STUDY DRUG INSTRUCTIONS:

Study Drug: Meloxicam/Placebo

How Often: You will take each dose daily for 5 days starting 6 days (Day -6) before you are scheduled to undergo the first apheresis procedure (Day 0) and continuing until 2 days before apheresis (Day -2).

SPECIAL INSTRUCTIONS:

- Meloxicam/Placebo can be taken with or without food.
- Tablets should be swallowed whole and not crushed, chewed or dissolved.
- Missed and/or vomited doses should not be made up. If you forget to take a dose or vomit after a dose, please wait until the next scheduled dose.

DOSING LOG

CYCLE X

Meloxicam/Placebo
For each dose take : X pills/capsules/tablets

Please indicate the date, time, amount taken and any comments.

	Date	Amount Taken AM dose	Comments
Ex:	6/1/09	8am - 1	Vomited AM dose
Day -6			
-5			
-4			
-3			
-2			
-1			
Day 0			
1			
2			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			

SYMPTOMS/SIDE EFFECTS

Please record any side effects experienced during this cycle. Include the date the particular symptom started and when it ended. Please evaluate the severity of the symptom according to the following scale:

Mild: Awareness of sign or symptom; easily tolerated and did not affect ability to perform normal daily activities. Symptom did not require medication or therapeutic intervention.

Moderate: Significant discomfort which interfered with ability to perform normal daily activities. Symptom was easily resolved with at home medication or simple therapeutic intervention.

Severe: Marked discomfort with an inability to carry out normal daily activities. Symptom required new medication and/or therapeutic intervention in order to resolve.

Please Note: The severity should reflect the most severe level experienced during the time period.

Symptom	Start Date	End Date	Severity