THE UNIVERSITY OF KANSAS

CANCER CENTER

Investigator - Initiated Trial

2015-IIT-BMT-MM-AutoSCT

An exploratory trial to estimate the proportion of patients with tumor cell contaminated, flow positive leukapheresis products collected with and without bortezomib as in-vivo purging prior to autologous stem cell harvest for multiple myeloma

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: 2015-IIT-BMT-MM-AutoSCT

Protocol Title: An exploratory trial to estimate the proportion of patients with tumor cell contaminated, flow positive leukapheresis products collected with and without bortezomib as in-vivo purging prior to autologous stem cell harvest for multiple myeloma

Protocol Version and Date: Protocol Version 5.0 dated 11-21-2016

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Date

Print Name and Title: _____

Site Number: N/A

Site Name: The University of Kansas Cancer Center / University of Kansas Medical Center

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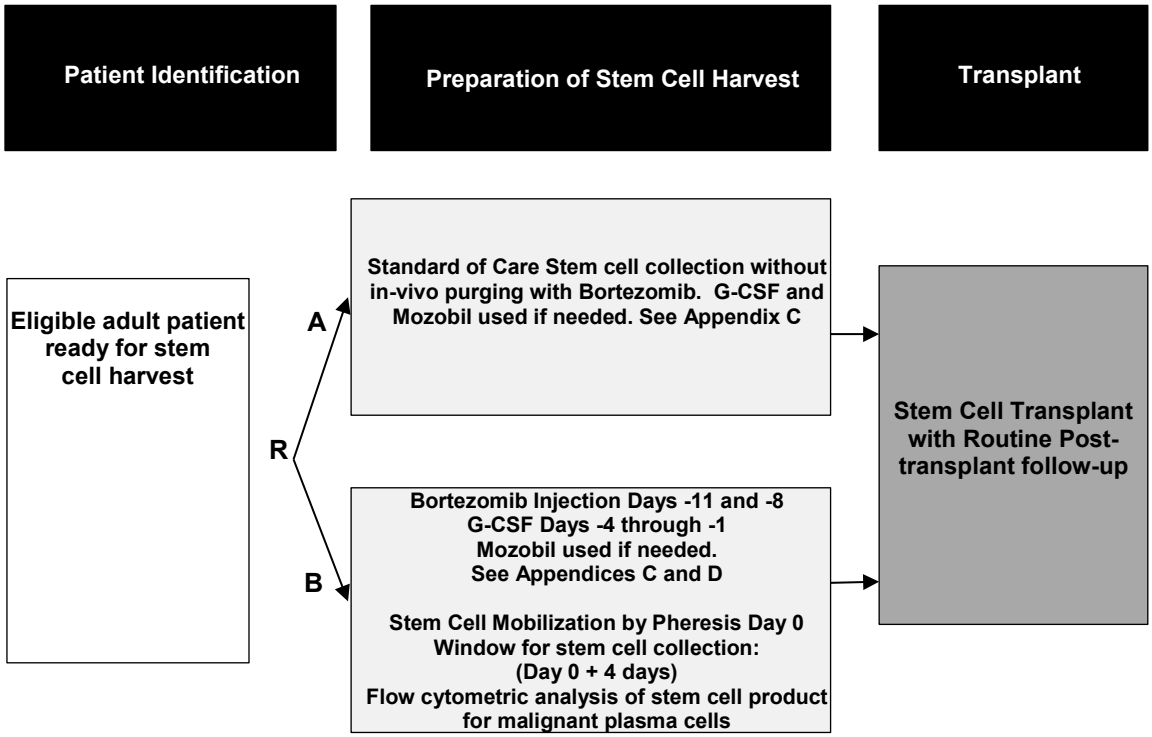
ABBREVIATION LIST

AE	Adverse Event
ALT	Alanine Aminotransferase
ARDS	Acute Respiratory Distress Syndrome
ASBMT	American Society for Blood and Marrow Transplantation
AST	Aspartate Aminotransferase
BA (study)	Bioavailability
BE (study)	Bioequivalence
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CFR	(United States) Code of Federal Regulations
CR	Complete Response
CRF	Case Report Form
CRIS	Comprehensive Research Information System
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CV	Cardiovascular
DFS	Disease-Free Survival
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
DSMC	Data and Safety Monitoring Committee
EBMT	European Blood and Marrow Transplantation
ECG	Electro-cardiogram
ECOG	Eastern Cooperative Oncology Group
E_{MAX}	Maximum Effect
EOT	End of Treatment
EU	European Union
FDA	United States Food and Drug Administration
G-CSF	Granulocyte colony-stimulating factor
GCP	Good Clinical Practice(s)
GI	Gastro-Intestinal
GGT	Gamma-glutamyltransferase
HCT	Hematopoietic Cell Transplantation
HSC	Human Subjects Committee
Hgb	Hemoglobin
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMWG	International Myeloma Working Group
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	Intra-uterine Device
IV	Intravenous
KPS	Karnosky Performance Status
KU	University of Kansas
KUCC	The University of Kansas Cancer Center
LDH	Lactic Dehydrogenase
MCL	Mantle Cell Lymphoma
MedDRA	Medical Dictionary for Regulatory Activities
MFC	Multi-parametric Flow Cytometry

µL	Microliter
MM	Multiple Myeloma
MR	Minor Response
MRD	Minimal Residual Disease
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NEC	Not Elsewhere Classifiable
NHL	Non-Hodgkins Lymphoma
ORR	Overall Response Rate
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear
PCR	Polymerase Chain Reaction
PFS	Progression Free Survival
PI	Principal Investigator
PK	Pharmacokinetics
PR	Partial Response
QA	Quality Assurance
SAE	Serious Adverse Event
SQ	Subcutaneous
TTP	Time to Progression
ULN	Upper Limit of Normal
USP	United States Pharmacopeia
WBC	White Blood Cell (count)

STUDY SCHEMA



STUDY SUMMARY

Title	An exploratory trial to estimate the proportion of patients with tumor cell contaminated, flow positive leukapheresis products collected with and without bortezomib as in-vivo purging prior to autolgous stem cell harvest for multiple myeloma.
Short Title	2015-IIT-BMT-MM-AutoSCT
Phase	Phase 2
Methodology	open label; randomized
Study Duration	2 years
Study Center(s)	Single-center
Objectives	Estimate proportion of subjects with plasma cell contamination of harvested stem cell product in standard and treatment arms.
Number of Subjects	100 patients
Diagnosis and Main Inclusion Criteria	Multiple myeloma patients undergoing autologous stem cell transplantation

Study Product(s), Dose, Route, Regimen	**Group A**: Standard of Care Stem cell collection without in-vivo purging with Bortezomib. G-CSF and Mozobil used if needed. See Appendices C and D **Group B**: Bortezomib ; SQ; Days -11 and -8; 1.3 mg/m^2 followed by G-CSF Days -4 through -1, prior to stem cell harvest (Day 0) G-CSF and Mozobil used if needed. See Appendices C and D o Stem cell collection can occur up to 12 days after the last injection of bortezomib, if needed, for participant safety and convenience. Window: Day 0 (+4 days)
Duration of Administration	2 doses
Reference Therapy	Standard of Care Stem cell collection without in-vivo purging with Bortezomib. G-CSF and Mozobil used if needed. See Appendices C and D
Interim Monitoring	Internal DSMC for IITs at KU
Statistical Methodology	Descriptive, numerical, only; Estimate the proportion of products Positive for contamination under the standard of care stem cell collection and under in-vivo purging with Bortezomib stem cell collection. Confidence intervals will be provided.

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

High dose chemotherapy with autologous stem cell transplant has resulted in improved overall survival and is currently considered an effective first line therapy applicable to the majority of patients with multiple myeloma. However disease relapse is inevitable and remains the primary cause of mortality in this cohort.

1.1.1 Myeloma Cell Contamination:

Myeloma cells contamination of the leukapheresis product has been demonstrated in previous studies, but, the contamination rate or percentage of patients with contaminated graft undergoing autologous stem cell transplantation is not well known.

Mariette et al, 1994, used the immunoglobulin heavy chain gene radioactive fingerprinting polymerase chain reaction (PCR) method to detect clonal cells in the graft from 10 patients. The sensitivity of the technique allowed the detection of one clonal cell among 10(4) normal blood mononuclear cells. A clonal band was detected in 4 of 11 leukapheresis samples (36%).

In another paper by Miklos et al. Blood 2014, myeloma cell contamination was checked by PCR not by flow, and was present at a median level of 62.7 myeloma molecules/million PBMCs (range 0-11,951) after Cycle of chemo and 43.6 myeloma molecules/million PBMCs (range 0-4,489) after Cycle 2 of chemotherapy. 2 out of 38 observations were free from tumor cell contamination (personal communication).

The impact of contaminated leukapheresis product is disputed, however evidence from prospective study by Gertz et al (Gertz et al., 1997) suggest that myeloma graft contamination leads to poor disease free survival. This is further supported by a recent clinical study, which showed low graft contamination ($< 4.5 \times 10^5$ plasma cell/kg of patient's body weight) did result in improved PFS (Vogel, Kopp, Kanz, & Einsele, 2005). In that series the number of contaminating plasma cells in the harvested product was 0.08% (median) with a range of 0-2.26%. Authors did not state how many patients did not have tumor contamination (0% plasma cells in the harvested graft).

1.1.2 Methods of Measurement of Minimal Residual Disease:

In the International Myeloma Workshop held in Kyoto, Japan (2013), two methods to measure MRD were given emphasis, viz. flow cytometry and real time PCR for gene sequencing.

Multiparametric flow cytometry (MFC) is a novel way of checking for minimal residual disease.

As per the recently published ASBMT myeloma guidelines (Shah, Callander, Ganguly et al, 2015), MRD testing after auto-HCT in MM can reveal patients at risk for poorer outcomes and should be considered for disease evaluation (Grade B). If MRD testing is attempted, multiparametric flow cytometry following European Myeloma Network consensus guidelines should be the method of choice.

1.1.3 Level of Detection:

Rawstron AC et al published the consensus guidelines on multiparametric flow cytometry in multiple myeloma and related disorders which established the presently accepted methods for measuring minimal residual disease in myeloma samples (Rawstron et al. 2008). In their

guidelines paper they recommended, that it is possible to detect neoplastic plasma cells by flow cytometry above the clinically relevant threshold of 0.01%. It is recommended that at least 100 neoplastic plasma cell events should be acquired for accurate enumeration. Hence, according to the consensus 1) at least 0.01% clonal plasma cells and 2) at least 100 cells per product would be considered as positive for minimal residual disease.

1.1.4 Purging:

Unfortunately multicenter studies with ex-vivo purging using CD 34 positive cell selection of the harvest product did not improve progression free survival (Stewart et al., 2001).

In vivo purging has been tried using various chemo-immunotherapeutic agents in diseases like lymphoma. PCR or minimal residual disease (MRD) negative products have been associated with improved progression free survival. In vivo purging has not been done in patients with multiple myeloma. In patients with lymphoma, in vivo purging and PCR negative products have been shown to improve disease-free survival (DFS).

For the purpose of the transplant, hematopoietic stem cells are mobilized from the bone marrow niche by using either chemotherapy with G-CSF or G-CSF alone. Bortezomib is approved for treatment in all stages of myeloma. In a single arm study at our institution we had shown successful collection and mobilization of stem cells using Bortezomib/G-CSF strategy.

In a recent study (Oakervee et al, 2007), use of bortezomib as induction therapy prior to stem cell transplantation did not adversely impact the mobilization and actually resulted in robust stem cell collection in all the enrolled patients. Bortezomib thus does not have any stem cell toxic effects. This study aims to determine the effectiveness of a combination of bortezomib and G-CSF in a tumor free stem cell mobilization.

Ex vivo purging of stem cell products by bortezomib with or without rituximab has successfully decreased the tumor contamination. 24-hour incubation with bortezomib at 80 nmol/L induced 80% to 90% growth inhibition in the myeloma cell lines, however, it also markedly inhibited (80%) the growth of normal $CD34^+$ cells. In contrast, a lower dose of bortezomib (20 nmol/L) for 16 hours was sufficient to induce 70% to 90% growth inhibition in myeloma cell lines, with only 20% growth inhibition in normal $CD34^+$ cells (Yang H et al. 2011).

In an attempt to decrease tumor contamination in the stem cell product by in vivo purging, bortezomib was chosen as the drug of choice, as we already have experience in using bortezomib in the similar setting for stem cell mobilization (Abhyankar et al. 2013)

We have already presented our experience of in vivo purging in lymphoma (Singh et al. 2015), and planning to extend the similar concept in plasma cell disorders.

1.2 Study Agent(s) Background and Associated Known Toxicities

VELCADE® (bortezomib) for Injection

1.2.1 Scientific Background

VELCADE® (bortezomib) for Injection is a small molecule proteasome inhibitor developed by Millennium Pharmaceuticals, Inc., (Millennium) as a novel agent to treat human malignancies. Bortezomib is currently approved by the United States Food and Drug Administration (FDA) for the treatment of patients with multiple myeloma (MM). It

is also indicated for the treatment of patients with mantle cell lymphoma (MCL) who have received at least 1 prior therapy. In the European Union (EU), bortezomib in combination with melphalan and prednisone is indicated for the treatment of patients with previously untreated MM who are not eligible for high-dose chemotherapy with bone marrow transplant. Bortezomib is indicated as monotherapy for the treatment of progressive MM in patients who have received at least 1 prior therapy and who have already undergone or are unsuitable for bone marrow transplantation.

By inhibiting a single molecular target, the proteasome, bortezomib affects multiple signaling pathways. The anti-neoplastic effect of bortezomib likely involves several distinct mechanisms, including inhibition of cell growth and survival pathways, induction of apoptosis, and inhibition of expression of genes that control cellular adhesion, migration and angiogenesis. Thus, the mechanisms by which bortezomib elicits its antitumor activity may vary among tumor types, and the extent to which each affected pathway is critical to the inhibition of tumor growth could also differ. Bortezomib has a novel pattern of cytotoxicity in National Cancer Institute (NCI) in vitro and in vivo assays (Adams et al., 1999). In addition, bortezomib has cytotoxic activity in a variety of xenograft tumor models, both as a single agent and in combination with chemotherapy and radiation (Steiner et al., 2001; Teicher et al., 1999; Cusack et al., 2001; LeBlanc et al., 2002; Pink et al., 2002). Notably, bortezomib induces apoptosis in cells that over express bcl-2, a genetic trait that confers unregulated growth and resistance to conventional chemotherapeutics (McConkey et al., 1999).

The mechanisms of action leading up to apoptosis have been more clearly defined and include initiation of the unfolded protein response and direct/indirect effects on various molecular targets including cell cycle control proteins p27 and p21, cyclins, signal transduction molecules, transcription factors c-jun and HIF-1, tumor suppressor protein p53, angiogenesis factors, and many others. Bortezomib is thought to be efficacious in multiple myeloma via its inhibition of nuclear factor κB (NF-κB) activation, its attenuation of interleukin-6 (IL-6)-mediated cell growth, a direct apoptotic effect, and possibly anti-angiogenic and other effects (Hideshima et al., 2001).

1.2.2 Nonclinical Pharmacology

Pharmacokinetic (PK) and pharmacodynamic studies were conducted in the rat and cynomolgus monkey. Upon intravenous (IV) bolus administration, bortezomib displays a rapid distribution phase ($t_{1/2\alpha}$ <10 minutes) followed by a longer elimination phase ($t_{1/2\beta}$ 5–15 hours). Bortezomib has a large volume of distribution (range 5–50 L/kg). The plasma PK profile is well described by a 2-compartment model.

The pharmacodynamic action of bortezomib is well established and can be measured through an ex vivo assay (20S proteasome activity) (Lightcap et al., 2000). This assay was used to determine the duration of drug effect in lieu of the PK data in the early preclinical toxicology studies as well as to set a guide for dose escalation in humans. Following dosing with bortezomib in the rat and cynomolgus monkey, proteasome inhibition in peripheral blood had a half-life less than 24 hours, with proteasome activity returning to pretreatment baseline within 24 hours in monkey and within 48 to 72 hours in rat after a single dose of bortezomib. Further, intermittent but high inhibition (>70%) of proteasome activity was better tolerated than sustained inhibition. Thus, a twice-weekly clinical dosing regimen was chosen in order to allow return of proteasome activity towards baseline between dose administrations.

1.2.3 Nonclinical Toxicity

Single-dose IV toxicity studies were conducted with bortezomib in the mouse, rat, dog, and monkey to establish the single-dose maximum tolerated dose (MTD). The MTDs were 0.25 mg/kg (1.5 mg/m^2) and 0.067 mg/kg (0.8 mg/m^2) in the 2 most sensitive species, rat and monkey, respectively.

Repeat-dose multi-cycle toxicity studies of 3 and 6 months in the rat and 9 months in the monkey, each with 8-week recovery periods, were conducted to characterize the chronic toxicity of bortezomib when administered by the clinical route and regimen of administration. The MTD in the 6-month rat study was 0.10 mg/kg (0.6 mg/m^2) and the key target organs were the gastrointestinal (GI) tract, hematopoietic and lymphoid systems. The MTD in the 9-month monkey study was 0.05 mg/kg (0.6 mg/m^2) and the key target organs were the GI tract, hematopoietic and lymphoid systems, peripheral nervous system, and kidney. Full or partial reversibility was observed for each of the toxicities described to date.

In general, the nature of the toxicity of bortezomib is similar across species, and target organs of toxicity in animals have been largely predictive of human toxicity. The toxicity of bortezomib in animals is characterized by a steep dose-response with mortality seen at dosages above the MTD. The cause of death at acutely lethal dosages is considered to be related to indirect cardiovascular (CV) effects of hypotension and vascular changes with secondary bradycardia and the cause of death in long-term studies has been attributed to GI or hematologic toxicity. The pharmacologic effects of bortezomib on the CV system have been extensively characterized and have demonstrated that indirect effects on CV function occur only at acutely lethal dosages and are abrogated by routine supportive care.

Additional detailed information regarding the nonclinical pharmacology and toxicology of BORTEZOMIB may be found in the Investigator's Brochure.

1.2.4 Clinical Pharmacokinetics and Pharmacodynamics

The clinical pharmacology characterization of bortezomib has been determined from phase 1 studies in subjects with solid tumors and hematological malignancies, and confirmed in phase 2 studies in subjects with multiple myeloma.

Bortezomib demonstrates multi-compartmental pharmacokinetics. Following intravenous administration of 1.0 mg/m^2 and 1.3 mg/m^2 dose, the mean first-dose maximum observed plasma concentrations of bortezomib were 57 and 112 ng/mL, respectively in 11 patients with multiple myeloma and creatinine clearance values > 50 mL/min participating in a pharmacokinetics study. In subsequent doses, mean maximum observed plasma concentrations ranged from 67 to 106 ng/mL for the 1.0 mg/m^2 dose and 89 to 120 ng/mL for the 1.3 mg/m^2 dose. The mean elimination half-life of bortezomib upon multiple dosing ranged from 40 to 193 hours. Bortezomib is eliminated more rapidly following the first dose. Mean Total Body Clearances were 102 and 112 L/h following the first dose for doses of 1.0 mg/m^2 and 1.3 mg/m^2, respectively, and ranged from 15 to 32 L/h following subsequent doses for doses of 1.0 and 1.3 mg/m^2, respectively. Clinical experience has shown that the change in clearance does not result in overt toxicity from accumulation in this multi-dose regimen in humans.

In subjects with advanced malignancies, the maximum pharmacodynamic effect (inhibition of 20S activity) occurred within 1-hour post dose. At the therapeutic dose of 1.3 mg/m^2 in subjects with multiple myeloma, the mean proteasome inhibition at 1-hour post dose was approximately 61%.

The time course of proteasome inhibition in subjects is characterized by maximum inhibition observed within the first hour after administration, followed by partial recovery of proteasome activity over the next 6 to 24 hours to within 50% of the pretreatment activity. On the Day 1, 4, 8, and 11 schedule, variable (10%–30%) levels of proteasome inhibition have been observed at next scheduled dosing. In theory, this advantage allows cells to recover proteasome activity for normal cellular housekeeping functions between doses.

The relationship between bortezomib plasma concentrations and proteasome inhibition can be described by a maximum effect (E_{max}) model. The E_{max} curve is initially very steep, with small changes in plasma bortezomib concentration over the range of 0.5 to 2.0 ng/mL relating to large increases in the percent inhibition (0–60%). After that, a plateau occurs where marginal increases of proteasome inhibition are observed in spite of large changes in plasma bortezomib concentrations.

1.2.5 Clinical Experience

It is estimated that as of June 2011, more than 300,000 patients have been treated with bortezomib, including patients treated through Millennium-sponsored clinical trials, Investigator-Initiated Studies, the US NCI Cancer Therapy Evaluation Program (CTEP), and with commercially available drug. Bortezomib has been commercially available since 13 May 2003.

The overall goal of the Millennium phase 1 program was to determine the MTD and dose-limiting toxicity (DLT) of bortezomib in a number of therapeutic settings involving subjects with various advanced malignancies. In a Phase I trial in patients with refractory hematologic malignancies, the MTD for a twice weekly for 4 weeks of a 42 day cycle was 1.04 mg/m^2/dose, with DLTs of thrombocytopenia, hyponatremia, hypokalemia, fatigue, and malaise (Orlowski et al., 2002). The toxicity was greatest during the third and fourth weeks of therapy. In the 3-week schedule of bortezomib monotherapy (4 doses, given on Days 1, 4, 8, and 11 of a 21-day treatment cycle), the DLT occurred at 1.56 mg/m^2/dose (3 subjects with Grade 3 diarrhea and 1 with peripheral sensory neuropathy). Therefore, the MTD at this schedule was 1.3 mg/m^2/dose. In a 35-day treatment cycle with 4 weekly doses of bortezomib monotherapy, the MTD was 1.6 mg/m^2/dose and DLT included hypotension, tachycardia, diarrhea, and syncope.

In phase 1 clinical studies, anti-tumor activity was reported in subjects with NHL, multiple myeloma, Waldenström's Macroglobulinemia, squamous cell carcinoma of the nasopharynx, bronchoalveolar carcinoma of the lung, renal cell carcinoma, and prostate cancer.

The safety and efficacy of bortezomib in subjects with multiple myeloma were investigated in two phase 2 clinical studies, studies M34100-024 (subjects with first relapse) (Jagannath et al, 2004) and M34100-025 (subjects with second or greater relapse and refractory to their last prior therapy) (Richardson et al, 2003). In M34100-025, 202 heavily pre-treated subjects with refractory multiple myeloma after at least 2 previous treatments received bortezomib, 1.3 mg/m^2 on Days 1, 4, 8, and 11 of a 21-day treatment cycle. The European Group for Blood and Marrow Transplant (EBMT) response criteria, as described by Blade (Blade et al., 1998) were utilized to determine disease response. CRs were observed in 4% of subjects, with an additional 6% of patients meeting all criteria for CR but having a positive immunofixation test. PR or better was observed in 27% of subjects, and the overall response rate (CR, PR and

minor response [MR] combined) was 35%. Seventy percent of subjects experienced stable disease or better.

The phase 3 study (M34101-039) (Richardson et al, 2005), also referred to as the APEX study, was designed to determine whether bortezomib provided benefit (time to progression [TTP], response rate, and survival) to patients with relapsed or refractory MM relative to treatment with high-dose dexamethasone. The study was also designed to determine the safety and tolerability of bortezomib relative to high-dose dexamethasone, and whether treatment with bortezomib was associated with superior clinical benefit and quality of life relative to high-dose dexamethasone. A total of 669 patients were enrolled and 663 patients received study drug (bortezomib: 331; dexamethasone: 332). Patients randomized to bortezomib received 1.3 mg/m^2 I.V. push twice weekly on days 1, 4, 8, and 11 of a 3-week cycle for up to eight treatment cycles as induction therapy, followed by 1.3 mg/m^2 bortezomib weekly on days 1, 8, 15, and 22 of a 5-week cycle for three cycles as maintenance therapy. Patients randomized to dexamethasone received oral dexamethasone 40 mg once daily on days 1 to 4, 9 to 12, and 17 to 20 of a 5-week cycle for up to four treatment cycles as induction therapy, followed by dexamethasone 40 mg once daily on days 1 to 4 followed of a 4-week cycle for five cycles as maintenance therapy. The European Group for Blood and Marrow Transplant (EBMT) response criteria, as described by Blade (Blade et al., 1998) were utilized to determine disease response. There was a 78% increase in TTP for the bortezomib arm. Median TTP was 6.2 months for the bortezomib arm and 3.5 months for the dexamethasone arm (*P*<.0001). CR (complete response) + PR (partial response) was 38% with bortezomib vs. 18% with dexamethasone (*P*<.0001). CR was 6% with bortezomib vs. <1% with dexamethasone (*P*<.0001). The CR + nCR rate was 13% with bortezomib vs. 2% with dexamethasone. In patients who had received only one prior line of treatment (bortezomib: 132; dexamethasone: 119), CR + PR was 45% with bortezomib vs. 26% with dexamethasone (*P*=.0035). With a median 8.3 months of follow-up, overall survival was significantly longer (*P*=.0013) for patients on the bortezomib arm vs. patients on the dexamethasone arm. The probability of survival at one year was 80% for the bortezomib arm vs. 66% for the dexamethasone arm, which represented a 41% decreased relative risk of death in the first year with bortezomib (*P*=.0005). In patients who had received only one prior line of treatment, the probability of survival at one year was 89% for the bortezomib arm vs. 72% for the dexamethasone arm, which represented a 61% decreased relative risk of death in the first year with bortezomib (*P*=.0098). Updated response rates and survival data were reported for M34101-039 (Richardson ASH, 2005). The updated CR (complete response) + PR (partial response) rate was 43% with bortezomib. The CR + nCR rate was 16% with bortezomib. With a median 22 months of follow-up, overall survival was significantly longer for patients on the BORTEZOMIB arm vs. patients on the dexamethasone arm. The median overall survival was 29.8 months (95% CI: 23.2, not estimable) for the bortezomib arm vs 23.7 months (95% CI: 18.7, 29.1) for the dexamethasone arm (hazard ratio = 0.77, *P*= 0.0272). The probability of survival at one year was 80% for the bortezomib arm vs. vs 67% for the dexamethasone arm (*P*=0.0002).

1.2.6 Potential Risks of Bortezomib

To date, more than 300,000 patients have been treated with bortezomib in both clinical trials investigating its use in hematological malignancies and solid tumors, and in patients who were treated with commercially available bortezomib.

Prescribing physicians and health care practitioners are referred to their locally approved product label for bortezomib regarding indications and usage, contraindications, warnings, and precautions.

The known anticipated risks of bortezomib therapy are presented in Table 1 later in this protocol (see section with title *DRUG INFORMATION*). These risks are grouped according to the combined frequency observed in an integrated analysis of AEs in sponsored clinical studies of single-agent bortezomib dosed at 1.3 mg/m^2 twice weekly on a 21-day schedule, in patients with multiple myeloma and mantle cell lymphoma.

1.3 Other Agents

G-CSF (Filgrastim) or Filgrastim G-CSF Biosimilar (Zarxio^{TM})

Filgrastim is human granulocyte colony-stimulating factor (G-CSF) produced by recombinant DNA technology. G-CSF stimulates the production, maturation, and activation of neutrophils and activates neutrophils to increase their migration and cytotoxicity. Toxicities that are likely to occur include myalgias and medullary bone pain. The bone pain can be generally controlled with non-narcotic analgesia. Less likely side effects include fluid retention, pericardial effusion, local inflammation at the injection site and transient laboratory abnormalities including mild elevations in uric acid, lactic dehydrogenase (LDH), alkaline phosphatase, and leukocytosis. Rare but serious side effects include reported cases of spleen swelling resulting in splenic rupture, adult respiratory distress syndrome (ARDS), and allergic reactions.

1.4 Rationale

The study plans to estimate the contamination rate of harvested stem cell products collected per standard of care approach and after using two doses of bortezomib as a part of in vivo purging method used in a prior published study (Abhyankar et al. 2013).

Multiparametric flow cytometry will be used to measure minimal residual disease (MRD) in the harvested product of patients with multiple myeloma.

Tumor contamination of the stem cell product is common in patients undergoing stem cell transplantation. Use of multi parametric flow cytometry in measuring low percentage of plasma cells contaminating harvested stem cell product has never been used.

MFC is the standard of care in measuring MRD. Contamination rate of >0.01% with >100 plasma cells per flow will be considered as positive for study purpose per guidelines (Rawstron AC et al, 2008).

Bortezomib has been used as anti-myeloma drug during ex vivo purging. Role of bortezomib used prior to stem cell collection in lowering tumor contamination of the stem cell product is an intriguing concept that has not been tested.

The rationale for bortezomib prior to the G-CSF is as follows:

Bortezomib causes thrombocytopenia; similar to the manner in which cyclophosphamide is given followed by G-CSF for stem cell mobilization. Usually a cycle of bortezomib for myeloma therapy consists of 4 doses, but this may result in more significant drop in the platelet count. Low platelet counts may preclude adequate stem cell collection. The mobilization with bortezomib and G-CSF will be approximately 3 -4 weeks after completion of the initial therapy for the primary disease.

Patients meeting eligibility criteria and who give informed consent will be randomized to receive mobilization using bortezomib at 1.3 mg/m^2 on day -11 and day -8 followed by G-CSF at 10 mcg/kg from day -4 to day -1 vs treatment with G-CSF 10 mcg/kg alone on days -4 through -1. For both groups stem cell collection will be done on day 0 (window: + 4 days) per standard of care regimen and protocol with processing of 4 - 6 blood volumes. Stem cell enumeration by flow cytometry will be done prior to the collection. The number of CD 34 positive cells (stem cell phenotype) / kg obtained each day of collection will be noted. G-CSF will be continued daily until adequate numbers of stem cells are obtained for the performance of a single autologous transplant (> 2.5 million CD 34 cells/kg of recipient weight). Subsequently the data for engraftment of neutrophils and platelets will be monitored as is routine for all patients undergoing autologous transplant.

Multiparametric Flow Cytometry will be used to determine presence (>0.01%) or absence (<0.01%) of malignant plasma cells in the harvested products. A positive flow cytometry also will have to have >100 plasma cells per analysis.

Recently the International Myeloma Working Group (IMWG) panel approved definitions of immunophenotypic CR and molecular CR to be incorporated into the IMWG criteria. In addition, several studies have prospectively employed multiparameter flow cytometry (MFC) and shown improved outcome in MRD- negative patients after auto-HCT. The European Myeloma Network (EMN) and ASBMT respectively developed a consensus for plasma cell enumeration, sample preparation, gating and immunophenotype for clonality assessment and guidelines for MRD assessment in patients with myeloma undergoing autologous stem cell transplantation (Rawstron AC et al, 2008 and Shah, Callander and Ganguly et al, 2015).

1.5 Correlative Studies

None

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 To estimate the proportion of subjects with plasma cell contamination (defined as >0.01% and at least 100 cellular events) (Rawstron AC et al, 2008 and Shah, Callander and Ganguly et al, 2015) of harvested stem cell product by multi parametric flow cytometry from patients with myeloma undergoing autologous stem cell collection by standard of care mobilization using GCSF with or without Mozobil.

2.1.2 To estimate the proportion of subjects with plasma cell contamination (defined as >0.01% and at least 100 cellular events) (Rawstron AC et al, 2008 and Shah, Callander and Ganguly et al, 2015) of harvested stem cell product by multi parametric flow cytometry from patients with myeloma undergoing autologous stem cell collection after two doses of bortezomib as in vivo purging plus standard of care using GCSF with or without Mozobil

2.2 Secondary Objectives

2.2.1 To estimate the proportion of subjects who have a successful collection of stem cells (> 2 million CD34 cells/Kg of body weight) for autologous transplant in both treatment groups.

2.2.2 To estimate the percentage of CD 34 positive cells in peripheral blood on the days of collection

3.0 SUBJECT ELIGIBILITY

3.1 Inclusion Criteria

Subjects must meet all of the inclusion criteria to participate in this study.

3.1.1 Ability to understand, and the willingness to sign a written Informed Consent Form

3.1.2 Diagnosis of multiple myeloma undergoing planned autologous stem cell transplantation

3.1.3 Age ≥ 18 years

3.1.4 KPS 70 or above, ECOG 0, 1 or 2 (**APPENDIX A)**

3.1.5 Adequate organ and marrow function as defined below:

- leukocytes	≥ 3,000/mcL
- absolute neutrophil count	≥ 1,500/mcL
- platelets	≥ 100,000/mcL
- total bilirubin	within normal institutional limits **NOTE**: For this study, subjects with bilirubin levels > 1.5 ULN are excluded from this enrollment in this study.
- AST (SGOT)	≤ 2.5 X institutional upper limit of normal
- ALT (SPGT)	≤ 2.5 X institutional upper limit of normal

3.1.6 Women of child-bearing potential and men with partners of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 30 days following completion of therapy. Should a woman or partner become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician and the investigator immediately.

3.1.6.1 A woman of child-bearing potential is any female (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:

- Has not undergone a hysterectomy or bilateral oophorectomy; **OR**
- Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months)

3.2 Exclusion Criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation.

3.2.1 Current or anticipated use of other investigational agents. **NOTE** the following clarification for this study:

Prohibited Concurrent Therapy:
Participation in clinical trials with other investigational agents, not included in this trial, within 14 days of the start of this trial until 2 weeks after subject has received the last dose of bortezomib for mobilization. Hypersensitivity to bortezomib, boron or mannitol or G-CSF.

3.2.2 Subject has received > 6 months of lenalidomide (Revlimid®) therapy prior to stem cell collection.

3.2.3 Subject has known brain metastases. Presence of brain metastases should be excluded from this clinical trial because of poor prognosis and because patients often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.4 Grade 3 or higher peripheral neuropathy

3.2.5 Bilirubin levels > 1.5 ULNl

3.2.6 Uncontrolled inter-current illness including, but not limited to

- ongoing or active infection
- symptomatic congestive heart failure
- unstable angina pectoris
- cardiac arrhythmia
- psychiatric illness/social situations that would limit compliance with study requirements

3.2.7 Pregnant or nursing: There is a potential for congenital abnormalities and for this regimen to harm nursing infants.

4.0 TREATMENT PLAN

4.1 Treatment Dosage and Administration

Treatment will be administered only to eligible subjects under the supervision of the investigator or identified sub-investigator(s). Subjects may be treated on an out-patient basis, if possible.

Study treatment will be prepared under the supervision of a pharmacist, or appropriately qualified and trained personnel. The amount (in mg) of drug to be administered will be determined based on body surface area. Body surface area is to be calculated based on body weight using a standard nomogram (See **APPENDIX B).**

Treatment Regimen Group A
Standard of Care Stem cell collection without in-vivo purging with Bortezomib. G-CSF and Mozobil used if needed. Physician discretion will be used to advise on the plan which may include the continuation of Mozobil. The provider has the discretion of collection outside the algorithm included in Appendix C (See **Appendices C and D**).

Treatment Regimen Group B

Bortezomib will be given SQ at 1.3 mg/m^2 on day -11 and day -8 followed by GCSF given SQ on day -4 thru day -1 and continued until the collection is completed. Mozobil used if needed. Physician discretion will be used to advise on the plan which may include the continuation of Mozobil. The provider has the discretion of collection outside the algorithm included in Appendix C (See **Appendices C and D**).

NOTES:

1. There must be at least 72 hours between each dose of bortezomib.
2. Multiparametric Flow Cytometry to be performed on samples for

   BOTH groups **A** AND **B**
3. Stem cell collection can occur up to 12 days after the last injection of bortezomib, if needed, for participant safety and convenience. Window: Day 0 (+4 days).

4.2 Toxicities and Dosing Delays/Dose Modifications

Before each drug dose, the subject will be evaluated for possible toxicities that may have occurred after the previous dose(s). Toxicities are to be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03.

Subjects who experience bortezomib-related neuropathic pain and/or peripheral sensory neuropathy after the first injection are to be managed as standard of care. No dose reduction will be necessary for Grades 1 and 2 AEs. If patient complains of Grade 3 or 4 neuropathic pain that is treatment-emergent after first dose, patient will be withdrawn from the study.

Time to engraftment will be monitored for all subjects. If a subject has delayed or non-engraftment of neutrophils and / or platelets the etiology of the delay will be determined and if believed to be possibly related, then the study will be halted.

Subjects with mild hepatic impairment (bilirubin $\leq 1.5 \times$ ULN) do not require a starting dose adjustment. Please note that subjects with bilirubin levels > 1.5 ULN are excluded from enrollment in this protocol.

4.3 Concomitant Medications/Treatments

Investigators should consider using antiviral prophylaxis in subjects being treated with bortezomib.

Prohibited Concurrent Therapy

Participation in clinical trials with other investigational agents, not included in this trial, within 14 days of the start of this trial until 2 weeks after subject has received the last dose of bortezomib for mobilization.

Precautions and Restrictions

It is not known what effects bortezomib has on human pregnancy or development of the embryo or fetus. Therefore, female subjects participating in this study should avoid becoming pregnant, and male subjects should avoid impregnating a female partner. Nonsterilized female subjects of reproductive age and male subjects should use effective methods of contraception through defined periods during and after study treatment as specified below.

Female subjects must meet 1 of the following:

- Postmenopausal for at least 1 year before the screening visit, or
- Surgically sterile, or
- If they are of childbearing potential, agree to practice 2 effective methods of contraception from the time of signing the informed consent form through 30 days after the last dose of bortezomib, or agree to completely abstain from heterosexual intercourse.

It is strongly recommended that at least 1 of these 2 methods be highly effective (see examples below).

Highly effective methods	Other effective methods (barrier methods)
Intra-uterine devices (IUD)	Latex condom
Hormonal contraceptives (birth control pills/oral contraceptives, injectable contraceptives, contraceptive patches, or contraceptive implants)	Diaphragm with spermicide Cervical cap Sponge

If one of the highly effective methods cannot be used, using 2 effective methods at the same time is recommended.

Male subjects, even if surgically sterilized (i.e., status post vasectomy) must agree to 1 of the following:

- Practice effective barrier contraception during the entire study treatment period and through a minimum of 30 days after the last dose of study drug, or completely abstain from heterosexual intercourse.

4.4 Duration of Therapy

Subjects will be on study from the first study treatment and will complete the study when an adequate number of stem cells are harvested. Subjects will be followed as per standard transplant protocol until engraftment of neutrophils (first of 3 days when absolute neutrophil count is equal or greater than 500/uL) and platelets (day to platelet counts of more than 20,000/uL). Usually this is accomplished by 30 days after transplantation. Subjects will be followed for adverse events until 30 days post-collection.

Participation in this clinical trial will not preclude enrollment in any other cooperative or consortium trial if subjects are in the standard of care arm.

4.5 Removal of Patients from Protocol Therapy

Subjects will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw, and in some cases is required to withdraw subjects from the study for any of the following reasons:

- Intercurrent illness
- Occurrence of an unacceptable adverse event
- Subject request
- Protocol violations

- Non-compliance
- Administrative reasons
- Failure to return for follow-up
- General or specific changes in the subjects condition unacceptable for further treatment in the judgment of the investigator
- Failure of mobilization following investigational treatment (see NOTE below for definition of failure of mobilization

NOTE: Failure of mobilization is defined as a patient body failure to collect CD34+ cells at least 2 million/Kg after standard procedure of using G-CSF with or without Plerixafor.

- At the time of withdrawal, the primary reason for a subject's withdrawal from the study is to be recorded in the source documents. The subject will be followed as per standard transplant protocol.

5.0 STUDY PROCEDURES

5.1 Screening/Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining Informed Consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before Informed Consent was obtained.

All screening procedures must be performed within 30 days prior to registration unless otherwise stated. The screening procedures include:

5.1.1 Informed Consent

5.1.2 Medical history

Complete medical, surgical and oncology history as well as history of infections are obtained at screening. Any changes from Screening (e.g. worsening severity or abnormal findings) are considered to be adverse events (AEs).

5.1.3 Demographics

Demographic profile will include date of birth, gender, race, ethnicity and zip code.

5.1.4 Review subject eligibility criteria

Review of eligibility criteria to ensure subject qualification for study entry.

5.1.5 Review previous and concomitant medications

All prior medication taken by the subject within 30 days before starting the study is to be recorded. At minimum, the start year of the medication should also be recorded. Concomitant medications taken by the subject during the study are to be recorded up until 30 days after the participant's last protocol therapy (collection). If a reportable adverse event (see section with title *Adverse Events*) occurs within 30 days after the participant's last protocol therapy (collection), recording of concomitant medications should continue until resolution of the adverse event.

5.1.6 Physical exam including vital signs, height and weight

Vital signs (temperature, pulse, respirations, blood pressure), height, weight, and assessment of all major body systems

5.1.7 Performance status

Performance status evaluated prior to study entry. Specific criteria for assessing performance status can be found in **APPENDIX A**.

5.1.8 Adverse event assessment

Baseline assessment of subject status for determining adverse events. See section with title *Adverse Events* monitoring and reporting.

5.1.9 Hematology

Hematology to include hemoglobin (Hgb), platelets, total white blood cell count (WBC) and differential.

5.1.10 Serum chemistries

Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT, AST, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin.

5.1.11 Pregnancy test (for women of child bearing potential)

5.2 Procedures During Treatment

- Day -1: Hematology and CMP
- Day 0: Stem Cell Harvest / CD34 enumeration

NOTES:

1. Multiparametric Flow Cytometry (MFC) analysis to be performed Day 0 only
2. CD34 enumeration to be performed per standard of care (Every day of stem cell collection)
3. Stem cell collection can occur up to 12 days after the last injection of bortezomib, if needed, for participant safety and convenience. Window: Day 0 (+4 days)..

5.3 Follow-up Procedures

Subjects will be followed after stem cell harvest usually within a week to start the high dose therapy and autologous stem cell transplantation. Thereafter subjects are followed per institutional transplant protocols. Study ends 30 days after the participant's last protocol therapy (collection) for the purpose of AE reporting.

5.4 Schedule of Events

Bortezomib Along With G-CSF For Hematopoietic Stem Cell Mobilization In Subjects Undergoing Autologous Transplantation For Myeloma

Study Calendar	Screening/ Baseline	Day -11	Day -8	Day -4	Day -3	Day -2	Day -1 / EOT	Day 0 (+ 4 Days)
Informed Consent	X							
Medical History Demographics	X							
Performance Status	X							
Physical Exam	X							
Concomitant Medication Review	X							
CBC/Serum Chemistries	X						X	
Pregnancy Test	X							
Adverse Events	X	X	X	X	X	X	X	X
Bortezomib		X	X					
GCSF				X	X	X	X	X
Stem Cell Collection / CD34 Enumeration[1]								X[1, 2]

Footnote:

1. Multiparametric Flow Cytometry (MFC) analysis to be performed for both study groups on Day 0 only. CD34 enumeration to be performed every day of stem cell collection.
2. Stem cell collection can occur up to 12 days after the last injection of bortezomib, if needed, for participant safety and convenience. Window: Day 0 (+4 days).

5.5 Removal of Subjects from Study Treatment and Study

Subjects can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons.

The reason(s) for discontinuation will be documented and may include:

5.5.1 Subject voluntarily withdraws from treatment (follow-up permitted);

5.5.2 Subject withdraws consent (termination of treatment and follow-up);

5.5.3 Subject is unable to comply with protocol requirements;

5.5.4 Subject demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator);

5.5.5 Subject experiences toxicity that makes continuation in the protocol unsafe;

5.5.6 Treating physician judges continuation on the study would not be in the subject's best interest;

5.5.7 Subject becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event; see section with title *Adverse Events*);

5.5.8 Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;

6.0 ADVERSE EVENTS

Text below in italics is verbatim from "Guidance for Industry and Investigators. Safety Reporting Requirements for INDs and BA/BE Studies", issued December 2012 by U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research. The guidance may be retrieved from:

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf?source=govdelivery

6.1 Definitions

6.1.1 **Adverse Event** [21 CFR 312.32(a)]

An adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

This study will use the descriptions and grading scales from Common Terminology Criteria for Adverse Events version 4.03 (CTCAE v4.03) for hematologic and non-hematologic toxicities. Detailed information may be found on the Cancer Therapy Evaluation Program (CTEP) website:

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

Information for adverse events, whether reported by the subject, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported in the CRF as described in the following sections.

Adverse events experienced by subjects will be collected and reported from time of signing of informed consent, throughout the study, and within 30 days of the last dose of protocol therapy (collection). Subjects who experience an ongoing adverse event related to a study procedure and/or study medication beyond 30 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the principal investigator. Study subjects should also be instructed to report any new serious post-study event(s) that might reasonably be related to participation in this study.

NOTE: For this study, *planned* hospital admission will NOT be considered a Serious Adverse Event.

Medical conditions/diseases, or cancer related symptoms present before starting study treatment are considered adverse events only if they worsen after initial screening. Adverse clinical events occurring before starting study drug but after signing the Informed Consent form are to be recorded in the subject's medical record. All significant cancer-related symptoms that have occurred in the last 30 days prior to start of study drug must also be recorded in the medical record.

Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, or require therapy. In this case they will be recorded in the medical record, along with the associated signs, symptoms or diagnosis.

As far as possible, each adverse event will also be described by:

- its duration (start and end dates),
- grading of severity,
- its relationship to the study drug,
- the action(s) taken,
- outcome.

6.1.2 Suspected Adverse Reaction [21 CFR 312.32(a)]

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Suspected adverse reactions are the subset of all adverse events for which there is a reasonable possibility that the drug caused the event. Inherent in this definition, and in the requirement to report suspected adverse reactions, is the need for the sponsor to

evaluate the available evidence and make a judgment about the likelihood that the drug actually caused the adverse event.

Factors to be considered in assessing the relationship of the adverse event to study drug include:

- The temporal sequence from study drug administration: The event should occur after the study drug is given. The length of time from study drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on discontinuation (de-challenge), recurrence on reintroduction (re-challenge): Subject's response after drug discontinuation (de-challenge) or subject's response after study drug re-introduction (re-challenge) should be considered in the view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases: Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication or treatment: The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them may be suspected to cause the event in question.
- The pharmacology and pharmacokinetics of the study drug: The pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the test drug(s), coupled with the individual subject's pharmacodynamics should be considered.

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

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

6.1.3 Unexpected [21 CFR 312.32(a)]

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application… "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the Particular drug under investigation.

This definition relies entirely on a listing of the adverse events or suspected adverse reactions in the investigator brochure…as the basis for determining whether newly acquired information generated from clinical trials or reported from other sources is unexpected. This means that events not listed for the Particular drug under investigation in the investigator brochure are considered "unexpected" and those listed are considered "expected." When new adverse event information is received, it is the sponsor's responsibility to determine whether the event is "unexpected" for safety reporting purposes.

6.1.4 Serious [21 CFR 312.32(a)]

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

6.1.5 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

6.2 Reporting Requirements for Adverse Events

6.2.1 Submitting Serious Adverse Events Reports to IRB

For serious adverse events, the clinical research site will follow local IRB policies and procedures.

NOTE: For this study, *planned* hospital admission will NOT be considered a Serious Adverse Event.

6.2.2 Study Investigator Notification of Adverse Events

All **expected** and **unexpected** serious adverse events occurring after the subject has signed the Informed Consent and has started protocol treatment must be reported to the study principal investigator within 24 hours of becoming aware of the event:

PI Name: Siddhartha Ganguly, MD
Office Phone: 913-588-6030
Fax: 913-588-3996

NOTE: For this study, *planned* hospital admission will NOT be considered a Serious Adverse Event.

6.2.3 DSMC Notification of SAEs

All **expected** and **unexpected** serious adverse events occurring after the subject has signed the Informed Consent and has started protocol treatment must be reported by phone or email to the KUCC DSMC within 24 hours of becoming aware of the event to:

Phone: 913-588-4791
Fax: 913-945-4394
Email: mpark@kumc.edu

NOTE: For this study, *planned* hospital admission will NOT be considered a Serious Adverse Event.

A follow-up written report in the form of a MEDWATCH Form FDA 3500A is required within 5 days.

6.2.4 Recording Adverse Events and Documentation in VELOS

All **expected** and **unexpected** adverse events and serious adverse events occurring after the patient has signed the Informed Consent and has started protocol treatment must be fully recorded in the subject's case record form.

NOTE: For this study, *planned* hospital admission will NOT be considered a Serious Adverse Event.

All AEs and SAEs regardless of causality must be entered in the KU implementation of eVelos, also called the Comprehensive Research Information System (CRIS). All SAEs regardless of causality must be entered into eVelos within 24 hours. Unexpected and expected adverse events must be entered within 5 days and include: new unexpected adverse events; worsening baseline conditions; clinically significant laboratory findings; disease-related signs and symptoms that were not present at baseline, and any event of findings that the Investigator feels is clinically significant.

Documentation must be supported by an entry in the subject's file. A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product, action taken and outcome.

6.2.5 Reporting of Unexpected, Related SAEs for Concomitant Medications

For concomitant medications, all unexpected, related serious adverse experiences will be forwarded to the product manufacturer by the investigator using the Voluntary MEDWATCH Form FDA 3500.

6.2.6 Summary of Expedited Serious Adverse Event Reporting

	Relationship to Study Drug	KUCC DSMC	IRB	PI	Velos
Unexpected SAE	Related	24 hrs	Follow local IRB reporting requirements	24 hrs	24 hrs
Unexpected SAE	Not-related	24 hrs		24 hrs	24 hrs
Expected SAE	Related	24 hrs		24 hrs	24 hrs
Expected SAE	Not-related	24 hrs		24 hrs	24 hrs

7.0 DRUG INFORMATION

7.1 Agent Bortezomib

Please refer to Investigator's Brochure for more comprehensive information.

- Other names for the drug(s): Bortezomib
- Classification - type of agent: Proteasome inhibitor
- Mode of action: Anti Myeloma

Velcade® (bortezomib) for injection is a sterile lyophilized powder for reconstitution and is supplied in vials containing bortezomib and mannitol at a 1:10 ratio. For example, vials containing 3.5 mg of bortezomib contain 35 mg of mannitol.

Storage and stability: Bortezomib will be supplied in vials as open-label stock. Both the box label and vial label will fulfill all requirements specified by governing regulations Vials containing lyophilized bortezomib for injection should be stored according to the label requirements. For the United States, store at USP Controlled Room Temperature which is 25ºC (77ºF); for Europe, do not store above 30ºC (86ºF); excursions permitted from 15 to 30ºC (59 to 86ºF). To date, stability data indicate that the lyophilized drug product is stable for at least 18 months when stored under the recommended conditions. Stability studies are ongoing, and Millennium Pharmaceuticals, Inc. will notify the investigator should this information be revised during the conduct of the study.

Preparation: Bortezomib is cytotoxic. As with all cytotoxic drugs, caution is required when preparing and handling bortezomib solutions. Cytotoxic drugs should only be handled by staff specially trained in the safe handling of such preparations. The use of gloves and other appropriate protective clothing is recommended. In case of skin contact, wash the affected area immediately and thoroughly with soap and water for at least 15 minutes. If product contacts eye, immediately flush eye thoroughly with water for at least 15 minutes. Always contact a physician after any form of body contact. All materials that have been used for preparation should be disposed of according to standard practices. A log must be kept of all disposed materials.

Drug is available in sterile, single use vials containing 3.5 mg of bortezomib. Each vial of bortezomib for injection should be reconstituted under a laminar flow biological cabinet (hood) within eight hours before dosing with 1.4 mL of normal (0.9%) saline, Sodium Chloride Injection USP, so that the reconstituted solution contains bortezomib at a concentration of 2.5 mg/mL. Prior to reconstitution the vials should remain in the cartons to protect them from light. Dissolution is completed in approximately 10 seconds. The reconstituted solution is clear and colorless, with a final pH of 5 to 6. Reconstituted bortezomib should be administered promptly and in no case more than 8 hours after reconstitution.

- Route of administration for this study: Subcutaneous Injection (SQ)
- Incompatibilities: NA
- Availability: commercially available
- Side effects:
    - To date, more than 300,000 patients have been treated with bortezomib in both clinical trials investigating its use in hematological malignancies and solid tumors, and in patients who were treated with commercially available bortezomib.
    - Prescribing physicians and health care practitioners are referred to their locally approved product label for bortezomib regarding Indications and Usage, Contraindications, Warnings, and Precautions.

The known anticipated risks of bortezomib therapy are presented in Table 1 below. These risks are grouped according to the combined frequency observed in an integrated analysis of AEs in sponsored clinical studies of single-agent bortezomib dosed at 1.3 mg/m^2 twice weekly on a 21-day schedule, in patients with multiple myeloma and mantle cell lymphoma.

Table 1 Known Anticipated Risks of Bortezomib by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class **Observed Incidence**	**Preferred Term**
Blood and Lymphatic System Disorders	
Most common	Thrombocytopenia*, anemia*
Very common	Neutropenia*
Common	Lymphopenia, pancytopenia*, leukopenia*, febrile neutropenia
Cardiac Disorders	
Common	Tachycardia, atrial fibrillation, palpitations, cardiac failure congestive*
Uncommon	Cardiogenic shock*, atrial flutter, cardiac tamponade*±, bradycardia, atrioventricular block complete, arrhythmia, cardiac arrest*, cardiac failure, arrhythmia, pericardial effusion, pericarditis, pericardial disease±, cardiopulmonary failure±
Ear and Labyrinth Disorders	
Uncommon	Deafness, hearing impaired
Eye Disorders	
Common	Blurred vision, conjunctivitis, conjunctival hemorrhage
Gastrointestinal Disorders	
Most common	Constipation, diarrhea*, nausea, vomiting*
Very common	abdominal pain (excluding oral and throat)
Common	Dyspepsia, pharyngolaryngeal pain, gastroesophageal reflux, abdominal distension, gastritis, stomatitis, mouth ulceration, dysphagia, gastrointestinal hemorrhage*, lower gastrointestinal hemorrhage*± rectal hemorrhage

The known anticipated risks of bortezomib therapy are presented in Table 1 below. These risks are grouped according to the combined frequency observed in an integrated analysis of AEs in sponsored clinical studies of single-agent bortezomib dosed at 1.3 mg/m^2 twice weekly on a 21-day schedule, in patients with multiple myeloma and mantle cell lymphoma.

Table 1 Known Anticipated Risks of Bortezomib by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class **Observed Incidence**	**Preferred Term**
Gastrointestinal Disorders (cont.) Uncommon	Eructation, gastrointestinal pain, tongue ulceration, retching, upper gastrointestinal hemorrhage*, hematemesis*, oral mucosal petechiae, ileus paralytic*, ileus, odynophagia, enteritis, colitis, esophagitis, enterocolitis, diarrhea hemorrhagic, acute pancreatitis*, intestinal obstruction
General Disorders and Administration Site Conditions	
Most common	Fatigue, pyrexia
Very common	Chills, edema peripheral, asthenia
Common	Neuralgia, lethargy, malaise, chest pain, mucosal inflammation*
Uncommon	Injection site pain, injection site irritation, injection site phlebitis, general physical health deterioration*, catheter-related complication
Hepatobiliary Disorders	
Uncommon	Hyperbilirubinemia, hepatitis*±
Immune System Disorders	
Uncommon	Drug hypersensitivity, angioedema
Infections and Infestations	
Very common	Upper respiratory tract infection, nasopharyngitis, pneumonia*, Herpes zoster*
Common	Lower respiratory tract infection*, sinusitis, pharyngitis, oral candidiasis, urinary tract infection*, sepsis*, bacteremia*, cellulitis*, Herpes simplex, bronchitis, gastroenteritis*, infection

The known anticipated risks of bortezomib therapy are presented in Table 1 below. These risks are grouped according to the combined frequency observed in an integrated analysis of AEs in sponsored clinical studies of single-agent bortezomib dosed at 1.3 mg/m^2 twice weekly on a 21-day schedule, in patients with multiple myeloma and mantle cell lymphoma.

Table 1 Known Anticipated Risks of Bortezomib by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class **Observed Incidence**	**Preferred Term**
Infections and Infestations (Cont.)	
Uncommon	Septic shock*, catheter-related infection*, skin infection*, Herpes zoster disseminated*, lung infection∗, infusion site cellulitis, catheter site cellulitis, infusion site infection, urosepsis*, Aspergillosis*, tinea infection, Herpes zoster ophthalmic, Herpes simplex ophthalmic, meningoencephalitis herpetic±, varicella, empyema±, fungal esophagitis±
Injury, Poisoning, and Procedural Complications	
Common	Fall
Uncommon	Subdural hematoma
Investigations	
Common	Weight decreased, alanine aminotransferase (ALT) increased, aspartate aminotransferase (AST) increased, blood alkaline phosphatase increased, liver function test abnormal, blood creatinine increased*
Uncommon	Gamma-glutamyltransferase (GGT) increased, oxygen saturation decreased*, blood albumin decreased, ejection fraction decreased*
Metabolism and Nutritional Disorders	
Very common	Decreased appetite, anorexia, dehydration*
Common	Hyperglycemia, hypoglycemia, hyponatremia, hypokalemia, hypercalcemia*
Musculoskeletal and Connective Tissue Disorders	
Very common	Bone pain, myalgia, arthralgia, back pain
Common	Muscular weakness
Uncommon	Limb discomfort
Neoplasms, Benign, Malignant, and Unspecified (including cysts and polyps)	
Uncommon	Tumor lysis syndrome*

The known anticipated risks of bortezomib therapy are presented in Table 1 below. These risks are grouped according to the combined frequency observed in an integrated analysis of AEs in sponsored clinical studies of single-agent bortezomib dosed at 1.3 mg/m^2 twice weekly on a 21-day schedule, in patients with multiple myeloma and mantle cell lymphoma.

Table 1 Known Anticipated Risks of Bortezomib by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class **Observed Incidence**	**Preferred Term**
Nervous System Disorders	
Most common	Peripheral neuropathy (including all preferred terms under the MedDRA High-level term Peripheral neuropathy NEC)
Very common	Paresthesia, dizziness excluding vertigo, headache
Common	Polyneuropathy, syncope, dysesthesia, dysgeusia, postherapeutic neuralgia
Uncommon	Convulsion, loss of consciousness, ageusia, encephalopathy, paralysis*,autonomic neuropathy, reversible posterior leukoencephalopathy syndrome± posterior reversible encephalopathy syndrome □
Psychiatric Disorders	
Very common	Anxiety, insomnia
Common	Confusional state
Uncommon	Delirium
Renal and Urinary Disorders	
Common	Renal impairment*, renal failure*, hematuria
Uncommon	Micturition disorder
Respiratory, Thoracic, and Mediastinal Disorders	
Very common	Cough, dyspnea
Common	Epistaxis, dyspnea exertional, pleural effusion*, rhinorrhea, hypoxia*, pulmonary edema*
Uncommon	Hemoptysis*, acute respiratory distress syndrome*, respiratory failure*, pneumonitis*, lung infiltration, pulmonary alveolar hemorrhage*, interstitial lung disease*, pulmonary hypertension*, pleurisy, pleuritic pain

The known anticipated risks of bortezomib therapy are presented in Table 1 below. These risks are grouped according to the combined frequency observed in an integrated analysis of AEs in sponsored clinical studies of single-agent bortezomib dosed at 1.3 mg/m^2 twice weekly on a 21-day schedule, in patients with multiple myeloma and mantle cell lymphoma.

Table 1 Known Anticipated Risks of Bortezomib by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class **Observed Incidence**	**Preferred Term**
Skin and Subcutaneous Tissue Disorders	
Very common	Rash
Common	Rash pruritic, rash erythematous, urticaria, petechiae
Uncommon	Cutaneous vasculitis, leukocytoclastic vasculitis±
Vascular Disorders	
Common	Hypotension*, orthostatic hypotension
Uncommon	Cerebral hemorrhage*

Source: Velcade® (Bortezomib) Investigator's Brochure Edition 15.

Most common = ≥ 30%, Very common = 10% to 29%, Common = 1% to 9%, Uncommon = < 1%.

* Fatal outcomes have been reported.

± Indicates a Preferred term not listed in the source table, however the event is deemed medically important and so is included.

Effective MedDRA update to version 14.0, the term 'reversible posterior leukoencephalopathy syndrome' updated to 'posterior reversible encephalopathy syndrome (PRES)'.

Table 7-1 Reports of Adverse Reactions From Post marketing Experience

System Organ Class Preferred Term	Observed Incidence[a]
Blood and lymphatic system disorders	
Disseminated intravascular coagulation	Rare
Cardiac Disorders	
Atrioventricular block complete	Rare
Cardiac tamponade	Rare
Ear and labyrinth disorders	
Deafness bilateral	Rare
Eye Disorders	
Ophthalmic herpes	Rare
Optic neuropathy	Rare
Blindness	Rare
Gastrointestinal Disorders	
Acute pancreatitis	Rare
Ischemic colitis	Rare
Hepatobiliary disorders	
Hepatitis	Uncommon
Liver failure	Unknown
Infections and infestations	
Herpes meningoencephalitis	Rare
Septic shock	Rare
Progressive multifocal leukoencephalopathy	Very Rare
Immune System Disorders	
Angioedema	Rare
Nervous System Disorders	
Autonomic neuropathy	Rare
Dysautonomia	Unknown
Encephalopathy	Rare
Respiratory, thoracic and mediastinal disorders:	
Acute diffuse infiltrative pulmonary disease[b]	Rare
Acute respiratory distress syndrome (ARDS)	Rare
Interstitial pneumonia	Rare

Table 7-1 Reports of Adverse Reactions From Post marketing Experience

System Organ Class Preferred Term	Observed Incidence[a]
Respiratory, thoracic and mediastinal disorders (Cont.):	Rare
Lung infiltration	
Pneumonitis	Rare
Pulmonary hypertension	Rare
Skin and subcutaneous system disorders	
Acute febrile neutrophilic dermatosis	Unknown
Toxic epidermal necrolysis	Unknown

Source: Velcade® (Bortezomib) Investigator's Brochure Edition 15. Addendum 1.

a Incidence is assigned using the following convention: very common (≥ 1/10); common (≥ 1/100 and < 1/10); uncommon (≥ 1/1000 and < 1/100); rare (≥ 1/10,000 and < 1/1000); very rare (< 1/10,000, including isolated reports).

b Acute diffuse infiltrative pulmonary disease is a MedDRA Lower Level Term which corresponds to a Preferred Term of Interstitial lung disease.

Other medical events of interest that are considered not causally related to bortezomib include hepatic failure and QT prolongation. Fatal outcomes have been reported.

Women of childbearing potential should avoid becoming pregnant while being treated with bortezomib. Genotoxicity testing has shown that bortezomib is negative in the in vitro Ames assay and in the in vivo micronucleus assay, but it is a clastogen in the in vitro chromosomal aberration assay.

Additional details on the potential risks of bortezomib may be found in the current Investigator's Brochure.

- Nursing implications: SQ injection on Days -11 and -8 at KUCC BMT treatment center

7.1.1 Return and Retention of Study Drug

Bortezomib is commercially available and available through the University of Kansas Hospital Pharmacy

7.1.2 Drug Accountability/Subject Compliance

All drugs will be administered to eligible subjects under the supervision of the investigator or identified sub-investigator(s).

8.0 MEASUREMENT OF EFFECT

8.1 Antitumor Effect- Hematologic Tumors

Positive or negative by flow cytometry of the stem cell harvest product

8.2 Safety / Tolerability

Analyses will be performed for all subjects having received study therapy. The study will use the CTCAE version 4.03:

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

9.0 DATA AND SAFETY MONITORING

9.1 Oversight and Monitoring Plan

The Data Safety Monitoring Committee (DSMC) of the KUCC is responsible for monitoring subject safety for this trial.

The Data Safety Monitoring Committee will include at least two external members. These individuals will not have any appointment, volunteer or otherwise, or membership with either the KU Cancer Center or University of Kansas Medical Center.

The external DSMC members have been identified as:

Rony Aboujawde, MD
Mosaic Life Care, St Joseph, MO
Rony.abou-jawde@mymlc.com

Dr. Leo Shunyakov
Central Cancer Care, Bolivar, MO
cancercare@gmail.com

These members will serve as ad-hoc voting members for this protocol. This protocol will be reviewed at the beginning of each committee meeting and these external members will attend the meetings by phone.

The DSMC is responsible for:

- Review of all clinical trials conducted by the KUCC for progress and safety
- Review of all adverse events requiring expedited reporting as defined in the protocol
- Submission of recommendations for corrective action to the PI and the Deputy Director of the KUCC or designee
- Notification of external sites participating in multi-institution clinical trials coordinated by the KUCC of adverse events requiring expedited reporting and subsequent committee recommendations for study modifications.
- The University of Kansas Cancer Center Quality Assurance Unit will audit study activity and reported data on at least a quarterly basis for any collaborating sites

9.2 Safety Review and Oversight Requirements

a) Serious Adverse Event

Serious adverse events that require expedited reporting will be reviewed by the DSMC Chair or designee who will determine if immediate action is required. If determined to be

necessary by the DSMC, all participating sites will be notified of the event and of any resulting action within one working day of this determination.

b) Review of Adverse Event Rates
Once per month, adverse event rates will be monitored by the DSMC Coordinator. If any study has had 2 or more of the same SAE reported within one month, or more than 6 of the same SAE in 6 months, the DSMC will review summaries of SAEs, and discuss events in detail with the PI. The DSMC chair or designee determines whether further action is required. The DSMC Coordinator ensures that collaborating investigators and IRBs for all participating sites are notified of any resulting action.

c) Study Safety and Progress – Quarterly Review
An overall assessment of toxicities as described in the protocol is reviewed at quarterly DSMC meetings. This review enables DSMC members to assess whether significant risks are occurring that would warrant study suspension/closure or protocol amendment.

9.3 Data Safety Monitoring Committee (DSMC) Reporting

All Data Safety Monitoring Committee (DSMC) reports, including those recommending continuation of the study, are forwarded to the KUMC Human Subjects Committee (HSC). Any DSMC recommendations for modifications to the trial are forwarded to the Deputy Director of KUCC or their designee. The PI is notified of this recommendation in order that he/she may alert all investigators about the potential action. At this time the PI may submit to the Deputy Director of KUCC or their designee additional information that could affect the committee's decision. The Deputy Director of the KUCC or their designee will notify the PI if he/she concurs with the DSMC's recommendation, including suspension or closure. The PI will notify all investigators involved with the study, the IRB, the sponsor and the funding agency and provide written documentation of these notifications to the DSMC.

NOTE: The Data Safety Monitoring Committee will include at least two external members. These individuals will not have any appointment, volunteer or otherwise, or membership with either the KU Cancer Center or University of Kansas Medical Center.

The external DSMC members have been identified as:

Rony Aboujawde, MD
Mosaic Life Care, St Joseph, MO
Rony.abou-jawde@mymlc.com

Dr. Leo Shunyakov
Central Cancer Care, Bolivar, MO
cancercare@gmail.com

These members will serve as ad-hoc voting members for this protocol. This protocol will be reviewed at the beginning of each committee meeting and these external members will attend the meetings by phone.

10.0 REGULATORY CONSIDERATIONS

10.1 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 Principal Investigator 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.

10.2 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.

10.3 Ethics and Good Clinical Practice (GCP)

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

1. ICH Consolidated Good Clinical Practice: Guidelines (E6)

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM073122.pdf

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

http://www.ecfr.gov/cgi-bin/text-idx?SID=3ee286332416f26a91d9e6d786a604ab&mc=true&tpl=/ecfrbrowse/Title21/21tab_02.tpl

With attention to the following specific regulations:

- Title 21 Part 50 – Protection of Human Patients
- Title 21 Part 56 – Institutional Review Boards
- Title 21 Part 312 – Investigational New Drug Application Responsibilities of Sponsors and Investigators

3. State laws
4. Institutional research policies and procedures

http://policy.ku.edu/research/human-subjects

AND

http://www.kumc.edu/human-research-protection-program/institutional-review-board/policies-and-regulations.html

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.

11.0 REGISTRATION PROCEDURES

11.1 General Guidelines for KUCC and Other Participating Organizations

Institutions will register eligible subjects through the KUCC Clinical Research Office central registration process. Registration must occur prior to the initiation of therapy, with treatment assignment (for both randomized and non-randomized studies) provided by KUCC. Any subject not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

The completed source documentation provided for eligibility verification and registration must be kept in the subject binder for monitoring purposes and documentation of subject eligibility.

Issues that would cause treatment delays should be discussed with the Principal Investigator. If a subject does not receive protocol therapy following registration, notify the KU Cancer Center Project Director or designee so that the subject's status can be changed in the CRIS system.

11.2 Registration Process for KUCC and Other Participating Centers

The Coordinating Center (KUCC), specifically the Project Director or designee is accessible for registration Monday through Friday from 8:00 AM to 5:00 PM Central Time.

The registration procedures are as follows:

1. Obtain written informed consent from the subject prior to the performance of any study related procedures or assessments. NOTE – if tests required at screening were performed as part of standard of care prior to signing consent for this study, the results from those tests are allowed in this study IF those tests were performed within the timeframe listed in the section of this protocol with title *Screening/Baseline Procedures*.

2. Complete the appropriate baseline demographic information in CRIS and any required registration forms using the eligibility assessment documented in the subject's medical/research record. To be eligible for registration to the study, the subject must meet each inclusion and none of the exclusion criteria listed in this protocol.

3. Fax or send via e-mail the eligibility checklist (checklist to be created from each version of this protocol and maintained by study project director and clinical team) and all pages of the consent form to the appropriate KUMC study project director (see below).

   Fax Number: 913-588-8279

   Project Director: Kelly Daniels - kdaniels2@kumc.edu
   Associate Project Director: Renee Sol - rsol@kumc.edu

4. The KU Project Director or designee will a) validate eligibility and b) register the subject on the study, assigning the subject to a treatment group

5. The KU Project Director or designee will send an email confirmation of the registration to the person initiating the registration immediately following the registration.

12.0 STUDY MANAGEMENT

12.1 Investigator Files and Retention of Documents

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 and clinic charts, laboratory and pharmacy records, ECG, signed ICFs, subject diaries and pathology reports. All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

12.2 Case Report Forms

Electronic case report forms (eCRFs) will be completed for each subject enrolled and entered into eVelos. All eCRFs will be customized by the KU Biostatistics Department for this study, and will be complete and accurate. The medical chart and any other clinical worksheets, procedural reports, etc. are the source of verification of the data captured into the study database.

12.3 Study Monitoring

Data collection and analysis will be independently reviewed by a researcher with suitable expertise designated by the Associate Vice Chancellor for Research. The results of that review will be reported to the Associate Vice Chancellor for Research.

The study will be monitored at appropriate intervals to assure compliance to GCP and to assess the data quality and study integrity. The frequency of monitoring may vary depending on enrollment rate and the quality of data collected.

The investigator and staff are expected to cooperate and provide all relevant study documentation in detail at each site visit on request for review. The study monitor will have direct access to source data for data verification. Data verification will be conducted by comparing the data entered into the CRFs with source data.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Design/Study Endpoints

This is an open label, randomized study intended to:

- Estimate the proportion of subjects with positive plasma cell contamination (expressed as percentage as mentioned in the consensus guidelines; >0.01% and at least 100 cellular events) (Rawstron AC et al, 2008 and Shah, Callander and Ganguly et al, 2015) of harvested stem cell product by multi parametric flow cytometry from patients with myeloma undergoing autologous stem cell collection 1) by standard of care mobilization using GCSF with or without Mozobil and 2) after two doses of bortezomib as in vivo purging plus standard of care using GCSF with or without Mozobil.
- Estimate the proportion of subjects who have a successful collection of stem cells (> 2 million CD34 cells/Kg of body weight) for autologous transplant in both treatment groups.
- Estimate the percentage of CD 34 positive cells in circulating peripheral blood as a measure of mobilization on the days of collection

13.2 Sample Size and Accrual

- N=100
- Patients will be randomized using sequential assignment of predetermined block randomized 1:1 treatment assignments provided by the study statistician prior to activation.
- Dichotomous yes or no response on tumor contamination of products in two study arms

Justification for Sample Size

YEAR	Number of Stem Cell Harvests	Number of Autologous Transplantations
2011	60	71
2012	78	94
2013	100	102
2014	87	89

NOTE: An average of 5-7% patients failed to collect with or without Mozobil historically over the last four years at the KU BMT program.

With 50 evaluable products in each treatment arm, 95% confidence interval width for the proportions of contaminated products will be no greater than 29% using the exact binomial method. Contamination is defined as >0.01% cancer cells and at least 100 cellular events (Rawstron AC et al, 2008 and Shah, Callander and Ganguly et al, 2015).

13.3 Data Analyses Plans

13.3.1 Descriptive statistics, such as means, standard deviations, frequencies, and proportions, will be generated for subject demographics and clinical characteristics, including disease and transplant-related factors. Estimates and 95% exact binomial confidence intervals will be calculated for the primary endpoint, the proportion of subjects in which the leukapheresis product was contaminated with tumor cells, within each treatment group. These estimates will provide critical information for designing future studies formally testing the effectiveness of this therapy. Attrition is not expected since the primary endpoint is an assessment of the leukapheresis product. All leukapheresis products are expected to be evaluable for contamination. Should a product NOT be evaluable, it would conservatively be considered contaminated in the primary analysis. Similar methods will be used to estimate and provide 95% exact binomial confidence intervals for the proportion of subjects who experience successful extraction of product for transplantation. Summary statistics will be provided for proportion of subjects who would have adequate number of CD 34 cells collected and the percentage of CD34 positive cells in peripheral blood on the days of collection.

13.3.2 Stopping Rules

Data collection and analysis will be independently reviewed by a researcher with suitable expertise designated by the Associate Vice Chancellor for Research. The results of that review will be reported to the Associate Vice Chancellor for Research.

In addition, as a routine and normal SOC for KU BMT Stem Cell lab, ongoing QA meetings are held and all patient collection data are reviewed in an ongoing basis per FACT guidelines (Foundation for the Accreditation of Cellular Therapy). KU BMT stem cell first week collection success over last 4 years had been 93%. Hence, if the collection rate falls below that i.e. if 7% or more patients fail to collect (as defined by failure to at least collect 2 million/Kg CD 34 + cells with GCSF and with or without Plerixafor mobilization procedure); root cause analysis is done by the team (SOC-per QA team). This trial stopping rule will be no exception to that policy as well.

Based on a binomial probability calculation, after 25 patients (half way through) are enrolled in each arm, we will calculate the collection rate and if more than 4 patients fail to mobilize in any arm, the trial will be stopped and further analysis and SOC root cause analysis would be done. Trial will only be resumed if it is determined that the failure to collect is due to the other factors and not due to bortezomib usage. The QA committee is entrusted with this analysis which is a requirement for FACT (Foundation for the Accreditation of Cellular Therapy).

NOTE: Failure of mobilization is defined as a patient's body's failure to collect CD34+ cells at least 2 million/Kg after standard procedure of using G-CSF with or without Plerixafor.

13.4 Interim Analysis and Data Supervision

Data collection and analysis will be independently reviewed by a researcher with suitable expertise designated by the Associate Vice Chancellor for Research. The results of that review will be reported to the Associate Vice Chancellor for Research.

In addition, as a routine and normal Standard of Care (SOC) process for KU BMT Stem Cell Lab, ongoing QA meetings are held, data is collected and analyzed for reporting purposes independent of any clinical trial. The data collected as a part of this trial will be entered by the trial data coordinator, and during the interim analysis will be available for analysis by the KU Biostatistics Department. Concurrently, data from the KU BMT Stem Cell Collection Lab will be made available to the KU Biostatistics Department, in order to match the results and document congruity. The AEs and SAEs are posted in the CRIS environment of KUCC website. AEs and SAEs are also noted by the KU BMT Stem Cell lab. Any discrepancy will be addressed during the interim analysis or at any time if deemed necessary by the DSMC, BMT QI committee or the KU Biostatistics Department. In essence, three independent groups will have access to the data, i.e. DSMC, KU BMT QI committee and the KU Biostatistics Department. Any discrepancy will be addressed immediately and will be brought to IRB attention.

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15.0 APPENDICES

APPENDIX A Performance Status

The following table presents the Karnofsky performance status scale:

Points	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity
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
60	Requires occasional assistance but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled; hospitalization indicated. Death not imminent
20	Very sick; hospitalization necessary; active support treatment necessary
10	Moribund; fatal processes progressing rapidly
0	Dead

Zubrod (ECOG) Performance Status

POINT	DESCRIPTION
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

APPENDIX B Body Surface Area

Body surface area (BSA) should be calculated using a standard nomogram that yields the following results in meters squared (m^2):

$$\text{BSA} = \sqrt{\frac{Ht(inches) \times Wt(lbs)}{3131}}$$

or

$$\text{BSA} = \sqrt{\frac{Ht(cm) \times Wt(kg)}{3600}}$$

APPENDIX C Standard Operating Procedures – Stem Cell Harvest

THE UNIVERSITY OF KANSAS HOSPITAL

DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

Title:	HPC or MNC APHERESIS USING THE COBE SPECTR
Index:	C-01
Author:	Kathleen Gaillard and Dean Merkel
Department Name:	BMT Apheresis & Cell Processing Lab
Category:	BMT COLLECTION
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C-01 HPC or MNC APHERESIS USING THE COBE SPECTRA

1.0 PRINCIPLE – CLINICAL SIGNIFICANCE

1.1 The purpose of the apheresis procedure is to harvest an adequate number of peripheral mononuclear cells (MNCs) for therapeutic patient procedures or Hematopoietic Progenitor (stem) cells (HPCs) for transplant.

1.2 Hematopoietic Progenitor (stem) cells capable of establishing hematopoiesis following high dose chemotherapy or radiation therapy are needed prior to the initiation of that treatment.

1.3 The collection dose of (MNCs) for therapeutic treatment varies with each treatment protocol.

1.4 These cells are collected from the peripheral blood by apheresis using the COBE Spectra. The Spectra removes cells from the mononuclear layer of the donor's centrifuged peripheral blood. The specific gravity of the cells allows them to be separated by centrifugation.

1.5 The Blood and Marrow Transplant Clinical Program determine donor selection according to the selection criteria. Donor suitability is evaluated and consent is obtained prior to harvest.

2.0 SPECIMEN

2.1 Hematopoietic Progenitor Cells or HPC, Apheresis

2.2 Mononuclear Cells or MNC, Apheresis

3.0 REAGENTS – SUPPLIES – EQUIPMENT- LABELS

3.1 **Critical**

- 3.1.1 COBE Spectra with disposable WBC blood tubing set
- 3.1.2 Astotherm Fluid Warmer with disposable blood tubing set
- 3.1.3 Spectra Tube Sealer
- 3.1.4 0.9% sodium chloride for injection (1000 ml bags)
- 3.1.5 Anticoagulant (ACD-A) (1000 ml bags)
- 3.1.6 Heparin (100 units/ml syringe and 1000 units/ml) for injection
- 3.1.7 Apheresis needle (17 g. with back-eye)
- 3.1.8 Collection product label containing unique alphanumeric identifier

3.2 **Non-critical**

- 3.2.1 Calcium Supplement
- 3.2.2 Syringes, blunt or interlink needles, & hemostats
- 3.2.3 0.9% sodium chloride for injection flush syringes
- 3.2.4 Face mask
- 3.2.5 Heparin lock caps
- 3.2.6 Alcohol pads
- 3.2.7 Sterile drapes & Sterile 4x4 gauze
- 3.2.8 Chloraprep (or equivalent)
- 3.2.9 Central Line Dressing Tray
- 3.2.10 Primapore or equivalent and 2 x 2 IV dressings
- 3.2.11 Insyte IV set
  - 3.2.11.1 IV Therapy may be called to place an 18g or 20g. IV into the patient's arm for the return site. Page IV Therapy at 917-7544.

4.0 QUALITY CONTROL: n/a

5.0 PROCEDURE

5.1 Treatment schedule will be according to physician orders. Release orders for each procedure.

5.2 **Power switch to on.** A short self-check will follow to ensure power supplies are at correct voltage.

5.2.1 Verify:

5.2.1.1 Cartridge clamps ready to load

5.2.1.2 Single stage channel filler is in place

5.2.1.3 Pause LED button is flashing

5.3 **Tubing Loading in the Spectra**

5.3.1 Place WBC tubing set on the centrifuge cover.

5.3.2 Remove inlet line coil from package (it will have three lines attached to a single junction). Hang line on left side of I.V. pole.

5.3.3 Place access saline line (green stripe) over top of system.

5.3.4 Remove return line from package (two lines attached at a common junction). Hang on left side of I.V. pole along with the access line.

5.3.5 Place return saline line over top of system.

5.3.6 Hang collection bag on far right hooks. Hang waste and plasma bags just to the left of the collection bag. Hang saline bag to the left of the waste bag and the anticoagulant bag to the left of the saline. From left to right: ACD-A; SALINE; PLASMA BAG, WASTE BAG; COLLECTION BAG.

5.3.7 Remove the return pump cartridge (on right) from the package and snap it into the cartridge clamp between the plasma and collect/replace pumps.

5.3.8 Remove the access pump cartridge (on left) and snap it into the cartridge clamp between the AC and inlet pumps.

5.3.9 Place AC line over top of the system.

5.3.10 Insure all lines are untangled and clear of pumps.

5.3.11 Press the continue key to load tubing into pump housings.

5.3.12 Verify all four pumps are loaded.

5.3.13 Put lines in collect/replace and plasma valves.

5.3.14 Place the sensor (on right) from package in return pressure sensor housing. Push down while turning clockwise to lock in place.

5.3.15 Place RBC line in RBC valve. Ensure line is completely inserted in RBC detector.

5.3.16 Place return and inlet air chambers from package in air detectors, making sure the filters are below the air detector housing. Rotate chambers until the waste-divert lines are facing front.

5.3.17 Put waste lines in waste valve assembly.

5.3.18 Use a flossing action to place line in centrifuge pressure sensor housing.

5.3.19 Place sensor (on left) from package in access pressure sensor housing by pushing downward and turning clockwise to lock in place.

5.3.20 Floss the return line in return valve so that it runs through the center of the valve.

5.3.21 Ensure the clamp on the plasma line is closed

5.3.22 Install WBC channel in centrifuge

5.3.22.1 Remove channel from package. Discard package.

5.3.22.2 Press the “unlock cover” button.

5.3.22.3 Slide the centrifuge cover back and lower centrifuge door.

5.3.22.4 Rotate centrifuge so loading port is facing front.

5.3.22.5 Rest the centrifuge collar holder on the outer rim of the filler.

5.3.22.6 Ensure that the four-lumen tubing is not twisted. Fold the channel in half and thread channel through lower loading port and pull it out from the top.

5.3.22.7 Position channel above filler so that the red line fits on the furthermost right groove and the clear collect line is aligned above the furthermost left groove. Lines with a Y junction will go in the middle grooves. The smallest line will sit atop the larger line.
5.3.22.8 Load centrifuge collar into centrifuge collar holder, closing cover over collar.
5.3.22.9 Lower filler latch to locked position.
5.3.22.10 Press channel into position as well as lines into the grooves of the filler chamber.
5.3.22.11 Place lower bearing in lower bearing holder.
5.3.22.12 Place upper bearing in upper bearing holder.
5.3.22.13 Place upper collar in upper collar holding, ensuring a point of the six-sided upper collar is facing out.
5.3.22.14 Floss the four-lumen tubing in the exit slot on the right side of the centrifuge chamber.
5.3.22.15 Rotate centrifuge to ensure that the tubing is not twisted and moves freely.
5.3.22.16 Close the centrifuge door and cover.
5.3.22.17 Place patient HIS sticker on the paper collection bag label under Autologous or Allogeneic label.

5.4 **Anticoagulant Use**

5.4.1 ACDA only is used for patients on any anticoagulant therapy such as Coumadin or Lovenox.
5.4.2 ACDA with 3000 units of heparin added is used for patients not on any anticoagulant therapy.
5.4.2.1 Add 3000 units of heparin to 1 liter of ACDA using aseptic technique.
5.4.3 Spike ACDA when ready to prime machine.

5.4.4 **Adding ACDA to Product Bag (if applicable)**

5.4.4.1 Products that will be given fresh do not require ACD-A. ACDA should be added only **AFTER** priming is completed.
5.4.4.2 If ACDA is to be added:
5.4.4.2.1 If using Spectra "Open" WBC set:
5.4.4.2.1.1 Using aseptic technique, insert a sample site coupler into the product bag.
5.4.4.2.1.2 Clean sample site coupler with Iodine pad and let dry. Use alcohol wipe to clean coupler.
5.4.4.2.1.3 Inject 20 ml of ACD-A or ACD-A with heparin to product bag for products that will be frozen.
5.4.4.2.2 If using Spectra "Closed" WBC set:
5.4.4.2.2.1 Using aseptic technique, remove and retain cap to product bag access port, and attach syringe with 20 mL ACD-A or ACD-A with heparin to port
5.4.4.2.2.2 Break "access" cartridge in access port tubing and inject syringe contents into product bag.
5.4.4.2.2.3 Clamp tubing above access cartridge and replace cap on access port.

5.5 **Connect Fluid and Priming WBC tubing set**

5.5.1 Select set from instrument panel 3 = WBC; 1= MNC
5.5.2 Clamp access and return lines. Roll both saline lines closed, making sure it is rolled completely in the locked position. Press Continue.

5.5.3 The anticoagulant spike is orange. Spike the anticoagulant bag.
5.5.4 Wipe the injection site on saline bag with alcohol, then spike the return needle into injection site, spike return spike into port.
5.5.5 Fill the drip chambers halfway.
5.5.6 Place AC line through AC detector.
5.5.7 Visually verify that fluid is flowing through the access, return, AC spike and drip chambers. Press Continue.
5.5.8 When prompted, roll open access and return saline lines. Press Continue.
5.5.9 When prompted, open clamp near access connection. Allow saline to prime the end of access by gravity. Close clamp again.
5.5.10 Open the clamp on the return line to prime the Astotherm tubing set and clamp off once again
5.5.11 Close roller clamp on green striped access saline line. Press Continue.
5.5.12 Perform Alarm tests by pushing "Yes" key.

5.6 **Entering Data and Patient Information**
5.6.1 Default Values
5.6.1.1 The COBE Spectra will start with the following values:
5.6.1.1.1 Inlet Vol.: 2 time patient total blood volume
5.6.1.1.2 Inlet: AC Ratio 12:1 for MNC
5.6.1.1.3 Collect Rate: 1 ml/min

5.7 **Quick Start.** The COBE Spectra uses a Quick Start Program to increase the plasma pump rate initially and reduce it within the first 15 minutes. This reduces the time required to establish interface.
5.7.1 Quick Start override may be used to achieve cell interface even faster than Quick Start. See the Start Run Mode in step 5.11.4.

Altering default values:

CHANGED VALUE	AFFECTED VALUE
Run Time	Inlet Volume - Collect Volume - AC Volume
Inlet Flow	Inlet Volume - AC Volume - AC Flow Rate - Plasma Flow Rate - Collect Volume
Collect Volume	*Collect Pump Flow Rate - AC Pump Flow Rate - Inlet Pump Flow Rate - Plasma Pump Flow Rate - Inlet Volume - AC Volume
Inlet Volume	Run Time - Collect Volume - AC Volume

*Things to consider when setting the collect volume include subject WBC and platelet counts as well as fluid balance. Low collect volumes with high WBC and/or platelet counts can cause platelet clumping. Low collect volumes over long procedure times can leave the subject volume expanded due to greater AC flow than collect flow.
**To change a white cell removal value, press the number correlating to the flow or volume to be changed. Parentheses will be around the number. Use the arrow keys or enter a new

number to change the values and press enter.

Changed Value	Allowed Range
Run Time	100-999 min
Inlet Flow	15-150 ml/min
Collect Volume	10-9999 ml
Inlet Volume	100-32,000 ml

- If the patient is known to have an abnormal number of platelets the AC Ratio needs to be adjusted. Decrease ratio for platelet clumping.
- For a polymorphonuclear cell collection, the collect rate will be much higher. The rate can be increased to 9.0 once the WBC layer has been reached. Watch the collect line closely to maintain correct interface.
- Maximizing the inlet flow will cut down the run time, but it also increases the ACD going to the patient. If the patient begins having symptoms of hypocalcemia, lower the inlet flow rate. If the symptoms continue, calcium-rich Tums may be given as a calcium supplement or the calcium infusion rate may be adjusted.

5.7 **Entering Subject Sex, Height and Weight**
- 5.7.1 Select Sex
- 5.7.2 Enter height in feet and/or inches
- 5.7.3 Enter weight in pounds; Total Blood Volume will be given

5.8 **Enter hematocrit**
- 5.8.1 Enter patient's last hematocrit, preferably on from the last 72 hours or perform a new blood count.

5.9 **Selecting a Procedure and Changing Default Values**
- 5.9.1 Select MNC procedure; inlet flow, inlet volume, time expected and collect volume will be calculated.
- 5.9.2 Approve or disapprove white cell removal values.
- 5.9.3 Instrument defaults to processing 2 times the total blood volume. The goal is to process approximately 4 to 6 blood volumes or as recommended by the patient's physician. If using ACD-A with heparin, AC ratio may be set to a maximum of 20:1.
- 5.9.4 Changes to variables may be made now or during the run.
- 5.9.5 Changes may be made by pressing the button above the value to be changed. This places brackets around the value to be changed. The value can be changed by entering a new value or using the up and down arrows to change the value. When one value is changed this will affect other values.

5.10 **Connect Donor**
- 5.10.1 **Access Line**
  - 5.10.1.1 Before connecting donor, check access and return lines for air. If air is present in these lines, do not connect subject. Remove air before starting procedure.
- 5.10.2 Clean venipuncture site with Chloraprep. Perform venipuncture for access, if

required, using a 17G x 1inch Apheresis needle with backeye.

5.10.3 If an Apheresis catheter is used, the red port is best for the access. Blue or white ports may be used if poor flow rates are encountered.

5.10.4 Use aseptic technique when accessing catheters.

5.10.5 Patient and apheresis staff must wear mask prior to removal of catheter caps.

5.10.6 Remove apheresis catheter cap and clean catheter hub with alcohol prep pads. Scrub end of catheter for 20 seconds, as friction, not the alcohol cleans the catheter hub.

5.10.7 Attach a sterile syringe to the catheter hub and remove 4 to 5 ml of blood to verify proper flow.

5.10.8 Always keep catheter pinch clamp closed when the catheter end is open.

5.10.9 Using a syringe or vacutainer adapter, draw sample(s) for pre-apheresis testing, if applicable.

5.10.10 Attach access tubing to red or blue catheter line or apheresis needle.

5.10.11 Open the pinch-clamp on the access line. Close roller clamp on access saline line.

5.10.12 **Return Line**

5.10.12.1 IV Therapy may be called to place an 18g. IV into the patient's arm for the return site. 20g IV set may be used if rate of inlet is less than 50 ml/min. Page IV Therapy at 917-7544.

5.10.12.2 Perform venipuncture for return site if required.

5.10.12.3 If an apheresis catheter is to be used, the blue or white port is best used for the return. Red port may be used if blue must be used for access.

5.10.13 Remove apheresis catheter cap and clean catheter hub with alcohol prep pads. Scrub end of catheter for 20 seconds, as friction, not the alcohol cleans the catheter hub.

5.10.14 Attach a sterile syringe to the catheter hub to remove the heparin from the line and verify proper catheter flow.

5.10.15 Attach the return tubing to the catheter or the return IV site.

5.10.16 Open the pinch-clamp on the return line.

5.10.17 Use roller clamp on return line to maintain a KVO normal saline drip during "Diverting Prime Saline" mode.

5.11 **Start Run Mode**

5.11.1 Press "Continue" to begin.

5.11.2 Once the RBC's start returning to the patient, the machine will beep. At this time completely close the roller clamp on the return line. Clear alarm. A saline drip for return is no longer required.

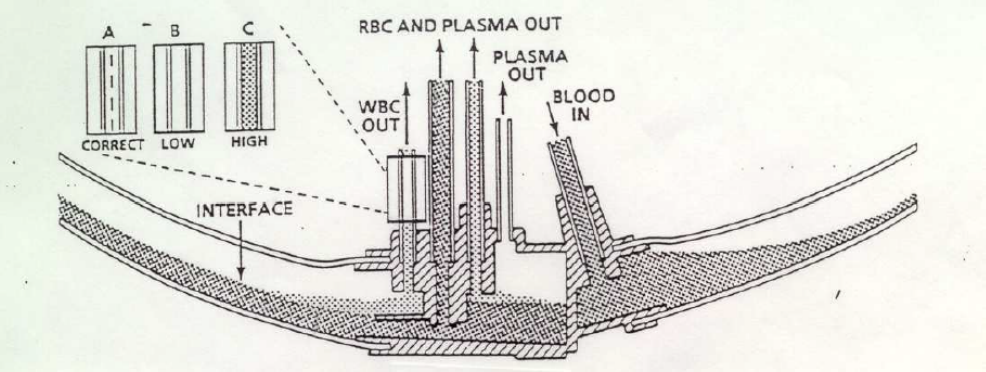
5.11.3 To establish RBC/plasma interface at beginning of run, changes in plasma pump flow rate will be frequent and in large increments. Watch through the centrifuge door view port, and observe the WBC collect line, which will be on the far left. For the MNC collection, look for a streaking effect spurting a fine line of RBC's. Once this interface has been reached, smaller and less frequent changes in plasma pump flow rate are required to maintain interface (0.2 to 0.5 ml/min changed every 2-5 min).

5.11.4 Quick Start override can be performed to achieve cell interface more quickly. Depress manual button twice. Increase the plasma pump by 10. When blood appears in the control line, decrease the plasma pump by 5. When blood appears in the collect line, decrease the plasma pump by 3. Watch and adjust the interface with small plasma pump adjustments as in step 5.11.3.

5.11.4.1 **REMEMBER: Too Dark** – "Drop" plasma pump flow rate

(decrease) **Too Light** – "Lift" plasma pump flow rate (increase)

5.11.4.2 If collecting too deep into the red cell layer, the WBC collect tube will be filled with red cells. If not collecting deep enough into white cell layer, WBC collect tube color will be clear with no RBC's present.



Correct Interface Position

5.12 **Interface:** When the desired interface is met, or at the end of Quick Start, increase the collect pump to 1.5 ml/min.

5.12.1 If the patient is experiencing any problems, periodically monitor the patient's blood pressure and pulse. Be aware of citrate reactions and be sure to inform the patient of side effects (tingling of the lips and jaw, heavy chest, cramping of fingers and feet). Monitor the patient periodically during the treatment. Record the patient's vitals and COBE Spectra settings on worksheet.

5.12.2 If patient experiences tingling of the lips and jaw, heavy chest or cramping of fingers and feet, the inlet rate may be decreased to decrease the amount of citrate the patient is receiving.

5.12.3 Inlet/AC Ratio adjustments may be made during the procedure depending upon the patient's platelet count. If the **patient has a low platelet count (<100,000), the ratio may be increased**. If the platelet count is **>400,000, the ratio may need to be decreased**. Always monitor the collect line and evaluate for possible platelet clumps throughout procedure. Clumping requires more anticoagulant; therefore a lower ratio would be necessary.

5.12.4 Run mode continues until target values are reached. There will be audio and visual warning when run mode is complete. Prepare take off supplies prior to the end of run.

5.12.5 At end of run, select 1 to begin rinseback or select 2 to continue run. To continue the run, you will need to select inlet volume or time and increase their values as desired.

5.13 **Start Rinseback Mode**

5.13.1 Select 1 to begin rinseback.

5.13.2 Press "Continue" to start rinseback. Open roller clamp on green-striped access saline. The Inlet Flow rate may need to be lowered to 50 ml/minute at this time to avoid alarms.

5.13.3 Clamp access line. A sample may be drawn at this time or at the end of rinseback for a post blood count.

5.13.4 Disconnect access line.

5.13.4.1 Catheter: Clean catheter lumen hub with alcohol wipes. Flush

catheter lumen with 20 ml normal saline. Prime catheter cap. Clean any excess blood from catheter lumen with additional alcohol wipes. Attached primed catheter cap to catheter hub. Flush catheter with heparin if desired, to the volume stated on the catheter. Remove syringe. Clamp catheter.

5.13.4.2 Apheresis needle: Discontinue IV per hospital protocol.

5.13.5 When instructed by the Spectra, clamp then disconnect collection bag using a heat sealer to create a minimum of 3 seals. Cut across the middle of the bottom seal to disconnect the bag. Press clear.

5.13.6 During rinseback, verify the patient's RBCs are being returned. The fluid in the WBC kit tubing should get lighter in color.

5.13.7 When rinseback is completed, open roller clamp on blue-striped return saline.

5.13.7.1 Flush the return line and blood warmer tubing until visible blood is flushed through the line.

5.13.8 Disconnect return line.

5.13.8.1 Catheter: Clean catheter lumen hub with alcohol wipes. Flush catheter lumen with 20 ml normal saline. Prime catheter cap. Clean any excess blood from catheter lumen with additional alcohol wipes. Attached primed catheter cap to catheter hub. Flush catheter with heparin if desired, to the volume stated on the catheter. Remove syringe. Clamp catheter.

5.13.8.2 IV: Discontinue IV per hospital protocol.

5.13.9 Record final volumes processed and end time on the procedure flowsheet.

5.13.9.1 Record product and anticoagulant volume on the product label. Obtain information by pressing: Menu, 1 Data Entry, 4 AC Data, AC Volume

5.13.10 Close all fluids and press Continue.

5.13.11 Take final vital signs and record.

5.13.12 Press Continue to unload.

5.14 **Removing the Disposable**

5.14.1 Remove disposable lines from the valves and sensors from the front of the Spectra, carefully place the tubing in appropriate biohazard disposal container to avoid splashing.

5.14.2 Disconnect return line needle from saline container. Dispose of needle in an approved sharps container.

5.14.3 Remove tubing and centrifuge channel from bearings and holders. Dispose in appropriate biohazard container.

5.14.4 Remove fluid containers and waste bag and dispose of them.

5.14.5 Turn power off.

5.14.6 Disinfect the Spectra instrument using Germicidal wipes or Asepticare spray and clean sensors with water only.

5.14.7 After completion of leukapheresis, the collected cells are to be processed and frozen according to procedures found in the cell processing laboratory.

CALCULATIONS - INTERPRETATION - RESULT REPORTING - NORMAL VALUES - CRITICAL VALUES

5.15 Refer to Procedure Notes for desired run and result endpoints. One to three collections should yield the required number of CD34+ cells desired, although this varies with each patient. Collection evaluation includes a cell count, viability, CD34 analysis and sterility of the collected product. F-12 Deviation Form must be completed for all components in which outcome analysis fall outside of the established tolerance limits.

5.16 Recipient engraftment outcome is evaluated monthly and reported in the Blood and Marrow Transplant Program Quality Assurance meeting. Any engraftment that is delayed or not accomplished within the set guidelines in G-04 Delayed or Failed Engraftment Protocol will be investigated by the QA committee.

5.17 Microbial Detection: 100% non-contaminated. The **patient's physician is notified immediately of any positive culture; refer to P-21 Microbial Contamination.** Any further action will be dictated by the physician including treatment, catheter care and the apheresis status. Contaminated products do not necessarily need to be destroyed. Decisions regarding the disposition of culture positive HPC's must be made by patient's transplant physician after considering the type of contamination and the potential benefits and the risks from the use of the product. Complete F-12 Deviation Form on all positive cultures. Documentation of recipient acknowledgement must be recorded prior to infusion.

5.18 A total bag cell count should typically yield 0.5 x 10^{10} to 8.0 x 10^{10} cells.

5.19 A viability percent of >95% is desired. Complete F-12 Deviation Form documenting viability of <95% and possible reason for the result.

5.20 The CD34% should be $\geq$0.1%. Notify physician if $\leq$ 0.1% for possible discontinuation of harvest.

5.21 A hematocrit of 1-4% in the collection bag is desired. Hematocrits may be elevated on products with very high nucleated cell counts. If the hematocrit is > 4% and the WBC count is <100,000 a manual spun hematocrit should be performed.

6. CALIBRATION – LINEARITY – AMR - CALIBRATION VERIFICATION: n/a

7. PROCEDURAL NOTES – LIMITATIONS

8.1 Use aseptic technique throughout procedure.

8.2 Harvesting and Processing Prescription:

8.2.1 F-01 Harvest and Processing Prescription is to be completed and signed by the patient's physician indicating donor and recipient information, mobilization type, processing and storage requirements and collection endpoint.

8.3 Venous Access:

8.3.1 Peripheral or central venous access will be determined prior to harvest by the collection staff and the BMT Nurse Coordinators. This information will be documented on F-01 Harvest and Processing Prescription. The nurse coordinators will arrange catheter placement, if needed, prior to collection. (Refer to section on Central Line Placement, procedure III-6 of BMT Program Clinical SOP's.)

8.4 Patient Information:

8.4.1 The use of ACDA during the apheresis procedure will chelate the patient's calcium and reduce the ionized and serum calcium level. Therefore, it is necessary to instruct the patient to increase their intake of calcium products to help prevent hypocalcemia. Possible reactions to the collection procedure are: cold/chills, tingling or numbness, vibration or heaviness in chest, feeling light-headed, increased pulse rate, shallow respiration, excess perspiration, hives, rash and tissue swelling. Calcium Gluconate given IV and blankets often decreases the effects of hypocalcemia. The order for calcium gluconate is documented in the patient's electronic medical record.

8.5 Consent:

8.5.1 Prior to starting the procedure, an informed consent form must be signed by the

donor. Donor consent must be obtained prior to the initiation of the recipient's myeloablative treatment. In the case of a minor donor (under the age of 18 years), it is necessary to attain consent from the minor's parent or legal guardian.

8.6 Health Assessment:

8.6.1 Before each apheresis procedure is started, an interim health assessment should be performed by the apheresis staff. This is to monitor the patient's general health, white blood count, hemoglobin, and platelet count and to document any changes in health and medications since the last visit. Documentation of Health Assessment is in the patients electronic medical record.

8.6.2 **The donor evaluation criteria for contacting the BMT physician or ARNP prior to or during the procedure is:**

8.6.2.1 Systolic blood pressure of <90 or >180
8.6.2.2 Diastolic blood pressure of <50 or >105
8.6.2.3 Pulse of <50 or >120
8.6.2.4 RR >28
8.6.2.5 SaO2 <90
8.6.2.6 Temperature of ≥101℉ or 38.3℃
8.6.2.7 Obvious symptoms of infection or inflammation at catheter site
8.6.2.8 Platelet count of <50
8.6.2.9 Hemoglobin of <8.0

8.6.3 Vital signs are performed at the completion of each procedure and at any time during the procedure, if needed.

8.6.4 The in-patient staff BMT physician performs a daily patient H & P evaluation of the donor and evaluates the prior day's collection results, if applicable.

8.6.5 After each collection procedure, a CBC is performed. Platelet and RBC transfusions are given per BMT transfusion guidelines.

8.6.6 The determination of completion of collections is made by the BMT physician upon notification of the collection results by the Cell Processing lab staff.

8.6.7 The donor is advised to follow-up with the BMT Clinic and their BMT Nurse Coordinator with any questions or concerns regarding post procedural care.

8.7 Calcium Replacement:

8.7.1 Calcium Gluconate is ordered and infused throughout procedure.

8.7.2 Rate may be titrated up if patient is having any symptoms of hypocalcemia.

8.8 Blood Type Check:

8.8.1 Blood type checks on a peripheral sample are required for the first Allogeneic collection, but are performed on all Autologous and Allogeneic collections. A blood type is performed on the peripheral blood sample when the donor is being harvested. This is to confirm previous blood bank typing or to establish a blood bank record. Any discrepancy is handled by the technologists and must be brought to the attention of the collection staff. Daily blood type validations are confirmed by the Cell Processing Lab.

8.9 Product:

8.9.1 **Labeling: Product must be labeled as outlined in G-03 Identification Protocol. Examples of labels are attached.**

8.9.1.1 HPC bag will be labeled with the following information:

8.9.1.1.1 Unique identifier
8.9.1.1.2 Proper name of the product
8.9.1.1.3 Recipient name and identifier if applicable

8.9.1.1.4 Date and time collection ended
8.9.1.1.5 Approximate volume of collection
8.9.1.1.6 Name and volume or concentration of anticoagulant
8.9.1.1.7 Name and volume or concentration of other additives
8.9.1.1.8 Donor identifier and donor name if applicable
8.9.1.1.9 Identity and address of collection facility
8.9.1.1.10 Recommended storage temperature
8.9.1.1.11 If applicable, Biohazard labels per G-03 Identification Protocol.
8.9.1.1.12 If applicable, Statement "For autologous use only" if autologous or "For use by Intended Recipient Only" if allogeneic.

8.9.2 **Product Storage**

8.9.2.1 Products are to remain hanging on the instrument stored at room temperature until picked up by the Cell Processing Lab staff.
8.9.2.2 Products are to be picked up prior to the patient leaving the room.
8.9.2.3 Product label/patient identification is again verified and is documented on the cell processing worksheet prior to transport of the product to the cell processing lab.
8.9.2.4 If a delay is anticipated, the apheresis staff may verify label/patient identification and transport of the product to the cell processing lab on completion of procedure.

8.9.3 **Transportation:**

8.9.3.1 Product must be transported to the Processing Laboratory as outlined in G-09 Transportation Policy for HPC within the Facility.
8.9.3.2 HPC's will be transported from the apheresis room to the cell processing laboratory at ambient temperature.
8.9.3.3 Sealing - The collection container should be sealed in a manner that minimizes the risk of cell loss and microbial contamination. Seal with heat sealer. Heat seal a minimum of three times.
8.9.3.4 Secondary container (cover bag) -The primary container should be placed in a secondary plastic bag and sealed to prevent leakage. Use zip-lock bags or other plastic bag that can contain any leakage and prevent exposure.
8.9.3.5 Outer container (small cooler) - The outer container should be made of material adequate to withstand leakage of contents, shock, and other incidents to ordinary handling in transportation. Use a small Igloo cooler (or equivalent) from the Cell Processing lab to transport the components.

8.9.4 **Expiration date of fresh products:** Fresh products are best used within 72 hours from time of completion of harvest. The patient's physician must approve release of the product if this time is exceeded.

8.9.5 **Release and exceptional release:**

8.9.5.1 Criteria for product release
8.9.5.1.1 Product label must be reviewed by two staff upon delivery of product.
8.9.5.1.2 Product must be labeled appropriately as described in G-03 Identification Protocol.
8.9.5.1.3 Product label must be complete.
8.9.5.1.4 Biohazard positive labeling must be present as described in G-

03 Identification Protocol.

8.9.5.1.5 Product must not exceed expiration date.

8.9.5.2 Exceptional release

8.9.5.2.1 If criteria for release are not verified, F-12 Deviation Form must be completed as per G-05 Deviation from Policy-Procedure.

8.9.5.2.2 The patient's physician must be notified before processing or infusion. **The decision to release/accept the unit is to be made by the attending physician.**

8.10 Procedure Duration, Objectives, & Acceptable Endpoints:

8.10.1 Each collection will be continued until prescribed blood volumes have been processed. Extended collections may be performed if requested by the patient's transplant physician. A BMT physician will see the patient daily and monitor the patient's collections. A physician will be available by pager to respond to any patient problems or patient concerns.

8.10.2 The objective of the Leukapheresis procedure is to harvest an adequate number of stem cells to accomplish adequate engraftment of the hematopoietic system for the HPC transplant recipient after myeloablative therapy. The collections should result in a viable, microbial free, CD34 enriched peripheral mononuclear product.

8.10.3 See prescription for individual collection end points.

8.10.4 If collection and processing end-points are not met, the transplant physician will be notified and remedial plans will be documented in the patient's medical record. The transplant physician or the nurse clinician will communicate with the patient concerning results.

8.11 Review of Collection Records:

8.11.1 Each collection day, the patient's procedure flowsheet is reviewed by the BMT Apheresis Manager for accuracy and completion. Review is documented on the back of the sheet.

8.11.2 Each collection day, the in-patient staff physician performs a patient evaluation and reviews the previous day's collection. This is documented in the patient's electronic medical record.

8.11.3 At the completion of all collections, the BMT staff physician will review the summary of all the collections and documents on F-04 Stem Cell Data Summary Sheet- Allogeneic or F-05 Stem Cell Data Summary Sheet-Autologous whether the processing end-point was met. If the end-point was not met, remedial action taken is to be documented on the summary or in the patient's electronic medical record.

8.11.4 .

8.12 Blood Warmer

8.12.1 The fluid warming set is a single use sterile device, designed to warm blood and intravenous fluid and deliver them the patient's intravenous return site at normothermic temperatures. Refer to C-05 Astotherm Blood Warmer Use with Spectra for instructions.

8.13 Adverse Reactions:

8.13.1 F-09 Harvest – Problem and Adverse Reactions Form must be completed noting any problems or adverse reactions encountered during the harvest. All actions taken and follow-up must be documented on this form. F-09 Harvest – Problem

and Adverse Reactions are to be reviewed by the BMT staff physician within 48 hours and filed in the patient's chart.

8.14 Disposables and Fluid Records:

8.14.1 All disposables and fluids are sterile and checked for integrity prior to use. Records of all lot numbers and expiration dates of all disposables and fluids are recorded on procedure flowsheet.

8.15 Donor Follow-up:

8.15.1 All donors are given post donation instructions.

8.15.2 For all autologous donors: if the patient is in need of care post-collection, they are followed and treated in the BMT clinic. The patient is referred back to the BMT clinic for follow-up regarding any post apheresis concerns or complications.

8.15.3 Follow-up of allogeneic donors is done by the apheresis staff 2-5 days post collection.

8.15.3.1 Documentation is on F-42 Allogeneic Donor Post Collection Follow-up and placed into the follow-up call binder.

8.15.3.2 Follow-up calls are logged in the patient collection log spreadsheet.

8.16 Biohazard Waste:

8.16.1 Biohazard waste must be placed into the appropriate biohazard container, (eg, plastic boxes for sharps, red bags for tubing, etc.) All biohazard waste is disposed of according to KU policy.

8.17 Backup Instrument:

8.17.1 If a backup or extra Spectra is needed due to an instrument malfunction or multiple patients, contact the Inpatient Dialysis Department (8-6069) to request the use of one of their instruments.

8.18 Disaster Preparedness

8.18.1 Refer to SOP G-15 Emergency and Disaster Preparedness

8.19 Cleaning and Sanitation

8.19.1 Environmental Services cleans the floors and removes trash on a daily basis. The COBE Spectra exterior surfaces and the patient's chairs are to be disinfected with Germicidal wipes or other hospital approved and provided cleaner after each procedure.

9. REFERENCES:

9.1 Gee, AP: Bone Marrow Processing and Purging: A Practical Guide. Boca Raton, FL. CRC Press 1991

9.2 Cobe Spectra Apheresis System Operator's Manual. Lakewood, CO. Terumo, BCT

10. APPENDICES: FORMS - LOGS – WORKSHEETS:

10.1 F-01 Harvest and Processing Prescription
10.2 F-09 Harvest – Problem and Adverse Reactions Form
10.3 F-12 Deviation Form
10.4 F-42 Allogeneic Donor Post Collection Follow-up
10.5 Document Control History

Appendix

Links

Please note: links are only correct at time of printing

Linked to Controlled Document

- **SOP**: **G-03: Identification Protocol** *v2.0* (Superseded)
- **SOP**: **P-21: Microbial Contamination** *v1.3* (Superseded)
- **SOP**: **G-09: Transportation of Non-Frozen Blood and Marrow Products to and from an Outside Institution** *v1.7* (Superseded)
- **Policy**: **G-15: Emergency and Disaster Preparedness Policy** *v1.0* (Authorised)
- **Policy**: **G-05: Deviations Policy** *v1.5* (Authorised)
- **SOP**: **C-05: Astotherm Blood Warmer Use** *v1.3* (Authorised)
- **Document**: **F-01: F-01 Harvest and Processing Prescription** *v1.9* (Authorised)
- **Document**: **F-09: F-09 Apheresis - Problem and Adverse Reaction Form** *v1.8* (Authorised)
- **Document**: **F-12: F-12 DEVIATION FORM** *v1.4* (Authorised)
- **Document**: **BMT Apheresis & Cell Processing Lab 511: F-42 Allogeneic Donor Post Collection Follow-up** *v1.0* (Authorised)
- **SOP**: **C-01: Leukapheresis Procedure** *v1.6* (Superseded)
- **SOP**: **G-03: Identification Protocol** *v2.1* (Superseded)
- **SOP**: **P-21: Microbial Contamination** *v1.4* (Authorised)
- **SOP**: **G-03: Identification Protocol** *v2.2* (Authorised)
- **SOP**: **G-09: Transportation of Non-Frozen Blood and Marrow Products to and from an Outside Institution** *v1.8* (Authorised)
- **SOP**: **C-01: Leukapheresis Procedure** *v1.8* (Superseded)

Document Revision History

Superseded on 09-Jan-2015 16:53 by Sunil Abhyankar

Version 1.8 superseded by version 1.9

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Authorized version 1.9 - Revised SOP.. The following users will be notified when a review is due for this document: Dean Merkel

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Reason: Removed the run worksheet and added EMR documentation.

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Version 1.7 superseded by version 1.8

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Authorised version 1.8 - Additional changes due to FACT.. The following users will be notified when a review is due for this document: Shaun Dejarnette, Dean Merkel

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Authorisation request sent to Sunil Abhyankar by Shaun Dejarnette on 15-Aug-2014 13:15.

Authorisation Requested on 15-Aug-2014 13:14 by Shaun Dejarnette

Authorisation request sent to Sunil Abhyankar by Shaun Dejarnette on 15-Aug-2014 13:14.

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Shaun Dejarnette completed task, "Review complete - no changes needed."

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Peer Review tasks were assigned to the following users: Shaun Dejarnette.

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Reason: Links added. New print required.

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Authorised version 1.7 - SOP updated post FACT inspection to include critical reagents, supplies etc and product storage time in Apheresis.. The following users will be notified when a review is due for this document: Shaun Dejarnette, Dean Merkel

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Reason: Updates to procedure post FACT audit.

Updated critical supplies reagents equipment.

Updated storage time in apheresis.

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Shaun Dejarnette completed task, "Reviewed and okayed."

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New SOP created

Peer Review Requested on 19-Nov-2013 11:09 by Kathleen Gaillard

Peer Review tasks were assigned to the following users: Shaun Dejarnette.

This review is to be completed by 27-Nov-2013

THE UNIVERSITY OF KANSAS HOSPITAL

DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

Title:	DETERMINATION OF PERIPHERAL CD34 COUNT
Index:	C-07
Author:	Kathleen Gaillard and Dean Merkel
Department Name:	BMT Apheresis & Cell Processing Lab
Category:	BMT COLLECTION
Document Type:	SOP
Version:	1.2
Document Status:	Authorised
Authorized By:	Sunil Abhyankar
Date Authorized	13-Jan-2014
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C-07 DETERMINATION OF ABSOLUTE PERIPHERAL CD34 COUNT

1. **PRINCIPLE - CLINICAL SIGNIFICANCE**
   1.1. The peripheral blood CD34 cell percentage may be predictive of the total yield of hematopoietic progenitor cells and may be used to determine when to initiate apheresis.
   1.2. Absolute CD34+ cell content of the peripheral blood is determined by performing a CD34 flow analysis on a peripheral blood sample and multiplying the percent CD34(+) by the peripheral blood WBC count.

2. **SPECIMEN:**
   2.1. Peripheral Blood

3. **REAGENTS – SUPPLIES – EQUIPMENT:** n/a

4. **QUALITY CONTROL:** n/a

5. **PROCEDURE**
   5.1. Report count as: CD34 cells / microliter (µl)
   5.2. Examples
   5.2.1. Multiply WBC Count/µl X CD34%
   5.2.1.1. WBC = 20.3 x 10^3 /µl or 20,300 cells/µl
   X 0.15% CD34(+)
   30.45 CD34 (+) cells/µl

   2) WBC = 13.7 x 10^3 /µl
   13,700 cells/µl
   X 0.10% CD34(+)
   13.7 CD34 (+) cells/µl

6. **CALCULATIONS - INTERPRETATION - RESULT REPORTING - NORMAL VALUES - CRITICAL VALUES:** n/a

7. **CALIBRATION – LINEARITY – AMR - CALIBRATION VERIFICATION:** n/a

8. **PROCEDURE NOTES – LIMITATIONS**
   8.1. Suggested peripheral absolute CD34 Count /µl for a successful collection is >20.
   8.2. Counts from 10 – 20 are acceptable to initiate collection.
   8.3. Collections from patients with counts <10 may be poor. However, in patients that have received large doses of chemotherapy previously, counts of 5 –10 may be the highest value reached and may be acceptable to initiate collection. A BMT physician should be consulted in this circumstance.

9. **REFERENCES:** n/a

10. **APPENDICES: FORMS - LOGS – WORKSHEETS:**
    10.1. Document Control History

Appendix

Document Revision History

Document Reviewed on 05-Jan-2015 16:02 by Dean Merkel

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Dean Merkel.

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Shaun Dejarnette verified review task: "" Notes: Reviewed and approved.

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Kathleen Gaillard approved change request: "Re-evaluate righ-sided margins - text runs off the page.
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Change Requested on 07-Jan-2014 17:20 by Shaun Dejarnette

Shaun Dejarnette requested a change: "Re-evaluate righ-sided margins - text runs off the page.
"

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THE UNIVERSITY OF KANSAS HOSPITAL

DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

Title:	Processing and Preparation of HPC or MNC, Apheresis for Freezing
Index:	P-01
Author:	Kathleen Gaillard and Dean Merkel
Department Name:	BMT Apheresis & Cell Processing Lab
Category:	BMT Processing
Document Type:	SOP
Version:	1.6
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P-01 PROCESSING & PREPARATION OF HPC or MNC, APHERESIS PRODUCT FOR FREEZING

1. PRINCIPLE – CLINICAL SIGNIFICANCE

1.1. The peripheral hematopoietic progenitor cells (HPCs) or mononuclear cells (MNCs) are collected and processed for freezing. The goal in this procedure is to concentrate the mononuclear cells collected from a leukapheresis procedure and safely cryopreserve them while maintaining their viability and integrity. Cryopreservation is required to preserve the viable hematopoietic progenitor cells for later transplant following myeloablative or sub-myeloablative therapy or MNCs for other therapies.

1.2. Dimethylsulfoxide (10%) is the cryoprotectant used to reduce the freeze-thaw injury to these cells.

1.3. Cryopreservation specific variables including the concentration of cells frozen, concentration of cryoprotectant, electrolyte medium, and the cryopreservation rate must be consistent to properly preserve cells.

2. SPECIMEN

2.1. Hematopoietic Progenitor Cells or HPC, Apheresis
2.2. Mononuclear Cells or MNC, Apheresis

3. REAGENTS – SUPPLIES - EQUIPMENT

3.1. Centrifuge with large buckets to handle 600 ml transfer packs
3.2. Balance
3.3. Expresser
3.4. Laminar flow hood
3.5. Blood tube sealer
3.6. Control rate freezer with temperature probe
3.7. Freezer canisters and frames
3.8. Freezer racks
3.9. Liquid (nitrogen) storage container
3.10. Cryopreservation freezing bags: 50,250,500 ml size (Origen or Miltenyi)
3.11. Dimethyl sulfoxide (DMSO)
3.12. Plasmalyte A
3.13. 600 mL transfer packs
3.14. 400 mL transfer packs
3.15. 140 cc, 60 cc & 3 cc syringes
3.16. Sterile over-wrap bag
3.17. Centrifuge bag “4R4424”
3.18. Hemostats, scissors, alcohol & betadine pads and marking pens
3.19. Female coupler adapters
3.20. Gloves and 18 gauge blunt fill needles
3.21. Liquid nitrogen
3.22. Sample site couplers
3.23. Sterile culture tubes
3.24. Microbial bottles (aerobic and anaerobic)

4. QUALITY CONTROL: n/a

5. PROCEDURE

5.1. Use sterile technique throughout procedure. Perform all procedures with a biosafety cabinet.

Spike collection bag port with a coupler and remove 2 ml of product with a 3ml syringe. Mix cells well in the bag before removing the 2 ml. Dispense 0.5 ml of the cells from the syringe into microbial culture each bottle for sterility test. Add remaining cells to a culture tube for cell count, viability and flow cytometry assays. The cell count is done in the Hematology laboratory according to their current method.

5.2. Label the appropriate number of freezer bags and canisters with patient name, patient number, patient DOB, date, product id #, contents and any other requirements as outlined in the Identification Protocol (G-03). All label checks must be performed by 2 staff prior to product transfer.

5.3. **QUARANTINE:** Products not meeting eligibility requirements should be stored in a designated quarantine area of the isothermal cryo-storage tanks. Quarantine areas are designated on diagrams posted on the front of the tanks. An inventory list of all quarantine products can be accessed and printed for review.
Note: see Analyzing Components section at the end of the procedure.

5.4. Transfer stem cell collection into a transfer pack. Heat seal and remove the excess tubing.

5.5. The products are double bagged for centrifugation. Place the transfer pack into a sterile over-wrap bag. Then place this into a "4R4424" centrifuge bag.

5.6. Balance the cell product in the Hettich 460RS centrifuge and centrifuge at 2420 rpm for 10 minutes or the Jouan at RPM of 2522) for 10 minutes. The temperature should be set at 20 degrees centigrade.

5.7. During the centrifugation time mix the 10 ml of DMSO and the 30 ml of Plasmalyte A. This can be done through one of the middle ports in the 500 ml cryo-freezer bag that has a 60 ml syringe attached. The plunger is removed from the syringe and the syringe can be used as a measuring device. Close the port with its clamp. Verify lot numbers on the daily processing reagent log.

5.8. Chill mixture in monitored BB refrigerator or between cool packs.

5.8.1. **It is desired to have a final concentration of cells in the cryo-bag of $<3.0 \times 10^8$ cells/ml. If the nucleated cell count is $>1.5 \times 10^{10}$ and $<3.0 \times 10^{10}$, use the above volumes of mixture. This will give a final cell concentration of $<3.0 \times 10^8$ cells/ml.**

5.8.2. **If nucleated cell count is $>3.0 \times 10^{10}$, double the mixture and divide the products to be frozen into 2 - 500 ml cryo-bags. If the cell count is $>6.0 \times 10^{10}$, triple the mixture and divide the products into 3 - 500 ml cryo-bags, etc. This will give a final cell concentration of $<3.0 \times 10^8$ cells/ml.**

5.8.3. **Optional: Use the table below to determine final volume per bag, volume of reagents and size of cryo-bags to use for various total cell counts.**

Total Cell Count	DMSO mls	Plasma Lyte A mls	Cells & Plasma mls	Final Volume mls	Bag Type & Number of Bags	Final Volume mls/bag(s)
$<1.5 \times 10^{10}$	5	15	30	50	1 – 250ml	50
$>1.0 \times 10^{10}$ and $<2.0 \times 10^{10}$	7.5	22.5	45	75	1 – 500ml	75
$>1.5 \times 10^{10}$ and $<3.0 \times 10^{10}$	10	30	60	100	1 – 500ml	100
$>3.0 \times 10^{10}$ and $<6.0 \times 10^{10}$	20	60	120	200	2 – 500ml	100
$>6.0 \times 10^{10}$ and $<9.0 \times 10^{10}$	30	90	180	300	3 – 500ml	100
Optional						
$>3.0 \times 10^{10}$ and $<4.5 \times 10^{10}$	15	45	90	150	2 – 250ml	75

5.9. Also during this time the freezer chamber should be pre-cooled. The instructions for using the freezer are in the procedure on Controlled Rate Freezing.

5.10. Fill out the completion of processing label and canister per Identification Protocol.

5.11. Fill out the stem cell processing worksheet with the necessary information.
5.12. When the product has finished centrifugation, attach a transfer pack to a port. Using a plasma expresser in a biosafety cabinet, expel most of the plasma until approximately 20-30 mls of specimen is left. Clamp line with hemostat. Mix the cell and plasma remaining in the bag thoroughly.
5.13. Spike a port with a plasma transfer coupler and attach a 60 or 140 cc syringe. Draw out the cells and plasma mixture. Unclamp hemostats to the plasma bag and rinse out bag with approximately what is needed to fill the syringe to 62 mls.
5.14. The Plasmalyte A & DMSO mixture should be pre-cooled until the concentrated cells are available.
5.15. Pre-cool the cell product with refrigerated cool packs prior to addition of cells to the pre-cooled freeze medium. The syringe of the concentrated cells is added to the labeled freezer bag containing the DMSO and Plasmalyte A mixture through the second port in the center of the bag and mix well.
5.16. Let the syringe remain attached to the bag. After mixing the contents well, remove all the air in the bag.
5.17. **NOTE: It is important not to delay freezing after the cells have been added to the DMSO mixture. If a delay is over 10 minutes, place the cells-DMSO mixture on cold packs. DMSO may be toxic to the cells at room temperature.**
5.18. Approximately 0.5 mls of this mixture is to remain in the cryobag pigtail and remain attached to the bag to be frozen with the stem cell product. These may be used for further testing if desired.
5.19. Sterility checks are performed at this time. Aseptically add approximately 0.25 - 0.5 ml to each microbial culture bottle for POST PROCESSING sterility.
5.20. The bag is then heat sealed 2-3 times near the base. Check bag for leaks and place in the labeled metal canister. The canister is then placed in the freezer with the sensor probe in place.
5.21. **Control Rate Freezer(CRF)**
  5.21.1. Input patient and product data into the CRF.
  5.21.2. Cool down the freezing chamber before putting in the sample. The controller will hold the freezer at a constant temperature until ready to start freezing the sample
  5.21.3. Pre-cool the freezer CBS
    5.21.3.1. Enter the patient information and start pre-cool as described in the Control rate freezing procedure (P-08 CONTROLLED RATE FREEZING AND STORAGE).
    5.21.3.2. Open precooled freezing chamber and place the canisters in the freezing chamber with temperature probe inserted in the center hole of the canister in contact with the product.
    5.21.3.3. Close chamber door. Press “continue” and the freeze program will continue.
    5.21.3.4. The program will run without assistance until sample temperature reaches -80°C.
5.22. **End of Freeze**
  5.22.1. When the product reaches -80 degrees Celsius it is ready to be put in liquid nitrogen storage tank at <160°C. Press the stop keypad and transfer the cryopreserved product to the storage tank. Quarantine products should be stored in designated locations.
  5.22.2. Record the starting and stopping times of the freeze on the processing worksheet.
  5.22.3. The freezer can be thawed using the warm program. This is not necessary unless the freezer is to be used again shortly.
  5.22.4. The control rate freezing curve should be reviewed for the proper freeze and electronically saved with the patients name, date and type of specimen. Document review on the processing worksheet. **Any disruption in the freezing or abnormal freezing curve should be documented on a Deviation Form.**
  5.22.5. An alphabetical inventory system, see Cryopreserved HPC Inventory Procedure (P-08).

Patient's information should be entered into this system after their components are processed. The assigned inventory numbers, tank number and date should be entered into this system by the patient's name, social security and medical record number. The reinfusion dates should be documented when the specimen is returned to the patient.

5.23. **Analyzing Components**

5.23.1. Testing: All samples for lab testing must be labeled with the donor's name, MR#, date, and product identifier.

5.23.2. Cell Count

5.23.2.1. An aliquot of the peripheral stem cell collection is taken to the Hematology lab and for a cell count. The WBC count result of the sample is multiplied by the milliliters of the collection bag after apheresis. This will give the total nucleated cell count for the harvest. A smear is made and Wright stained. A differential is performed and the total mononuclear cell count is calculated. All documentation of results and calculations are made on the processing worksheet.

5.23.3. Flow Analysis

5.23.3.1. An aliquot of the peripheral cell collection is taken to the Flow Cytometry Lab where a CD34 or other flow analysis is performed.

5.23.3.2. CD34 or other flow analysis is performed and the result is given as a percentage of the total cells. This value is multiplied by the total nucleated cell count and the total CD34+ or CD3+ cell count is determined for that harvest.

5.23.3.3. Divide this result by the patient or recipient's weight in kilograms and the cells / kilogram is calculated. Enter the CD34 results into the Mysis lab computer system. It is ordered and resulted as the test code CD34.

5.23.4. Microbial Detection

5.23.4.1. A sample (0.25 - 0.50 mls) of the collection is added to a microbial vial for sterility testing. The microbial vial is placed in an incubator at 37 degrees Celsius for a maximum of 14 days. The cultured samples are continually monitored for microbial growth. All cultures are sent to and processed by the **Microbiology lab** for tested for organism presence, identification, and sensitivity.

5.23.4.2. The **BMT physician is notified immediately of the positive culture**. Any further action with the patient will be dictated by the physician. Complete a deviation form for any positive cultures.

5.23.4.3. All results and physician notification is documented in the lab computer. This information should also be entered into the patient inventory of the freezer inventory book under the comment section.

6. CALCULATIONS - INTERPRETATION - RESULT REPORTING - NORMAL VALUES - CRITICAL VALUES

6.1. Flow Analysis:

6.1.1. CD34% on the PSC collected sample: >0.10%

6.1.2. Percent recovery of CD34+ cells on a selected manipulated sample: >20%

6.2. Microbial Detection: 100% non-contaminated

6.2.1. **Notify the BMT physician immediately of the positive culture.** Contaminated products do not necessarily need to be destroyed. Decisions regarding the disposition of culture positive HPC's must be made by patient's transplant physician after considering the type of contamination and the potential benefits and the risks from the use of the product. Complete a Deviation Form on positive cultures.

6.3. Viability: viability checks: >90% viable

6.4. Cell counts:

6.4.1. Cell counts are useful in determining the absolute CD34 count, but there are no expected results. Total nucleated cell count results generally range from 1.0 x 10^{10} to 10.0 x 10^{10} cells.

7. CALIBRATION – LINEARITY – AMR - CALIBRATION VERIFICATION: n/a

8. PROCEDURE NOTES - LIMITATIONS

8.1. **Storage of Components Prior to Processing:**

8.1.1. Products may be stored until processing. Components may be kept overnight for next day processing. Except for components held for CD34 selection, processing should be performed within 24 hours. Components held for CD34 selection should be processed within 36 hours, but may be longer in the event of an international product. All components must be stored between 2°-8°C in the Ste m Cell Processing lab's monitored blood bank refrigerator.

8.2. **Disposables and Freezing Mixture Records:**

8.2.1. All disposables and fluids are sterile and checked for integrity prior to use. Record all disposables and fluids used on the processing worksheet. Lot numbers and expiration dates of all disposables and fluids will be listed on the daily supply log.

8.2.2. Only validated freezing bags designed for HPC freezing and storage are to be used.

8.3. **DMSO:**

8.3.1. The most widely used cryoprotectant is dimethyl sulfoxide (DMSO), although it has not been formally been approved by the FDA for this use. DMSO is cited in medical literature as the most common freezing mixture for cryopreserving hematopoietic cells. Adverse reactions to this agent can occur, particularly in the case of HPC transplants, when large volumes are infused. It is recommended that only the purest contaminant-free DMSO be used as a reagent in the preparation of a freezing mixture

8.4. **Plasmalyte A:**

8.4.1. Used as a buffered fluid replacement medium for cryopreservation.

8.5. **Biohazard Waste:**

8.5.1. All hazardous materials must be disposed of as directed by the institution's hazardous waste plan. Biohazard waste should be placed into the appropriate biohazard container, (eg, plastic boxes for sharps, red bags for tubing, etc.).

8.6. **NOTE: Products must never be filtered with a leuko-reduction filter or irradiated.**

8.7. **NOTE:** Document any encountered deviations, processing problems, or discrepancies on F-12 Deviation Form. This completed form should be brought to the attention of the Medical Director for review. A copy of the completed form should be placed into the patient's Cell Processing chart for future reference in the event of a component delay or failure.

8.8. **NOTE:** All units will be kept indefinitely unless patient expires. Units from expired patients will be discarded per BMT physician's approval by the hospital's biohazard waste removal procedure. Refer to P-15 Cryopreserved Hematopoietic Progenitor Cells Disposal.

8.9. **LIMITATIONS**

8.9.1. Final cell concentration of product to be cryopreserved should be $\leq$3.0 x 10^{8} cells/ml.

8.9.2. Toxicity has been reported to be associated with the use of DMSO cryoprotectant. Reducing the amount of DMSO required is desirable. By reducing the volume of component to be frozen, we are reducing the volume of cryoprotectant needed.

8.9.3. All ABO discrepancies must be resolved prior to release of products and the staff physician must be notified.

9. REFERENCES

9.1. Gorin NC: Cryopreservation and storage of stem cells, in Areman, Deeg, Sacher (eds): Bone Marrow and Stem Cell Processing: A Manual of Current Techniques. Philadelphia, PA. Davis, 1992.

9.2. Gee, AP: Bone Marrow Processing and Purging: A Practical Guide. Boca Raton, FL. CRC Press 1991.

9.3. Hematopoietic Cell Transplantation, Second Edition, E.D. Thomas, K.G. Blume, S.J. Forman, Blackwell Science, Inc., Malden, MA, 1999.

10. APPENDICES: FORMS - LOGS – WORKSHEETS

10.1. F-02 Peripheral Stem Cell Processing Worksheet

10.2. F-12 Deviation Form

10.3. Document Control History

Appendix

Links

Please note: links are only correct at time of printing

Linked to Controlled Document

- **SOP**: **P-21: Microbial Contamination** *v1.4* (Authorised)
- **SOP**: **P-06: Preparation of Bone Marrow for Freezing** *v1.6* (Authorised)
- **SOP**: **P-01: Processing and Preparation of HPC, Apheresis for Freezing** *v1.5* (Superseded)

Document Revision History

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New SOP created

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Peer Review tasks were assigned to the following users: Shaun Dejarnette.
This review is to be completed by 03-Jan-2014

APPENDIX D Guidance For Use Of Heparin And Mozobil In Performing Large Volume Apheresis

Indications And Usage Of Heparin Anticoagulant

A) Heparin may be used as an anticoagulant additive to ACDA on patients having a stem cell collection procedure in certain instances. Guidelines for heparin usage are to be determined on each patient by CD34 assessment and a clinical assessment.
   1) For patients on anticoagulant therapy, i.e., Coumadin, Lovenox, etc, do not use heparin as an anticoagulant.
   2) For patients with heparin induced antibodies, do not use heparin as an anticoagulant.
   3) All large volume collections procedures are dependent upon patient's tolerance for procedure and other clinical/medical conditions, i.e., platelet counts, hypervolemia, dialysis, etc.

Circulating Peripheral Cd34 Count And Mozobil® Usage

A) Patients needing cells for one or multiple peripheral stem cell transplants. 2.5 x $x10^6$ CD34/Kg is the desired goal for each transplant.
   1) If the CD34 count is **less than 5 cells/ul**, do not perform collection
      a. Give 10 mcg/kg G-CSF now.
      b. Administer Mozobil® that evening.
      c. Perform another peripheral CD34 count on the following morning.
      d. If on **second day the CD34 count is less than 5 cells/ul**, do not perform collection.
      e. The physician is to advise on the plan which may include the continuation of Mozobil®.
   2) If the CD34 count is **greater than 5 and less than 10 cells/ul**, do not perform collection
      a. Give G-CSF now.
      b. Administer Mozobil® that evening.
      c. G-CSF will also be given 1 hour pre-collection the following morning, if the pre-collection WBC count is < 65K.
      d. Perform a larger volume collection (approximately 5 - 6 blood volumes) utilizing heparin/ACDA as the anticoagulant. Each blood volume processed can reduce the patient's platelet count by 10%.
   3) If the CD34 count is **greater than 10 cells/ul,** Perform collection as in 2)d. above.
   4) The provider has the discretion to perform a collection outside of this algorithm.

Post-Collection Results And Mozobil Usage

A) If on the first day of collection the collected product contains LESS THAN 65% of the desired dose, use Mozobil® that evening. G-CSF will also be given 1 hour pre-collection the following morning if the WBC count is <65,000.

B) Goal	**65% of goal**
2.5 x 10^6 CD34/Kg	(<1.63 x 10^6 CD34/Kg)
5.0 x 10^6 CD34/Kg	(<3.25 x 10^6 CD34/Kg)
7.5 x 10^6 CD34/Kg	(<4.88 x 10^6 CD34/Kg)

Mozobil Dosage And Administration

1) Notify BMT Pharmacist. To reach correct pharmacist call BMT Triage Nurse at **913-588-9821**. The triage nurse will direct the call to the pharmacist.
2) BMT Pharmacist to consult and write order for use of Mozobil®.
3) Repeat Mozobil® dose up to 4 consecutive days.
4) Select dose based on 0.24 mg/kg actual body weight (0.012 X patient's actual body weight in kg = volume to be administered in mL).
5) Maximum dose is 24 mg or 1 vial. (1.2 mL of a 20 mg/mL solution).
6) Administer by subcutaneous injection approximately 10 - 14 hours prior to initiation of apheresis.
7) Renal impairment: If creatinine clearance is ≤ 50 mL/min, decrease dose by one-third to 0.16 mg/kg.

Adverse Reactions

A) Most common adverse reactions (≥ 10%): diarrhea, nausea, fatigue, injection site reactions, headache, arthralgia, dizziness, and vomiting.

APPENDIX E Standard Operating Procedures – Cytoplasmic Staining

CYTOPLASMIC STAINING PROCEDURE

1. PRINCIPLE/CLINICAL SIGNIFICANCE

   A. Principle

   This procedure facilitates antibody access to intracellular structures and leaves the morphological scatter characteristics of the cells intact. There are several antigens that are expressed predominantly within the cell that have excellent sensitivity and specificity for lymphoid or myeloid differentiation. Nuclear terminal deoxynucleotidyl transferase (nTdT), myeloperoxidase (MPO), BCL-2, Zap-70, CD22, CD3, CD79a, pan-Cytokeratin, and immunoglobulin determination are commonly tested using this procedure.

   B. Clinical Significance

   Intracellular markers are useful in the identification and classification of acute leukemias, and in the prognostication of chronic lymphocytic leukemia.

   - nTdT marks immature B and T cells
   - cyCD3 marks immature T cells
   - cyCD22 marks immature B cells
   - MPO marks myeloid cells
   - cyCD79a marks immature and mature B cells
   - pan-Cytokeratin marks small cell carcinomas
   - Cytoplasmic immunoglobulin marks plasma cell neoplasms
   - ZAP-70 is a prognostic indicator in chronic lymphocytic leukemia
   - BCL-2 is differentiates between reactive hyperplasic and follicular lymphoma.

2. SPECIMEN

   A. Specimen Type

   1. Peripheral Blood[1,2]
      - EDTA specimens are viable up to 24 hours at room temperature.
      - Heparin specimens are viable up to 48 hours at room temperature.
      - ACD specimens are viable up to 72 hours at room temperature.
      - Transport specimen in biohazard transport bag and hold at room temperature (18-25ºC).
      - Do not refrigerate.
   2. Bone Marrow Aspirate
      - 1-2mL of bone marrow in a heparinized syringe or collection tube at room temperature
      - Transport specimen in biohazard transport bag and hold at room temperature (18-25ºC).
      - Do not refrigerate.
   3. Bone Marrow Biopsies
      - If bone marrow procedure was a dry tap, the bone marrow biopsy can be processed for flow.

- Place into RPMI or saline and transport in a biohazard transport bag and hold at room temperature (18-25ºC).
- If biopsy is in saline, replace the saline with RPMI immediately and hold until processing.
- Do not refrigerate.

4. Fluids – CSF, pleural, bronchial lavage, etc.
   - Natural cellular suspension or supplemented with heparin in a collection or test tube.
   - Transport specimen in a biohazard transport bag and hold at room temperature (18-25ºC).
   - Do not refrigerate.
5. Tissue – lymph node, spleen, other soft tissue, etc.
   - Tissue or cellular suspension held in supplemented RPMI.
   - Note: Mince tissue and express through wire mesh screen to dislodge cells.
   - Transport specimen in biohazard transport bag and hold at 2-8ºC.[1]
6. Post Stem Cell Apheresis Collection Product (PSC).

B. Specimen Rejection
   1. Incomplete data or patient identification on label. Pathologist will be consulted. Test will be canceled, if appropriate, in LIS and clinician will be notified.
   2. Improperly collected specimen: clotted, auto-lysed, cracked or leaking tube. Indicate if clot present. Pathologist will be consulted. Test will be canceled, if appropriate, in LIS and clinician will be notified.
   3. Quantity not sufficient to perform test. Pathologist will be consulted. Test will be canceled, if appropriate, in LIS and clinician will be notified.
   4. Peripheral blood
      - EDTA specimens greater than 24 hours old.
      - Heparin specimens greater than 48 hours old.
      - ACD specimens greater than 72 hours old.

      If peripheral blood is greater than the designated acceptable range, then a viability will be performed and reported. A text box will be added to the FCS Express layout informing the pathologist of the viability and age of the specimen. The reporting is at the discretion of the pathologist.
   5. Tissue and Body Fluid specimens that have a suboptimal viability of < 50%. A text box will be added to the FCS Express layout informing the pathologist of the viability and reporting is at the discretion of the pathologist.
   6. CSF specimens greater than 4 days old will have a text box added to the FCS Express layout informing the pathologist of the age of the specimen and reporting is at the discretion of the pathologist.
   7. Inappropriate orders: The ordering physician will be contacted to verify the correct order and documentation will be included when the test is canceled.

3. REAGENTS/SUPPLIES/EQUIPMENT

A. Reagent Handling and Storage
   1. Conjugated Monoclonal Antibodies
      - Store at 2-8ºC.
      - Do not freeze.
      - Stable until the expiration listed on the bottle.

- Use according to manufacturer's instructions or validated procedure.
- Contains sodium azide to inhibit bacteria growth. Chronic exposure has shown to have mutagenic effects. PPE should be worn at all times. Wash thoroughly after handling.
- Sodium azide is harmful if swallowed. Wear protective clothing. If swallowed, seek medical advice immediately and show container or label. Contact with acid liberates very toxic gas. Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

2. Fix and Perm Cell Permeabilization Reagents
   - Invitrogen Corporation, Cat# GAS-003
   - Store at room temperature.
   - Stable until expiration on kit.
   - Reagent A contains Formaldehyde.
   - Precautions: Formaldehyde is toxic, allergenic and a suspected carcinogen. Avoid contact with eyes, skin and clothing.
   - In case of contact, rinse immediately with plenty of water and contact physician.
3. RPMI 5% FBS with Heparin
   - Cardinal Health, Catalog No. M7201-56
   - Store at 2-8ºC.
   - Fetal Bovine Serum (FBS) – Sigma Aldrich, Catalog No. F4135-500mL
     - When received, thaw and aliquot out 50mL conical tubes of FBS. Label and refreeze. Store in freezer until outdate.
   - Penicillin/Streptomycin – Sigma Aldrich, Catalog No. P0781-20mL
     - Store in Freezer until outdate.
   - Heparin – 1,000 USP units/mL, 2mL vial
     - Store at room temperature.
     - Obtain from Bone Marrow bench.
   - Prepare using sterile technique; remove 35mL of RPMI and add 25mL of thawed FBS, 10mL of thawed Pen/Strep solution, and one vial of heparin to the RPMI. Store media in refrigerator. Stable for 6 months or until pH has changed. pH change is indicated when the salmon colored media has changed to a yellow or pink solution.
   - Store at 4ºC for 6 months.
   - May cause irritation to skin and eyes. Wash with large amounts of water (eye wash). Call a physician if necessary.
   - Use appropriate PPE: gloves, lab coat, and protective eye wear.
4. Dulbecco's Phosphate Buffered Saline (PBS) (10X)
   - Thermo Scientific, Catalog No. SH30378.02
   - Store 15-30ºC.
   - Stable until the expiration listed on the bottle.
   - Dilute to 1X prior to use with DI water.
   - pH the 1X solution with pH indicator strips. It should fall within the range of 7.2-7.4. Adjust as necessary with 1N NaOH or 1N HCl.
   - Expiration is one month from date of preparation.
   - Irritant to eyes, respiratory system, and skin. In case of contact, rinse immediately with plenty of water.
5. RBC Lysis Buffer (10X)

- BioLegend, Catalog No. 420301.
- Store at 2-8ºC.
- Stable until the expiration listed on the bottle.
- Measure 100mL of RBC Lysis Buffer. QS to 1L with DI water.
- pH the 1X solution with pH indicator strips. It should fall within the range of 7.1-7.4. Adjust as necessary with 1N NaOH or 1N HCl.
- Warm the 1X solution to room temperature prior to use.
- Stable for one month.

6. 1N NaOH
   - Sigma, Catalog No. S2770
   - Store at room temperature.
   - Irritant to eyes, respiratory system, and skin. In case of contact, rinse immediately with plenty of water and contact physician.
7. 1N HCl
   - Sigma, Catalog No. H9892
   - Store at room temperature.
   - Irritant to eyes, respiratory system and skin. In case of contact, rinse immediately with plenty of water and contact physician.
8. BD FACS Flow
   - BD Biosciences, Catalog No. 342003
   - Store at room temperature.
   - Stable until the expiration date listed on box.
9. BD FACS Clean
   - BD Bioscience, Catalog No. 340345
   - Store at room temperature.
   - Stable until the expiration date listed on box.
10. DI Water – Reagent Grade Type 1 Water
    - Ricca Chemical Co., Catalog No. 9150-5
    - Store unopened until expiration date listed on box.
    - After opening, use within 30 days.
    - Store at room temperature.
11. Bleach Concentrate (6% sodium hypochlorite)
    - Store at room temperature.
    - Corrosive: Irritant to respiratory tract, skin, and eyes (flush with plenty of water – use eye wash/safety shower). Ingestion causes nausea and vomiting. Call a physician.
    - Use appropriate PPE: glove, lab coat, and protective eye wear.
12. Bleach-Rite
    - Fisher Scientific, Catalog No. 14-412-53.
    - Store at room temperature. Do not expose to excessive heat or sunlight or UV light or else bleach efficacy may deteriorate.
    - 1:10 dilution of bleach and detergent.
    - Stable until expiration date.
    - Irritant to eyes. Rinse with copious amounts of water for 15-20 minutes.
    - Use appropriate PPE: glove, lab coat, and protective eye wear.

B. Materials and Equipment
   1. 12x75mm polystyrene test tubes
   2. Pipettes (20uL, 200uL, 1000uL)

3. Pipette tips
4. Repeat pipettor
5. Disposable transfer pipettes – large and small tipped
6. Test tube rack
7. 17x100mm test tubes
8. Petri dish
9. Tissue sieve
10. 10mL syringe plunger
11. Scalpel and/or surgical scissors
12. 100um cell strainer
13. pH indicator strips
14. Centrifuge
15. Vortex mixer
16. Timer
17. Mechanical rocker
18. BD FACS Lyse Wash Assistant – Serial No. K14610025
19. BD FACSCanto II – Serial No. V96300795, Serial No. V33896201741
    - Laser safety: The FACSCanto II flow cytometer is a Class I laser product. The laser is fully contained within the structure and calls for no special work area safety requirements. Nevertheless, regulations require that a warning be posted to avoid tampering with the instrument. The protective shields surrounding the laser source should never be removed or altered except by the technical service personnel. Removal of any of these safety shields would place the operator at risk of scattered or direct exposure to laser radiation.

4. QUALITY CONTROL

A. BD Cytometer Setup and Tracking Beads - Catalog No. 641319
   - Store the bead vial at 2-8^{0}C
   - Protect from light.
   - Do not freeze.
   - Dilute one drop of CS&T Beads with 450ul of BD FACSFlow reagent.

B. Leukemia/Lymphoma QC panel - CD-Chex Plus
   - Stored at 2-8ºC. Do not freeze.
   - Once open, tubes are good for 30 days.
   - A positive control panel is run twice a month. Control results should fall within control ranges. If control(s) are out of range, the test system must be evaluated for source of error. Determine cause by evaluating and troubleshooting. Repeat, remake, and/or check them. Note in the "Comments" section of the CD-Chex Plus QC Log any corrective action that has occurred.
   - All control specimens must be tested in the same manner and by the same personnel as patient samples.
   - QAP monthly report compares KU data to QC data reported by peer lots. Results reviewed against group results for precision accuracy. Investigate when SDIs and CV are >2.0.

C. BD CompBeads – Anti-Mouse Ig/k Negative Control Compensation Particles Set. This panel is run weekly or whenever there has been service on the instrument.

D. Gating Controls: Population of interest by light scatter and anti-leukocyte antibody (CD45).

E. pH Control Log – pH checks of reagents should be recorded on the monthly pH control log.

5. PROCEDURE

A. Label each tube with the antibodies per panel, along with two patient identifiers.
B. Pipette appropriate volume of adjusted cell suspension into each tube (50uL).
C. Pipette appropriate volume of the conjugated antibodies directed to the cell surface markers of interest into the appropriate tubes.
D. Vortex and incubate for 15 mins in the dark at room temperature.
E. Add 100uL of Reagent A (Fixative Medium).
F. Vortex each tube for 2-3 seconds and incubate at room temperature for 15 minutes.
G. Wash once in 2 mL of PBS.
H. Centrifuge the tubes for 3 mins at 400xG or 1600 rpms and decant supernatant.
I. Vortex until cell pellet is fully resuspended.
J. Add 100uL of Reagent B (Permeabilization Medium) and recommended volume of the conjugated intracellular antibodies.
K. Vortex 1-2 seconds and incubate for 20 minutes at room temperature in the dark.
L. Wash twice in 2 mL of PBS.
M. Centrifuge for 3 minutes at 400xG or 1600 rpms and decant supernatant.
N. Resuspend cells in 0.5mL of PBS.
O. Store at 2-8ºC in the dark until panel is run.
P. Acquire data on the BD FACSCanto II with the DIVA software.
Q. Analyze data with the FCS Express software off-line.

6. CALCULATIONS/INTERPRETATION/RESULT REPORTING/NORMAL VALUES/CRITICAL VALUES

A. The results are analyzed by the flow cytometry technologists and delivered to the pathologist for interpretation. The pathologist will complete the final report and notify the physician if indicated.

7. CALIBRATION/LINEARITY/AMR/CALIBRATION VERIFICATION

A. Instrument calibration is performed by BD Technical Service on a semi-annual basis.
B. Application Settings are performed after every major maintenance or service issue and performed after a new CS&T baseline is established.
C. Compensation is performed on weekly basis.

8. PROCEDURE NOTES/LIMITATIONS

A. There are multiple components within the reagent kit. The laboratory only uses within kit lot components of reagents unless otherwise specified by the manufacturer.
B. DO NOT USE REAGENTS IF A PRECIPITATE FORMS OR DISCOLORATION OCCURS.

C. Reagents are stable for the period shown on the package label when stored as directed.
D. If the CS&T beads do not pass, the instrument should not be operated. Refer to the troubleshooting information in the BD Cytometer Setup and Tracking Application Guide.
E. When performing a Zap-70, if a defined population of T-cells is not present, or if a Zap-70 negative population is not present, a patient control must be done.
F. If instruments are inoperable:
   1. For analyzer problems, call BD Bioscience at 1-877-232-8995 and log the problem in the Action Log with a reference number.
   2. If one instrument is down, use the other instrument only and contact technical service.
   3. If both instruments are down the hematology supervisor or medical staff on call for hematology should be notified at this time.
   4. All testing should be sent to St. Luke's by courier. Call the Flow Lab at St. Luke's at 816-932-6265 to let them know we are sending specimens.
   5. Have St. Luke's fax flow cytometry requisition with indication of necessary information to be filled in.
   6. Use St. Luke's courier until 7:00 pm (816-932-3850). After hours use KU's courier, Quicksilver (321-5959).
   7. Follow reference lab's protocol for collection and handling of specimens.

9. REFERENCES

1. Stewart, C. C., Nicholson, J.K.A. "Immunophenotyping." Page 181-182. New York: A John Wiley & Sons, Inc., 2000. Print.
2. Hawley, T. S., Hawley, R. G. "Flow Cytometry Protocols." Third Edition. Pages 297 and 301. New York: Humana Press. Print.
3. Keren, D F.., McCoy Jr., J. Phillip, Carey, J. L. "Flow Cytometry in Clinical Diagnosis." 4th Edition, ASCP Press, 2007.
4. Gorczyca, W. "Flow Cytometry in Neoplastic Hematology." United Kingdom: Taylor & Francis.
5. Cornfield, D; Liu, Z; Gorczyca, W; Weisberger, J. "The potential role of flow cytometry in the diagnosis of small cell carcinoma."
6. Archives in Pathology & Laboratory Medicine, Vol. 127, April 2003.
7. Invitrogen Corporation – Fix & Perm Cell Permeabilization Reagent package Insert.
8. BD FACSCanto II Instructions For Use, #644450, April 2009.

10. APPENDICES

A. Attachments
   1. pH Log
B. Bench Manual
   1. Flow Cytometry panels

SOP Version 1.3
Added to Protocol Version 2.0

APPENDIX F Multiparametric Flow Cytometry (MFC) – Validation Model Multiple Myeloma (MM)-Minimal Residual Disease (MRD)

2015-IIT-BMT-MM-AutoSCT

Multiparametric Flow Cytometry (MFC) - Validation Model - MM-MRD

MM MRD Panel

Tube #	Name	FITC	PE	PerCP5.5	PE-Cy7	APC	APC-H7	v450-PB	v500c
1	MM1	27	117	138	19	38	45	56	20 BV510
2	MM2	81	28	138		38	45		
4	cyMM	cyKappa	cyLambda	138	19	38	45		

APPENDIX G 8-Color Leukemia / Lymphoma Immunophenotyping Processing Procedure

1. PRINCIPLE /CLINICAL SIGNIFICANCE

   A. Principle of the Procedure

   Immunophenotypic analysis involves the identification and classification of the cells by the presence of surface antigens or intracellular antigens, which are identified by fluorochrome-labeled monoclonal antibodies directed against those antigens. Panels of antibodies are utilized to determine the origin and stage of differentiation of hematopoietic neoplasms including acute and chronic leukemias and lymphomas.

   B. Clinical Significance

   Immunophenotypic analysis by flow cytometry is routinely utilized for diagnostic and therapeutic decision making for types of acute and chronic leukemias and lymphomas.

2. SPECIMEN

   A. Specimen Type

   1. Peripheral Blood[1,2]
      - EDTA specimens are viable up to 24 hours at room temperature.
      - Heparin specimens are viable up to 48 hours at room temperature.
      - ACD specimens are viable up to 72 hours at room temperature.
      - Transport specimen in biohazard transport bag and hold at room temperature (18-25^{o}C).
      - Do not refrigerate.
   2. Bone Marrow Aspirate
      - 1-2mL of bone marrow in a heparinized syringe or collection tube at room temperature
      - Transport specimen in biohazard transport bag and hold at room temperature (18-25^{o}C).
      - Do not refrigerate.
   3. Bone Marrow Biopsies
      - If bone marrow procedure was a dry tap, the bone marrow biopsy can be processed for flow.
      - Place into RPMI or saline and transport in a biohazard transport bag and hold at room temperature (18-25^{o}C).
      - If biopsy is in saline, replace the saline with RPMI immediately and hold until processing.
      - Do not refrigerate.
   4. Fluids – CSF, pleural, bronchial lavage, etc.
      - Natural cellular suspension or supplemented with heparin in a collection or test tube.
      - Transport specimen in a biohazard transport bag and hold at room temperature (18-25^{o}C).
      - Do not refrigerate.

5. Tissue – lymph node, spleen, other soft tissue, etc.
   - Tissue or cellular suspension held in supplemented RPMI.
   - Note: Mince tissue and express through wire mesh screen to dislodge cells.
   - Transport specimen in biohazard transport bag and hold at 2-8°C.[1]
6. Post Stem Cell Apheresis Collection Product (PSC).

B. Specimen Rejection
   1. Incomplete data or patient identification on label. Pathologist will be consulted. Test will be canceled, if appropriate, in LIS and clinician will be notified.
   2. Improperly collected specimen: clotted, auto-lysed, cracked or leaking tube. Indicate if clot present. Pathologist will be consulted. Test will be canceled, if appropriate, in LIS and clinician will be notified.
   3. Quantity not sufficient to perform test. Pathologist will be consulted. Test will be canceled, if appropriate, in LIS and clinician will be notified.
   4. Peripheral blood
      - EDTA specimens greater than 24 hours old.
      - Heparin specimens greater than 48 hours old.
      - ACD specimens greater than 72 hours old.

      If peripheral blood is greater than the designated acceptable range, then a viability will be performed and reported. A text box will be added to the FCS Express layout informing the pathologist of the viability and age of the specimen. The reporting is at the discretion of the pathologist.
   5. Tissue and Body Fluid specimens that have a suboptimal viability of < 50%. A text box will be added to the FCS Express layout informing the pathologist of the viability and reporting is at the discretion of the pathologist.
   6. CSF specimens greater than 4 days will have a text box added to the FCS Express layout informing the pathologist of the age of the specimen and reporting is at the discretion of the pathologist.
   7. Inappropriate orders: The ordering physician will be contacted to verify the correct order and documentation will be included when the test is canceled.

3. REAGENTS/SUPPLIES/EQUIPMENT

A. Reagent Handling and Storage
   1. Conjugated Monoclonal Antibodies
      - Store at 2-8°C.
      - Do not freeze.
      - Stable until the expiration listed on the bottle.
      - Use according to manufacturer's instructions or validated procedure.
      - Contains sodium azide to inhibit bacteria growth. Chronic exposure has shown to have mutagenic effects. PPE should be worn at all times. Wash thoroughly after handling.
      - Sodium azide is harmful if swallowed. Wear protective clothing. If swallowed, seek medical advice immediately and show container or label. Contact with acid liberates very toxic gas. Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

2. Propidium Iodide
   - Sigma, Catalog No. P4170
   - Store at 2-8ºC.
   - Dissolve 0.005g into 500mL PBS. Store at 2-8ºC.
   - Prepared PI is stable for 2 years.
   - PI is known to show possible mutagenic effects. PPE should be worn at all times. Wash thoroughly after handling.
3. RPMI 5% FBS with Heparin
   - Cardinal Health, Catalog No. M7201-56
   - Store at 2-8ºC.
   - Fetal Bovine Serum (FBS) – Sigma Aldrich, Catalog No. F4135-500mL
     - When received, thaw and aliquot out 50mL conical tubes of FBS. Label and refreeze. Store in freezer until outdate.
   - Penicillin/Streptomycin – Sigma Aldrich, Catalog No. P0781-20mL
     - Store in Freezer until outdate.
   - Heparin – 1,000 USP units/mL, 2mL vial
     - Store at room temperature.
     - Obtain from Bone Marrow bench.
   - Prepare using sterile technique; remove 35mL of RPMI and add 25mL of thawed FBS, 10mL of thawed Pen/Strep solution, and one vial of heparin to the RPMI. Store media in refrigerator. Stable for 6 months or until pH has changed. pH change is indicated when the salmon colored media has changed to a yellow or pink solution.
   - Store at 4ºC for 6 months.
   - May cause irritation to skin and eyes. Wash with large amounts of water (eye wash). Call a physician if necessary.
   - Use appropriate PPE: gloves, lab coat, and protective eye wear.
4. Dulbecco's Phosphate Buffered Saline (PBS) (10X)
   - Thermo Scientific, Catalog No. SH30378.02
   - Store 15-30ºC.
   - Stable until the expiration listed on the bottle.
   - Dilute to 1X prior to use with DI water.
   - pH the 1X solution with pH indicator strips. It should fall within the range of 7.2-7.4. Adjust as necessary with 1N NaOH or 1N HCl.
   - Expiration is one month from date of preparation.
   - Irritant to eyes, respiratory system, and skin. In case of contact, rinse immediately with plenty of water.
5. RBC Lysis Buffer (10X)
   - BioLegend, Catalog No. 420301.
   - Store at 2-8ºC.
   - Stable until the expiration listed on the bottle.
   - Measure 100mL of RBC Lysis Buffer. QS to 1L with DI water.
   - pH the 1X solution with pH indicator strips. It should fall within the range of 7.1-7.4. Adjust as necessary with 1N NaOH or 1N HCl.
   - Warm the 1X solution to room temperature prior to use.
   - Stable for one month.

6. 1N NaOH
   - Sigma, Catalog No. S2770
   - Store at room temperature.
   - Irritant to eyes, respiratory system, and skin. In case of contact, rinse immediately with plenty of water and contact physician.
7. 1N HCl
   - Sigma, Catalog No. H9892
   - Store at room temperature.
   - Irritant to eyes, respiratory system and skin. In case of contact, rinse immediately with plenty of water and contact physician.
8. BD FACS Flow
   - BD Biosciences, Catalog No. 342003
   - Store at room temperature.
   - Stable until the expiration date listed on box.
9. BD FACS Clean
   - BD Bioscience, Catalog No. 340345
   - Store at room temperature.
   - Stable until the expiration date listed on box.
10. DI Water – Reagent Grade Type 1 Water
    - Ricca Chemical Co., Catalog No. 9150-5
    - Store unopened until expiration date listed on box.
    - After opening, use within 30 days.
    - Store at room temperature.
11. Bleach Concentrate (6% sodium hypochlorite)
    - Store at room temperature.
    - Corrosive: Irritant to respiratory tract, skin, and eyes (flush with plenty of water – use eye wash/safety shower). Ingestion causes nausea and vomiting. Call a physician.
    - Use appropriate PPE: glove, lab coat, and protective eye wear.
12. Bleach-Rite
    - Fisher Scientific, Catalog No. 14-412-53.
    - Store at room temperature. Do not expose to excessive heat or sunlight or UV light or else bleach efficacy may deteriorate.
    - 1:10 dilution of bleach and detergent.
    - Stable until expiration date.
    - Irritant to eyes. Rinse with copious amounts of water for 15-20 minutes.
    - Use appropriate PPE: glove, lab coat, and protective eye wear.

B. Materials and Equipment
   1. 12x75mm polystyrene test tubes
   2. Pipettes (20uL, 200uL, 1000uL)
   3. Pipette tips
   4. Repeat pipettor
   5. Disposable transfer pipettes – large and small tipped
   6. Test tube rack
   7. 17x100mm test tubes
   8. Petri dish
   9. Tissue sieve
   10. 10mL syringe plunger
   11. Scalpel and/or surgical scissors

12. 100um cell strainer
13. pH indicator strips
14. Centrifuge
15. Vortex mixer
16. Timer
17. Mechanical rocker
18. BD FACS Lyse Wash Assistant – Serial No. K14610025
19. BD FACSCanto II – Serial No. V96300795, Serial No. V33896201741
    - Laser safety: The FACSCanto II flow cytometer is a Class I laser product. The laser is fully contained within the structure and calls for no special work area safety requirements. Nevertheless, regulations require that a warning be posted to avoid tampering with the instrument. The protective shields surrounding the laser source should never be removed or altered except by the technical service personnel. Removal of any of these safety shields would place the operator at risk of scattered or direct exposure to laser radiation.

4. QUALITY CONTROL

A. BD Cytometer Setup and Tracking Beads - Catalog No. 641319
   - Store the bead vial at 2-8^{0}C
   - Protect from light.
   - Do not freeze.
   - Dilute one drop of CS&T Beads with 450ul of BD FACSFlow reagent.

B. Leukemia/Lymphoma QC panel - CD-Chex Plus
   - Stored at 2-8^{o}C. Do not freeze.
   - Once open, tubes are good for 30 days.
   - A positive control panel is run twice a month. Control results should fall within control ranges. If control(s) are out of range, the test system must be evaluated for source of error. Determine cause by evaluating and troubleshooting. Repeat, remake, and/or check them. Note in the “Comments” section of the CD-Chex Plus QC Log any corrective action that has occurred.
   - All control specimens must be tested in the same manner and by the same personnel as patient samples.
   - QAP monthly report compares KU data to QC data reported by peer lots. Results reviewed against group results for precision accuracy. Investigate when SDIs and CV are >2.0.

C. BD CompBeads – Anti-Mouse Ig/k, Negative Control Compensation Particles Set. This panel is run weekly or whenever there has been service on the instrument.

D. Gating Controls: Population of interest by light scatter and anti-leukocyte antibody (CD45).

E. pH Control Log – pH checks of reagents should be recorded on the monthly pH control log.

5. PROCEDURE

A. Manual Method:

1. Concentrate cellular elements of the specimen by transferring an aliquot (1-2mL) of well-mixed sample to a 17x100mm tube.
   - If BM specimen, break up spicules with a small tipped pipette.
   - If tissue specimen, use tissue sieve and rubber tipped syringe plunger to gently dissociate.
2. Add 5% FBS RPMI and centrifuge at 400g or 1600rpms for 5 minutes to wash the cells.
3. If tissue or fluid specimen, proceed to step 9.
4. Aspirate the supernatant.
5. Add 10mL of 1X RBC Lysis Buffer to specimen and place on mixer for 10 minutes.
6. Centrifuge at 400g or 1600 rpms for 5 minutes.
7. Decant supernatant. Note: If incomplete lysis has occurred – repeat steps 4-6.
8. Add PBS to specimen to wash cells. Centrifuge at 400g or 1600 rpms for 5 minutes.
9. Decant supernatant and resuspend the cells in 1 mL of 5% FBS RPMI.
10. Obtain a cell count using CountBright Beads (see appropriate procedure). Adjust the concentration to 10.0 x 10^6 WBC/mL (working solution).
11. Pipette the appropriate antibodies into labeled tubes.
12. Add 100uL of working solution of cells to the antibody panel.
    - If setting up any MRD panel, use 200uL of working solution if the cell concentration is < 10.0 x 10^6 WBC/mL.
13. Incubate the panel for 15 to 30 minutes in the dark at room temperature.
14. If tissue or fluid specimen, add 2 mLs of RBC Lysis Buffer and incubate in the dark for 10 minutes. Centrifuge at 400g or 1600 rpms for 3 minutes. Decant tubes.
15. Add 2 mL of PBS to each tube and vortex gently.
16. Centrifuge at 400g or 1600 rpms for 3 minutes.
17. Decant supernatant and add 0.5 mL of PBS to each tube and gently vortex.
18. Remove the PI viability tube (if appropriate) and place 1 mL of Propidium Iodide into the PI tube. Vortex and place back into carousel rack. PI will incubate while the panel is running.
19. Run the panel on the BD FACSCanto II using the Diva Software. Gate on the CD45 bright population and/or population of interest. Analyze data using FCS Express software.
20. After analysis, tubes can be disposed of in a biohazard container.
21. Original specimen is kept at room temperature for 7 days.

B. Lyse Wash Assistant Method (LWA):

1. Concentrate cellular elements of the specimen by transferring an aliquot (1-2 mL) of well-mixed sample to a 17 x 100mm tube.
   - if BM specimen, break up spicules with a small tipped pipette .
   - if tissue specimen, use tissue sieve and rubber tipped syringe plunger to gently dissociate.

2. Add 5% FBS RPMI and centrifuge at 400g or 1600 rpm for 5 minutes to wash the cells.
3. If tissue or fluid specimen, proceed to step 6.
4. Aspirate the supernatant.
5. Add PBS to specimen to wash cells. Centrifuge at 400g or 1600 rpms for 5 minutes.
6. Decant supernatant and resuspend the cells in 1 mL of 5% FBS RPMI.
7. Obtain a cell count using CountBright Beads (see appropriate procedure). Adjust the concentration to 10.0 x 10^6 WBC/mL (working solution).
8. Pipette the appropriate antibodies into labeled tubes.
9. Add 100uL of working solution of cells to the antibody panel.
   - If setting up an AML/ALL MRD panel, use 200uL of working solution if the cell concentration is < 10.0 x 10^6WBC/mL.
10. Gently vortex tubes and load the panel into a carousel rack.
11. Place carousel on the LWA and close the lid.
12. Make sure all reagent volumes are full.
13. Select the *KU/incub/lyse/wash* protocol and then *Run*.
14. When the LWA cycle is completed, open lid and remove carousel and place on the BD FACSCanto II.
15. Remove the PI viability tube (if appropriate) and place 1 mL of Propidium Iodide into the PI tube. Vortex and place back into carousel rack. PI will incubate while the panel is running.
16. Run the panel on the BD FACSCanto II using the Diva Software. Gate on the CD45 bright population and/or population of interest. Analyze data using FCS Express software.
17. After analysis, tubes can be disposed of in a biohazard container.
18. Original specimen is kept at room temperature for 7 days.

6. CALCULATIONS/INTERPRETATION/RESULT REPORTING/NORMAL VALUES/CRITICAL VALUES

   A. The results are analyzed using FCS Express software, by the flow cytometry technologists and delivered to the pathologist for interpretation. The pathologist will complete the final report and notify the physician if indicated.

7. CALIBRATION/LINEARITY/AMR/CALIBRATION VERIFICATION

   A. Instrument calibration is performed by BD Technical Service on a semi-annual basis.
   B. Application Settings are performed after every major maintenance or service issue and performed after a new CS&T baseline is established.
   C. Compensation is performed on a weekly basis.

8. PROCEDURE NOTES/LIMITATIONS

A. To ensure that immunoglobulin staining is intrinsic and not extrinsic, CD19 is stained in the same tube with Kappa and Lambda. Also, the specimen is washed with PBS prior to staining to alleviate non-specific binding.[2]
B. The laboratory uses antibodies appropriate for the clinical situation. Pathologists review as necessary.
C. The use of Hyaluronidase has been validated in BAL's and fluids. If these specimens are thick and mucoid add a few crystals of Hyaluronidase (found in Hematology's freezer). Wait until the sample liquefies then proceed with above procedure.
D. There are numerous variables that may affect the staining properties or the identification of specific cell populations and thus result in inaccurate results. These include the following:
    1. Patient's disease state or therapy, such as immune-suppressive drugs, may affect the normal antibody staining characteristics.
    2. Purity of the gated cell population.
    3. Resolution between the positive and negative populations.
    4. Non-specific staining and auto-fluorescence.
    5. Cell count of population of interest may be too low to collect significant results.
E. If the CS&T beads do not pass, the instrument should not be operated. Refer to the troubleshooting information in the BD Cytometer Setup and Tracking Application Guide.
F. If instruments are inoperable:
    a. For analyzer problems, call BD Bioscience at 1-877-232-8995 and log the problem in the Action Log with a reference number.
    b. If one instrument is down, use the other instrument only and contact technical service.
    c. If both instruments are down the hematology supervisor or medical staff on call for hematology should be notified at this time.
    d. All testing should be sent to St. Luke's by courier. Call the Flow Lab at St. Luke's at 816-932-6265 to let them know we are sending specimens.
    e. Have St. Luke's fax flow cytometry requisition with indication of necessary information to be filled in.
    f. Use St. Luke's courier until 7:00 pm (816-932-3850). After hours use KU's courier, Quicksilver (321-5959).
    g. Follow reference lab's protocol for collection and handling of specimens.

9. REFERENCES For this Appendix

1. Stewart, C. C., Nicholson, J.K.A. "Immunophenotyping." Page 181-182. New York: A John Wiley & Sons, Inc., 2000. Print.
2. Hawley, T. S., Hawley, R. G. "Flow Cytometry Protocols." Third Edition. Pages 297 and 301. New York: Humana Press. Print.
3. Keren, D F.., McCoy Jr., J. Phillip, Carey, J. L. "Flow Cytometry in Clinical Diagnosis." 4th Edition, ASCP Press, 2007.
4. BD Cytometer Setup and Tracking Application Guide for BD FACS Digital Flow Cytometers. BD Biosciences. com. 23-12881-00, Rev.01 10/2010.
5. BD FACSCanto II Instructions For Use, #644450, April 2009.
6. BD Bioscience – BD FACSCanto II Operators Course Workbook, 23-10926-00, August 2009.
7. BD Bioscience – BD FACS Lyse Wash Assistant User's Guide, 23-11113-00 Rev. A, January 2010.

APPENDIX H Summaries of Changes

Summary of Changes for Revised Protocol Version 5.0 dated 11-21-2016

- Previous Protocol Version 4.0 dated 04-06-2016
- Revised Protocol Version 5.0 dated 11-21-2016

General Comments

This protocol version incorporates the protocol clarification letter dated 05-18-2016 (allows use of filgrastim biosimilar), removes an inclusion criterion, and includes administrative updates & clarifications.

Key Protocol Changes:

Section	Summary of Change	Rationale
3.1.5	Removed Inclusion Criterion: "Creatinine - within normal institutional limits"	Patients with increased creatinine/kidney disease would not be contraindicated for stem cell collection. Bortezomib is the preferred myeloma drug for patients with kidney disease and using Bortezomib for mobilization may be of benefit for patients with kidney disease. Patients who meet all other enrollment criteria but have kidney disease and/or increased creatinine should not be excluded from the clinical trial.
4.0	Added clarification for Treatment Group A & B: "Physician discretion will be used to advise on the plan which may include the continuation of Mozobil. The provider has the discretion of collection outside the algorithm included in Appendix C."	Clarification
Throughout	Added clarification for Adverse Event reporting: The study ends 30 days after the participant's last protocol therapy (collection) for the purpose of AE reporting. If a reportable AE occurs within 30 days after a participant's collection, recording of concomitant medications should continue until resolution of the AE.	Clarification - The last day should be the collection date because not all patients make it to AutoSCT.
Throughout	Added that Filgrastim G-CSF biosimilar (Zarxio) may/will be substituted for filgrastim	Clarification (institutional standard)

Administrative Protocol Changes:

Section	Summary of Change	Rationale
All	Added new protocol version and date	Protocol tracking
Page 2	Updated biostatistician	Personnel change
Appendix H	Added latest protocol changes to this section	Clarity/Consistency/QA
All	Formatting	Administrative

Consent Form Changes:

Section	Summary of Change	Rationale
Header	Removed protocol version/date	Administrative / Prevents maintenance when consent is unaffected by protocol amendments

*****Re-consent/notification of participants is not required.*****

Summary of Changes for Revised Protocol Version 4.0 dated 04-06-2016

- Previous Protocol Version 3.0 dated 03-14-2016
- Revised Protocol Version 4.0 dated 04-06-2016

General Comments
This revised protocol version 4.0 dated 04-06-2016, incorporates the addition of one exclusion criterion, a note (repeated in several places in the section on adverse events) regarding planned hospital admission, and a study staff change as listed below:

- Patients who have received more than 6 months of lenalidomide (Revlimid®) therapy prior to stem cell collection are excluded from this study.
- **NOTE:** For this study, planned hospital admission will NOT be considered a Serious Adverse Event.
- Brea Lipe, MD was removed as a sub-investigator
- Other minor version-related administrative protocol changes were also completed.
- Changes to the consent form were completed for clarity of standard of care study processes related to stem cell collection and timing
- **It is our opinion, because the changes clarify study procedures undergone by the study participants; any currently enrolled participants must re-sign this version of the consent form after IRB approval.**

Key Protocol Changes:

Section	Summary of Change	Rationale
3.2	Added Exclusion Criterion –Patients who have received more than 6 months of lenalidomide (Revlimid®) therapy prior to stem cell collection are excluded from this study.	Excessive exposure to Lenalidomide (Revlimid®) prior to stem cell harvest is known to adversely affect stem cell mobilization. Due to that fact, heavily pre-treated patients (> 6 months of Lenalidomide exposure) will not be included in this trial. This added exclusion increases participant safety
Sections: 6.1.1 6.2.1 6.2.2 6.2.3 6.2.4	Added the following note: **NOTE:** For this study, planned hospital admission will NOT be considered a Serious Adverse Event. (Repeated in multiple positions for clarity for study team during clinical operations)	Clarification of study reporting for this disease.

Administrative Protocol Changes:

Section	Summary of Change	Rationale
Title Page	Added information regarding current protocol version	Clarity and Protocol tracking
Header	Updated to include current protocol version and date	Protocol Tracking/ Clarification/Version Control
Footer	Updated to include current protocol version and date	Protocol Tracking /Clarification/Version Control
Table of Contents	Updated page numbers and section titles	Protocol consistency per changes in current version
Section 14.0 References	Added reference # 46, pertinent to added exclusion criterion	Clarity/Consistency
Appendix H – Summaries of Changes	Added latest changes to this section	Clarity/Consistency/QA

ALL Consent Form Changes

Section	Summary of Change	Rationale
Header	Updated notation regarding protocol version and date	Clarity for Study Team/Per SOPs
What is being tested in this study?	Changed “twelve” to “12”	Correction per grammar rules
What will I be asked to do? / Group B	Added note that bortezomib doses are per institutional standard	Clarification/ Standard of Care treatment for this disease
Study Treatment	Added the word “NOTE” to notation regarding 72 hours required between each dose or bortezomib. Added note that bortezomib doses are per institutional standard	Clarity/Emphasis Clarification/ Standard of Care treatment for this disease
Study Calendar Treatment Period/ Day 0	Changed collection window days from “ 4 to 8 days” to “8 to 12 days”	Calculation of days in window was incorrect – now corrected. This window was previously approved in Protocol version 3.0 added at that time per standard of care for stem cell collection and for Participant safety and convenience
Study Procedures and Tests / Bortezomib	Added note that bortezomib doses are per institutional standard	Clarification/ Standard of Care treatment for this disease

Summary of Changes for Revised Protocol Version 3.0 dated 03-14-2016

- Previous Protocol Version 2.0 dated 02-11-2016
- Revised Protocol Version 3.0 dated 03-14-2016

General Comments

- This revised protocol version 3.0 dated 03-14-2016, incorporates only the addition and clarification of the following:
  - Clarification of Window for stem cell collection (Day 0 + 4 days)
  - Clarification that mozobil will be used if needed for BOTH groups A and B (per Standard of Care (SOC)
  - Addition of Appendix G - 8-Color Leukemia / Lymphoma Immunophenotyping Processing Procedure
  - Addition of Appendix H – Summaries of Changes
- Other administrative protocol changes related to this change, including template language and quality assurance updates, were also completed.
- Changes to the consent form were completed for clarity of standard of care study processes related to stem cell collection timing
- **It is our opinion, because the changes clarify study procedures undergone by the study participants; any currently enrolled participants must re-sign this version of the consent form after IRB approval.**

Key Protocol Changes for Revised Protocol Version 3.0 dated 03-14-2016:

Section	Summary of Change	Rationale
Schema Study Summary 1.4 – Paragraph 8 4.1 5.2 5.4	Clarification of Window for stem cell collection (Day 0 + 4 days) Clarification that mozobil will be used if needed for BOTH groups A and B (per Standard of Care (SOC)	Clarification / Participant safety and convenience
15.0	Added Appendix G - 8-Color Leukemia / Lymphoma Immunophenotyping Processing Procedure Added Appendix H – Summaries of Changes	Clarity Per KUCC Quality Assurance, going forward, summaries of changes should be included in the protocol document.

Administrative Protocol Changes for Revised Protocol Version 3.0 dated 03-14-2016:

Section	Summary of Change	Rationale
Title Page	Added information and corrected section header regarding previous and current protocol modifications	Clarity and Protocol tracking
Header	Updated to include current protocol version and date	Protocol Tracking/ Clarification/Version Control
Footer	Updated to include current protocol version and date	Protocol Tracking /Clarification/Version Control
Table of Contents	Updated page numbers and section titles	Protocol consistency per changes in current version

Consent Form Changes for Revised Protocol Version 3.0 dated 03-14-2016:

Section	Summary of Change	Rationale
Header	Changed Amendment/Version Information	Auditing reference/version control
What is being tested in this study? Paragraph regarding Group B	Added that stem cell collection may occur approximately eight to twelve days after the second dose of bortezomib.	Clarification / 4 –day window clarification added per standard of care for stem cell collection and for Participant safety and convenience
What is being tested in this study?	Moved and bolded note that both Group A and B participants will receive G-CSF and may have Mozobil if needed	Clarification
What will I be asked to do? Group B	Added that stem cell collection may occur up to 12 days after second dose of bortezomib.	See rationale for “What is being tested in this study?”, above
Study Treatment	Group B – modified language in bullet 3 per the clarification to the window for standard of care for stem cell collection. Added bullet 4 to indicate mozobil will be used for this group also, if needed	See rationale for “What is being tested in this study?”, above Clarification
Study Calendar	Changed/expanded treatment-period column headings (only) per the clarification to the window for standard of care for stem cell collection.	Clarification and see rationale for “What is being tested in this study?”, above.
Blood tests	Deleted “Day – 1” per the clarification to the window for standard of care for stem cell collection.	Consistency