

**Title: Evaluation of Melphalan + Bortezomib (Velcade®) As A Conditioning
Regimen For Autologous And Allogeneic Stem Cell Transplants In Multiple
Myeloma After Cytoreductive Therapy**

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This is an investigator-initiated study. The principal investigator Dr. Melissa Alsina (who may also sometimes be referred to as the sponsor-investigator), is conducting the study and acting as the sponsor. Therefore, the legal/ethical obligations of the principal investigator include both those of a sponsor and those of an investigator.

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CLINICAL TRIAL PROTOCOL SYNOPSIS

Protocol Title:	Evaluation Of Melphalan + Bortezomib (Velcade®) As A Conditioning Regimen For Autologous And Allogeneic Stem Cell Transplants In Multiple Myeloma After Cytoreductive Therapy
Primary Study Objectives	<ul style="list-style-type: none">□ To determine the 2 year-progression free survival (PFS) in multiple myeloma patients ≤ 60 years of age following allogeneic transplant for those with an available HLA-matched donor using a conditioning regimen of melphalan + fludarabine + Bortezomib (Velcade®)□ To determine the 2 year-PFS in multiple myeloma with an autologous stem cell transplant using a conditioning regimen of melphalan + Bortezomib (Velcade®) for patients > 60 years of age and patients ≤ 60 years of age who decline allogeneic stem cell transplant or for whom a suitable HLA-identical donor was not identified□ To determine the 2 year-PFS in multiple myeloma with an autologous stem cell transplant using a conditioning regimen of melphalan + Bortezomib (Velcade®) followed by 1 year maintenance with bortezomib for patients > 60 years of age and patients ≤ 60 years of age who decline allogeneic stem cell transplant or for whom a suitable HLA-identical donor was not identified
Secondary Study Objectives	<ul style="list-style-type: none">□ To determine the overall survival (OS) in multiple myeloma with autologous or allogeneic stem cell transplants using the above conditioning regimens□ To determine the response rates in multiple myeloma using the above regimens.□ To determine minimal residual disease status using allele specific oligonucleotides (ASO-PCR) by PCR and flow-cytometry for multiple myeloma cells.□ To correlate minimal residual disease status with 2 year PFS and OS.□ To determine overall regimen safety.□ To determine the incidence of acute and chronic GVHD in multiple myeloma with allogeneic stem cell transplant using the above conditioning regimen.□ To examine quality of life in patients treated with allogeneic and autologous stem cell transplants using the above conditioning regimens.□ To examine effects of Melphalan and bortezomib on protein and gene expression profiles in peripheral blood mononuclear cells
Study Design	This is an investigator initiated, single institution, open-label, phase II trial
Accrual Objective	150 patients will need to be consented to accrue 95 evaluable patients
Study Period	The duration of patient participation will be anticipated to be through the completion of the study, approximately 4 years (enrollment for two years and follow up for two years).
Inclusion Criteria	<ul style="list-style-type: none">□ Voluntary written informed consent before performance of any study-related procedure not part of normal medical care, with the

understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.

- Female subject is either post-menopausal or surgically sterilized or willing to use an acceptable method of birth control (i.e., a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study.
- Male subject agrees to use an acceptable method for contraception for the duration of the study
- Multiple Myeloma Criteria (International Uniform Response Criteria for Multiple Myeloma) Section 7.7.
 - Patients with responsive disease after **any** line of induction therapy or early first relapse. Early first relapse is defined as patients that achieve a CR from induction that lasts for more than six (6) months and relapse but are still in VGPR without re-treatment. These patients may be placed on anti-myeloma therapy while awaiting transplant workup for a maximum of 3 months.
- Patients greater than or equal to 18 years of age are eligible. There is an upper age limit of 60 years for allogeneic transplants. There is no upper age limit for autologous transplant.
- Patients must have a histologically confirmed diagnosis.
- Patients must have undergone a complete psychosocial evaluation and have been considered capable of compliance.
- Meet the following criteria for allogeneic hematopoietic cell transplant:
 - Must have an identified donor match defined as: HLA-A, HLA-B, HLA-C, DRB1 8/8 allele matched sibling, family member, or unrelated donor and be ≤ 60 years of age. Single antigen or allele mismatched donors are not permitted.

Exclusion Criteria

- Patients who do not achieve at least a partial response (PR) by the criteria mentioned above with induction therapy.
- Patient has a platelet count of $<30 \times 10^9/L$ within 30 days before initiation of study therapy and is not improved to $>30 \times 10^9/L$ before initiation of study therapy
- Patient has \geq Grade 2 peripheral neuropathy within 30 days before initiation of study therapy.
- Patient has an absolute neutrophil count of $<1.0 \times 10^9/L$ within 30 days before initiation of study therapy and is not improved to $>1.0 \times 10^9/L$ before initiation of study therapy
- Patient has hypersensitivity to Bortezomib (Velcade®), boron or mannitol.
- Patients with active leptomeningeal involvement are ineligible. Patients with a history of previous CSF tumor involvement without symptoms or signs are eligible provided the CSF is now free of disease on lumbar puncture, and MRI of the brain shows no evidence of tumor progression

following completion of appropriate therapy. Patients with severe symptomatic CNS disease of any etiology are ineligible.

- Patients are not eligible to receive high dose chemotherapy as per standard BMT criteria
- Patients with any previous malignancy other than non-melanoma skin cancer are ineligible, unless the patient is without evidence of disease \geq 5 years after the treatment for the cancer was completed.

Study Intervention

Autologous Peripheral Blood Stem Cell Rescue

Stem cell mobilization with G-CSF (granulocyte colony-stimulating factor) at a dose of 10 $\mu\text{g/kg/day}$ as per institutional standards. CD34^+ peripheral blood stem cells will be collected following the administration of G-CSF as per institutional standards. A minimum of 2×10^6 CD34^+ peripheral blood stem cells per kilogram of recipient's body weight must be collected prior to proceeding to autologous stem cell transplant in patients who are in CR/VGPR and 4×10^6 CD34 cells /kg for patients who are in PR and may receive tandem autologous transplants.

Day -4 Melphalan 100 $\text{mg/m}^2/\text{day}$ IV over 30 minutes.

Day -3 Melphalan 100 $\text{mg/m}^2/\text{day}$ IV over 30 minutes, followed by Bortezomib (Velcade®) (1.3 mg/m^2) as an intravenous push over 3 to 5 seconds.

Day -2,-1 NO THERAPY

Day 0 Infusion of autologous stem cells

Allogeneic Peripheral Blood Stem Cell Rescue

The intended goal for CD34^+ peripheral blood stem cell collection from matched donor will be from of 5 to $10 \times 10^6/\text{kg}$ of body weight of the recipient for the allogeneic stem cell transplant. For the allogeneic matched-related donors peripheral blood stem cells will be harvested with GCSF mobilization and infused fresh to the recipients. Allogeneic donor stem cells may also be cryopreserved if they cannot be infused fresh. For the matched unrelated donors National Marrow Donor Program protocols will be followed.

Day -6 Fludarabine 30 mg/m^2

Day -5 Fludarabine 30 mg/m^2

Day -4 Fludarabine 30 mg/m^2 + Melphalan 70 $\text{mg/m}^2/\text{day}$ IV over 30 minutes.

Day -3 Fludarabine 30 mg/m^2 + Melphalan 70 $\text{mg/m}^2/\text{day}$ IV over 30 minutes followed by Bortezomib (Velcade®) (1.3 mg/m^2) as an intravenous push over 3 to 5 seconds.

Day -2,-1 NO THERAPY

Day 0 Infusion of allogeneic peripheral blood stem cells

TABLE OF CONTENTS

Abbreviations List.....	7
1.0 INTRODUCTION AND STUDY RATIONALE:	9
1.1 Overview of the Disease	9
1.2 Drug Resistance in Multiple Myeloma	9
1.3 Autologous Peripheral Blood Stem Cell Transplantation in Multiple Myeloma ..	11
1.4 Allogeneic Peripheral Blood Stem Cell Transplantation in Multiple Myeloma..	12
1.5 Melphalan + Bortezomib (Velcade®) as Condition Regimen?	14
1.6 Molecular Remission in Multiple Myeloma – what does it mean?	15
1.7 Quality of Life in Multiple Myeloma and RIC	17
2.0 STUDY OBJECTIVES.....	18
2.1 The primary objectives of this study are:.....	18
2.2 The secondary objectives of this study are:	18
3.0 INVESTIGATIONAL PLAN.....	19
3.1 Selection of Patients.....	19
3.1.1 Inclusion Criteria	19
3.1.2 Exclusion Criteria	20
3.2 Study Treatment.....	22
3.2.1 Stem Cell Mobilization	22
3.2.2 Administration and schedule of chemotherapy:	22
3.2.3 Treatment Assignment	26
3.2.4 Supportive Treatment.....	27
3.2.5 Treatment Compliance.....	27
3.3 Duration of Patient Participation	28
3.4 Termination of Treatment and/or Study Participation	29
3.5 Efficacy, Pharmacodynamic/Pharmacogenomic, and Safety Measurements	29
3.6 Recommended Monitoring Parameters.....	31
3.7 Study Endpoints.....	34
4.0 ADVERSE EVENTS.....	35
4.1 Definitions.....	35
4.2 Procedures for AE and SAE Reporting	36
4.3 Monitoring of Adverse Events and Period of Observation.....	38
4.4 Reporting Drug Exposure During Pregnancy and Birth Events	38
5.0 STATISTICAL PROCEDURES	40
5.1 Sample Size Estimation	40
5.2 Selection of patients.....	41
5.3 Populations for Analysis.....	41
5.4 Procedures for Handling Missing, Unused, and Spurious Data.....	41
5.5 Statistical Methods.....	41
5.6 Safety and Toxicity Analysis	42
5.7 Procedures for Reporting Deviations to Original Statistical Analysis Plan	42

6.0	ADMINISTRATIVE REQUIREMENTS.....	43
6.1	Good Clinical Practice	43
6.2	Ethical Considerations	43
6.3	Patient Information and Informed Consent.....	43
6.4	Patient Confidentiality	43
6.5	Protocol Compliance.....	43
6.6	Data Safety and Monitoring.....	44
6.7	Drug Accountability.....	46
6.8	Premature Closure of the Study	46
6.9	Record Retention	47
7.0	SUPPLEMENTAL INFORMATION	48
7.1	Study Flow Chart	48
7.2	Body Surface Area Calculation	49
7.3	New York Heart Association Classification of Cardiac Disease.....	50
7.4	Declaration of Helsinki	50
7.5	Common Toxicity Criteria Version 4.0	54
7.6	Eastern Cooperative Oncology Group (Ecog) Performance Status Scale	54
7.7	International Uniform Response Criteria for Multiple Myeloma	55
7.8	Durie and Salmon Multiple Myeloma Staging Criteria	57
8.0	DRUG INFORMATION	58
8.1	MELPHALAN.....	58
8.2	Bortezomib (Velcade®) for Injection	59
8.3	Clinical Trial Materials	72
8.4	Preparation, Handling, and Storage of Drugs	73
9.0	References.....	76
Appendix A.	Quality of Life Survey	83
Appendix B	Protocol Algorithm	86
Appendix C	Body Surface Area and Creatinine Clearance Calculations.....	86
Appendix D	Pregnancy Reporting Form.....	87

ABBREVIATIONS LIST

Abbreviation	Definition
°C	degrees Celsius
μM	micromolar
20S	20S proteasome subunit
AE	adverse event
ANC	absolute neutrophil count
Bcl-2	B-cell lymphoma-2; a gene that inhibits apoptosis
BSA	body surface area
CAM	cell adhesion molecules
CAM-DR	cell-adhesion mediated drug resistance
cm	centimeter
CR	Complete Response
CTC	(NCI) Common Toxicity Criteria
CTEP	Cancer Therapy Evaluation Program
dL	deciliter
DLT	Dose Limiting Toxicity
DNA	deoxyribonucleic acid
EFS	Event-free survival
FDA	Food and Drug Administration
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony-Stimulating Factor
GLP	Good Laboratory Practice
HDC	High-dose chemotherapy
ht	height
IκB	I kappa B kinase; cytokine response kinase that activates transcription factor NF-kappa b at serine 32 and 36
ICAM-1	intercellular adhesion molecule 1
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IL-6	Interleukin-6
IL-10	Interleukin-10
IND	Investigational New Drug
IRB	Institutional Review Board
IV	intravenous

Abbreviation	Definition
IκBα	I kappa B alpha-associated protein kinase
Kg	kilogram
Ki	inhibitory constant
Lbs	pounds
LRP	lung-resistance protein
m ²	square meters
Mg	milligram
Min	minute
mL	milliliter
mm ³	cubic millimeters
mmol	millimole
MP	Melphalan and prednisone
MRP	multidrug resistance-associated protein
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NF-κB	nuclear factor-κB
Ng	nanogram
nM	nanomole
OS	Overall survival
p21	p21(ras) farnesyl-protein transferase
p27	cyclin-dependent kinase inhibitor
p53	tumor suppressor protein with molecular weight of 53 kDa
PBSCT	Peripheral blood stem cell transplantation
Pgp	P-glycoprotein
SAE	serious adverse event
US	United States
USP	United States Pharmacopeia
VAD	Vincristine, adriamycin and dexamethasone
VCAM-1	vascular cell adhesion molecule 1
w/w	weight-to-weight ratio
Wt	weight

1.0 INTRODUCTION AND STUDY RATIONALE:

1.1 Overview of the Disease

Multiple myeloma represents approximately 10% of all hematologic malignancies, and accounts for 1% of all cancers in the United States.¹ As the second most common hematologic malignancy, mortality rates from multiple myeloma have increased over the previous three decades. An estimated 19,900 new cases with 10,790 deaths resulting from this disease are expected in 2007.² Incidence rates are highest among the elderly, men (male:female ratio of 1.1:1), and African Americans. Mortality rates are highest among the elderly and blacks. The median age at diagnosis is 70 years and the median survival with treatment is approximately 3 years.² Even with conventional treatment using melphalan + prednisone (MP) or vincristine + adriamycin + decadron (VAD) chemotherapy or high-dose chemotherapy (HDC) followed by stem cell transplantation, multiple myeloma still remains an incurable disease. References 2 and 3 review the recommended treatment approaches for multiple myeloma.^{3,4} Total response rates of 40-60% can be achieved with VAD or MP, however less than 10% are true complete responses (CR).⁵⁻⁷ The 5 and 10-year survival rates with standard treatment for myeloma are 29% and 5%, respectively.⁵ In an effort to improve the rather dismal prognosis for patients with multiple myeloma, treatment protocols using high-dose chemotherapy (HDC) regimens followed by autologous stem cell transplants have been undertaken at several institutions.^{5,6,8,9} HDC followed by autologous peripheral blood stem cell transplant has shown disease-free and overall survival advantages when compared to standard dose chemotherapy, but has failed to induce long term responses in the majority of these patients.

1.2 Drug Resistance in Multiple Myeloma

The pathogenesis of primary resistance to chemotherapy is poorly understood, but likely involves factors intrinsic to the myeloma cells, as well as those posed by the bone marrow microenvironment. Regarding the latter, it is well known that adhesion of myeloma cells to stroma renders these cells resistant to chemotherapy.¹⁰ This is a form of *de novo* drug resistance, and these phenotypes have been described for multiple chemotherapeutic agents.¹¹⁻¹³

Interestingly, when myeloma cells are adherent to fibronectin this resistance is overcome by the proteasome inhibitor, Bortezomib (Velcade®). This suggests a role for Bortezomib (Velcade®) to overcome *de novo* drug resistance and sensitize multiple myeloma cells to chemotherapy. Several investigators have shown that Bortezomib (Velcade®) sensitizes myeloma cells to chemotherapy, in particular melphalan.¹⁴

On multiple levels, the bone marrow microenvironment is uniquely influential in imparting selective tumor survival advantages among many varying hematologic malignancies, one of which includes multiple myeloma. Numerous *in vitro* models have demonstrated the major impact of bone marrow stromal cells in regulating malignant cell growth and inducing drug resistance.^{11, 14-18} Regulation of myeloma cell growth occurs via the effects of soluble mediators such as: IL-6, oncostatin M, leukemia inhibitory factor, IL-10, and insulin-like growth factor 1, which are all elaborated by the bone marrow microenvironment. Protection against drug induced cytotoxicity occurs via direct contact with stromal elements, and this most probably accounts for

poor response rates to chemotherapy in those patients with primary refractory multiple myeloma. Several mechanisms involving this phenomenon, in both hematologic and non-hematologic cell lines, have been proposed. NF- κ B is one important and, perhaps, the major regulatory pathway that mediates resistance of myeloma cells when adhered to fibronectin. Increases of NF- κ B activity in multiple myeloma correlates with an increase in tumor cell survival.¹⁹⁻²²

Regulation of anti-apoptotic products via NF- κ B activation is one example of cell-adhesion mediated drug resistance (CAM-DR). Anti-apoptotic signal transduction pathways are activated and gene products, which are regulated by the NF- κ B superfamily, are altered through myeloma cell interactions with elements of their bone marrow microenvironment- most specifically, fibronectin.¹⁰ Hideshima *et al* has proposed a mechanism explaining de novo myeloma cell drug resistance imparted by the bone marrow microenvironment.¹⁵ This pathway involves tumor necrosis factor- α mediated expression of NF- κ B by the stromal elements. Bortezomib (Velcade®) has been shown to reduce binding interactions between myeloma cells and bone marrow stromal cells.¹⁵ Bortezomib (Velcade®) has also been shown to sensitize highly resistant myeloma cell lines to chemotherapy.¹⁴ Ma *et al* demonstrated that Bortezomib (Velcade®) sensitized resistant myeloma cell lines to melphalan and other chemotherapeutic agents, thereby overcoming drug resistance by enhancing apoptosis.¹⁴ It has been established that levels of phosphorylated I κ B α , a cytoplasmic NF- κ B inhibitory protein, increase in myeloma cells following exposure to chemotherapy.¹⁴ Phosphorylated I κ B α becomes a selective target for ubiquitination and subsequent cytoplasmic degradation by the proteasome- a process that is inhibited by Bortezomib (Velcade®).

Other mechanisms of intrinsic or acquired drug resistance include: both quantitative and qualitative changes in intracellular drug targets, increases in p27^{kip1} levels, overexpression of multidrug resistance-associated protein (MRP), P-glycoprotein (Pgp), and lung-resistance protein (LRP).²³⁻²⁷

A recent, non-randomized, multicenter, Phase II trial evaluated the efficacy of Bortezomib (Velcade®) in patients with relapsed, refractory multiple myeloma.²³ Ninety-two percent of the 193 evaluable patients who were enrolled in this study were previously treated with three or more regimens known to be active in multiple myeloma (median number of previous therapies was 6). Bortezomib (Velcade®) alone was given at a dose of 1.3 mg/m² twice weekly for two weeks on a 21-day cycle. The median duration of treatment was 3.8 months. Follow-up after 24 weeks of treatment revealed an overall response rate of 35%. Of these, 4% developed a complete response (no evidence of demonstrable myeloma protein) and 6% developed a near-complete response (myeloma protein only demonstrable by immunofixation). Furthermore, the median duration of response was 1 year with a median overall survival of 16 months.

One other Phase II study, by Jagannath *et al*, evaluated 54 multiple myeloma patients who failed or relapsed after first-line therapy.²⁴ Two doses of Bortezomib (Velcade®) (1.0 mg/m² and 1.3 mg/m²) were given twice weekly, of a 3-week cycle, for 8 cycles. The complete and partial response rates among those who received Bortezomib (Velcade®) 1.3 mg/m² were 38%, while the complete and partial response rates among those who received Bortezomib (Velcade®) 1.0 mg/m² were 30%.

1.3 Autologous Peripheral Blood Stem Cell Transplantation in Multiple Myeloma

High-dose chemotherapy followed by autologous peripheral blood stem cell rescue is a preferred primary treatment approach for patients with multiple myeloma who have adequate organ function to be eligible for it.^{3, 25, 26} Estimated overall response rates and CR rates following this approach are 75-90% and 20-40%, respectively; and, estimated median survival is 4 to 5 years.^{3, 25, 27-29} Compared with conventional chemotherapy, high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation affords a 13-month longer median duration of overall survival and a higher rate of seven year event-free survival in some studies (16% versus 8%).^{30, 32} Also, overall response rates, CR rates, and overall survival rates resulting from high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation greatly exceed those from conventional chemotherapy.²⁵⁻²⁷

In the large MRC VII trial, Child *et al* reported rates of CR of 44% in patients treated with HDT and autologous PBSCT, while those treated with conventional chemotherapy achieved rates of CR of 8% ($p < 0.001$).²⁶ The median overall survival was 54.1 months in those patients receiving HDT and PBSCT compared to 42.3 months in patients who received conventional chemotherapy. Higher rates of progression-free survival, 32 months versus 20 months ($p < 0.001$), were also seen in the HDT group. Further evidence supporting the benefits of HDT followed by autologous PBSCT over conventional chemotherapy were reported by Attal *et al* from the IFM-90 trial.²⁵ Complete response rates or very good partial response rates were achieved by 38% of the patients receiving HDT and PBSCT versus 14% in the conventional chemotherapy arm ($p < 0.001$). After a median follow-up of 7 years, event-free survival was superior in the HDT arm (28 months versus 18 months), and a 7-year EFS of 16% versus 8% ($p = 0.01$). A median overall survival of 57 months was reported for the HDT group, while that of the conventional chemotherapy group was 44 months (7-year overall survival 43% versus 25%, $p = 0.03$). Based upon this data and others³⁰⁻³⁴, HDT followed by autologous PBSCT significantly enhances complete response rates and EFS; while, in the case of the IFM-90 and MRC VII trials HDT followed by autologous PBSCT improves OS. Achievement and maintenance of durable disease control in multiple myeloma strongly correlates with early development of CR that is considerably enhanced by HDT followed by PBSCT.

Despite these positive results, very few people have been cured to date. Although less toxic and more available than allogeneic transplantation, autologous peripheral blood stem cell transplantation is problematic for the following two reasons: lack of a graft-versus-myeloma effect and contamination of the graft with myeloma cells. Efforts to further reduce tumor cell contamination through tandem autologous transplantations have occurred, and results have been reported.³⁴⁻³⁹ Recently, in a landmark trial, the French Myeloma Intergroup reported an overall survival benefit among patients undergoing tandem autologous stem cell transplantation.³⁹ Patients who failed to achieve a very good partial response after a single autologous transplant seemed to benefit the most from tandem autologous transplantation. Both the seven-year event free survival, 20% versus 10% ($p < 0.03$), and seven-year overall survival, 42% versus 21% ($p = 0.01$), were double among those in the tandem transplant cohort. Among patients failing to achieve a very good partial response within three months after a single autologous transplantation, the estimated overall seven-year survival advantage was nearly fourfold in those receiving a tandem transplant.³⁹

In a randomized trial, Vesole *et al* applied tandem autologous PBSCT to a large number of multiple myeloma patients, and subsequently demonstrated the safety and benefits of extensions in event-free survival (EFS) and overall survival (OS) resulting from this treatment. Furthermore, they demonstrated increased CR rates from 24% to 43% following the first PBSCT and second PBSCT, respectively.³⁶ Barlogie *et al* demonstrated the safety and feasibility, as well as improved outcomes resulting from tandem PBSCT. In Total Therapy I, Barlogie *et al* demonstrated improved response rates and OS among patients who received tandem autologous transplants as compared with matched controls (receiving standard therapy) or those receiving single autologous transplants. At ten years of follow-up, the median OS and EFS of the 152 patients who received a tandem autologous transplant were 79 months (33%) ($p < 0.001$) and 37 months (15%) ($p < 0.001$), respectively. The median OS and EFS, among the comparative 152 matched controls who received standard therapy, were 43 months (15%) and 16 months (5%), respectively.³⁴

Other randomized trials comparing single autologous transplants to tandem autologous transplants have been published with mixed results.⁴⁰⁻⁴⁴ These studies, however, differ in design and have shorter intervals of median follow-up. Therefore, overall conclusions are challenging and require cautious interpretation.³

1.4 Allogeneic Peripheral Blood Stem Cell Transplantation in Multiple Myeloma

Presently, allogeneic stem cell transplantation is considered to be the only potentially curative therapy for patients with MM. The potential advantages of an allogeneic transplantation are: 1) allogeneic hematopoietic progenitor cells are devoid of malignant plasma cells and 2) allogeneic immune cells can mediate a graft-versus-myeloma effect.⁴⁵ Durable molecular complete remissions have been observed in patients who have undergone allogeneic transplantation for MM.⁴⁶⁻⁴⁹ Furthermore, the risk of relapse is also reduced with allogeneic hematopoietic cell transplantation (HCT) when compared with autologous stem cell transplantation (ASCT).⁵⁰

In some studies, allogeneic HCT has resulted in significantly longer event-free survival (EFS), increased overall survival (OS), and increased numbers of durable complete response (CR) rates.⁵¹⁻⁵³ Kuruvilla *et al*⁵⁴ reported OS and EFS of 39 and 31% at 10 years in a retrospective review of their experience in a 72 patient cohort at a single center. Their TRM at 1 year was 22% while using a myeloablative conditioning regimen. The United States Intergroup S9321⁵⁵ trial reported a 39% plateau in the survival curve at 7 to 10 years in 36 patients undergoing allogeneic stem cell transplantation with melphalan and TBI and only 18 remained in remission; however, transplant-related mortality (TRM) was reported to be 53% among those receiving an allogeneic HCT. Giralt *et al.* analysed 22 patients from 1996 to 2000, with relapsed/refractory multiple myeloma who received an allogeneic transplant after conditioning with melphalan (140 – 180 mg/m²) and fludarabine (30 mg/m² x 4 days or 25 mg/m² x 5 days).⁵⁶ Non-relapse mortality was 40±10%, however, for patients transplanted within three years of diagnosis the NRM was lower (23% vs 55%). The OS and PFS were 30%±11% and 19%±10% at two years.

The benefits of allogeneic HCT are outweighed by the high treatment-related mortality rates associated with standard intensity myeloablative regimens.⁵⁷⁻⁵⁹ Novel reduced-intensity conditioning (RIC) regimens and improvements in supportive care, have reduced TRM significantly and led to favorable outcomes after allogeneic BMT.

Due to improvements in supportive care and better patient selection, the European Group for Blood and Marrow Transplantation (EBMT) reported TRM rates that approximated 20%.⁶⁰ The development and use of RIC regimens in treating patients with MM has reduced immediate TRM.⁶¹⁻⁶³ RIC reduces the severity and rates of acute graft-versus-host disease, while still maintaining a graft-versus-myeloma effect.

Reynolds et al.⁶⁴ compared sibling allogeneic and autologous transplant in 56 patients with multiple myeloma (Auto 35, allo 21) using TBI/Bu/Cy and showed that the two-year progression free survival was more (60% vs 30%) in the allogeneic group, even though it did not reach statistical significance. Within the allogeneic group, patients ≤ 50 years of age had a 3-year PFS of 79%. The 100 day mortality in the allogeneic arm was 19%. Crawley et al.⁶⁵ from EBMT compared RIC regimens with myeloablative ones retrospectively in myeloma and found a reduced NRM (24% vs 37%) in the RIC arm which was offset by a higher relapse risk (HR 2.0). Maloney et al.⁶⁶ used a tandem approach to cytoreduce with an autologous transplant prior to a planned RIC allogeneic transplant from HLA-identical siblings with 2 Gy TBI in previously treated stage II/III myeloma. 52% patients were refractory or relapsed disease. They achieved almost 50% 2 year PFS. Mortality not related to relapse was 2% with autologous and 15% with allogeneic HCT. Lee et al.⁶⁷ suggested that although RIC allotransplant induces high rates of CR and near CR, even in refractory disease, it appears to result in a durable response only if it is applied early in the disease in high risk patients, when they still are chemosensitive. Kroger et al.⁶⁸ reported on 120 patients of multiple myeloma given allotransplants (related 85, unrelated 35) with melphalan/fludarabine RIC. 38 received a planned autotransplant prior to allotransplant, 58 had relapsed disease after an autograft and 24 were given an allograft after a response to chemotherapy alone. The 2-year estimated event free survival was 60% for related donors and 81% for unrelated donors, respectively. Cumulative TRM at one year was 18%. Relapse after prior autograft (66% vs 33%, $p < 0.001$), chemorefractory disease at transplantation (55% vs 38%) and late transplantation > 2 years after diagnosis (43% vs 42%) were the most significant factors for a higher relapse rate. Patients who had chronic GVHD had two times less probability of relapse. Georges et al.⁶⁹ treated 24 poor risk (failure of prior autograft, failure of 1 or more frontline therapies, preexisting comorbidities) patients with unrelated RIC allografts using a conditioning regimen of Flu/ 2 Gy TBI. 13 patients had planned auto-allografts whereas 11 proceeded directly to unrelated transplantation. 3-year PFS was 33% for all patients. However, the tandem auto-allo group had PFS 51% compared to 11% in those who proceeded to allotransplants directly. Thus, majority of patients going into RIC allo directly were refractory whereas the planned auto-allo group had cytoreduction to PR in majority of patients prior to the allograft. The 3 year NRM was 21%. Bruno et al.⁷⁰ treated 162 patients with newly diagnosed myeloma who were ≤ 65 years. All patients received an autotransplant after VAD induction. Patients who had an HLA-identical sibling received a planned RIC allograft with 2 Gy TBI 2-4 months after the first autotransplant. Those without a HLA-identical sibling received a second autologous transplant. 3 year PFS was around 50% at which point the auto-allo and the double-auto curves separated with the auto-allo arm achieving a plateau. At median follow up of 45 months, the median event-free survival was 80 months and 54 months in those with HLA-identical sibling and those without, respectively. Even though the treatment related mortality did not differ significantly in both groups, disease related mortality was significantly higher in the double-auto group (43% vs 7%, $p < 0.001$). Bruno et al.⁷¹ from GITMO transplanted 22 patients

with unrelated allografts after conditioning with Flu/ 2Gy TBI and found 2 year PFS of 79% in patients transplanted upfront and 25% in relapsed patients, respectively. Six patients died of TRM and 3 of disease progression. They concluded that “disease control appears more pronounced when patients are treated soon after diagnosis”.

Rosinol *et al*⁷² presented data at the Myeloma International meeting, 2007 on a study comparing a second autotransplant with RIC-allotransplant in patients not achieving CR or near CR with a first autotransplant. The CR rate was significantly higher with allo-RIC (33% vs 11%, $p=0.02$). Although the EFS (26 vs 19 mo) and the OS (57 vs not reached in allo-RIC) were not significantly different, there is a plateau in the allo-RIC group.⁹⁴ The recent CTN 0102 trial⁷³ comparing tandem transplant using melphalan 200 mg/m² versus RIC- allotransplant using 200 cGy TBI and HLA-matched sibling donors following an auto-transplant with melphalan 200 mg/m² has completed accrual and is awaiting analysis.⁹⁶

1.5 Melphalan + Bortezomib (Velcade®) as Condition Regimen?

Roussel *et al* treated 35 patients with stage II/III myeloma with Bortezomib (Velcade®) + melphalan (200 mg/m²) and at 3 months achieved 31.4% CR and 31.4% VGPR.⁷⁴ They used Bortezomib (Velcade®) 1 mg/m² on day -6, -3, +1 and +4 with melphalan 200 mg/m² on day -2. Kim *et al* treated five patients with a similar regimen consisting of Bortezomib (Velcade®) 1 mg/m² + Mel 50 mg/m² on day -4 and Bortezomib (Velcade®) 1mg/m² + Mel 150 mg/m² on day -1 and achieved 4 CRs.⁷⁵

Preliminary results of the use of Melphalan + Bortezomib (Velcade®) conditioning regimen in a tandem setting for refractory myeloma at H. Lee Moffitt Cancer Center (Aleksun & Alsina *et al*)⁷⁶ has yielded very good results. This phase I/II dose escalation study of Bortezomib (Velcade®) followed by high dose melphalan+ Bortezomib (Velcade®) as conditioning regimen for autologous PSCT in refractory myeloma patients at our institution is accruing patients. Currently, out of 11 evaluable patients, evaluation at three months post tandem transplant, 36 % of patients achieved a CR, 27% a VGPR and 27 % a PR for a response rate of 90%. One patient developed progressive disease following the first transplant. In our study 2 cycles of Bortezomib (Velcade®) were given prior to transplant.

The importance of Bortezomib (Velcade®) with melphalan sequence in the conditioning regimen for autologous transplant for myeloma was highlighted by the study by Kaufman *et al* showing that Bortezomib (Velcade®) used after melphalan may increase apoptosis of bone marrow plasma cells as compared to being used before melphalan.⁷⁷

The role of Bortezomib (Velcade®) for conditioning in RIC allogeneic transplant in myeloma has not been tested till date. However, Bortezomib (Velcade®) has been used to maintain remission status after reduced intensity allogeneic transplant for multiple myeloma.⁷⁸ It is our hypothesis that addition of Bortezomib (Velcade®) to the RIC-allogeneic conditioning regimen of melphalan+fludarabine will increase the anti-myeloma potency of the conditioning regimen and delay relapse.

1.6 Molecular Remission in Multiple Myeloma – what does it mean?

The treatment of multiple myeloma is passing through a period of rapid evolution. The introduction of novel agents like proteasome inhibitors and immunomodulatory agents in the induction phase of treatment has resulted in response rates equivalent to those achieved with high dose chemotherapy and autologous stem cell transplant. Palumbo et al.⁷⁹ evaluated the feasibility and efficacy of the combination of melphalan, prednisone, and thalidomide (MPT) in 49 newly diagnosed patients with multiple myeloma. They showed that 18% of patients achieved immunofixation-negative complete remission (CR), 6% achieved immunofixation-positive near CR, 4% achieved a very good partial response, and 45% achieved a partial response, with a 50-89% reduction in monoclonal paraprotein. Six percent did not respond and 10% showed progressive disease. The median time to maximum response was 4 months. The Kaplan-Meier estimates of event-free survival and overall survival at 2 years were 64% and 91%, respectively. However, the durability of these responses is unknown. In a retrospective analysis of a phase II study the Mayo group identified 21 patients who received Thal/Dex for induction but did not proceed to ASCT for reasons of age, comorbidity and patients' refusal. They found that the median time to disease progression in patients with multiple myeloma who receive initial therapy with Thal/Dex and who do not undergo ASCT is 18 months.⁸⁰ Barlogie et al have reported in a phase-2 study that Bortezomib (Velcade®) could be safely combined with multi-agent chemotherapy, effecting near-complete remission status and 2-year survival rates in more than 80% of patients.⁸¹ In addition, the present definitions of complete remission (CR) do not correlate with increased survival.⁸²⁻⁸⁴ This led to questions whether the definitions of CR really reflected minimal residual disease. It is estimated that, at the time of presentation, patients have $10^{12} - 10^{13}$ myeloma cells.⁸⁵ At strictly defined CR they may have up to 10^9 myeloma cells.⁸⁶ The International Myeloma Working Group in 2006 defined stringent CR as negative immunofixation on the serum and urine, disappearance of any soft tissue plasmacytoma and $\leq 5\%$ plasma cells in the bone marrow as well as normal FLC ratio ($< 4:1$ or $> 1:2$) and absence of clonal cells in the bone marrow by immunohistochemistry or immunofluorescence.⁸⁷

The two main aspects of this definition are 1) measurement of the malignant clone of plasma cells and 2) measurement of the secreted products of the malignant clone. The ability to detect minimal residual disease is limited by the sensitivity of the assays to measure these two markers.

Rawstron et al.⁸⁸ used a flow cytometric technique to detect malignant plasma cells ($CD19^-$ or $CD19^+ CD56^+$) with a sensitivity of .01% (1 in 10^4). In their study flow cytometry was better than fluorescent-PCR analysis using consensus primers for IgH rearrangement in detecting minimal residual disease.

Using patient specific primers for the IgH-PCR assay initially developed by Cremer et al.⁸⁹, Lipinski et al could detect a single copy of target DNA in a background of DNA from 330,000 cells.⁹⁰ Raab et al compared four methods of real-time PCR assay to detect MRD after a non-myeloablative allogeneic transplantation in 11 patients of multiple myeloma. They found that ASO-forward and -reverse primers together with the clone specific TaqMan probe were the most sensitive approach with a detection limit of $1/10^4 - 1/10^5$ cells.⁹¹ Although, this type of approach detects a probe in 95 – 100% cases of acute and chronic B-lymphocytic leukemias, it will find a patient-specific probe only in 60-80% cases of myeloma.⁸⁶

Using a qualitative ASO-PCR technique, Corradini et al. showed that molecular remissions are rare in myeloma patients in continuing complete hematologic remission whether by single or tandem autografting (7%). In contrast, in the allograft setting, 50% of the hematologic CR patients also had a molecular remission.⁹² Similar findings were reported by Martinelli et al, who showed a 50% molecular CR rate after allotransplantation in hematologic CRs, compared with 16% after autotransplantation. In that study no difference in molecular CR rate was found in patients receiving a single versus tandem autotransplants. However, achieving a molecular remission was associated with a prolonged relapse-free survival (35 versus 110 months; $p < .005$).⁹³ In a retrospective study of seventy patients in continuing CR after myeloablative allogeneic transplant from a matched sibling donor, Corradini et al⁹⁴ showed that persistently negative PCR was associated with 0% cumulative risk of hematologic relapse at five years, whereas a persistently positive PCR was associated with a 100% risk of hematologic relapse. Patients whose PCR status changed (mixed group) had an intermediate risk of 33%.

Bakkus et al⁹⁵ performed a study on 67 patients using a quantitative ASO-PCR technique to measure tumor load in the bone marrow at 3 to 6 months after high dose chemotherapy and auto-transplantation. A method of maximally selected log-rank statistics was used to test if a cutoff value existed in remaining tumor load post-transplantation that correlated with good or bad prognosis. The estimated threshold for good versus bad prognosis was 0.015%, i.e., 15 malignant plasma cells in 100,000 bone marrow cells. Patients in the good prognosis group had a median survival of 64 months versus 16 months for those in the bad prognosis group ($p = .001$). Multivariate analysis showed that grouping by PCR results was an independent prognostic factor for progression-free survival. The estimated hazard ratio associated with the good prognosis group was 3.91. An interesting finding of this study was, the quantitative PCR assay defined 7 patients with hematologic CR in the bad prognosis group, while 12 of the 43 patients who only achieved a partial hematologic remission were assigned to the good prognosis group. These findings strongly suggest that the amount of paraprotein remaining after high dose therapy is not an accurate reflection of the remaining tumor load. This finding brings into question the second aspect of the definition of hematologic CR. It is quite possible that immature malignant plasma cells/ B cells may not produce paraprotein but nevertheless may cause progressive disease, whereas mature plasma cells may produce significant amount of monoclonal protein and yet behave in an indolent fashion. The plasma cell labeling index of these clonal cells would be a good indicator of the malignant nature of the disease.

Voena et al⁹⁶ also successfully used ASO-PCR to monitor graft-versus myeloma effect after allogeneic transplantation in multiple myeloma. For an excellent review of the use of allogeneic transplantation in multiple myeloma with the goal of achieving molecular remission see Kroger.⁹⁷

Quantitative flow cytometry has been used by many investigators to monitor minimal residual disease. Rawstron et al⁹⁸ used CD19 and CD56 to discriminate between normal and neoplastic plasma cells in the bone marrow and showed that the presence of only normal plasma cells in the bone marrow at 3 months post autologous transplant and at a subsequent 3-6 months assessment consisted of a low risk group. Patients who had neoplastic plasma cells at 3 months post-transplant or normal plasma cells at 3 months followed by neoplastic cells at the second assessment 3-6 months later had high risk disease and significantly higher risk of disease.

progression($p < 0.0001$). Five year survival rates were 54% for the high risk group as compared to 100% for the low risk ($p = 0.036$). Liu et al⁹⁹ also reported on a retrospective study suggesting presence of abnormal plasma cells(APC), defined by aberrant CD56 expression, prior to autologous transplant significantly correlated with poorer PFS. Sarasquete et al.¹⁰⁰ compared quantitative flow cytometry (FCM) and real-time ASO-PCR after autologous transplant in multiple myeloma and concluded that even though minimal residual disease (MRD) evaluation by ASO-PCR is slightly more sensitive and specific than FCM, it is applicable in lower proportion of patients and is more time consuming, while both techniques provide similar prognostic information. Recently, Paiva et al¹⁰¹ showed that MRD negativity by multi-parametric flow cytometry, defined as < 1 MM-PC in 10^4 N-PC (MM-PC multiple myeloma plasma cells, N-PC normal plasma cells) at 100 days post autologous transplantation was the most important independent prognostic marker for PFS and overall survival by a multivariate analysis.

1.7 Quality of Life in Multiple Myeloma and RIC

Gulbrandsen et al.¹⁰² compared quality of life in 221 patients with multiple myeloma treated with autologous transplantation to age- and gender-adjusted population norms. At diagnosis, pain, fatigue, reduced physical functioning, limitations in role functioning, and reduced quality of life were found and were significantly different from population norms. Quality of life improved from baseline to 12 months post-transplant, with small to moderate effect sizes noted. The percentage of patients scoring at or below the 10th percentile of norms was reduced for global quality of life, fatigue, and pain. Similar results were found for patients who were alive at 36 months. However, reduced physical and role functioning were evident in the majority of patients.

Diez-Campelo et al.¹⁰³ compared quality of life in 47 hematologic cancer patients receiving either RIC allogeneic transplantation or autologous transplantation. Patients undergoing autologous transplantation reported worse quality of life on days 7 and 28, and better quality of life on days 270 and 365. Thus, it appears that RIC regimens may offer patients an advantage in quality of life immediately following transplant. Further data are needed to corroborate these findings.

2.0 STUDY OBJECTIVES

2.1 The primary objectives of this study are:

- To determine the 2 year-progression free survival (PFS) in multiple myeloma patients ≤ 60 years of age following allogeneic transplant for those with an available HLA-matched donor using a conditioning regimen of melphalan + fludarabine + Bortezomib (Velcade®)
- To determine the 2 year PFS in multiple myeloma with an autologous stem cell transplant using a conditioning regimen of melphalan + Bortezomib (Velcade®) for patients > 60 years of age and patients ≤ 60 years of age who decline allogeneic stem cell transplant or for whom a suitable HLA-identical donor was not identified.
- To determine the 2 year PFS in multiple myeloma with an autologous stem cell transplant using a conditioning regimen of melphalan + Bortezomib (Velcade®) followed by 1 year maintenance with bortezomib for patients > 60 years of age and patients ≤ 60 years of age who decline allogeneic stem cell transplant or for whom a suitable HLA-identical donor was not identified.

2.2 The secondary objectives of this study are:

- To determine the overall survival (OS) in multiple myeloma with autologous or allogeneic stem cell transplants using the above conditioning regimens
- To determine the response rates in multiple myeloma using the above regimens.
- To determine minimal residual disease status using allele specific oligonucleotides (ASO-PCR) by PCR and flow-cytometry for multiple myeloma cells.
- To correlate minimal residual disease status with 2 year PFS and OS.
- To determine overall regimen safety.
- To determine the incidence of acute and chronic GVHD in multiple myeloma with allogeneic stem cell transplant using the above conditioning regimen.
- To examine quality of life in patients treated with allogeneic and autologous stem cell transplants using the above conditioning regimens.
- To examine effects of Melphalan and bortezomib on protein and gene expression profiles in peripheral blood mononuclear cells

3.0 INVESTIGATIONAL PLAN

3.1 Selection of Patients

Consent for entry into study (and HLA-typing if age ≤ 60 years) will be taken when a myeloma patient is thought to be potentially eligible by a transplant physician either before or at anytime during their induction treatment. Eligibility will be determined prior to initiation of any study specific therapy.

A separate consent will be taken for obtaining bone marrow biopsy sample for monitoring minimal residual disease (i.e for making primers for ASO-PCR and flow cytometry) when the patient is first seen at H. Lee Moffitt Cancer Center. This sample is not considered study specific therapy for this trial unless the patient consents to participation in this trial and is determined eligible for the trial.

Number of patients to be treated

A total of 95 eligible patients will be treated, of which 26 patients will be enrolled in Group A (≤ 60 years and consent for HLA typing and for whom a suitable HLA-identical donor was identified) and 69 in Group B Expansion (> 60 years age, ≤ 60 years but refuse consent for HLA typing or for whom a suitable HLA-identical donor was not identified).

3.1.1 Inclusion Criteria

- Voluntary written informed consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.
- Female subject is either post-menopausal or surgically sterilized or willing to use an acceptable method of birth control (i.e., a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study.
- Male subject agrees to use an acceptable method for contraception for the duration of the study
- Multiple Myeloma Criteria (International Uniform Response Criteria for Multiple Myeloma):
 - Patients with responsive disease after **any** line of induction therapy or early first relapse. Early first relapse is defined as patients that achieve a CR from induction that lasts for more than six (6) months and relapse but are still in VGPR without re-treatment. These patients may be placed on anti-myeloma therapy while awaiting transplant workup for a maximum of 3 months.

A **complete response (CR)** will be defined as the following:

- Complete disappearance of serum and/or urine monoclonal protein by immunofixation, disappearance of any soft tissue plasmacytomas and $\leq 5\%$ plasma cells in the bone marrow.

A stringent CR (sCR) is defined as the above criteria plus normal free light chain (FLC) ratio and absence of clonal cells in the bone marrow by immunohistochemistry or immunofluorescence.

A very good partial response (VGPR) is defined as

- Serum/ urine M-protein detectable by immunofixation but not by electrophoresis or
- $\geq 90\%$ reduction in the level of the serum monoclonal paraprotein plus urine M-Protein < 100 mg in 24 hours

A partial response (PR) will be defined as the following:

- $\geq 50\%$ reduction in serum M-spike and $\geq 90\%$ reduction in urine M-protein or to < 200 mg per 24 hours.

If the serum and urine M-protein are unmeasurable, a

- $\geq 50\%$ reduction in the difference between the involved and uninvolved free light chain (FLC)

If serum free light chains are also unmeasurable, a

- $\geq 50\%$ reduction bone marrow plasma cells provided diagnostic bone marrow plasma cells were $\geq 30\%$

In addition to above, if present at baseline, a

- $\geq 50\%$ reduction in size of soft tissue plasmacytoma

- Patients greater than or equal to 18 years of age are eligible. There is an upper age limit of 60 years for allogeneic transplants. There is no upper age limit for autologous transplant.
- Patients must have a histologically confirmed diagnosis.
- Patients must have undergone a complete psychosocial evaluation and have been considered capable of compliance.
- Meet the following criteria for allogeneic hematopoietic cell transplant:
 - Must have an identified donor match defined as: HLA-A, HLA-B, HLA-C, DRB1 8/8 allele matched sibling, family member, or unrelated donor and be ≤ 60 years of age. Single antigen or allele mismatched donors are not permitted.

3.1.2 Exclusion Criteria

Patients meeting any of the following exclusion criteria are not to be enrolled in the study.

- Patients who do not achieve at least a partial response (PR) by the criteria mentioned above with induction therapy.

- Patient has a platelet count of $<30 \times 10^9/L$ within 30 days before initiation of study therapy and is not improved to $>30 \times 10^9/L$ before initiation of study therapy
- Patient has \geq Grade 2 peripheral neuropathy within 30 days before initiation of study therapy.
- Patient has an absolute neutrophil count of $<1.0 \times 10^9/L$ within 30 days before initiation of study therapy, and is not improved to $>1 \times 10^9/L$ before initiation of study therapy
- Myocardial infarction within 6 months prior to enrollment or has New York Hospital Association (NYHA) Class III or IV heart failure (see section 7.3), uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at screening has to be documented by the investigator as not medically relevant in order for the subject to be considered eligible. LVEF $< 40\%$
- Patient has hypersensitivity to Bortezomib (Velcade®), boron or mannitol.
- Female subject is pregnant or breast-feeding. Confirmation that the subject is not pregnant must be established by a negative serum β -human chorionic gonadotropin (β -hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women.
- Serious medical or psychiatric illness likely to interfere with participation in this clinical study.
- Patient has received other investigational drugs with 30 days before initiation of study therapy.
- Patients with a DLCO less than 50% (adjusted) of normal or with symptomatic obstructive or restrictive lung disease are ineligible.
- Patients with a total bilirubin greater than 2.0 mg/dL excluding Gilbert's syndrome and SGOT or SGPT greater than two and a half times normal (unless due to primary malignancy), or a history of severe hepatic dysfunction are ineligible.
- Calculated creatinine clearance ≤ 30 ml/min within 30 days before initiation of study therapy.
- Patients with current uncontrolled bacterial, viral or fungal infection (currently taking medication with evidence of progression of clinical symptoms or radiologic findings) are ineligible.
- Patients who are HIV positive are ineligible.
- Patients with active leptomeningeal involvement are ineligible. Patients with a history of previous CSF tumor involvement without symptoms or signs are eligible provided the CSF is now free of disease on lumbar puncture, and MRI of the brain shows no evidence of tumor progression following completion of appropriate therapy. Patients with severe symptomatic CNS disease of any etiology are ineligible.
- Patients with uncontrolled insulin-dependent diabetes mellitus defined as a random glucose level of > 400 in the 30 days prior to initiation of study therapy; or

uncompensated major thyroid or adrenal dysfunction are ineligible.

- Patients with an ECOG performance status of ≥ 2 (Karnofsky $< 50\%$) are ineligible (See section 7.6).
 - Patients with an ECOG performance status of 2 to 3 (Karnofsky 30-50%), secondary to bone pain, may be enrolled.
 - Patients with an ECOG performance status of 2 to 3 (Karnofsky 30-50%), secondary to a potentially reversible disease-related problem, may be enrolled.
- Patients with any previous malignancy other than non-melanoma skin cancer are ineligible, unless the patient is without evidence of disease ≥ 5 years after the treatment for the cancer was completed.

3.2 Study Treatment

3.2.1 Stem Cell Mobilization

Autologous Transplant

Patients will undergo stem cell mobilization with G-CSF (granulocyte colony-stimulating factor) at a dose of $10 \mu\text{g/kg/day}$ as per institutional standards. CD34^+ peripheral blood stem cells will be collected following the administration of G-CSF as per institutional standards. A minimum of $2 \times 10^6 \text{ CD34}^+$ peripheral blood stem cells per kilogram of recipient's body weight must be collected prior to proceeding to autologous stem cell transplant in patients who are in CR/VGPR and $4 \times 10^6 \text{ CD34}^+$ cells/kg for patients who are in PR and may receive tandem autologous transplants. If there is failure to mobilize autologous stem cells or the patient is at high risk for poor mobilization, physicians at their discretion can use plerixafor or other agents for stem cell mobilization. The stem cells from autologous patients will be cryopreserved and stored in Cell Core Facility until the day of transplant.

Stem Cell Mobilization for Allogeneic Transplant

The intended goal for CD34^+ peripheral blood stem cell collection from matched donor will be from 5 to $10 \times 10^6/\text{kg}$ of body weight of the recipient for the allogeneic stem cell transplant. For the allogeneic matched-related donors peripheral blood stem cells will be harvested with G-CSF mobilization and infused fresh to the recipients. Allogeneic donor stem cells may also be cryopreserved if they cannot be infused fresh. For the matched unrelated donors National Marrow Donor Program protocols will be followed.

3.2.2 Administration and schedule of chemotherapy:

High Dose Chemotherapy followed by Autologous Stem cell Rescue

Following the collection of the defined number of CD34^+ peripheral blood stem cells, patients will receive the following pre-transplant conditioning regimen: Day -4 melphalan 100 mg/m^2 intravenously over 30 minutes, Day -3 melphalan 100 mg/m^2 intravenously over 30 minutes immediately followed by Bortezomib (Velcade®) (1.3 mg/m^2) as an intravenous

push over 3 to 5 seconds followed by a standard saline flush or through a running IV line. Day -2, -1 no therapy, and Day 0 autologous PBSC rescue with a minimum of 2 million CD34⁺ peripheral blood stem cells per kilogram of body weight.

Subjects will be followed according to standard institutional protocol for autologous stem cell transplant.

Autologous Peripheral Blood Stem Cell Rescue

Day -4	Melphalan 100 mg/m ² /day IV over 30 minutes.
Day -3	Melphalan 100 mg/m ² /day IV over 30 minutes, followed by Bortezomib (Velcade®) (1.3 mg/m ²) as an intravenous push over 3 to 5 seconds.
Day -2,-1	NO THERAPY
Day 0	Infusion of autologous stem cells

Maintenance Therapy

Patients enrolled in Group B expansion will receive therapy as described above followed by maintenance bortezomib starting between Day 90 and Day 120 post transplant. Patients will receive subcutaneous bortezomib at 1.3mg/m² given weekly, 4 weeks on, 4 weeks off for 1 year.

Subcutaneous Administration:

Drug is available in sterile, single use vials containing 3.5 mg of VELCADE. Each vial of VELCADE 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 VELCADE at a concentration of 2.5 mg/mL for subcutaneous administration.

Subcutaneous Administration Precautions:

- The drug quantity contained in one vial (3.5 mg) may exceed the usual dose required. Caution should be used in calculating the dose to prevent overdose.
- When administered subcutaneously, sites for each injection (thigh or abdomen) should be rotated.
- New injections should be given at least one inch from an old site and never into areas where the site is tender, bruised, erythematous, or indurated.
- If local injection site reactions occur following VELCADE administration subcutaneously, a less concentrated VELCADE solution (1 mg/mL instead of 2.5 mg/mL) may be administered subcutaneously. Alternatively, the IV route of administration should be considered.
- In clinical trials of VELCADE IV, local skin irritation was reported in 5% of patients, but extravasation of VELCADE was not associated with tissue damage. In a clinical trial of

subcutaneous VELCADE, a local reaction was reported in 6% of patients as an adverse event, mostly redness.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]	[REDACTED]		
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[illegible]

An 8/8 HLA-A, B, C, DRB1 matched related/unrelated donor will be selected for eligible patients who are less than or equal to 60 years of age. A target of 5 -10 million/kg of CD34 cells will be collected from the donor. The patient will receive Fludarabine 30 mg/m² (CrCL 30-70ml/min, reduce to 24mg/m²) from day -6 to -3 and melphalan 70 mg/m² on day -4 and -3 intravenously followed by Bortezomib (Velcade®) (1.3 mg/m²) as an intravenous push over 3 to 5 seconds followed by a standard saline flush or through a running IV line on day -3. On day 0 the patient will receive the donor cells.

Allogeneic Peripheral Blood Stem Cell Rescue

Day -6 Fludarabine 30 mg/m²

Day -5	Fludarabine 30 mg/m ²
Day -4	Fludarabine 30 mg/m ² + Melphalan 70 mg/m ² /day IV over 30 minutes.
Day -3	Fludarabine 30 mg/m ² + Melphalan 70 mg/m ² /day IV over 30 minutes followed by Bortezomib (Velcade®) (1.3 mg/m ²) as an intravenous push over 3 to 5 seconds.
Day -2,-1	NO THERAPY
Day 0	Infusion of allogeneic peripheral blood stem cells

Doses for melphalan and fludarabine are based on actual body surface area. Fludarabine will be dose reduced for patients with a creatinine clearance of 30-70 mL/min/1.73 m²

Bortezomib (Velcade®) will be administered only to eligible patients under the supervision of the investigator or identified sub-investigator(s). Patients may be treated on an out-patient basis, if possible. The pharmacist will prepare the drug under aseptic conditions.

Doses for Bortezomib (Velcade®) will be determined based on actual body surface area. Body surface area is to be calculated based on body weight using a standard nomogram (see 7.2). The dose should be calculated on Day -4 of each auto-transplant and day -6 of allo-transplant; the dose administered should be recalculated at the start of the next transplant for patients receiving two transplants.

The appropriate amount of Bortezomib (Velcade®) will be drawn from the injection vial and administered as an intravenous (IV) push over 3 to 5 seconds followed by a standard saline flush or through a running IV line. Vials are for single use administration.

3.2.2.1 Renal Insufficiency Dose Adjustment

Adult patients with moderate impairment of renal function (creatinine clearance 30-70 mL/min/1.73 m²) should have dose reduced from 30mg/m² to 24mg/m², a 20% dose reduction of fludarabine. Fludarabine should not be administered to patients with severely impaired renal function (creatinine clearance less than 30 mL/min/1.73 m²).

Drugs will be administered to eligible patients under the supervision of a BMT attending physician. Patients may be treated on an out-patient basis, if possible. The pharmacist will prepare the drugs under aseptic conditions.

3.2.3 Treatment Assignment

Group A: Allogeneic

All patients who are less than or equal to 60 years of age and achieve a CR/VGPR with induction therapy will be offered an allogeneic stem cell transplant. If they consent for HLA-typing, insurance approval will be required for HLA-typing (HLA A, B, C and DR typing) and search for a related or unrelated donor.

Group B Expansion: Autologous Only

If patients do not consent for HLA-typing, insurance refusal prevents HLA-typing/allogeneic transplant, or a suitable HLA-identical donor is not identified, they will be offered an

autologous stem cell transplant. Patients who are more than 60 years of age will be offered an autologous transplant if they achieve either a CR/VGPR.

Patients in PR after Initial Induction:

Patients who have a PR after cytoreductive induction therapy will receive an autologous transplant. If the first autologous transplant results in CR/VGPR within 3-6 months post transplant, patients in Group A will receive an allogeneic transplant (Group A only). If the first autologous transplant results in CR/VGPR within 3-6 months post transplant, patients in Group B Expansion will not proceed to a second autologous transplant. Patients who do not achieve VGPR/CR after the initial transplant will come off trial and be treated at the discretion of their physician.

3.2.4 Supportive Treatment

The following medications/supportive therapies are recommended during study participation, as applicable:

- Granulocyte colony-stimulating factor (G-CSF) 5mcg/kg/day from day +7 for autologous transplants. No G-CSF will be administered for allogeneic transplants unless otherwise clinically indicated.
- The antiemetic regimen(s) will be determined by the attending physician(s).
- Antimicrobials: the exact antimicrobial regimen(s) will be determined by the study investigator(s) or treating physician(s), and will be in accordance with the standard supportive care guidelines established by the Division of Bone Marrow Transplantation at H. Lee Moffitt Cancer Center & Research Institute for patients receiving peripheral blood stem cell transplantations.
- Graft-versus-host disease prophylaxis will be according to current Moffitt BMT Program institutional protocols for standard of care.
- Total parenteral nutrition may be utilized for supportive nutrition.
- Opioid analgesics may be utilized for supportive pain control.
- Packed red blood cell transfusions would be utilized for supportive care.
- Platelet transfusions would be utilized for supportive care.
- Vasoactive medications may be utilized for supportive care.
- In an effort to prevent hepatotoxicity, ursodiol will be given at 300 mg twice a day if <90 kg, or 300 mg in a.m. and 600 mg in p.m. if >90 kg

Prohibited Concurrent Therapy

Any investigational agents within 30 days of enrollment.

3.2.5 Treatment Compliance

All drugs will be administered to eligible patients under the supervision of the investigator or identified subinvestigator(s). The pharmacist will maintain records of drug receipt (if applicable), drug preparation, and dispensing, including the applicable lot numbers, patients' height, body weight, and body surface area (see Appendix 8.1), total drug administered in milliliters and milligrams, and date and time of administration. A +/- 10% discrepancy between the calculated dose and dose administered and the reason for the discrepancy will be recorded in the source documents. Any discrepancy between the calculated dose and dose administered and the reason for the discrepancy must be recorded in the source documents.

Precautions and Restrictions

It is not known what effects VELCADE has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Non-sterilized female patients of reproductive age and male patients should use effective methods of contraception through defined periods during and after study treatment as specified below. Female patients 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 VELCADE, 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 patients, even if surgically sterilized (ie, 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.**

3.3 Duration of Patient Participation

The duration of patient participation will be anticipated to be through the completion of the study, approximately 4 years (enrollment for two years and follow up for two years).

3.4 Termination of Treatment and/or Study Participation

Patients 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 patients from the study for any of the following reasons:

- Subjects who failed to harvest a minimum of 2 million CD34⁺ peripheral blood stem cells per kilogram of body weight.
- Patient request to stop follow up will be honored; however, patients will be informed about the implications of having received high dose melphalan, Bortezomib (Velcade®) ± allograft.
- Administrative reasons
- Failure to provide follow-up information
- Changes in the patient's condition that make the patient ineligible for further treatment
- Intercurrent illness except those normally related to stem cell transplant
- Occurrence of an unacceptable adverse event except those normally related to stem cell transplant
- Non-compliance
- Failure to return for follow-up
- Progressive disease at any time

At the time of withdrawal, all study procedures outlined for the End of Study visit should be completed. The primary reason for a patient's withdrawal from the study is to be recorded in the source documents.

3.5 Efficacy, Pharmacodynamic/Pharmacogenomic, and Safety Measurements

3.5.1 Efficacy Measurements

Time to disease progression, overall survival time, and time to other events will be assessed by the method of Kaplan and Meier with standard errors computed using Greenwood's formula and 95% confidence bands constructed by the method of Hall and Wellner.²

Response rates, CR rate, and other rates or proportions will be estimated using the International Response criteria. Exact 95% confidence intervals will be computed using the method of Clopper and Pearson.¹

Quality of life will be examined longitudinally using Generalized Estimating Equation (GEE).

3.5.2 Safety Measurements

Toxicities will be recorded as outlined in section 5.6 and proportions estimated. .

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

3.6 Recommended Monitoring Parameters

Monitoring Parameters

The following studies and time frames are guidelines for monitoring toxicity and efficacy.

The following laboratory tests should be done within **30-days** prior to stem cell collection or initiation of conditioning for allogeneic transplant recipients. Patients with known positive viral titers need not be retested:

- 1) CMV, HSV IgG
- 2) Hepatitis B and Hepatitis C panels
- 3) Hepatitis C NAT
- 4) HIV NAT
- 5) VZV antibody
- 6) HTLV-1 antibodies
- 7) HIV-1 and HIV-2 NAT
- 8) RPR
- 9) Urine β -hCG (females of childbearing potential)
 - a woman will be considered of childbearing potential unless she is status-post hysterectomy or tubal ligation or she has had no menstrual periods in the preceding 12 months
- 10) CBC with differential
- 11) Comprehensive metabolic panel
- 12) Magnesium level

The following vital organ evaluations should be performed within **12weeks** (for autologous) and within 4-6 weeks (for allogeneic transplants) of initiating the transplant conditioning regimen:

- 1) Pulmonary function tests with arterial blood gas analysis and DLCO (adjusted)
- 2) MUGA or ECHO
- 3) Creatinine Clearance estimation (24-hour urine collection or calculation)
- 4) Comprehensive metabolic panel

The following evaluation should be performed, if appropriate, anytime following the initial evaluation but prior to initiating the transplant conditioning regimen:

- 1) Dental consult

All of the following should be obtained within 4-6 weeks prior to G-CSF priming or initiation of conditioning for the allogeneic transplant recipients:

- 1) SPEP with immunofixation
- 2) serum quantitative immunoglobulins
- 3) 24-hour urine for total protein, UPEP with immunofixation
- 4) Serum free light chains
- 5) skeletal bone survey
- 6) Bone marrow biopsy and aspirate

The following evaluations should be performed within 30 days prior to the initiation of the transplant conditioning regimen:

- 1) CBC with differential
- 2) Comprehensive metabolic panel

Routine laboratory evaluations such as: CBC, metabolic panels, renal function tests, hepatic function tests, etc. will be performed after the initiation of the transplant conditioning regimen. The frequency and duration will be determined by the treating physician based upon the patient's clinical status.

Disease status post-transplant will be evaluated:

- 1) Approximately 3 months post-transplant, as measured from Day 0 following re-infusion of PBSC's and approximately every three-six months thereafter. Disease status will be evaluated by obtaining all of the following: SPEP with immunofixation, serum quantitative immunoglobulins, 24-hour urine for total protein, UPEP with immunofixation, and serum free light chains and complete skeletal survey (to be done at 3 months post transplant and once per year thereafter, at the discretion of the treating physician). Responses to high-dose chemotherapy, Bortezomib (Velcade®), and PBSCT (CR, PR, MR, NC or PD) must be confirmed by the criteria outlined in Section 7.7.
- 2) If the monoclonal protein (serum, urine or both) decreases to zero following transplantation, then a bone marrow biopsy and aspirate will then be performed to confirm a CR. A bone marrow biopsy will also be done for non-secretory myeloma. MRD studies with ASO-PCR will be done for all patients who had an adequate (with more than 10% plasma cells) baseline sample collected for ASO-PCR who achieve a CR at any point.
- 3) Monitoring of minimal residual disease in patients who achieve a CR and have an adequate baseline bone marrow sample (>10% plasma cells) will be done by ASO-PCR and quantitative flow cytometry every 6-12 months \pm 15 days from the bone marrow biopsy done to confirm CR.
- 4) Following relapse or progression of primary disease patient will be treated off study as per treating physician.

- 5) For patients enrolled in Group B expansion on bortezomib maintenance, a CBC and CMP will be done on day 1 of each bortezomib cycle, in addition to above evaluations.

Following allogeneic stem cell transplants, in addition to the above studies, routine follow up of chimerism, pulmonary function tests, CMV viremia, galactomannan assay, IgG, blood cultures will be done as per institutional guidelines.

3.7 Study Endpoints

Primary Endpoints

1. the 2 year PFS with allogeneic stem cell transplant in multiple myeloma using melphalan+fludarabine+ Bortezomib (Velcade®) as the conditioning regimen
2. the 2 year PFS with autologous stem cell transplant in multiple myeloma using melphalan+ Bortezomib (Velcade®) as the conditioning regimen
3. the 2 year PFS with autologous stem cell transplant in multiple myeloma using melphalan+ Bortezomib (Velcade®) as the conditioning regimen followed by maintenance therapy with bortezomib for 1 year

Secondary Endpoints

1. OS in patients with multiple myeloma treated with Bortezomib (Velcade®) containing conditioning regimen and autologous as well as allogeneic transplantation.
2. molecular CR rates patients with multiple myeloma treated with Bortezomib (Velcade®) containing conditioning regimen and autologous as well as allogeneic transplantation.
3. Overall safety,
4. incidence of acute and chronic GVHD in patients with multiple myeloma following allogeneic stem cell transplant using the above conditioning regimen.
5. Quality of life in patients with multiple myeloma following allogeneic stem cell transplant using the above conditioning regimen

4.0 ADVERSE EVENTS

All serious adverse events (SAEs) (regardless of expectedness, causality, and whether commercial or investigational Velcade is used) will be reported to Millennium Pharmacovigilance (or designee).

The sponsor-investigator is responsible to meet all regulations and requirements applicable to the sponsor-investigator.

4.1 Definitions

Adverse Event Definition

An **adverse event** (AE) is any untoward medical occurrence in a patient administered a pharmaceutical product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An adverse event can be any unfavorable and unintended sign (eg, including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the drug, whether or not it is considered to be drug related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of drug.

For this protocol an abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline

Serious Adverse Event Definition

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

- Results in **death**.
- Is **life-threatening**. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, ie, it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires inpatient **hospitalization or prolongation of existing hospitalization**. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (eg, surgery performed earlier than planned).
- Results in **persistent or significant disability/incapacity**. Disability is defined as a substantial disruption of a persons' ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is **medically important event**. An important medical event is an event that may not result in death, be immediately life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient, require medical or surgical intervention to prevent one of the outcomes listed in the definitions for

SAEs above, or involves suspected transmission via a medicinal product of an infectious agent.

- Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
- With respect to suspected transmissions via a medicinal product of an infectious agent; any organism, virus, or infectious particle (e.g., prion protein transmitting Transmissible Spongiform Encephalopathy), whether pathogenic or non-pathogenic, is considered an infectious agent.

Clarification should be made between the terms “serious” and “severe” because they ARE NOT the same. The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is NOT the same as “serious,” which is based on patient/event outcome or action criteria described above and is usually associated with events that pose a threat to a patient’s life or functioning. A severe adverse event does not necessarily need to be considered serious. For example, persistent nausea of several hours duration may be considered severe nausea but not an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild, but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

4.2 Procedures for AE and SAE Reporting

Adverse events (AEs) may be spontaneously identified by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures must be reported to Millennium Pharmacovigilance (or designee).

AEs which are **serious** must be reported to Millennium Pharmacovigilance (or designee) from first dose of Velcade up to and including 30-days after administration of the last dose of Velcade. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. Any SAE that occurs at any time after completion of Velcade treatment or after the designated follow-up period that the investigator and/or sub-investigator considers to be related to any study drug must be reported to the Millennium Pharmacovigilance (or designee). Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient’s stable or chronic condition or intercurrent illness(es).

Since this is an investigator-initiated study, the principal investigator Dr. Melissa Alsina, (who may also sometimes be referred to as the sponsor-investigator), is conducting the study and acting as the sponsor. Therefore, the legal/ethical obligations of the principal investigator include both those of a sponsor and those of an investigator.

Institution must report all SAEs, regardless of expectedness or relationship with any study drug, to Millennium Pharmacovigilance (or designee) as soon as possible, but no later than five (5) calendar days of the sponsor-investigator's observation or awareness of the event. Millennium Pharmacovigilance (or designee) may request follow-up information to a reported SAE, which the sponsor-investigator will be responsible for providing to Millennium Pharmacovigilance (or designee).

The SAE report must include event term(s), serious criteria, and the investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration.

Intensity for each adverse event, including any lab abnormality, will be determined by using the NCI CTCAE, version 4.0, as a guideline, wherever possible. The criteria are provided in the study manual and also are available online at <http://ctep.cancer.gov/reporting/ctc.html>.

Relationship to all study drugs for each SAE will be determined by the investigator responding yes or no to the question: Is there a reasonable possibility that the adverse event is associated with the study drug?

Institution will provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study or study drug(s), including, but not limited to, telephone conversation logs, as soon as possible but no later than 5 calendar days of such communications.

Millennium's will send to the Investigator-Sponsor a quarterly listing of the SAE reports received for site verification. Institution and/or Principal Investigator will be responsible for forwarding such reports to Sub-investigator(s).

SAEs will be identified by the investigators or BMT Research Staff and will be reported to the IRB according to current IRB policy. Exceptions to this will be the following:

- Hospitalizations post transplant for neutropenic or nonneutropenic fevers
- Hospitalizations for non-life-threatening events (life-threatening or unexpected events will be reported as we are notified)
- Hospitalizations to receive blood or platelet transfusions for anemia or thrombocytopenia
- Hospitalizations for chemotherapy or radiation therapy (or its complications) used for the treatment of disease relapse post transplant
- Extended outpatient (EOP) admissions or 23 hour observation admissions
- Relapse-related deaths (to be reported in the annual research progress report)
- Hospitalizations after relapse of primary disease
- Hospitalizations for diagnostic work up only. An SAE report will be generated if the diagnosis made otherwise fits the definition of an SAE.
- Mucositis, enteritis, myelosuppression, infections or GVHD. These are expected posttransplant complications.

Note: for open label studies in which there is a comparator arm, Millennium expects SAEs only for subjects on Millennium's drug.

**SAE and Pregnancy Reporting Contact Information (North America Reporting)
US and Canada**

Suggested Reporting Form:

- US FDA MedWatch 3500A:
<http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm>
- Any other form deemed appropriate by the sponsor-investigator

4.3 Monitoring of Adverse Events and Period of Observation

Adverse events, both serious and non-serious, and deaths that occur during the patient's study participation will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

For IIS studies that include patients undergoing hematopoietic cell transplantation, review and consideration will be given by Millennium Pharmacovigilance for reporting of adverse events that are designated in the protocol as commonly observed after hematopoietic cell transplantation, not to be reported to Millennium, unless serious per SAE definition **and** considered related to VELCADE. These serious events must be reported to the company within the timelines specified for immediate reporting.

- Toxicity/Adverse Events' will be scored according to the NCI Common Toxicity Criteria 4.0 (<http://ctep.cancer.gov/reporting/ctc.html>) grading scale. The following toxicities occur at a high rate with high dose chemotherapy and transplantation and will not be reported unless the AE in question is both serious and related to Velcade. AE's that are Serious and related to Velcade will be reported per the timelines above. Any hematologic toxicity (>77%)
 - The use of intravenous antibiotics (41%)
 - Mucositis (17%)
 - Diarrhea (5%)
 - Nausea or vomiting (6%)
 - Infection (40%)

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5.0 STATISTICAL PROCEDURES

5.1 Sample Size Justification

A total of 95 evaluable subjects who achieve CR/VGPR by induction therapy or autologous transplant will be treated in 2 years. They will be stratified in two groups, A and B, based on age ≤ 60 years, consent for HLA typing, and identification of a suitable HLA-identical donor (Group A) and age > 60 years as well as those ≤ 60 years who do not consent for HLA typing or for whom a suitable HLA-identical donor is not identified (Group B Expansion). As of August 15, 2011, 30 subjects were accrued to Group B, and Group B has been closed. Given new emerging data of importance of maintenance therapy post transplant for PFS in myeloma, Group B will be expanded to accrue 39 additional patients (Group B expansion arm), that will be treated with 1 year maintenance post transplant. This cohort will be compared to the initial 30 pts accrued and we hypothesize that the maintenance intervention will improve 2 year PFS from the projected 50% to 70% with the maintenance intervention.

A follow-up period of 2 years will start from the entry of the last patient. Consent for entry into study (and HLA-typing if age ≤ 60 years) will be taken when a myeloma patient meeting the eligibility criteria is referred to H. Lee Moffitt Cancer Center, either before or at anytime during their induction treatment. A separate consent will be taken for obtaining bone marrow biopsy sample for monitoring minimal residual disease (i.e., for making primers for ASO-PCR and flow cytometry) when the patient is first seen at H. Lee Moffitt Cancer Center and at subsequent intervals as specified in Section 7.1. All the patients who are ≤ 60 years of age and have available HLA-identical related or unrelated donor will undergo an allogeneic transplant. If any patient changes his mind or is ineligible for allogeneic transplant after consent for HLA-typing is given, autologous transplant will be offered if eligible and the patient will be enrolled in arm Be.

The primary goal of this parallel phase II study is to assess 2-year progression-free survival (PFS) for each arm (Group A, Group B, and Group B expansion arm). With 90% power and 10% type I error rate, the sample size is selected using the Minmax two-stage design. For Group A, a total of 26 subjects (13 in first and additional 13 in second stage) will be evaluated to test the null 2-year PFS of 50% versus the alternative of 75% or higher. Group A will be terminated early if 6 or fewer out of 13 subjects ($\leq 6/13$) are progression-free at 2 years. If the trial proceeds to the second stage, a total of 26 subjects will be studied. If the total number of 2-year progression free subjects is less than or equal to 16 ($\leq 16/26$), the null hypothesis fails to be rejected. For Group B expansion arm, a total of 39 subjects will be evaluated to test the null 2-year PFS of 50% versus the alternative of 70% or higher. Group B expansion arm will be terminated early if 11 or fewer out of 23 subjects ($\leq 11/23$) are progression free at 2-years. If the trial proceeds to the second stage, a total of 39 subjects will be studied. If the total number of 2-year progression free subjects is less than or equal to 23 ($\leq 23/39$), the null hypothesis fails to be rejected.

The accrual will not be halted after reaching the accrual goal of the 1st stage due to the long follow-up time for the primary endpoint. A subject who lost follow-up within 2 years will be considered as a failure which is progression of disease within 2 year. Due to an expected drop out rate of 10% from ineligibility, it is anticipated that 29 and 43 subjects will be accrued to Group A and B expansion arm in order to achieve accrual goal.

All patients will be followed and monitored for disease progression and survival until death. The primary endpoints will be analyzed on all patients who start the transplant procedure based on donor availability and age. Safety will be determined by monitoring morbidity, mortality and engraftment characteristics. Toxicities will be monitored as per definitions in Common Toxicity Criteria (version 4.0).

5.2 Selection of patients

Selection of patients between autologous and allogeneic arm for patients less than or equal to 60 years will be on the basis of donor availability.

5.3 Populations for Analysis

The population for analysis will include all patients registered on this study. The only exclusions will be for patients later found to be ineligible. The clinically evaluable population is defined as all patients who receive any amount of the conditioning regimen and will be used for safety analyses and efficacy analyses. Patients consented at diagnosis or at anytime during induction therapy that are found not eligible for transplant upon full evaluation and do not receive treatment intervention, will not be considered part of the clinically evaluable population.

5.4 Procedures for Handling Missing, Unused, and Spurious Data

All data will be subjected to range and logical checks by computer programs written by the biostatistician with guidance from the P.I. Questionable data will be referred to the P.I. for resolution. Those patients with missing data will be excluded from analyses requiring those data. There will be no data imputation used.

5.5 Statistical Methods

Summary tabulations will be presented that will display the mean, standard deviation, median, minimum, and maximum for continuous variables, and the frequency and percent per category for categorical data. The differences between two arms will be assessed by parametric or nonparametric method for continuous variables, Fisher exact tests for categorical variables. Significance will be established if the two-sided p-value of the test is less than 0.05.

5.5.1 Primary Endpoint

- The primary endpoint of this study is to estimate the 2-year progression-free survival (PFS) for each study arm. Survival time will be measured from the date of transplant to the date of progression, death or the last follow-up, whichever comes first. Two-year PFS and 95% confidence interval will be computed using the Kaplan-Meier method with and Greenwood's formula with log-log transformation.

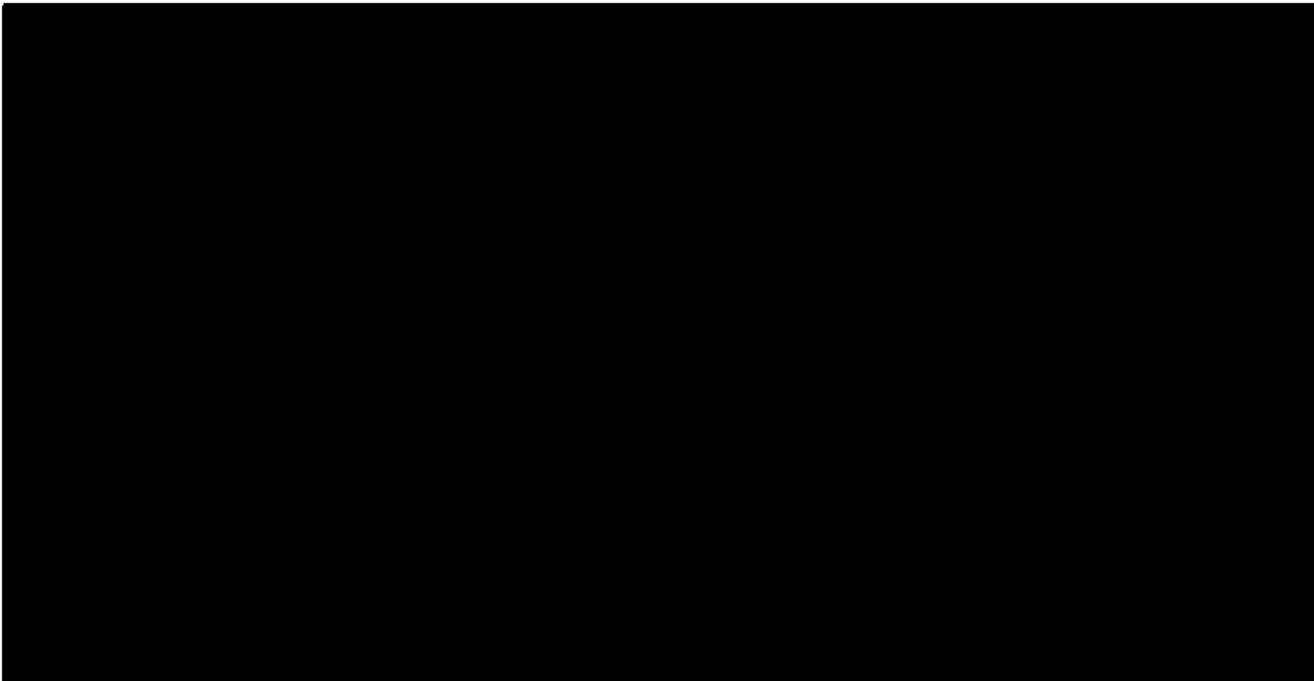
5.5.2 Secondary Endpoints

- Time to disease progression, overall survival, and time to other events will be assessed by the Kaplan-Meier method. The difference in time-to-event will be tested by a two-sided log-rank test.
- The cumulative incidence curves of competing risks including treatment related mortality (TRM) and relapse will be estimated and compared using the Gray method.

- Response rate, CR rate, and other rates or proportions will be estimated. The exact 95% confidence intervals will be computed using the method of Clopper and Pearson.
- Correlative analysis of the minimal residual disease status with survival will be carried out using the Cox proportional hazards model. Longitudinal change in subjects' quality of life will be examined using the Generalized Estimating Equation (GEE).
- Subjects in Group B expansion arm will receive maintenance therapy (velcade) for 1 year while those in initial Group B did not. The comparisons between three groups (Allo, Group B, and Group Be) will be explored at two-sided 5% significance level, and no multiplicity adjustment is planned.

5.6 Safety and Toxicity Analysis

Toxicity will be assessed according to CTC 4.0 criteria and any grade 4 non-hematological toxicity up to 100 days will be recorded in all patients. Accrual will be halted if the number of subjects who experience any grade 4 non-hematological toxicity up to 100 days is equal to or exceeds b_n out of n subjects. This is a Pocock-type stopping boundary that assumes the grade 4 toxicity rate of 0.25 is acceptable. If true grade 4 toxicity rate is equal to 0.25, the probability of crossing the boundary is 0.05. Beyond 100 days, all hematological and non-hematological toxicities > grade 3-4 will be recorded up to progression of disease/ death within 2 years follow up. Treatment related mortality will be assessed and documented in all patients.



5.7 Procedures for Reporting Deviations to Original Statistical Analysis Plan

Additional analyses, as suggested by the data or by results reported by others, may be performed when determined to be of interest by the P.I. Any such analyses will be clearly identified as being post-hoc and/or data directed in any reports of the results of this trial and any conclusions based on these analyses will be tentative and require confirmation by studies designed to study that issue.

6.0 ADMINISTRATIVE REQUIREMENTS

6.1 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

6.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (see section 8.5). The Institutional Review Board (IRB) / Institutional Ethics Committee (IEC) will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients (including diary cards), safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the investigator. Millennium requests that informed consent documents be reviewed by Millennium or designee prior to IRB/IEC submission.

6.3 Patient Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

6.4 Patient Confidentiality

In order to maintain patient privacy, all data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number. The investigator will grant monitor(s) and auditor(s) from Millennium or its designees and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the data capture records and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

6.5 Protocol Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB/IEC and the appropriate regulatory authority(ies). Changes to the protocol will require approval from Millennium and written IRB/IEC and SRC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB/IEC may provide, if applicable regulatory

authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB/IEC. The investigator will submit all protocol modifications to Millennium and the regulatory authority(ies) in accordance with the governing regulations. Any departures from the protocol must be fully documented in the source documents.

6.6 Data Safety and Monitoring

The Data Safety & Monitoring Plan (DSMP) will ensure that this trial is well designed, responsibly managed, appropriately reported, and that it protects the rights and welfare of patients. The following internal and external review and monitoring processes provide oversight and active monitoring of this trial:

- The Principal Investigators (PI)
- The Scientific Review Committee (SRC)
- The Protocol Monitoring Committee (PMC);
- The Research Compliance Division (RCD) of the Cancer Center's Compliance Office;
- The University of South Florida, Institutional Review Board (USF IRB).

The protocol includes a section that specifies the following with respect to Adverse Event reporting: what constitutes an adverse event (versus what is a serious adverse event), the entities to which adverse events should be reported, the timing of this reporting, and the person or persons responsible for reporting. This includes prompt (within one day of knowledge of the event) reporting to the IRB for unanticipated risks to subjects and reporting in writing within five working days to the IRB and sponsor.

6.6.1 Initial and Ongoing Monitoring and Review

Principal Investigator (PI)

The PI of the study has primary responsibility for ensuring that the protocol is conducted as approved by the SRC and the IRB. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to the Scientific Review Committee (SRC), Protocol Monitoring Committee (PMC) and IRB as required, and that all adverse events are appropriately reported.

The Scientific Review Committee (SRC)

The Cancer Center's internal Scientific Review Committee (SRC) provides for a formal internal peer review of all protocols and general scientific oversight of interventional clinical research. The Committee has a defined membership representing all of the major research divisions of the Cancer Center, including biostatisticians. All new protocol submissions must contain the required elements of the protocol, and must include a DSMP prior to approval by the Committee. The plan has to be appropriate for the phase and risk of the proposed study.

The Protocol Monitoring Committee (PMC)

The Protocol Monitoring Committee (PMC) will monitor this trial for safety, protocol compliance, adverse event reporting, and data integrity. The membership of the PMC includes physician representation from each program area and a biostatistician. In addition to the existing stopping rules, the PMC is authorized to suspend a trial for non-compliance with a DSMP or as a result of audit findings deemed unacceptable.

The PMC will report significant findings to the IRB, the sponsor, and the applicable regulatory body. Interim meetings are scheduled to address specific issues that require immediate attention to ensure safety of research participants.

The Research Compliance Division of the Cancer Center's Compliance Office (RCD)

The Cancer Center's Research Compliance Division (RCD) of the Corporate Compliance Department coordinates internal audits of all investigator-initiated trials conducted at the Cancer Center and its affiliates. The frequency of the audits is driven by the rate of accrual on a specific trial as well as the perceived patient risk for participating in the trial. Internal audits are conducted by the RCD in accordance with applicable regulatory standards.

The purpose of the internal audit program is to:

- Ensure protocol compliance and the validity and integrity of data, thereby promoting patient safety and maintaining scientific validity
- Recommend modification of research practices as necessary and provide education on issues that are critical to good research practices

The following elements of trial documentation may be incorporated into this review:

- Source documentation verification of eligibility and compliance with the protocol
- Compliance with adverse and serious adverse event reporting standards
- Regulatory review of IRB compliance and external reporting requirements
- Drug/device accountability and handling
- Completeness and quality of data

RCD auditors report findings to the PI and PMC, as appropriate, for review and audit determinations along with a statement from the PI. The PI is encouraged to proactively implement corrective actions to remedy any deficiencies noted in the audit. The PMC determines the findings as one of the following: *Acceptable*, *Acceptable with corrective action*, *Tabled for Additional Information*, or *Unacceptable*. The PI and IRB are then informed of the audit determinations made by the PMC. The PI and IRB are then informed of the audit determinations made by the PMC. Audit results may then be presented to the Corporate Compliance Steering Committee, chaired by the Center Director, and to the Joint Corporate Compliance Committee of the Board

Institutional Review Board (IRB)

The trial will not be initiated without approval of the University of South Florida Institutional Review Board (IRB). All administrative requirements of the governing body of the

institution will be fully complied with. This protocol, consent procedures, and any amendments will be approved by the IRB in compliance with current regulations of the Food and Drug Administration prior to initiation unless necessary to protect the safety and welfare of subjects; in which case, the IRB will be notified within 24 hours of implementing the change. The IRB will be kept informed by the investigator as to the progress of the study as well as to any serious or unusual adverse events.

6.6.2 On-site Audits

Regulatory authorities, the IEC/IRB and/or Millennium's clinical quality assurance group may request access to all source documents, data capture records, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

6.7 Drug Accountability

Accountability for the drug at all study sites is the responsibility of the principal investigator. The investigator will ensure that the drug is used only in accordance with this protocol. Drug accountability records indicating the drug's delivery date to the site (if applicable), inventory at the site (if applicable), use by each patient, and return to Millennium or disposal of the drug (if applicable and if approved by Millennium) will be maintained by the clinical site. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers.

All materials containing Bortezomib (Velcade®) will be treated and disposed of as hazardous waste in accordance with governing regulations.

6.8 Premature Closure of the Study

This study may be prematurely terminated, if in the opinion of the investigator or Millennium, there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the investigator or Millennium by the terminating party.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients
- Failure to enter patients at an acceptable rate
- Insufficient adherence to protocol requirements
- Insufficient complete and/or evaluable data
- Plans to modify, suspend or discontinue the development of the drug

Should the study be closed prematurely, all study materials must be returned to Millennium.

6.9 Record Retention

The investigator will maintain all study records according to ICH-GCP and applicable regulatory requirements.

6.10 Product Complaints

A product complaint is a verbal, written, or electronic expression which implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact MedComm Solutions (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium quality representative.

A medication error is a preventable event that involves an identifiable patient and that leads to inappropriate medication use, which may result in patient harm. While overdoses and underdoses constitute medication errors, doses missed inadvertently by a patient do not. Individuals who identify a potential medication error situation should immediately contact MedComm Solutions (see below) and report the event.

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7.0 SUPPLEMENTAL INFORMATION

7.1 Study Flow Chart

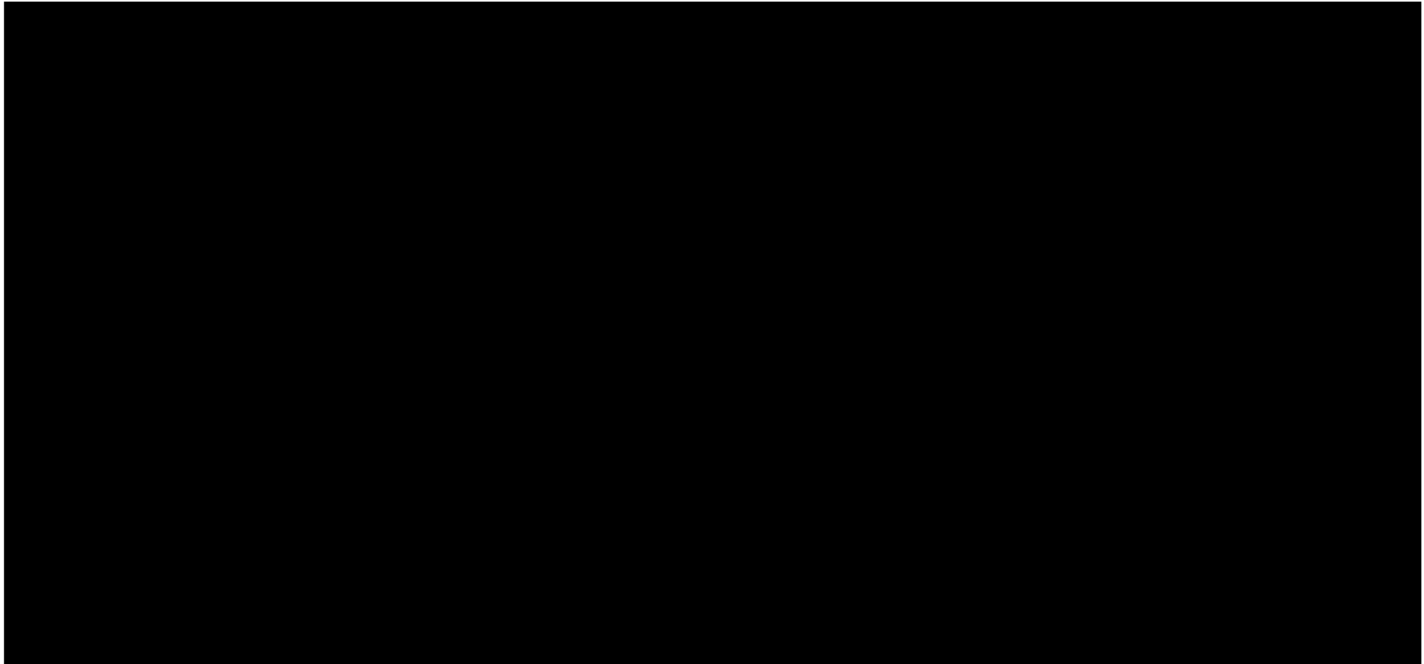
Tests and Procedures	Prior to Transplantation		30	100	180	Every 6mos After day 180 assessment
	Within 4-6 weeks prior to allo-HCT AND within 12 weeks prior to auto-HCT	≤30 Days				
Visit Window			± 7 days	± 14 days	± 28 days	± 28 days
History & Physical	X	X	X	X	X	X
Weight, PS, Height (at baseline)	X	X				
CBC w/ differential ¹	X ¹	X	X	X	X	X
Chemistry panels ⁴ (must include: creatinine, AST, ALT, alk phosphatase, total bilirubin, calcium, , glucose, albumin)	X ¹	X	X	X	X	X
Bone marrow biopsy & aspirate with cytogenetics and MRD by ASO-PCR ²	X			X ²	X ²	X ²
SPEP with immunofixation	X			X	X	X
UPEP with immunofixation & 24- hour urine for total protein	X			X	X	X
Quantitative immunoglobulins and serum free-light chains	X			X	X	X
Bone survey	X			X		
Quality of life	X ³		X	X	X	X

¹within 30 days of initiating study therapy

²only to confirm hematologic complete remission and every 6- 12 months to evaluate molecular remission in patients in hematologic complete remission in whom a baseline bone marrow for ASO primers was obtained and bone marrow contained >10% plasma cells

³within 30 days of initiating study therapy for auto-HCT or conditioning regimen for allo-HCT

⁴For patients enrolled in Group B expansion on bortezomib maintenance, a CBC and CMP will be done on day 1 of each bortezomib cycle



7.3 New York Heart Association Classification of Cardiac Disease

The following table presents the NYHA classification of cardiac disease:

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

7.4 Declaration of Helsinki

World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes

- research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
 3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
 4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
 5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
 6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
 7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
 8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
 9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

1. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
3. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
4. The design and performance of each experimental procedure involving human subjects

- should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
5. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
 6. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
 7. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
 8. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
 9. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
 10. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
 11. The subjects must be volunteers and informed participants in the research project.
 12. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
 13. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician

should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

14. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
15. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
16. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
17. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
18. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

1. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
2. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.
3. At the conclusion of the study, every patient entered into the study should be assured of

access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

4. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
5. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

7.5 Common Toxicity Criteria Version 4.0

<http://ctep.cancer.gov/reporting/ctc.html>

7.6 Eastern Cooperative Oncology Group (Ecog) Performance Status Scale

GRADE	SCALE
0	Fully active, able to carry out all pre-disease performance without restriction. (Karnofsky 90-100)
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, <i>e.g.</i> , light housework, office work. (Karnofsky 70-80)
2	Ambulatory and capable of all self-care but unable to carry out work activities. Up and about more than 50% of waking hours. (Karnofsky 50-60)
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. (Karnofsky 30-40)
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. (Karnofsky 10-20)

7.8 Durie and Salmon Multiple Myeloma Staging Criteria

STAGE I

Low myeloma cell mass ($< 0.6 \times 10^{12}$ cells/m²)

All of the following criteria

Hgb > 10 g/dL

Serum calcium ≤ 12 mg/dL

Normal bone structure or solitary lesion by X-rays

Low M component production values

IgG < 5 g/dL

IgA < 3 g/dL

Bence-Jones proteinuria < 4 g/24 hours

STAGE II

Intermediate myeloma cell mass ($0.6\text{--}1.2 \times 10^{12}$ cells/m²)

Criteria fit neither Stage I nor Stage III

STAGE III

High myeloma cell mass ($> 1.2 \times 10^{12}$ cells/m²)

Any of the following criteria

Hgb < 8.5 g/dL

Serum calcium (corrected) > 12 g/dL

Advanced lytic bone lesions

High M component production values

IgG > 7 g/dL

IgA > 5 g

International Staging System for Multiple Myeloma

Stage I — B2M < 3.5 mg/L and serum albumin ≥ 3.5 g/dL

Stage II — neither stage I nor stage III

Stage III — B2M ≥ 5.5 mg/L

8.0 DRUG INFORMATION

8.1 MELPHALAN

Melphalan is an alkylating agent and a synthetic derivative formed from the amino acid phenylalanine and mechlorethamine. Its primary mechanism of action involves the bifunctional alkylation of proteins and nucleic acids through the transfer of ethylamine groups causing DNA crosslinking and inactivation. Melphalan has significant activity in multiple myeloma. Melphalan is a bis-chloroethylamine nitrogen mustard that has long been used in high-dose chemotherapy regimens for multiple myeloma (refer to section 1.1 High-Dose Chemotherapy in Myeloma). The mechanism of alkylation by melphalan involves an initial step in which a positively charged reactive aziridinium moiety reacts with a nucleophile (DNA or protein) to yield an initial alkylated product.¹²⁵ A second aziridinium ring is formed from the remaining chloroethyl group resulting in a second alkylation. Therefore, melphalan causes the formation of interstrand and intrastrand DNA crosslinks or DNA-protein crosslinks. It is postulated that the cellular cytotoxicity resulting from melphalan exposure depends primarily on the formation of DNA interstrand crosslinks. One major mechanism of resistance to melphalan involves an increased capacity to repair the DNA damage induced by this alkylating agent.

Clinical Pharmacokinetics and Pharmacodynamics

The clinical pharmacology of high-dose intravenous melphalan has been reviewed.¹³¹ Intravenous melphalan has a volume of distribution of 44 L which appears to approximate total body water. In myeloma patients, over 60% of melphalan is bound to albumin and over 20% to α_1 -acid glycoprotein. The drug has a biphasic elimination pattern with an alpha half-life of 6 to 8 minutes and a beta half-life of 40 to 60 minutes. There does not appear to be any evidence of dose-dependent pharmacokinetics. Melphalan has both renal and non-renal mechanisms of clearance. Approximately 21 to 34% of the drug is excreted unchanged in the urine. At doses of 140-180 mg/m², melphalan has a $t_{1/2\alpha}$ = 5-15 min and $t_{1/2\beta}$ = 17-75 min, which allows for the infusion of peripheral blood stem cells within 8-24 hours. Ninety percent of melphalan is bound to plasma proteins. There is no significant effect of renal function on pharmacokinetics, however, a 50% dose reduction is recommended for patients with a BUN \geq 30 mg/dL (secondary to an increase in myelosuppression). The major toxicities of high-dose melphalan include myelosuppression (often resulting in neutropenic fever), nausea, vomiting, mucositis, and an increased risk of secondary acute leukemia. Hemorrhagic cystitis, often seen with cyclophosphamide and ifosfamide, is uncommon with melphalan, and forced diuresis is not required. Clinical Experience

Melphalan has been the cornerstone of high-dose treatment of multiple myeloma. Melphalan in doses of up to 200 mg/m² have been given without stem cell support to previously untreated patients with a CR rate of 32% and a median survival of 47 months.^{133 134,135} CR rates following high dose melphalan/methyl-prednisolone and autologous bone marrow transplant have been as high as 75% with an estimated probability of survival at 54 months of 63%.¹³³

Potential Risks and Benefits of Melphalan

The most common side effects of melphalan observed in subjects are bone marrow suppression (this suppression may be delayed and prolonged, with an onset of 4-7 days and an extended

nadir of 2-3 weeks with stem cell rescue) often manifesting as leukopenia and thrombocytopenia and often resulting in neutropenic fever, nausea, vomiting, diarrhea, mucositis, alopecia, weakness, fatigue, general discomfort, an increased risk of secondary acute leukemia, and anorexia.

Less common side effects of melphalan observed in subjects are irreversible bone marrow failure, abnormal liver function tests, acute hepatitis, jaundice, veno-occlusive disease of the liver, anaphylaxis, urticaria, pruritis, edema, bronchospasm, tachycardia, dyspnea, hypotension, skin hypersensitivity, skin ulceration at injection site, skin necrosis rarely requiring skin grafting, vasculitis, hemolytic anemia, allergic reaction, pulmonary fibrosis, interstitial pneumonitis, cardiotoxicity, phlebitis, and extravasation. Hemorrhagic cystitis, often seen with cyclophosphamide and ifosfamide, is uncommon with melphalan, and a forced diuresis is not required.

8.2 Bortezomib (Velcade®) for Injection

Scientific Background

Bortezomib (Velcade®) for Injection is a small molecule proteasome inhibitor developed by Millennium Pharmaceuticals, Inc., (Millennium) as a novel agent to treat human malignancies. Bortezomib (Velcade®) is currently approved by the United States Food and Drug Administration (US FDA) and it is registered in Europe for the treatment of multiple myeloma patients who have received at least one prior therapy.

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8.3 Clinical Trial Materials

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

ALKERAN® (melphalan) injectable is available commercially from GlaxoSmithKline as a single-use vial of freeze-dried melphalan hydrochloride equivalent to 50 mg melphalan and one 10 ml vial of sterile diluent. When reconstitution is complete, the final solution results in a concentration of 5 mg/ml and should be used promptly. Melphalan injectable can be further diluted in D5W and is reportedly stable over 24 hours. Melphalan injectable is administered over 30 minutes.

FLUDARA (fludarabine) FOR INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. Each vial of sterile lyophilized solid

cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2.

Reconstitution with 2 mL of Sterile Water for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration

8.4 Preparation, Handling, and Storage of Drugs

BORTEZOMIB (VELCADE®)

Vials containing lyophilized Bortezomib (Velcade®) for Injection should be stored according to the label requirements. For the United States, store at USP Controlled Room Temperature which is 25°C (77°F); excursions permitted from 15 to 30°C (59 to 86°F). For Europe, do not store above 30°C (86°F). 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 the sponsor will notify the investigator should this information be revised during the conduct of the study.

Bortezomib (Velcade®) is cytotoxic. As with all cytotoxic drugs, caution is required when preparing and handling Bortezomib (Velcade®) 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 (Velcade®). Each vial of Bortezomib (Velcade®) for Injection should be reconstituted under a laminar flow biological cabinet (hood) within eight hours before dosing with 3.5 mL of normal (0.9%) saline, Sodium Chloride Injection USP, so that the reconstituted solution contains Bortezomib (Velcade®) at a concentration of 1 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 (Velcade®) should be administered promptly and in no case more than 8 hours after reconstitution. 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.

In fulfillment of Form FDA 1571, Section 12, Item 7, the sponsor-investigation claims exemption from the requirements for environmental assessment under the provisions of 21 CFR 25.31(3). Bortezomib (Velcade®) is intended for use in clinical studies on research in which waste will be controlled; in addition, the amount of waste expected to enter the environment may reasonably be expected to be nontoxic.

VELCADE Administration

Patients enrolled in Group B expansion will receive bortezomib as part of the conditioning regimen for autologous transplant, followed by maintenance bortezomib starting between Day 90 and Day 120 post transplant. Patients will receive subcutaneous bortezomib at 1.3mg/m² given weekly, 4 weeks on, 4 weeks off for 1 year.

Drug will be administered only to eligible patients under the supervision of the investigator or identified subinvestigator(s). Patients may be treated on an outpatient basis, if possible. The drug 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 or calculation (see Appendix 8.1). The dose should be calculated on Day 1 of each cycle; the dose administered should remain the same throughout each cycle but should be recalculated at the start of the next cycle. If a patient experiences a notable change in weight within a cycle, as determined by an unscheduled weight assessment, then the patient's dose should be recalculated at that time based on clinical judgment.

There must be at least 72 hours between each dose of VELCADE.

INTRAVENOUS AND SUBCUTANEOUS ROUTE OF ADMINISTRATION HAVE DIFFERENT RECONSTITUTED CONCENTRATIONS. CAUTION SHOULD BE USED WHEN CALCULATING THE VOLUME TO BE ADMINISTERED.

MELPHALAN (Alkeran®)

Melphalan hydrochloride for injection is commercially available as a sterile, nonpyrogenic, lyophilized powder. The powder for injection also contains povidone. A sterile diluent containing water for injection, sodium citrate, propylene glycol, and alcohol is provided by the manufacturer for reconstitution. Melphalan hydrochloride powder for injection is reconstituted by adding 10 mL of the diluent provided by the manufacturer to a vial labeled as containing 50 mg of melphalan using a 20-gauge or larger needle to provide a solution containing 5 mg/mL. The diluent should be added rapidly and the vial should be shaken vigorously until a clear solution is obtained. Reconstituted solutions of melphalan hydrochloride should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. The reconstituted solution should not be refrigerated, since a precipitate may form at 5 °C. The reconstituted solution of melphalan hydrochloride should be diluted further with 0.9% sodium chloride injection to provide a solution with a concentration not exceeding 0.45 mg/mL. Dilution of reconstituted solutions of the drug should be performed immediately because a citrate derivative of melphalan has been detected in the solution within 30 minutes of reconstitution of melphalan hydrochloride for injection. Because approximately 1% of the labeled strength of melphalan hydrolyzes every 10 minutes following dilution with 0.9% sodium chloride, the solution of melphalan hydrochloride should be administered soon after dilution. Melphalan is administered by IV infusion, usually over 15-20 minutes. Administration of melphalan hydrochloride should be completed within 60 minutes of reconstitution.

The manufacturer recommends that procedures for proper handling and disposal of antineoplastic drugs (e.g., use of gloves) be used, since adverse dermatologic effects associated with exposure to the drug may occur. If melphalan solution comes in contact with the skin or mucous membranes, the affected area should be washed thoroughly with soap and water. Following reconstitution with sterile diluent, melphalan hydrochloride solution containing 5 mg of melphalan per mL is stable for up to 90 minutes at room temperature; this reconstituted solution should not be refrigerated since a precipitate may form at 5°C. The reconstituted solution should be diluted further with 0.9% sodium chloride injection to provide a solution with a concentration not exceeding 0.45 mg/mL. This diluted solution is stable for 60 minutes at room temperature.⁹⁸

MELPHALAN is cytotoxic. As with all cytotoxic drugs, caution is required when preparing and handling MELPHALAN 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.

FLUDARA FOR INJECTION should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2 mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP. Reconstituted FLUDARA FOR INJECTION contains no antimicrobial preservative and thus should be used within 8 hours of reconstitution. Care must be taken to assure the sterility of prepared solutions. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

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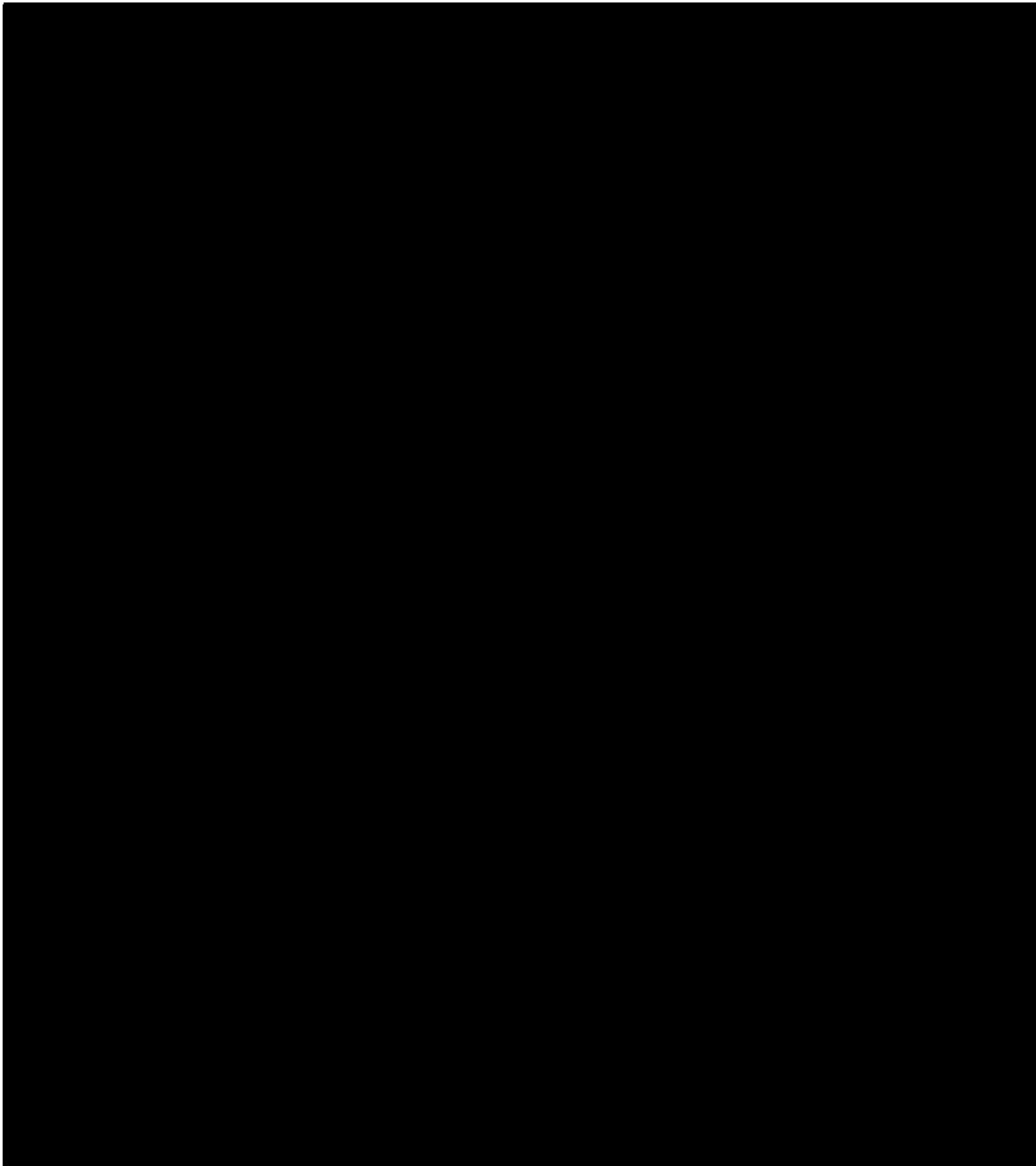
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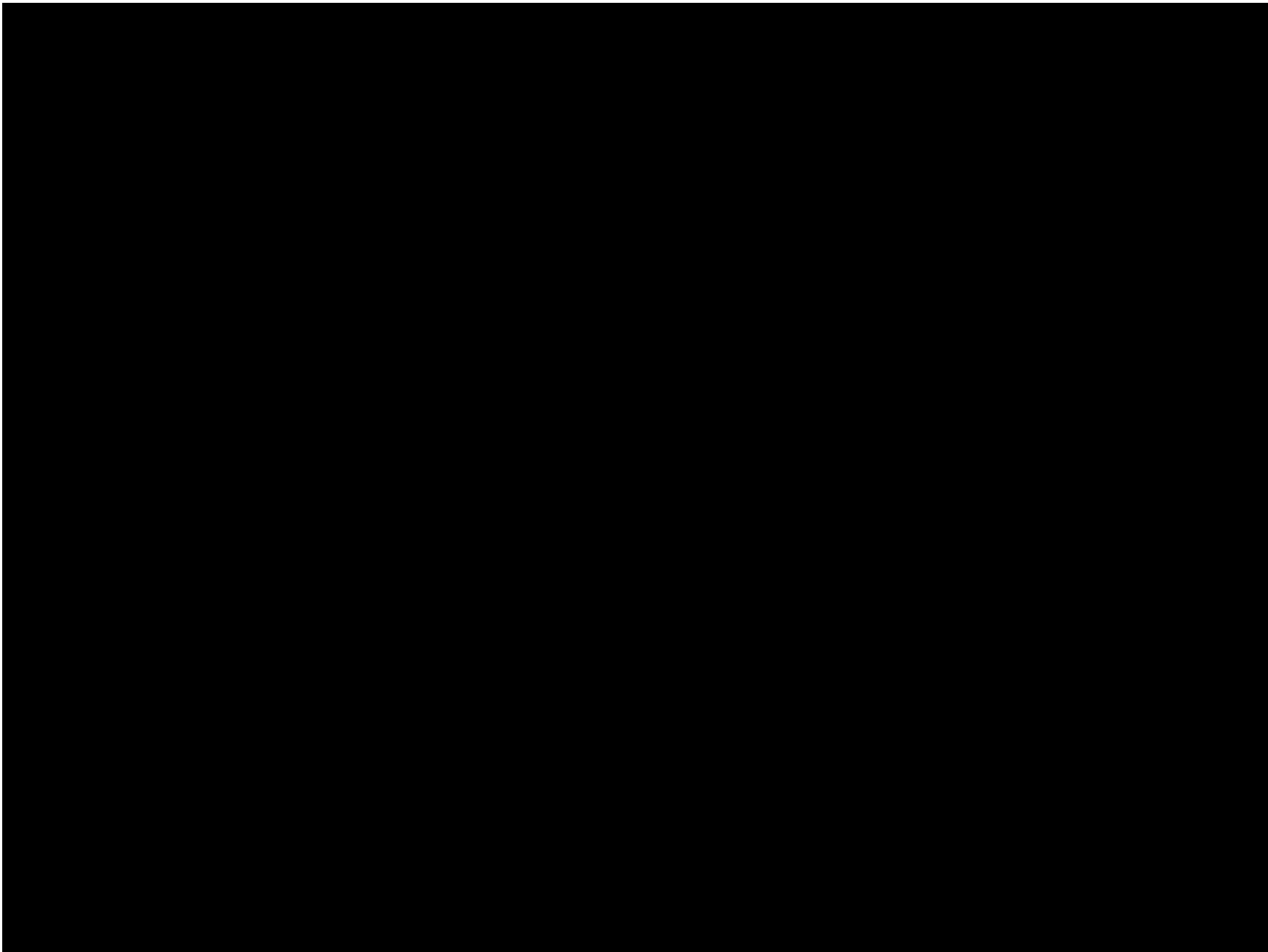
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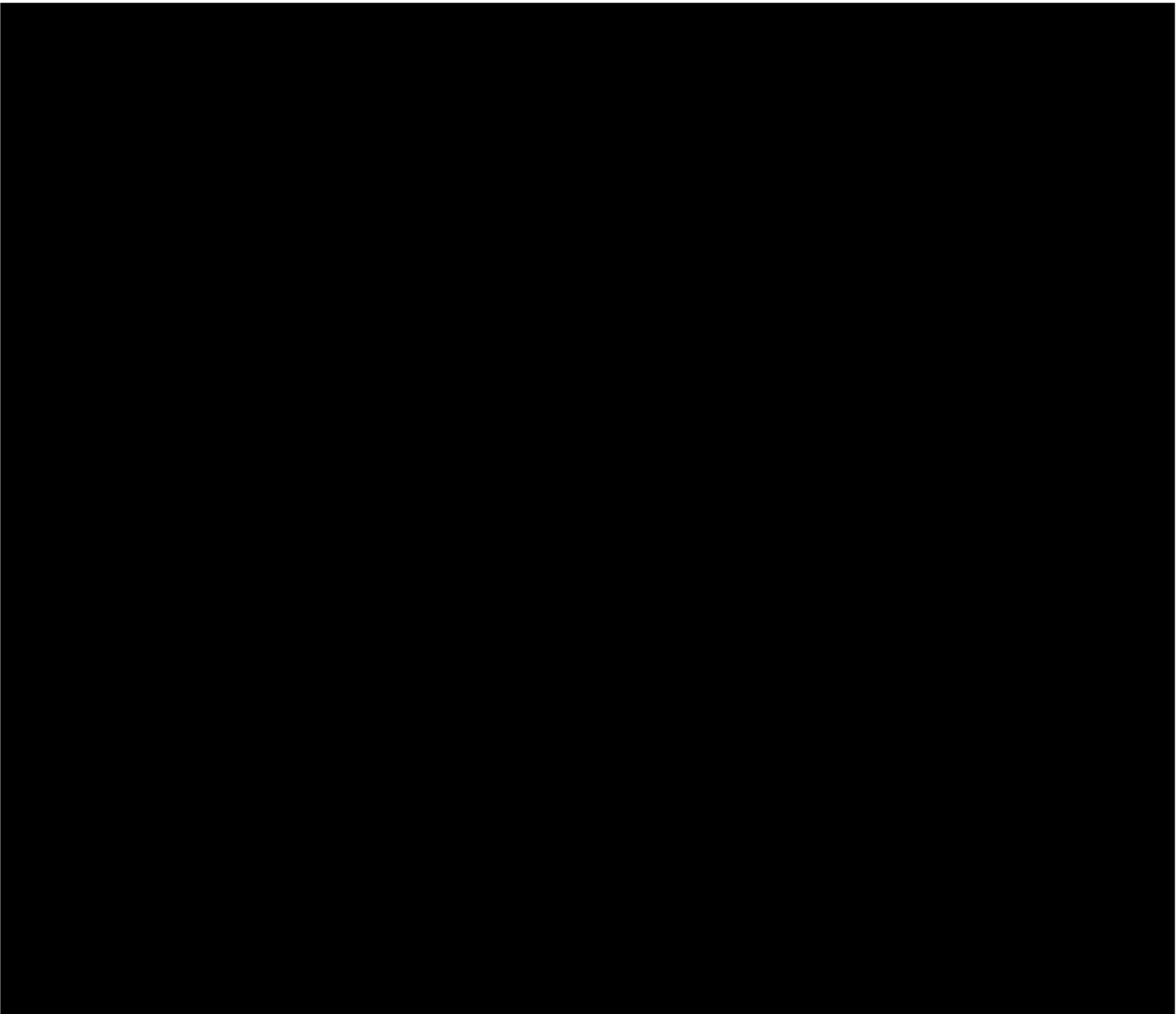
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