Protocol Number	CUMC AAAJ7800
TITLE	Phase II Study of the Combination of Bendamustine and Dexamethasone in Patients with Relapsed AL Amyloidosis

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PROTOCOL SUMMARY

Title: Phase II study of the bendamustine and dexamethasone in patients with relapsed AL amyloidosis

Objectives:

- The primary objective of this study is to estimate the partial hematologic response rate (PHR)

- The secondary objectives of this study are to:
  - Determine overall hematologic response rate
  - Determine the organ response rate
  - Determine time to treatment failure
  - Assess toxicity of the regimen
  - Determine overall survival (OS)
  - To assess by RT-PCR the expression in clonal plasma cells of *cyclin D1* (*CCND1*) and *SEL1L,* before treatment. To assess the bone marrow cytokine profile before and after treatment with bendamustine.

Patient population: (Specific inclusion and exclusion criteria are detailed in section 4.2)

Individuals are eligible for this study if they have:

- Primary systemic amyloidosis (biopsy proven), ***OR***
- Light chain deposition disease (biopsy proven), ***OR***
- Meet the IMWG definition of *symptomatic myeloma* with symptoms attributable ***only*** to associated amyloid. The patient cannot otherwise meet criteria for diagnosis of symptomatic MM with other disease-related symptoms not attributable to amyloid deposition.
- Either are not a candidate for autologous stem cell transplant (ASCT), have declined option of ASCT, or have relapsed after prior ASCT.

Number of patients: 13 patients in the first stage, 16 patients in the second stage (max 29)

Study design and methodology:

This Phase IIa clinical trial uses a two-stage optimal Simon design.[1] In the first stage, 13 patients are enrolled. If at least three patients experience PHR, the trial proceeds to the second stage, otherwise, the trial terminates with no further interest in the treatment. In the second stage, 16 additional patients are treated. If a total of 9 or more out of the total of 29 patients experience PHR, the treatment will be considered worthy of further development. Patients who finish at least 2 cycles are eligible for evaluation of PHR. Patients with primary progressive disease after only receiving 1 cycle of therapy will be included in the response analysis.

Treatments administered:

- Bendamustine 100 mg/m^2 IV on day 1 and 2 of each cycle
- Dexamethasone 40 mg PO or IV on days 1, 8, 15, 22 of each cycle

- Cycle will be 28 days. Treatment may be continued until progression of disease or a maximum of 6 cycles beyond CHR, after which time patients will be observed until other non-protocol therapy is needed.

Efficacy data collected:

- Serial serum and urine M-protein assessment
- Serum and urine electrophoresis/immunofixation
- Serum free light chain (Freelite) assay
- Serial echocardiogram assessment of cardiac wall thickness and left ventricular ejection fraction
- Serial BNP/NT-proBNP and troponin I/T levels
- Serial assessment of non-light chain 24-hr urinary protein excretion
- Serial assessment of liver function tests and size of liver
- Change in bone marrow plasmacytosis

Correlative studies:

- Baseline samples will be sent to Dr. Comenzo's lab for correlative studies. The correlative studies are optional.

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LIST OF ABBREVIATIONS

AE	**adverse event**
AL	**systemic light-chain amyloidosis**
ALT	**alanine aminotransferase/glutamic pyruvic transaminase/GPT**
ANC	**absolute neutrophil count**
AST	**aspartate aminotransferase/glutamic oxaloacetic transaminase/GOT**
ASCT	**Autologous stem cell transplant**
BNP	**brain natriuretic peptide**
BP	**bendamustine and prednisone**
BUN	**blood urea nitrogen**
CLL	**chronic lymphocytic leukemia**
CHR	**complete hematologic response**
CR	**complete response/remission**
CTCAE	**NCI common terminology criteria for adverse events (version 4.0)**
DLT	**dose-limiting toxicity**
dFLC	**difference between the involved and uninvolved free light chain**
ECG	**12 lead electrocardiogram**
ECOG	**Eastern Cooperative Oncology Group**
ER	**endoplasmic reticulum**
FDA	**Food and Drug Administration**
G-CSF	**granulocyte colony-stimulating factor (e.g. filgrastim)**
GM-CSF	**granulocyte-macrophage colony-stimulating factor (e.g. sargramostim)**
HIV	**human immunodeficiency virus**
IV	**intravenous(ly)**
IRB	**institutional review board**
LLN	**lower limit of normal**
MDex	**melphalan and dexamethasone**
mg/m2	**milligrams per square meter**
MM	**multiple myeloma**
MP	**melphalan and prednisone**
MR	**minor response**
NCI	**National Cancer Institute**
NHL	**non-Hodgkin lymphoma**
NIH	**National Institutes of Health**
OrRR	**organ response rate**
OS	**overall survival**
OHR	**overall hematologic response**
PLT	**platelet**
PR	**partial response**
qRT-PCR	**quantitative real-time polymerase chain reaction**
SAE	**serious adverse event**
SD	**stable disease**
SJS	**Stevens Johnson Syndrome**
SPEP	**serum protein electrophoresis**
T4	**thyroxine**
TEN	**toxic epidermal necrolysis**

TTF	**time to treatment failure**
ULN	**upper limit of normal**
UPEP	**urine protein electrophoresis**
VGPR	**very good partial response**

1 BACKGROUND AND RATIONALE

1.1 Systemic Light Chain Amyloidosis

Systemic light-chain amyloidosis (AL) is a protein conformation disorder due to a clonal plasma cell dyscrasia.[2] In both AL and multiple myeloma (MM), clonal plasma cells in the bone marrow produce monoclonal immunoglobulins.[3,4] Unlike myeloma, the AL plasma cell clone in most patients is the equivalent of a monoclonal gammopathy of undetermined significance.[5,6] In AL, abnormal monoclonal immunoglobulin light chains unfold and aggregate to form insoluble fibrils that are deposited in the kidneys, heart, liver, GI tract and peripheral nervous system.[7] The deposits lead to multiorgan dysfunction and death. The annual incidence of AL is estimated to be six to nine per million. The prognosis of AL is poor, with a median survival without therapy of approximately 13 months from diagnosis and a 10-year survival rate of 5%. Survival can be prolonged with effective treatment.[8,9]

Therapy of AL is generally based on treatment regimens used in MM. The aim of therapy is to eliminate the clonal plasma cells in order to reduce the production of abnormal light chains. Reduction in the amyloid-precursor light chains in the serum can lead to resorption of amyloid deposits in tissues and restoration of organ function. Achievement of a hematologic response is associated with improved outcome, and a complete hematologic response (CHR) has been shown to prolong survival.[10] Rapid response to treatment may be important as the benefit of therapy on organ function typically does not become evident before months after hematologic response. Patients may not survive long enough if therapy is slow-acting.

High-dose melphalan with stem cell transplant is effective in AL and in eligible patients may be the therapy of choice.[8,11] Toxicities and treatment-related mortality are high in patients with involvement of multiple organs, which has led investigators to propose and develop a risk-adapted approach to patient selection for stem cell transplant.[12] Standard therapy for patients who do not undergo autologous stem cell transplant (ASCT) remains oral melphalan and dexamethasone (MDex),[13] which achieves a hematologic response rate of 67%, including 33% complete response/remission (CR).

Median survival for those who do not respond to initial therapy with melphalan and stem cell transplant is 2 years, and in those with cardiac involvement who do not respond to any melphalan-based initial therapy the median survival is 10 months.[14] There is an urgent need in AL for better treatment options that would result in substantial and rapid activity, with good tolerability, particularly for patients with persistent plasma cell disease and progressive organ dysfunction despite initial therapy and for those who experience relapsed disease, two situations for which no standard treatment exists.

Because the immunoglobulin light chains secreted by clonal plasma cells in AL have an abnormal configuration, novel agents that interfere with intracellular protein quality control

processes will likely have a role in the treatment of AL. Interference with cellular quality controls in cells that secrete abnormal proteins can cause increased intracellular stress and may trigger cell death. Recently, for example, in the phase I portion of a phase I/II trial with the proteasome-inhibitor bortezomib in AL patients with relapsed disease, 63% overall and 38% CHR rates were achieved.[15] Nevertheless those patients were not heavily pretreated. Dr. Palladini and colleagues enrolled 24 patients with relapsed/refractory AL Amyloidosis who were previously treated with Melphalan, bortezomib, and thalidomide (Ann Hematol 2012 Vol 91: 89-92). In this protocol, patients received lenalidomide and dexamethasone. Hematologic response defined as partial response or higher was seen in 9 out of 24 subjects (41%). There were no complete responses seen. In a follow-up study (Palladini et al. Haematologica 2013; Vol 98 p 433-5), twenty-one patients who had relapsed or refractory AL Amyloidosis received treatment with cyclophosphamide, lenalidomide, and dexamethasone. Hematologic response was seen in 62% of patients, but there was only one complete response (5%).

Since the immunoglobulin light chain proteins made by the clonal plasma cells in AL are abnormal in sequence and conformation, they may require optimal intracellular protein quality control management. Impairment of intracellular chaperone and protein-processing quality controls could cause increased intracellular stress due to protein misfolding and aggregation, thereby leading to cell death. Bendamustine may interfere with protein chaperone and quality controls in AL plasma cells by modulating expression of genes involved in those processes.

1.2 Bendamustine

Bendamustine is a bifunctional alkylating agent. It is a mechlorethamine derivative with structural similarity to chlorambucil and other drugs from the nitrogen mustard class, as well as a benzimidazole ring, which may act as an antagonist to purines and amino acids. It has good oral bioavailability but has been studied almost exclusively in the intravenous formulation. It undergoes extensive first-pass metabolism by cytochrome P450 1A2 to active metabolites gamma-hydroxy bendamustine and N-desmethyl-bendamustine, but clinical activity appears to be associated primarily with the parent compound. The t(1/2) of bendamustine is approximately 40 minutes. While bendamustine has 2 moieties with possible antitumor effect, it is unclear to what extent the benzimidazole ring enhances the efficacy of the drug.[16] Numerous studies including *in vitro* assays have reported, however, that bendamustine has little cross-resistance with other alkylating agents and remains active even in extensively pretreated patients.

Bendamustine has been approved in Europe for treating MM, non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL), breast cancer, and Hodgkin lymphoma. It is of note that bendamustine was developed and approved in East Germany in 1971. Dose-limiting toxicity (DLT) is primarily hematologic. Treatment-associated infections have been reported in some studies; however, nonhematologic adverse events (AEs) have rarely been dose limiting. The most common nonhematologic AEs include fatigue, nausea, xerostomia, and pyrexia.

Pharmacokinetic studies have shown that Bendamustine is >95% protein bound, but this binding is not affected by age (>70years), low serum albumen levels (<3.1 g/dl) or the presence of advanced tumors [33].

Bendamustine was approved by the US Food and Drug Administration (FDA) for the treatment of CLL in March 2008 and for the treatment of rituximab-refractory, indolent B-cell NHL in October 2008.

FDA approval for use in CLL was based on findings from a randomized, open-label, Phase III study comparing bendamustine with chlorambucil as single-agent therapy in treatment naive patients with CLL (Binet stage B or C) [29, 30]. Bendamustine was administered intravenously at a dose of 100 mg/m2 on days 1 and 2, while chlorambucil was administered orally at 0.8 mg/kg daily, both over 4-week cycles for up to 6 cycles. At interim analysis (the data used for FDA approval), bendamustine was associated with a greater overall response (68% vs 39%; P <0.001), median progression-free survival (21.7 vs 9.3 months; P <0.001) and median duration of remission (18.9 vs 6.1 months; P <0.001) compared with chlorambucil. FDA approval for rituximab refractory, indolent B-cell NHL followed a Phase III, open-label, single-arm study evaluating bendamustine monotherapy in patients who did not respond to rituximab or who had progressive disease within 6 months of rituximab therapy. Bendamustine 120 mg/m^2 was administered intravenously on days 1 and 2 of a 21-day cycle for up to 8 cycles. At interim analysis, the overall response rate was 84%, including 29% CR. The median progression-free survival was 9.7 months.

The efficacy of bendamustine has also been reported in the treatment of MM in a large number of patients and several clinical trials and was first used in 1969 in Germany. A randomized phase III study compared bendamustine and prednisone (BP) to standard melphalan and prednisone (MP) treatment in previously untreated patients with MM [31]. To be included, patients had to have histologically and cytologically proven stage II with progressive disease or stage III MM. They were randomly assigned to receive BP (n=68) or MP (n=63). The primary endpoint was the time to treatment failure (TTF). Secondary endpoints included survival, remission rate, toxicity and quality of life. The overall response rate was 75% in the BP and 70% in the MP group. A significantly higher number of patients treated with BP achieved a complete remission than did patients receiving MP (32 vs. 13%; P =0.007), and the maximum response was achieved more rapidly in patients treated with BP compared to those receiving MP (6.8 vs. 8.7 cycles; P <0.02). TTF and remission duration were significantly longer in the BP group. Patients receiving BP had higher quality of life scores and reported pain less frequently than patients receiving MP. The authors concluded that BP is superior to MP with respect to complete remission rate, TTF, cycles needed to achieve maximum remission and quality of life and should be considered the new standard in first-line treatment of MM patients not eligible for transplantation.[17]

A retrospective analysis evaluated 39 patients with relapsed or refractory MM [32], who have been treated with bendamustine as salvage therapy. After a median 2 lines of prior therapy (range: 1-5) patients received a median 3 (range: 1-10) cycles of bendamustine. Bendamustine dose was 80-150 mg/m^2 on days 1 and 2 of a monthly cycle. Bendamustine was administered as monotherapy in 39% of patients, whereas 61% received concomitant steroids. Toxicity was mild to moderate. Response rates were as follows: 3% very good partial response (VGPR), 33% partial response (PR), 18% minor response (MR), 26% stable disease (SD) and 20% progressive disease. The median event-free and overall survivals (OS) were 7 and 17 months, respectively. The authors concluded that in patients with advanced MM, bendamustine is effective and associated with mild toxicity.[18]

In another study, 17 patients with relapsed MM with a median age of 70 years (51-82) were treated with bortezomib 1-1.3 mg/m^2 on days 1, 4, 8 and 11; bendamustine 60 mg/m^2 on days 1 and 8; and dexamethasone 24 mg orally days 1-3 and days 8-10. Response was evaluated according to the Southwest Oncology Group (SWOG) criteria. The overall response rate (CR+PR+MR) was 88%. CR: (n=2; 12%), PR: (n=10; 58%), MR (n=3; 18%; No Change (n=2; 12%). A total of 28 patients with a median age (range) of 64 years (40-78) have been enrolled in a phase I study of bendamustine in combination with thalidomide and prednisolone in patients with stage II or Ill refractory or relapsed MM after transplantation or conventional chemotherapy.[19] After at least 2 cycles of chemotherapy, 25/28 patients responded with 3 complete remission, 1 near CR, 5 VGPR, 15 PR, and 1 MR. Two patients had stable disease and one was refractory. After a median follow up of 15 months, event free survival and OS at 12 months were 34% and 92% respectively.

Our group performed a phase 1 trial using bendamustine combined with lenalidomide. Our primary objective was to determine the maximum tolerated dose and safety profile of bendamustine and lenalidomide when administered with dexamethasone for patients with relapsed or refractory MM. Patients aged ≥18 years with confirmed, measurable MM that was refractory to or progressed after 1 or more prior therapies received bendamustine by intravenous infusion on days 1 and 2, oral lenalidomide on days 1-21, and oral dexamethasone on days 1, 8, 15, and 22 of each 28-day cycle. Treatment was continued until a plateau of best response, as defined by the International Myeloma Working Group, was reached. Study drug doses were escalated through 4 levels, with 3-6 patients enrolled at each level depending on whether any DLT occurred. Secondary endpoints included preliminary efficacy, as evidenced by objective responses, time to disease progression, and OS. To date, 13 patients have been enrolled; 12 patients have been treated (median age 61 years [range, 38-75 years]). The maximum tolerated dose of bendamustine and lenalidomide has not been identified at this point; currently, patients are enrolling on dose level 3 with 100 mg/m^2 bendamustine and 10 mg lenalidomide. Thus far, DLTs include 1 grade 4 neutropenia at dose level 2 and 1 grade 3 thrombocytopenia at dose level

3. Ten patients are currently eligible for response assessment. An objective response was observed in 70% of patients, including 3 VGPR and 4 PR. Two patients experienced stable disease and 1 exhibited progressive disease.[20] These data suggest that bendamustine, lenalidomide, and dexamethasone form a well-tolerated regimen in heavily pretreated MM patients.

DLT of bendamustine is primarily hematologic. Treatment-associated infections have been reported in some studies; however, nonhematologic AEs have rarely been dose limiting. The most common nonhematologic AEs include fatigue, nausea, xerostomia, and pyrexia.

Tumor Lysis Syndrome

Tumor lysis syndrome associated with bendamustine treatment has been reported in patients in clinical trials and in spontaneous reports. The onset tends to be within the first treatment cycle of bendamustine and, without intervention, may lead to acute renal failure and death. Preventive measures include maintaining adequate volume status, and close monitoring of serum chemistry, particularly potassium and uric acid levels. Allopurinol has also been used prior to or at the beginning of bendamustine therapy. However, there may be an increased risk of severe skin toxicity when bendamustine and allopurinol are administered concomitantly.

Skin Reactions

Skin reactions have been reported in clinical trials and post-marketing spontaneous reports. These events have included rash, toxic skin reactions, and bullous exanthema. Some events occurred when bendamustine was given in combination with other anticancer agents, so the precise relationship of the skin reactions to bendamustine treatment is uncertain.

In a study of bendamustine (90 mg/m^2) in combination with rituximab (study SDX-105-02), 1 case of toxic epidermal necrolysis (TEN) occurred. TEN has been associated with treatment with rituximab. Spontaneous reports of Stevens Johnson Syndrome (SJS) and TEN, some fatal, have been reported when bendamustine was administered concomitantly with allopurinol and other medications known to cause these syndromes. The relationship to bendamustine cannot be determined.

When skin reactions occur, they may be progressive and increase in severity with further treatment. Therefore, patients with skin reactions should be monitored closely. If skin reactions are severe or progressive, bendamustine treatment should be withheld or discontinued.

1.3 Study Rationale and Selection of Drug Doses

There are no established and approved second-line therapies for patients with systemic AL amyloidosis who fail initial melphalan-based treatment, be it high-dose melphalan with stem cell transplant or oral melphalan and dexamethasone (MDex). Therefore new treatments are needed for those who fail initial therapy and for those who initially respond but subsequently relapse.

The clonal plasma cells that cause AL produce abnormal immunoglobulin light chains that are the precursor proteins of amyloid fibrils. These light chain proteins are abnormal in sequence and conformation, and have a tendency to self-assemble and aggregate. Thus eradication or the control of the abnormal plasma cell clone is the precondition for successful treatment of AL amyloidosis. Bendamustine, a bifunctional alkylating agent with high anti-myeloma activity, achieves a PR in 70% of patients with relapsed/refractory MM. Based on this high anti-MM activity, we anticipate that bendamustine will also be very active in clonal plasma cell disorder associated with AL.

MM and AL are plasma cell dyscrasias which receive similar treatment, e.g. melphalan, steroids, high dose chemotherapy with autologous stem cell transplant. Substances testing positive for anti-MM activity are also used for the treatment of AL, and data on the toxicity and efficacy from MM trials are used for subsequent studies in amyloidosis.

Bendamustine is a drug which is approved and has been widely used in therapy for MM, NHL, breast cancer and other malignancies for 46 years. Based on several phase III trials, bendamustine received FDA approval for CLL (100 mg/m^2) and NHL (120 mg/m^2 d1 and d2) in the US. Due to the extensive experience and large amount of safety and toxicity data on bendamustine in hematologic malignancies, we think it is justified and safe to use bendamustine in a phase II trial for patients with AL with a strict stopping rule.

1.4 Correlative Studies:

Cyclin D1 Overexpression in AL

The correlative studies for this trial will be performed at Tufts University Medical Center, Boston, MA in Dr. Raymond Comenzo's laboratory. This group is actively investigating the characterization of clonal plasma cells in AL. Recent data from this lab and others indicate that these cells over-express *CCND1* in one-half of cases, often associated with t(11;14) and or gain 11q.[21,22] In a report at the American Society of Hematology Meeting (ASH 2009), clonal plasma cells (CD138+) obtained at diagnosis from 58 patients with AL were evaluated for CCND1 expression.[23] Sixteen cases were assessed by Affymetrix U133A 2.0 arrays (Affymetrix; Santa Clara, CA) and 42 cases by quantitative *real-time* polymerase chain reaction (qRT-PCR) as previously described.[7,24] With transcriptional profiles (n=16), *CCND1* median $\log_e$-transformed quantitative expression levels were 11.08 (range, 9.6-11.55) in five versus 4.163 (3.875-5.447) in eleven patients ($P < 0.01$). With qRT-PCR (n=42), relative *CCND1* expression levels were high in twenty [median of 4.21 (1.76-17.24)], and low in twenty-two patients, [median of 0.014 (0-0.99)] ($P < 0.0001$). Overall, 43% (25/58) of patients were CCND1^{hi} and did not differ from

CCND1^{lo} patients with respect to organ involvement, troponin I, urine total protein, creatinine or alkaline phosphatase levels. The median brain natriuretic peptide (BNP) of CCND1^{hi} patients was 252pg/ml (range 20-1880), significantly higher than that of CCND1^{lo} patients (109pg/ml (5-4210), $P < 0.05$). CCND1^{hi} patients tended to have more plasma cells (13% versus 8%, $P = 0.06$), higher serum free light chain levels (23.3 versus 14mg/dl, $P = 0.12$) and more kappa clones (6/25 versus 2/33, $P = 0.06$) than CCND1^{lo} patients. CCND1^{hi} patients also had significantly fewer intact immunoglobulin M-proteins (4/25 versus 22/33, $P <0.01$). Although there were no differences in treatment modalities used in terms of SCT or MDex as well as in the rates of CHR, there was a significant difference in OS (Figure 1). CCND1^{hi} patients survived a median of only 27 months compared to 60 months for CCND1^{lo} patients ($P = 0.02$), and had a risk of death of 2.86 (CI 1.18-6.94).

CCND1 over-expressing clones then usually produce only immunoglobulin light chains and not intact immunoglobulins because of loss of Ig heavy chain gene loci due to *t(11;14)*. Preliminary data from the Comenzo lab indicates that *CCND1* over-expressing clones also over-express genes associated with maintenance of protein solubility and protein quality control such as *PDIA6* and *SEL1L*, a phenomena possibly linked to the exclusive production of Ig light chains that form amyloid. The correlative studies of clonal bone marrow plasma cells selected from pre-treatment marrow aspirates will further test the hypothesis that *CCND1* over-expressing clones up-regulate *SEL1L*, a genes involved in ER-associated protein quality control. Using patient bone marrow specimens obtained at baseline, we will perform RT-PCR to assess the expression in clonal plasma cells of the genes *CCND1* and *SEL1L* and determine whether their expression is correlated.

Figure 1. Overall Survival is Significantly Decreased in CCND1^{hi} Patients

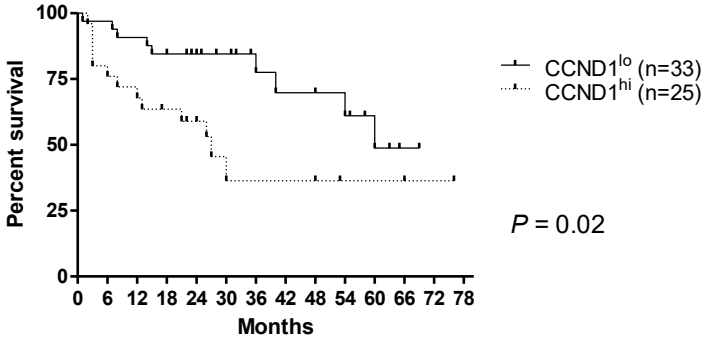

Figure 1. Overall Survival is Significantly Decreased in CCND1^{hi} Patients

Quantitative RT-PCR will be performed (in duplicate and on 2 separate days) using TaqMAN Gene Expression Assays with RPLP0 (Hs99999902_m1) primers and probes (Applied Biosystems; Foster City, CA) as internal control on an Mx 3000P platform and related software (Stratagene; La Jolla, CA). The comparative Ct method will be used and the amount of target normalized to the control ($2^{-\Delta\Delta Ct}$) assuming an efficiency of 2. Statistical analyses (paired t-test)

will be performed with GraphPad PRISM (GraphPad; La Jolla, CA) with $P < 0.05$ significance level for *CCND1*. Then CCND1^{hi} and CCND1^{lo} cases will be compared by paired t-test with respect to expression levels of *SEL1L*. These studies may lead to new therapies for AL by identifying new druggable targets.

With patients' consent baseline samples (5 ml) will be sent to Dr. Comenzo's lab for these correlative studies. The correlative studies are optional.

2 OBJECTIVES

2.1 Primary Objectives

Determine the CHR rate.

2.2 Secondary Objectives

- Determine the overall hematologic response (OHR) rate
- Determine the organ response rate (OrRR)
- Determine the time to treatment failure (TTF)
- Assess the toxicity of the regimen
- Determine the distribution of OS and PFS
- To assess by RT-PCR the expression in clonal plasma cells of *cyclin D1* (*CCND1*) and *SEL1L* before treatment. To assess the bone marrow cytokine profile before and after treatment with bendamustine.

3 STUDY DESIGN

3.1 Study Design

This Phase IIa clinical trial uses a two-stage optimal Simon design.[1] In the first stage, 13 patients are enrolled. If at least three patients experience hematologic PR or better, the trial proceeds to the second stage, otherwise, the trial terminates with no further interest in the treatment. In the second stage, 16 additional patients are treated. If a total of 9 or more out of the total of 29 patients evaluable for response experience hematologic PR and better, the treatment will be considered worthy of further development. Patients who finished at least 2 cycles are eligible for evaluation of response.

Patients who have not completed 2 cycles and therefore are not evaluable for response will be replaced. However, patients with primary progressive disease after only receiving 1 cycle of therapy will be included in the response analysis. A maximum of 40 patients will be consented to accrue the maximum of 29 patients evaluable for response.

3.2 Stopping Rule for Excess Toxicity

The trial will employ a stopping rule[25] for excess of drug toxicity defined as Grade 4 and 5 as defined in NCI CTCAE, Version 4.0 and related to the study medication. The number of patients experiencing toxicity during any cycle of treatment will be evaluated after 5, 10, 15, 20 and 25 patients have completed treatment. For instance, if 4 or more patients out of the first 10

experience toxicity as defined, the trial will be paused for evaluation by the DSMB. Accrual does not need to be stopped until 5, 10, etc., patients have completed their treatment per Table 1 below. Grade 4 thrombocytopenia and lymphocytopenia are expected events and will not be considered an excess of toxicity. The protocol allows for patients to be treated with CrCl 15 – 30 mL/min. For patients with a baseline CrCl within this range, grade 4 Chronic Kidney Disease defined as a CrCl < 15 will not be considered an excess of toxicity.

Table 1. Stopping rule for excess toxicity

# Patients	# Toxicities
5	3
10	4
15	5
20	6
25	7

3.3 Justification of Sample Size and Stopping Rule

The optimal two-stage Simon design was determined from the following parameters. For the treatment to be of further interest, the proportion of patients experiencing clinical response should be at least 0.40 (p_1). A proportion of responding patients of 0.20 or less is clearly unacceptable (p_0). The probability of promoting an unacceptable drug to Stage 2 (α) is 0.10, and the probability of rejecting an acceptable drug at the end of Stage 2 (β) is set to 0.15. The resulting trial has an expected sample size of 21.0 patients if the treatment is unacceptable. The trial is stopped if $P(\pi>0.1)>0.7$, where π is the true probability of toxicity in the patient population. In Monte Carlo simulations, it was determined that, if $\pi=0.2$, the probability of stopping early equaled 0.3, while, if $\pi=0.3$, the probability the trial stopped early was 0.72.

4 PATIENT SELECTION

4.1 Patient Population

Eligible patients will be 18 years old or older with AL with persistent or progressive clonal plasma cell disease after first line therapy.

4.2 Inclusion and Exclusion Criteria

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. All baseline evaluations, which assure that inclusion and exclusion criteria have been satisfied, must be reviewed by the Investigator or an authorized physician sub-investigator prior to enrollment. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent

must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

4.2.1 Inclusion Criteria

- Male or female patients aged ≥ 18 years old
- Histopathology of amyloidosis or light chain deposition disease based on detection by polarizing microscopy of green bi-refringent material in Congo red-stained tissue specimens or characteristic electron microscopy appearance **or** immunohistochemical stain with anti-light chain anti-sera
  - Demonstrate measurable disease as defined by one or more of the following:
    - Serum monoclonal protein ≥ 0.5 g/dL by serum electrophoresis
    - Urine monoclonal protein > 200 mg/dL in a 24 hr urine electrophoresis
  - Demonstrate clonal population of plasma cells in the bone marrow or abnormal FLC ratio
  - ECOG performance status of 0, 1, or 2
  - Patients had at least one prior regimen consisting of at least 1 cycle
  - If not previously transplanted, patient should be either ineligible for ASCT, or must have declined the option of ASCT. Patients who have previously had ASCT and have subsequently progressed are eligible, provided other entry criteria are met
  - Ability to provide written informed consent obtained prior to participation in the study and any related procedures being performed
  - Patients must meet the following laboratory criteria:
  - ANC ≥ 1.5 x 10^9/L
  - Hemoglobin ≥ 9 g/dl (May transfuse PRBC to meet parameter)
  - Platelets ≥ 100x 10^9/L (Must be independent of platelet transfusion)
  - Calculated CrCl ≥ 30 mL/min, patients with a CrCl ≥ 15 mL/min can be considered for the trial if patients are not in active renal failure and after discussion with the PI (Cockcroft-Gault Formula – see Appendix D)[34]
  - AST and ALT ≤ 2.5 x upper limit of normal (ULN)
  - Serum bilirubin <1.5 x ULN
  - Serum potassium WNL
  - Total serum calcium (corrected for serum albumin) or ionized calcium below ULN

4.2.2 Exclusion Criteria

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

- Patients meeting the criteria for symptomatic MM:
  - lytic lesions on skeletal survey or
  - plasmacytoma

  Patients meeting International Myeloma Working Group definition of **symptomatic myeloma** with symptoms ***only*** related to associated amyloidosis who would otherwise only meet the criteria for smoldering MM ***are*** potentially eligible.

- Myocardial infarction within 6 months prior to enrollment or has New York Heart Association (NYHA) Class IIIB or IV heart failure, uncontrolled angina, severe

uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities (not including 1st degree AV-block, Wenckebach type 2nd degree heart block, or left bundle branch block. Prior to study entry, any ECG abnormality at Screening has to be evaluated by the investigator or an authorized physician sub-investigator). Note: There is no lower limit of left ventricular ejection fraction below which patients are excluded from participation.

- Patients with NT-proBNP ≥ 1800 ng/L or BNP ≥ 400 ng/L, abnormal cTnT or cTnI [26, 27] can only be included after evaluation by cardiology to determine of the risk associate with the treatment. This evaluation needs to be discussed with the PI (Dr Lentzsch).
- Patient has received other ***investigational*** drugs within 14 days prior to enrollment
- Any form of secondary / familial amyloidosis
- Serious concurrent illness, which in the opinion of the investigator or an authorized physician sub-investigator would interfere with participation in this clinical study.
- Known HIV Infection.
- Inability to provide informed consent or to comply with the schedule of office and treatment visits
- 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 pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women (woman not of child-bearing potential is defined as any woman whose menstrual periods have stopped in the past 12 consecutive months or have had a complete hysterectomy or both ovaries surgically removed).
- Diagnosed or treated for another malignancy within 3 years of enrollment, with the exception of complete resection of basal cell carcinoma or squamous cell carcinoma of the skin, an in situ malignancy, low-risk prostate cancer, or cancer after **curative treatment.**

5 REGISTRATION PROCEDURES

5.1 Assignment of Study Numbers

Each patient enrolled will be assigned a unique and sequential study identifier.

Registration Guidelines

CUMC Research Participant Registration:

Confirm eligibility as defined in the section entitled Criteria for Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures, along with applicable institutional policies and federal regulations.

Research personnel trained to consent subjects for this protocol will be required to complete a protocol specific Eligibility Checklist.

All participants must be centrally registered at CUMC prior to initiation of study treatment.

Registration hours are available Monday through Friday from 9:00am – 5:00pm EST (excluding holidays). Same day patient registrations (and after hour registrations) will be accommodated on a case by case basis provided that the study team has expressed all time sensitive registration concerns/cases in a timely manner.

CPDM Central Registration Procedures:

Within 48 hours of obtaining consent, a completed/signed IRB approved informed consent HIPAA form, and demographics forms must be submitted to the CPDM Central Registration Office via an email to CPDMRegistration@columbia.edu or fax to 212.305.5292, with the subject line "AAAJ7800 Pending Subject Registration Request (PHI)". Upon receipt, applicable subject information as well as a "pending eligibility" status will be entered into HICCC's institutional database. This status will remain until further source documentation is made available to determine overall patient eligibility. Required materials for all pending registration submissions are as follows:

- Completed/signed IRB approved/stamped Informed Consent Forms, including additional study ICFs (i.e. tissue, DNA, etc.) as applicable
- The completed/signed IRB approved HIPAA Authorization form
- Completed/signed CPDM ICF checklist
- Completed/signed HICCC personal census form
- Completed/signed CPDM Demographics Note to File

In order to confirm eligibility status, Investigators/designees (i.e., study specific Clinical Research Coordinator/Research Nurse, etc.) must submit the following documentation to the Central Registration Office via email or fax:

- The completed/signed study specific Eligibility Checklist
- Completed/signed Velos Demographics Note to File
- Copies of source documentation necessary for each item to be verified on the CUMC specific Eligibility Checklist, including but not limited to:
    - Copy of required laboratory test and procedure reports (i.e., hematology, serum chemistry, pregnancy test when applicable, MRI reports, CT/bone scans, etc.)
    - Copy of pathology and surgical reports

- Copy of clinic note(s) or other appropriate medical records capturing the consent process information, along with providing source documentation of any other items needed for screening/eligibility that are not captured in other source document forms (e.g., positive investigator statements of unique eligibility items not captured via other direct source documentation, concomitant medication lists, etc)

- Protocol deviation/waiver approvals (if applicable)

- **Please note**: subject line of email or fax should include the following: "AAAJ7800 Complete Subject Registration Request (PHI)".

Upon Receipt of the above mentioned documentation, participant eligibility information will be verified by a qualified Central Registration Registrar. If any questions arise during the review process, queries in the form of emails will be addressed to the applicable study team personnel for clarification prior to enrollment. .All applicable finalized registration/eligibility information will then be entered into HICCC's institutional database by the Central Registration Registrar. Upon completion, an official subject registration notification email will be sent to the PI/research team which will include eligibility/enrollment status, as well as subject ID information. Protocol therapy may not be initiated prior to receipt of this notification from the Central Registration Office.

All screen fail/ineligible subjects, as well as subject's who withdraw consent prior to enrollment/initiation of protocol therapy must be submitted to the Central Registration office in a manner analogous to the procedures noted above. Applicable source documentation will be required within the corresponding submissions.

Affiliate Institution Research Participant Registration Process:

All Affiliate Institutions **must** register subjects with the coordinating center (CUMC) **prior** to any administration of study drug/intervention/local institution registration. Please see instructions below:

1. Within 48 hours of the consent visit, the Affiliate Institution CRN and/or CRC is required to submit the following documents to the coordinating center's designee (CUMC's study specific Clinical Research Coordinator or Clinical Research Nurse) via email at AAAJ7800@columbia.edu. The coordinating center's designee will review the documents for accurateness, and subsequently submit the documents to the CPDM Central Registration Office via email at CPDMRegistration@columbia.edu (or via fax at 212.305.5292), with a request to register the patient "pending eligibility." The title of the email should read, "AAAJ7800 Pending Subject Registration Request (PHI)". The following documents should be submitted with the pending registration request:

    a. Redacted Completed/signed IRB approved/stamped Informed Consent Forms, including additional study ICFs (i.e. tissue, DNA, etc.) as applicable

b. Redacted Signed HIPAA (or institutional equivalent)

c. MCT Velos Demographics Note to File form

2. The Participating Institution's investigator/research nurse/data manager/coordinator must contact the coordinating center's designee (CUMC's study specific Clinical Research Coordinator or Clinical Research Nurse) via telephone or email to communicate the following:

- Notify of pending registration request
- Confirm method of registration request submission (email or fax)
- Communicate expected time-line of registration request submission (i.e., same day, next day, within the hour, etc.)

3. For a full registration, the Affiliate Institution's investigator/research nurse/data manager/coordinator should then submit the following documents to the CUMC study specific designee:

- A signed Affiliate Site Eligibility Checklist (signed by treating physician/investigator)
- Copies of redacted source documentation necessary for each item to be verified on the CUMC specific Eligibility Checklist, including but not limited to:
    - Copy of required laboratory test and procedure reports (i.e., hematology, serum chemistry, pregnancy test when applicable, MRI reports, CT/bone scans, etc.)
    - Copy of pathology and surgical reports
    - Copy of clinic note(s) capturing the consent process information, along with providing source documentation of any other items needed for screening/eligibility that are not captured in other source document forms. (e.g., positive investigator statements of unique eligibility items not captured via other direct source documentation, concomitant medication lists, etc.)
    - Protocol deviation/waiver approvals (if applicable)
- **Please note**: subject line of email or fax should include the following: "AAAJ7800 Complete Subject Registration Request (PHI)".

4. Upon receipt of the above mentioned documents, the designated study specific Clinical Research Coordinator will review all documents and verify patient eligibility. If any questions arise during the review process, queries in the form of emails will be addressed to the applicable affiliate site study team personnel for clarification prior to enrollment. Upon

verification, the CUMC study specific designee will then forward all documents to the CPDM Central Registration Office for central registration (as described above). The CPDM Central Registration Registrar will review all applicable documents and communicate to the CUMC study specific designee in order to clarify any items. The CUMC study specific designee will communicate with the applicable site study team personnel for additional clarifications necessary prior to enrollment.

5. Upon receipt of the subject registration notification email, the CUMC study specific designee will forward the notification email (which will include the study specific subject ID) to the affiliate site's Principal Investigator, Consenting Professional, and applicable research personnel. This notification should be filed in the subject research record, medical record, and regulatory binders accordingly. Protocol therapy **may not** be initiated prior to receipt of this notification from the coordinating center.

6. All screen fail/ineligible subjects, as well as subject's who withdraw consent prior to enrollment/initiation of protocol therapy must be submitted to the Central Registration office in a manner analogous to the procedures noted above. Applicable source documentation will be required within the corresponding submissions.

6 TREATMENT PLAN

Treatment will be administered on an outpatient basis. Each treatment cycle will be 28 days. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

6.1 Study Agents

6.1.1 Bendamustine

Patients will start bendamustine at dose level 0 and according to CrCl on day 1 cycle 1 and actual body weight:

CrCl ≥ 60 mL/min: 100 mg/m2 IV on day 1 and 2 of each cycle

CrCl 59 – 30 mL/min: 90 mg/m^2 IV on day 1 and 2 of each cycle

CrCl 15 – 30 mL/min: 70 mg/m^2 IV on day 1 and 2 of each cycle

Available to qualifying subjects is the option to dose escalate to dose level (+)1:

120 mg/m^2 (if CrCl ≥ 60 mL/min at the time of inclusion into the study)

100 mg/m^2 (if CrCl 59 – 30 mL/min at the time of inclusion into the study)

Refer to section 6.7 for detailed dose modification information.

6.1.1.1 How Supplied

Bendamustine is an investigational agent supplied to investigators by Teva Pharmaceuticals (formerly Cephalon, Inc) (See Appendix G for Study Drug Request Form).The bendamustine supplied for this protocol is intended for clinical trial use only and is not commercially available.

For further details and molecule characterization, see the Cephalon IND application and/or Investigator's Brochure (See Appendix E for cross reference letter).

6.1.1.2 Route and Method of Administration

The dose should be administered intravenously over 60 minutes. To insure complete delivery of bendamustine, the IV infusion line must be flushed with 50 mL 0.9% sodium chloride for injection and a volume equal to the volume contained in the tubing according to institutional standards.

6.1.1.3 Expected Adverse Events

Common (occurs in more than 10% of people - more than 10 out of 100 people):

- Blood abnormalities, such as a decrease in the number of white blood cells, red blood cells, platelet, and/or neutrophils. White blood cells are cells of your immune system. A decrease in white blood cells and or neutrophils may lead to fever and/or a life threatening infection. The red blood cells carry oxygen to your organs. A decrease in red blood cells can lead to fatigue. A decrease in platelets may lead to bleeding and require stopping treatment. An elevated number of eosinophils, a type of white blood cell, may also be seen.
- Infections (such as pneumonia) requiring antibiotic treatment
- Nausea
- Vomiting
- Diarrhea
- Constipation
- Dehydration
- Fatigue
- Hair loss
- Fever
- Allergic reactions
- Chills
- Pruritus (itchy skin)
- Rash
- Phlebitis
- Hives
- Hypersensitivity
- Mouth sores and pain
- Dry mouth
- Cough
- Weight loss/ lack of appetite
- Potential risk of temporary discomfort, bleeding, hematoma and infection after venipuncture

Infrequent (occurs in 1% to 10% of people - from 1 to 10 out of 100 people):

- Blood clots in your arteries which may cause stroke or heart attack or other problems. These conditions can be life-threatening or fatal.

Rare (occurs in fewer than 1% of people – fewer than 1 out of 100 people):

- Kidney damage
- Reversible changes in liver function tests that may indicate liver damage

- Pulmonary edema (pulmonary edema is an abnormal build up of fluid in the lungs, which leads to swelling).
- Myelodysplastic syndrome, a pre-cancerous blood condition which could lead to leukemia
- Toxic Epidermal Necrolysis, which is a severe and frequently fatal skin reaction similar to a severe burn.
- Anaphylactic and anaphylactoid reaction (particularly in the second and subsequent cycles of therapy)
- Infection associated with septic shock and death
- Death
- Extravasation: During the intravenous infusion of bendamustine, there have been reports of drug escaping from the blood vessel into the tissues. This can cause redness, swelling, pain, infection and tissue damage.
- Severe skin reactions (if relationship to Bendamustine is suspected, discontinue Bendamustine)
- Tumor lysis syndrome (tends to be within the first treatment cycle and without intervention, may lead to acute renal failure and death)

Tumor Lysis Syndrome

Tumor lysis syndrome associated with bendamustine treatment has been reported in patients in clinical trials and in spontaneous reports. The onset tends to be within the first treatment cycle of bendamustine and, without intervention, may lead to acute renal failure and death. Preventive measures include maintaining adequate volume status, and close monitoring of serum chemistry, particularly potassium and uric acid levels. Allopurinol has also been used prior to or at the beginning of bendamustine therapy. However, there may be an increased risk of severe skin toxicity when bendamustine and allopurinol are administered concomitantly.

Skin Reactions

Skin reactions have been reported in clinical trials and postmarketing spontaneous reports. These events have included rash, toxic skin reactions, and bullous exanthema. Some events occurred when bendamustine was given in combination with other anticancer agents, so the precise relationship of the skin reactions to bendamustine treatment is uncertain.

In a study of bendamustine (90 mg/m^2) in combination with rituximab (study SDX-105-02), 1 case of TEN occurred. TEN has been associated with treatment with rituximab. Spontaneous reports of SJS and TEN, some fatal, have been reported when bendamustine was administered concomitantly with allopurinol and other medications known to cause these syndromes. The relationship to bendamustine cannot be determined.

When skin reactions occur, they may be progressive and increase in severity with further treatment. Therefore, patients with skin reactions should be monitored closely. If skin reactions are severe or progressive, bendamustine treatment should be withheld or discontinued.

6.1.2 Dexamethasone

Will be administered 40 mg orally or intravenously on days 1, 8, 15, 22 of each cycle. Dose reduction is outlined under Section 6.7.

6.1.1.4 How Supplied

Decadron, Hexadrol, Dexameth, Dexone, DXM, others.

Commercially available in 0.25, 0.5, 0.75, 1, 1.5, 2, 4, and 6 mg tablets.

6.1.1.5 Route of Administration
Oral: 40 mg orally will be administered on days 1, 8, 15, 22 of each cycle.
Individual patients will stay at the same dose level throughout study treatment unless they require dose reduction due to DLT.
Intravenous: Dexamethasone can be administered intravenously at the same dose level at any point in the study cycle if this is more convenient for the study subject and/or the study site.

6.1.1.6 Risks of Dexamethasone
The most common side effects of dexamethasone include infection, insomnia, seizures, muscle weakness, particularly the thigh muscles, irritability and mood swings, weight gain, increased appetite, diabetes mellitus (high blood sugar), high blood pressure, thromboembolism, peptic ulcers, pancreatitis (inflammation of the pancreas), mucositis and fluid retention.

Intravenous administration of dexamethasone may cause following side effects:

- Temporary discomfort from the needle stick
- Bruising
- Bleeding
- Infection (rare)

6.2 Other Agents

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as anti-diarrheal, analgesics, anti-emetics received from the first administration of study drugs until 30 days after the final dose are to be recorded in the subject's research record.

All patients will receive prophylaxis with either an H-2 blocker or proton pump inhibitor while on study medications. Prophylactic antibiotics (Bactrim, Diflucan) should be considered. All supportive care should be given according to institutional standards.

Use of insulin and/or oral hypoglycemics to control hyperglycemia related to dexamethasone therapy is allowed per physician discretion.

6.3 General Concomitant Medication and Supportive Care Guidelines

Myelosuppression: Growth factors, specifically filgrastim (granulocyte colony stimulating factor; G-CSF) or pegfilgrastim only (GM-CSF not allowed), **can** be used to treat neutropenia. The usual dose of filgrastim is 5 micrograms/kg/day given subcutaneously until the ANC is greater than 1,000 x 2 subsequent readings. G-CSF can be rounded to the nearest vial size. The usual dose of pegfilgrastim is a single dose of 6 mg administered subcutaneously. No additional doses of pegfilgrastim are permitted in the next 28-day period. Growth factors should be given 24-72 hours after the last chemotherapy. The major adverse even with filgrastim and pegfilgrastim is bone pain. Other side effects are rare and include nausea, fatigue, diarrhea, vomiting, constipation, fever, anorexia, headache, taste perversion, dyspepsia, myalgia, insomnia,

abdominal pain, arthralgia, generalized weakness, peripheral edema, and dizziness. Reversible elevations in LDH, alkaline phosphatase, and uric acid, not requiring therapy have also been observed.

Hyperglycemia: Hyperglycemia, sometimes severe, including diabetic coma or ketoacidosis can occur with dexamethasone therapy. Therefore blood sugars should be monitored in diabetic patients, borderline patients, and in patients at risk for hyperglycemia or diabetes.

Anemia: Patients with hemoglobin < 8 mg/dL and/or symptomatic receive packed red blood cells transfusion. The final decision should be made at the discretion of the investigator or an authorized physician sub-investigator.
Subjects are allowed to be treated with full dose anti-coagulation (full dose low molecular weight heparin or warfarin with a goal INR of 2 to 3) while on study, however the toxicity management guidelines as outlined below must be followed.

6.4 Dosing Delays/Modifications

Treatment modifications are based on adverse events, which are possibly, probably, or definitely related to drug. All AEs should be graded according to the Common Terminology Criteria for Adverse Events (CTCAE, v. 4.0). Due to the complex problems that may occur in this patient population, it is at the discretion of the investigator to decrease the dose level of the study drugs if medically indicated. The investigator should inform the lead institution's principal investigator regarding these decisions. Dose modifications and delays different from those stated in the protocol, for management of toxicities, will be permitted at the discretion of the Investigator.

6.4.1 Bendamustine Adjustments for Hematologic Toxicities

The following treatment adjustments for hematologic toxicities are recommended. The investigator or an authorized physician sub-investigator may modify the recommendations if the clinical situation dictates a change.

▪ *Uncomplicated Neutropenia*: On Day 1 of each cycle, if Grade 3 or 4 neutropenia is the only Grade 3 or 4 adverse event present, hold all study drugs, monitor CBC weekly. If neutropenia resolves to grade 1 or baseline within 7 days resume treatment at previous dose level and add G-CSF or Peg-G-CSF at Day 3 of cycle.
If neutropenia does not resolve to baseline or grade 1 within 7 days, start daily G-CSF without initiating next cycle of therapy. If neutropenia resolves with G-CSF at ≤ 7days of starting G-CSF, start next cycle without dose reduction and adding G-CSF with the next cycle. If neutropenia does not resolve with G-CSF at ≤ 7 days of adding G-CSF, patient is off study. Patients who experience a Grade 3 or 4 neutropenia on Day 1 and received pegfilgrastim in the previous cycle should be dose-reduced for the following cycle.

At any other time of each cycle (with protocol labs), if grade 3 or 4 neutropenia is the only grade 3 or 4 adverse event, add G-CSF. Do not reduce dose for next cycle. Add G-CSF on Day 3 of next cycle. If patient is already receiving G-CSF, then dose is reduced by one level with continued use of growth factor when next cycle is initiated.

▪ *Complex/Complicated Neutropenia*: Sustained (≥ to 7 days) Grade 3 or 4 neutropenia subsequent to G-CSF or associated with fever/infection (temperature ≥ 38.5°C AND requiring antibiotic treatment) hold study drugs and follow CBC weekly. When toxicity resolves to baseline or ≤ Grade 1, start new cycle at next lower dose level with G-CSF.

Adherence to the preceding guidelines regarding G-CSF use with neutropenia is strongly encouraged; however final decision regarding use of G-CSF rests with the study site investigator and his/her clinical judgment.

▪ *Thrombocytopenia:* Grade 3/4 (Plt < 50,000/mm^3) hold all study drugs, follow CBC and dose reduce to next lower dose level. If grade 4 thrombocytopenia occurs for more than 7 days and/or is associated with significant bleeding (e.g., requiring transfusion) patient is off study regardless of the dose level. When toxicity resolves to baseline or ≤ Grade 1, start new cycle at next lower dose level.
▪ *Lymphopenia:* Grade 3/4 is expected and will not be graded as an AE.
▪ *Anemia:* Grade 4, patient should be evaluated for other reasons of anemia such as hemolytic anemia, bleeding, etc.

All patients should get optimal supportive therapy such as platelet or red blood cell transfusion.

6.4.2 Bendamustine Adjustments for Other Toxicities

There are no reductions in the bendamustine dose if bendamustine is given at dose level -2. If adverse events occur at dose level -2 that require holding bendamustine, the dose will remain the same once treatment resumes. If bendamustine is held for more than 4 weeks, the study site investigator should consult with the Principal Investigator of the study to assess the appropriateness of continuing the patient on study. If an AE occurs at a higher dose, the bendamustine dose will be reduced according to the table in section 6.7.

Bendamustine Dose Management based on Adverse Events	
CTCAE Grade	**Bendamustine Treatment Adjustment**
Hepatic or other non-hematologic toxicity assessed as bendamustine related ≥ Grade 3	Hold bendamustine and follow the subject. If the toxicity resolves to ≤ Grade 1 restart the next cycle at the next lower dose level. IF AE occurred under dose level -2 of bendamustine and the dose is held for more than 4 weeks the patients will be off study.

6.4.3 Dexamethasone Treatment Adjustments

It is expected given the low dose of dexamethasone that few dose modifications related to dexamethasone will be necessary. Refer to the dose modifications table, Section 6.7, for the dose reduction levels for dexamethasone.

CTCAE Category	**Adverse Event**	**Dexamethasone**	**Treatment**

		Adjustment
Gastrointestinal (All patients will be treated with H2 Blockers, ranitidine, or omeprazole as per Section 6.2)	Dyspepsia, gastric or duodenal ulcer, gastritis Grade 1-2	If symptoms persist despite prophylaxis, decrease dexamethasone to dose level -1 permanently.
	Dyspepsia, gastric or duodenal ulcer, gastritis ≥ Grade 3 (requiring hospitalization or surgery)	Hold dexamethasone until symptoms are adequately controlled. Restart at dose level -1. If symptoms persist, discontinue dexamethasone and do not resume
	Acute pancreatitis	Discontinue dexamethasone and do not resume.
Cardiovascular	Edema ≥ Grade 3	Diuretics as needed, and decrease dexamethasone dose to dose level -1, if edema persists despite above measures, decrease dose to dose level -2; discontinue dexamethasone and do not resume if symptoms persist despite reduction to dose level -2.
Neurology	Confusion or Mood Alteration ≥ Grade 2 (interfering with function and/or ADLs)	Hold dexamethasone until symptoms resolve, restart at next lower dose level. If symptoms persist despite above measures, discontinue dexamethasone and do not resume.
Musculoskeletal	Muscle Weakness ≥ Grade 2 (symptomatic and interfering with ± function of ADLs)	Decrease dexamethasone to dose level -1, if weakness persists despite above measures, decrease dose to dose level -2; discontinue dexamethasone and do not resume if symptoms persist at dose level -2.
Metabolic	Hyperglycemia ≥ Grade 3 or higher	Treatment with insulin or oral hypoglycemics as needed. If uncontrolled despite above measures, decrease to dose level -1, decrement until levels are satisfactory.

6.5 Duration of Treatment

Therapy will be given until disease progression or for up to *6 courses* after a patient has a CHR. Patients will come off treatment for unacceptable toxicity, patient refusal, non-response, progression or non-compliance.

6.6 Duration of Follow-Up

Patients who have progressed will be followed for survival for a maximum of 3 years, with phone calls every 3 months. Patients who have not progressed, will be followed according to the Study Calendar in Section 6.8. Patients removed from study for unacceptable adverse events will be monitored until resolution or stabilization of the adverse event.

6.7 Dose Modifications

Dose reduction level for bendamustine:

If the AE is not described in 6.4 and cannot be attributed to a specific drug e.g. (hyperglycemia and dexamethasone, neutropenia and bendamustine) a dose reduction of both drugs should be performed.
Patients will start bendamustine at dose level 0 according to calculated creatinine clearance (CrCl) result on day 1 cycle 1 and actual body weight on day 1 of each cycle, and dose modifications will be based on their individual starting dose.
When CrCl ≥ 60 mL/min: start at 100 mg/m^2 IV on day 1 cycle 1
When CrCl 59 – 30 mL/min: start at 90 mg/m^2 IV on day 1 cycle 1
When CrCl 30 – 15 mL/min: start at 70 mg/m^2 IV on day 1 cycle 1
Dose modifications will be performed according to the starting dose.

	CrCl ≥ 60 mL/min	**CrCl 59 – 30 mL/min**	**CrCL 30 – 15 mL/min**
Dose Level +1	120 mg/m^2 days 1+2	100 mg/m^2 days 1+2	
Dose Level 0	100 mg/m^2 days 1+2	90 mg/m^2 days 1+2	70 mg/m^2 days 1+2
Dose Level -1	90 mg/m^2 days 1+2	70 mg/m^2 days 1+2	50 mg/m^2 days 1+2
Dose Level -2	70 mg/m^2 days 1+2	50 mg/m^2 days 1+2	

Dose reduction level for dexamethasone:

	Standard risk patient	**Patient with edema, cardiac symptoms or previous AE to dexamethasone**
Dose Level 0	40 mg/week	20 mg/week
Dose Level -1	20 mg/week	20 mg q 14 days
Dose Level -2	20 mg q 14 days	10 mg/week
Dose Level -3	D/C dexamethasone and continue bendamustine monotherapy	D/C dexamethasone and continue bendamustine monotherapy

Investigator has option of escalating dose of bendamustine to dose level +1 if no dose interruptions or serious adverse events (SAEs) in cycles 1 and 2. If the subject tolerates treatment well without serious side effects, dose escalation can be considered upon discussion with the Principal Investigator of the study who will make the final determination for dose escalation.

6.8 Study Calendar

All pre-study scans, x-rays and biopsy to be done up to 6 weeks before registration. EKG and Echocardiogram to be done up to 4 weeks before registration. Pre-study CBC (with differential and platelet count) and all required pre-study chemistries must be done up to 4 weeks before registration. Patients must begin therapy within 4 weeks of registration. If a cycle needs to be delayed due to the subject's inability to comply with the study calendar (i.e., hospitalizations, business and vacation travel plans, illness, transportation issues, holidays, family emergencies, etc.), a one week delay is available for rescheduling of day 1 treatment and procedures per the discretion of the treating physician investigator. Laboratory assessments required for treatment must be performed on day 1 of each cycle (up to 3 days prior to or on the day of treatment administration is permitted). UPEP may be performed up to two weeks prior to Day 1. After discontinuation of therapy, subjects who have not progressed should be followed every 3 months (± 1 month) for the first two years, every 6 months (± 1 month) for years 2-5, and annually thereafter until progression (see Section 6.6). The following parameters need to be evaluated by the treating physician **prior to start of therapy**: History and physical examination including graded neuropathy, VS and AE assessment, performance status (ECOG and Karnofsky), CBC, Calcium, sodium, potassium, serum creatinine, BUN, EKG, BNP/NT-proBNP, Troponin I/T.

Study parameters[15]	**Baseline**	**Every Cycle d1[15, 16]**	**Every Cycle d15 (± 2 days)[17]**	**Discontinuation of therapy[13]**	**Follow-up[14]**
History & Physical Examination, orthostatic VS, Height & Weight[1]	X	X[2]		X	X
VS, orthostatic VS, AE assessment		X[2]	X		
Performance Status (ECOG and Karnofsky)	X	X[2]		X	X
CBC (with differential and platelets)	X	X[2]	X	X	X
Calcium, sodium, potassium, serum creatinine, BUN	X	X[2]		X	X
Glucose	X	X			
Total Bilirubin, LDH	X	X		X	
AST/ALT	X	X			
Alkaline Phosphatase	X	X			
Albumin	X	X			
PT, PTT, INR	X	X			
BNP/NT-proBNP, Troponin I/T[3]	X	X[2]		X	X
B12 level	X	X[9]		X[9]	
Urinalysis	X	X		X	X
SPEP[4]	X	X		X	X

Study parameters[15]	**Baseline**	**Every Cycle d1[15, 16]**	**Every Cycle d15 (± 2 days)[17]**	**Discontinuation of therapy[13]**	**Follow-up[14]**
Serum free light chains[4]	X	X		X	X
24 hour urine collection for total protein with UPEP [4]	X	X		X	X
Immunofixation of serum and urine	X	X[10]		X[10]	X[10]
Bone Marrow Aspirate/Biopsy[5]	X			X[5]	
Cytogenetics (bone marrow)	X			X	
Beta 2 Microglobulin	X	X		X	
Quantitative Immunoglobulins[6]	X	X		X	
Pregnancy test[7]	X	X			
EKG[1]	X	X[2]		X	X
Echocardiogram[1]	X	X[11]		X[11]	X[11]
X-ray: skeletal survey with CXR	X				
CT scan/ultrasound abdomen	X	X[11]		X[11]	X[11]
Fecal Fat[8]	X	X		X	
Bendamustine & Dexamethasone		X[12]			

Correlative studies (Optional) 5mL pre-treatment bone marrow aspirate, shipped to Dr. Comenzo's lab (Boston)	X				

1. Physical exam including graded neuropathy, ECG and echocardiogram can be done up to 4 weeks before registration. Height is obtained at baseline only; weight is measured at baseline, day 1 (with a window of -3 days) of each treatment cycle, at discontinuation of therapy, and at follow-up assessment visits.
2. Results should be evaluated prior to start of drug treatment.
3. BNP or NT-proBNP and Troponin I or Troponin T can be used (investigator's choice) but initial test chosen should be the one used throughout the study for an individual patient.
4. After baseline testing, the appropriate marker should be followed to assess response. This might be either SPEP, free light chains and/or UPEP. Serum immunofixation should be performed in patients in which the M-protein spike became undetectable.
5. Bone marrow aspirate/biopsy at the end of treatment is obtained only to confirm achievement of CHR or if necessary for response assessment.
6. Includes lgG, IgA, IgM.
7. All females of childbearing potential should complete a serum pregnancy test within 7 days prior to day 1 of cycle 1. The pregnancy test should be repeated in week 1 of every cycle (except for cycle 1). Postmenopausal women must have been amenorrheic for ≥ 12 months in order to be considered "of non-childbearing potential."
8. Twenty four to 72 hr fecal fat testing is recommended (but not required) for patients with clinical signs/symptoms of intestinal malabsorption, diarrhea, impaired motility, etc. If baseline results of test are abnormal, then analysis should be repeated every 4 cycles and at discontinuation of therapy. NOTE: Colonoscopy/endoscopy may be useful for confirmation of bowel involvement by amyloid and assessment of bowel-related symptoms, but will not be used in assessment of organ response, and is thus NOT included in the requested baseline testing.
9. If baseline results of test are abnormal, then analysis should be repeated Day 1 of every cycle and at discontinuation of therapy.

10. Required to document CHR or PD
11. Only if baseline results are abnormal then EKG, TTE, and/or intra-abdominal imaging will be performed again every 4 cycles (not required to be performed on day1), at follow-up and discontinuation of therapy if clinically indicated. Echocardiogram must report LV ejection fraction, intra-ventricular septum wall thickness and left ventricular posterior wall thickness.
12. Bendamustine is administered IV on days 1 and 2 of each cycle. Dexamethasone is administered PO or IV on days 1, 8, 15, 22 of each cycle.
13. To be performed within 4 weeks after completion of the last cycle.
14. After discontinuation of therapy, subjects should be followed every 3 months (± 1 month) for the first two years, every 6 months (± 1 month) for years 2-5, and annually thereafter.
15. CBC, glucose, calcium, sodium, potassium, serum creatinine, BUN, VS, AE assessment should be performed on the day of treatment administration (a window of minus 3 days is allowed).
16. All other labs and tests should be performed on day 1 of each cycle (a window of minus 7 days is allowed) if not otherwise specified.
17. Day 15 of each cycle can be performed by a non-physician clinician.

Note: For subjects coming from great distances, follow-up may be performed by a local physician/oncologist if no treatment related toxicities have been noted in the prior evaluation performed at the treating institution, starting 6 months after the last treatment, and as long as the subject remains free of treatment related toxicities. The study coordinator will make direct telephone contact with the subject to assure the absence of any new symptoms and will make arrangements to receive records from the local physician for investigator review. If the subject experiences any toxicities that are considered attributable to study treatment, the subject must return to the treating institution for examination and follow-up with the study team.

7 MEASUREMENT OF EFFECT

Patients that received at least 2 cycles of therapy will be evaluable for response assessment such as SD, MR, PR, VGPR or CR. Patients who receive less than 2 complete cycles of treatment are not eligible for response assessment but will be evaluable for toxicity and for OS in secondary analyses. Patients with primary progressive disease after only receiving 1 cycle of therapy will be included in the response analysis.

7.1 Definition of Hematologic Response

The clonal protein may be in the form of an M spike (SPEP/UPEP) or monoclonal protein (immunoelectrophoresis) or elevated free light chain levels with an abnormal ratio. Some patients have all 3 findings, some have 2 and some only 1. Some patients have only urine M spikes and urine immunofixation findings. One percent of patients have no measurable abnormal clonal protein by any blood or urine test. Therefore, evaluation of the clonal plasma cell disease can be based on serum protein electrophoresis (SPEP, M spike), immunoelectrophoresis (quantitative immunoglobulins, monoclonal or M protein), or serum free light chain assay, and on bone marrow biopsy stained for isotypic plasma cells. Nevertheless free light chains are the most relevant prognostic marker and should be followed as the preferred response criteria. It is expected that around 15% of the patients have normal FLC or dFLC < 4 g/dL at baseline. For those patients standard criteria of multiple myeloma response evaluation using SPEP and UPEP should be applied.

- **Complete Response (CR):** Normalization of the free light chain levels and ratio, negative serum and urine immunofixation.
- **Very Good Partial Response (VGPR):** Reduction in the difference between involved and uninvolved free light chains (dFLC) to <4 mg/dL.
- A ≥ 90% reduction of the M protein with persistence by immunofixation will be considered a VGPR.
- **Partial Response (PR):** Reduction of the difference between involved and uninvolved free light chains (dFLC) of ≥ 50%.
- A reduction of ≥ 50% of the M-protein if M-spike is ≥ 0.5 g/dL will be considered a PR.
- **No Response (NR):** Less than a PR.
- **Progression of Disease (PD):**
    - •From CR: any detectable monoclonal protein or abnormal free light chain ratio (light chain ratio must double)
    - •From PR, 50% increase in serum M protein to >0.5 g/dL or 50% increase in urine M protein to >200mg/day (a visible peak must be present)
    - •Free light chain increase of 50% to >100 mg/L

- Bone marrow biopsy comparisons are part of the response assessment in order to confirm CHR or in case of not measurable m-spike or FLCs. It is for this reason that marrow biopsies are stained at baseline for CD138 (plasma cell marker) and isotypic light chains (kappa and lambda, by immunohistochemical or *in situ* hybridization techniques). This approach allows estimation of total plasma cells (CD138+) and relative kappa-to-lambda ratio based on isotypic staining. A normal marrow biopsy has less than 2% plasma cells in a diffuse distribution. The normal kappa-to-lambda ratio ranges from 4:1 to 1:1; estimates of the ratio outside of that range are considered indicative of clonal plasma cell disease.
- A CHR to therapy requires undetectability of the prior M spike and/or monoclonal protein and/or abnormal serum free light chain by any and all tests, and no evidence of the prior clonal plasma cell disease by bone marrow biopsy (normal marrow biopsy < 5% plasma cells).

7.2 Definition of Organ Response Rate (OrRR)

Amyloid-related organ response will be evaluated on the basis of the accepted criteria described below. The tests and studies listed in Section 7.1 will be repeated as appropriate to assess patients for both organ response and progression of disease.
Based on the criteria below, amyloid-related organ involvement will be scored as response, stable or progression every 4 cycles of therapy (or at the end of therapy if the patient receives more or less than 6 cycles).

Response

Kidneys: 30% reduction or drop below 0.5 g in 24-hour urine protein excretion in the absence of progressive renal insufficiency.[35]
Heart: NT-proBNP or BNP response (>30% and >300 ng/L decrease in patients with baseline NT-proBNP ≥ 650 ng/L or NYHA class response (≥ 2 class decrease in subjects with baseline NYHA class 3 or 4).
Liver: 50% decrease of an initially elevated alkaline phosphatase level or reduction in the size of the liver by at least 2 cm (determined by physical exam, ultrasound or computer tomography).
Neuropathy: clinical improvement supported by clinical history, neurologic exam, orthostatic vital signs, resolution of severe constipation or reduction of diarrhea to less than 50% of previous movements/day, and EMG studies if indicated.

Progression
Kidneys: 25% decrease in eGFR[35]
Heart: NT-proBNP or BNP progression (>30% and >300 ng/L increase) or cTN progression (≥33% increase) or Ejection fraction progression (≥10% decrease).
Liver: 50% increase in the alkaline phosphatase level above the lowest value.
Neuropathy: clinical worsening supported by history, worsening orthostatic vital signs and symptoms, and EMG studies if indicated.
Stable disease is defined when none of the criteria for improvement or for worsening disease are met. Subjects with progressively worsening renal function cannot be scored for NT-proBNP progression

8 STATISTICAL CONSIDERATIONS

8.1 Justification of Design

The optimal two-stage Simon[1] design was determined from the following parameters. For the treatment to be of further interest, the proportion of patients experiencing clinical response should be at least 0.40 (p_1). A proportion of responding patients of 0.20 or less is clearly unacceptable (p_0). The probability of promoting an unacceptable drug to Stage 2 (α) is 0.10, and the probability of rejecting an acceptable drug at the end of Stage 2 (β) is set to 0.15. Given the parameters above, 13 patients are enrolled in the first stage. If at least three patients experience a hematologic response defined as PR or higher, the trial proceeds to the second stage with additional 16 patients, otherwise, the trial terminates with no further interest in the treatment. If a total of 9 or more out of the total of 29 patients experience a hematologic PR or better, the treatment will be considered worthy of further development. In Monte Carlo simulations, it was

determined that, if π =0.2, the probability of stopping earlier is 0.5. Under this scenario, the expected number of patients is 21.

8.2 Data Analysis Plan

1. Primary Objective: *Determine the partial hematologic response (PHR)* Only patients who have received at least 2 cycles of therapy are eligible for response assessment. The proportion of patients with PHR two months post-treatment will be estimated, with a 95% exact binomial confidence interval.
2. Secondary Objective 1: *Determine overall hematologic response (OHR) rate* The proportion of response-evaluable patients experiencing OHR will be estimated, with a 95% exact binomial confidence interval.
3. Secondary Objective 2: *Determine the organ response rate (OrRR)* The proportion of response-evaluable patients experiencing OrRR will be estimated, with a 95% exact binomial confidence interval.
4. Secondary Objective 3: *Determine the distribution of time to failure (TTF).* Time to failure will be assessed for each patient from day of first treatment, where failure will be defined as disease progression or death. The survival function of TTF for response-evaluable patients will be estimated using the product-limit (Kaplan-Meier) estimator, along with 95% confidence bounds. The median time to failure will be estimated from the survival function. The analysis will be repeated on all patients who receive any therapy.
5. Secondary Objective 4: *Assess the toxicity of the regimen* Toxicities (adverse events at least possibly related to treatment) will be tabulated for patients who receive any therapy by NCI CTCAE grade and category.
6. Secondary Objective 5: *Determine the distribution of overall survival (OS)* Survival is assessed as time to death from first day of treatment The OS function for response-evaluable will be estimated using the product-limit (Kaplan-Meier) estimator, along with 95% confidence bounds. The median survival will be estimated from the survival function. The analysis will be repeated on all patients who receive any therapy.
7. Secondary Objective 6: *Assess* by RT-PCR the effects on expression in clonal plasma cells of genes associated with ER stress (GADD153, BiP) and cell-cycle regulation (CCND1, p16, p21). Change from baseline in these endpoints will be characterized graphically (e.g., by boxplots) and by descriptive statistics (e.g., mean, standard deviation, median). The null hypothesis of no change over time will be tested by a signed-rank test, or, if appropriate, single-sample t-test.

9 CORRELATIVE LABORATORY STUDIES

The laboratory of one of the co-investigators (Dr. Raymond Comenzo, Tufts Medical Center) will be performing correlative studies on the marrow plasma cells of consenting patients obtained before the first cycle of bendamustine and dexamethasone. Five (5) mL of bone marrow aspirate will be sent in one green to tube (heparin prefilled) at room temperature to

Dr. Comenzo's laboratory
Raymond Comenzo, MD
Tufts Medical Center,
800 Washington Street

Boston, MA 02111

These studies involve investigations of the character of the clonal plasma cells in AL. Recent data from this lab and others indicate that AL plasma cells over-express *CCND1* in almost one-half of cases, likely often associated with *t(11;14)* or *+11q*.[23] In several retrospective studies, over-expression of *CCND1* or *t(11;14)* have been associated with poorer survival in AL patients.[21,22]

CCND1 over-expressing clones usually produce only immunoglobulin light chains and not intact immunoglobulins because of loss of Ig heavy chain gene loci due to *t(11;14)*. Preliminary data from the Comenzo lab indicates that *CCND1* over-expressing clones also over-express genes associated with maintenance of protein solubility and protein quality control (*PDIA6*, *Sel1L*), a phenomena possibly linked to the exclusive production of Ig light chains that form amyloid. The correlative studies of clonal bone marrow plasma cells selected from pre-treatment marrow aspirates will further test the hypothesis that *CCND1* over-expressing clones up-regulate these genes involved in ER-associated protein quality control. Using patient bone marrow specimens obtained at baseline, we will perform RT-PCR to assess the expression in clonal plasma cells of the genes *CCND1* and *Sel1L.*

Quantitative RT-PCR will be performed (in triplicate and on 2 separate days) using TaqMAN Gene Expression Assays with RPLP0 (Hs99999902_m1) primers and probes (Applied Biosystems; Foster City, CA) as internal control on an Mx 3000P platform and related software (Stratagene; La Jolla, CA). The comparative Ct method will be used and the amount of target normalized to the control ($2^{-\Delta\Delta Ct}$) assuming an efficiency of 2. Statistical analyses (paired t-test) will be performed with GraphPad PRISM (GraphPad; La Jolla, CA) with $P < 0.05$ significance level for *CCND1*. Then $CCND1^{hi}$ and $CCND1^{lo}$ cases will be compared by paired t-test with respect to expression levels of *PDIA6 and SEL1A*. These studies may lead to new therapies for AL by identifying new druggable targets.

The correlative studies are optional.

10 ADVERSE EVENT REPORTING

10.1 Adverse Event Definitions

- *Adverse event*: Any untoward medical occurrence in a clinical study; regardless of the causal relationship of the event with the investigational drug or study treatment(s).
- *Associated with the use of the investigational drug or study treatment(s)*: There is a reasonable possibility that the adverse event may have been caused by the investigational drug or study treatment(s).
- *Disability*: A substantial disruption of a person's ability to conduct normal life functions.
- *Life-threatening adverse event*: Any adverse event that places the patient or subject, in the view of the investigator, at immediate risk of death from the event as it occurred (i.e., does not include an adverse event that, had it actually occurred in a more severe form, might have caused death).
- *Serious adverse event*: Any adverse event occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or

prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.
  - Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse event; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse event (e.g., for a preexisting condition not associated with a new adverse event or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse event.
- *Unexpected adverse event*: Any adverse event, the frequency, specificity or severity of which is not consistent with the risk information described in the clinical protocol(s).

10.2 Recording/Reporting Requirements

10.2.1 Eliciting Adverse Event Information

Clinical study subjects will be routinely questioned about adverse events at study visits.

10.2.2 Recording Requirements

All observed or volunteered adverse drug events (serious or non-serious) and abnormal test findings, regardless of treatment group or suspected causal relationship to the investigational drug or study treatment(s) will be recorded in the subjects' case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a serious adverse event) and; 2) an assessment of the casual relationship between the adverse event and the investigational drug or study treatment(s).

Adverse events or abnormal test findings felt to be associated with the investigational drug or study treatment(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.

Abnormal Test Findings
An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:
▪ The test finding is accompanied by clinical symptoms.
▪ The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy.
Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
▪ The test finding leads to a change in study dosing or discontinuation of subject participation in the clinical study.
▪ The test finding is considered an adverse event by the investigator-sponsor.

10.3 Reporting of Adverse Events

Safety Monitoring and Adverse Event Reporting

All participating sites are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants. All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the criteria specified in the protocol. Life-threatening toxicities must be reported immediately to Columbia University, the Coordinating Site (lead institution).

AEs and SAEs can be reported on the standard **MedWatch form (FDA Form 3500A)** http://www.fda.gov/downloads/Safety/MedWatch/HowToReport/DownloadForms/ucm082728.pdf

Name of Contact:	Suzanne Lentzsch, M.D.
Address:	Columbia University Medical Center Herbert Irving Comprehensive Cancer Center Herbert Irving Pavilion 161 Fort Washington Avenue New York, NY 10032
Email:	aaaj7800@columbia.edu
Fax:	212-305-3035 or 212-305-4667

An adverse event is any unfavorable and unintended sign, symptom, or disease temporarily associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment. Serious adverse events are defined as complications in the patient's clinical course that are outside the normal course for the underlying disease/condition and/or significantly increase the risk of compromising the patient's health.

Adverse Event Documentation

Regardless of relationship to study treatment, all serious and non-serious adverse events that occur during the protocol-defined AE and/or SAE reporting period are to be recorded on an Adverse Event Log during the study.

The following information should be collected for adverse events:

- Description of the adverse event including onset and resolution dates
- Severity of Adverse Event
- Relationship to study treatment or other causality
- Outcome of each event
- Whether event met SAE criteria

Multicenter Site Investigator Reporting Responsibility

Site investigators (affiliate sites) are responsible for knowing the policies of the local IRB, adhering to these policies, and maintaining a copy of the policies in the study file. The site

investigators are also responsible for the accurate documentation, investigation and follow-up of all possible study-related adverse events.

All serious adverse events that occur during the study, regardless of the relationship to the study drug, must be reported by the site investigators to the Coordinating Site within 24 hours and the Institutional Review Board (IRB) in accordance with institutional guidelines of being made aware of the SAE. For initial SAE reports, available case details are to be recorded on an SAE form. At a minimum, the following should be included:

- Patient number
- Date of event onset
- Description of the event
- Study treatment, if known

Relevant follow-up information is to be submitted to the Coordinating Site in the form of a follow-up SAE report within 10 business days (if available) of the original submission. The follow-up SAE report should include a re-evaluation of the original determination of grade and relationship of the event, along with all results supporting this assessment.

The Coordinating Site will maintain documentation of all participating sites' Adverse Event information and be responsible for communicating to all participating site investigators and notifying the funding agency or sponsor, as necessary. Participating site investigators will review any distributed AE reports, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

Reporting Adverse Events to the Responsible IRB

In accordance with applicable policies of the Columbia University's Institutional Review Board (IRB), the investigator-sponsor will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) at least possibly related to participation 2) increases risk; and 3) unexpected. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 5 calendar days following the investigator-sponsor's receipt of the respective information. Adverse events which are 1) associated with the investigational drug or study treatment(s); 2) fatal or life-threatening; and 3) unexpected will be reported to the IRB within 24 hours of the investigator-sponsor's receipt of the respective information.

Follow-up information to reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the sponsor-investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigator-sponsor will report the adverse event to the IRB as soon as possible, but in no event later than 5 calendar days, after the determination was made.

10.3.1

The Investigator will comply with all safety reporting regulations as set forth in the Code of Federal Regulations. The investigator being the sponsor of the study has sole responsibility for reporting of adverse events to the FDA. For informational purposes any correspondence to the FDA regarding adverse events or other safety issues will be simultaneously copied to us.clinops.sae@tevapharm.com or reported to Teva via facsimile at 215-619-3825. The Investigator will communicate the occurrence of serious adverse events to Teva within 24 hours of becoming aware of the event. Reporting of Adverse Events to Teva does not preclude the responsibility of the investigator to report adverse events to the FDA. Please note that the reporting period begins when a patient signs the informed consent, and ends 30 days after the discontinuation of dosing or completion of the patient's participation in the Study if the last scheduled visit occurs at a later time. In addition, the Investigator must notify Teva of any serious adverse events that may occur after this time period which he or she believes to be definitely, likely or possibly related to the Study Product. Institution acknowledges that Teva is required to comply fully and promptly with all regulatory safety reporting requirements regarding its products.

The MedWatch 3500A form should be utilized to report serious adverse events to the FDA.

A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity;
- Is a congenital anomaly or birth defect;
- Results in the development of drug dependency or drug abuse;
- Is a serious adverse event drug experience.

For the purpose of this Agreement, these terms shall have the same meaning as the terms used in the provisions of the Code of Federal Regulations governing drug and biologic safety reporting. See 21 CFR 314.80(a); 600.80(a). Additionally, the following will also be deemed to be adverse events for purposes of this Agreement: pregnancy exposure, infant exposure during breastfeeding, overdose, abuse, misuse, medication errors, lack of efficacy, infectious agents, as well as all reports of accidental pediatric exposure, and any other safety information as reasonably requested by Company. Investigator shall use his/her judgment to determine the relationship between the serious adverse event and the Study Product. In the event the IRB requests additional safety information from Investigator, Investigator shall notify Teva of such request within one (1) business day.

Investigator and Institution further agree to report all Adverse Events in compliance with all applicable legal and regulatory requirements, and in accordance with any requirements provided by Teva.

10.4 Guidelines for Processing IND Safety Reports

The U.S. Food and Drug Administration (FDA) regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any serious and unexpected adverse experiences that are possibly related to the investigational agent. The CUMC Principal Investigator will review all applicable IND Safety Reports and has the responsible for forwarding the IND Safety Reports to the Affiliate Institutions. The Affiliate Institution investigators are to review, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents. All Affiliate site INDSR submissions, along with IRB acknowledgment (per local policies and procedures) are to be forwarded to CUMC for placement within the trial master file.

10.5 Data and Safety Monitoring Committee

Every subject who receives treatment on this protocol will be monitored for toxicity by the Herbert Irving Comprehensive Cancer Center (HICCC), an NCI-approved Data Safety Monitoring Committee (DSMC). This protocol will adhere to the policies of the HICCC Data and Safety Monitoring Plan, version 2 guidelines in accordance with NCI regulations. The committee is led by Dr. Gregory Mears and consists of HICCC members. The DSMC meets monthly to review adverse event reporting and the timeliness of adverse event reporting. The PI will submit data and safety monitoring reports to the DSMC. Upon review, the HICCC DSMC will disseminate the review findings to the CUMC PI. CUMC, as the coordinating center for this trial, will subsequently distribute all DSMC review letters to affiliate sites for applicable local submission and regulatory binder maintenance.

According to DSMC guidelines, all protocols managed through CUMC review and monitoring systems are subject to the HICCC Data and Safety Monitoring Plan. Part of the plan provides for monthly review of all studies in each disease center. In addition, each subject will be assessed periodically according to the treatment schedule (flowchart) for the development of any toxicity. Dose modifications will be made based on toxicities described earlier.
All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 4.0 and recorded prior to each course of therapy. In addition, all toxicities will be reported to the IRB along with any Data Safety meeting minutes in the annual renewal report. Any required modifications necessary to ensure patient safety are discussed and will be submitted to the IRB. All protocol violations will also be reported to the IRB and all other designated regulatory agencies as required. All study data reviewed and discussed during these meetings will be kept confidential. Any breaches in research subject confidentiality will be immediately reported to the IRB.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by the CPDM Compliance Core on behalf of the HICCC DSMC. Additionally, the Compliance Oversight Committee of the IRB at Columbia University Medical Center may audit the study at any time per institutional policies and procedures.

The investigator-sponsor and Columbia University Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

A risk-based approach will be used by the Compliance Core to determine the frequency, number of subject charts, and data elements to be monitored. The Compliance Coordinator will review the study status and summarize enrollment, toxicities, SAEs/UPs, dose escalation, statistical endpoints (e.g., stopping rules), etc. for the full DSMC membership at the regularly scheduled meetings.

MCT Monitoring:

1. Initial, recurrent, and close-out on-site monitoring visits will also be conducted at remote clinical sites, as appropriate/feasible. Other sites will have monitoring performed remotely (see below for further details).

    a. The study Monitoring Visit Log will be completed and signed by the monitor and the PI/CRNP/CRN and/or CRC and will be filed in the regulatory binder.

2. The Compliance Coordinator will communicate with the participating site coordinator to schedule the monitoring visit and arrange for access to study materials and documentation.

3. The Compliance Coordinator will monitor IIT trials within 1 month after the first subject is enrolled at the participating site and throughout the life of the study to ensure that the study is being conducted in accordance with the protocol, GCP, applicable federal and local regulations, and per all applicable SOPs. The Compliance Coordinator is responsible to notify the participating site PI and CRNP/CRN/CRC of upcoming monitor visits and convey what information and documentation will be required for the visit(s). The Compliance Coordinator is responsible for verifying that informed consent is properly obtained, eligibility is met (via the central registration process), and all study procedures are conducted according to the study protocol. The Compliance Coordinator will also verify that the data reported in the CRF's accurately reflect source documents, that all toxicities have been reported to date, and that all SAE's/UPs/deviations/violations have been reported according to Coordinating Center, local IRB and HICCC DSMC requirements. The Compliance Coordinator will issue queries and ensure resolution in a timely and efficient manner.

4. An SIV (or) teleconference will be scheduled and conducted prior to study drug being made available (if applicable) and before any subjects are enrolled on a study at the participating site.

MCT Remote Monitoring:

1. When necessary (due to logistical constraints), participating sites will be monitored remotely by a designated Compliance Coordinator. Sites will be informed of this remote monitoring process on a site by site basis.

2. Participating sites will be monitored by the Compliance Coordinator on both a regulatory level, as well as a clinical data/source documentation review level.

3. Redacted source documents (applicable to supporting the protocol specific CRF data requirements) will be sent to the designated Compliance Coordinator via fax or secure email for all subjects enrolled at participating sites. Timelines for submission procedures will be defined on a case by case basis.

4. The Compliance Coordinator will review all submitted redacted source documents against the data entered on the protocol specific CRFs. The Compliance Coordinator will issue queries when/if necessary.

5. The participating site research staff will respond to queries within 30 days. If queries remain outstanding, the Compliance Coordinator will send a delinquent query reminder for the outstanding items.

6. The remote monitoring procedures will include review of applicable redacted source documentation and supporting applicable documents to determine compliance regarding:

    a. Informed consent procedures
    b. Eligibility criteria
    c. Protocol specific treatment compliance
    d. Protocol specific schedule of events (e.g., baseline visits, pre-treatment, on study, follow-up)
    e. Participating site IRB documents (e.g., IRB amendment approvals, annual renewals, SAE/UP submissions, violation/deviation submissions, INDSR submissions, etc).
    f. Required specimen submissions (e.g., tissue specimens, research blood specimens, etc.)
    g. Pharmacy accountability records
    h. Adherence to the CRF submission timeframes to CUMC (within the protocol specified timeframes)

7. Participating site performance reports will be sent to lead PI, HICCC DSMC, and participating sites on a quarterly basis. Reports will include information regarding data submission timeliness/accuracy, query resolution status, regulatory status, and overall participating site performance. These reports will be generated by the Compliance Coordinator and reviewed with the Compliance Core Manager prior to dissemination.

11.1 Guidelines for Affiliate site prospective protocol deviation requests:

For multisite-IIT trials in which CUMC is serving as the coordinating center, the participating site MUST submit a prospective deviation request to the CUMC lead PI for review and submission to the HICCC DSMC, HICCC PRMC, and CUMC IRB. Approvals must be obtained from all entities prior to implementation at the participating site. If a prospective protocol deviation request is submitted for review (from a participating site), the PI/site memo(s), HICCC DSMC approval(s) and correspondence, HICCC PRMC approval(s) and correspondence, and

CUMC IRB eligibility deviation letter(s) should be forwarded to the participating site for documentation. The participating site is also required to obtain prospective local IRB approval as per institutional policies/procedures prior to implementing the proposed deviation. All documents and determinations must be clearly documented in the study subject's medical record, research chart and regulatory binder, as described.

12 DATA HANDLING AND RECORD KEEPING

12.1 Data Recording/Case Report Forms

A Case Report Form will be completed for each subject enrolled into the clinical study through Velos eResearch. It is the investigator/sponsor's responsibility for ensuring that all clinical and laboratory data entered on the CRFs are complete, accurate and authentic.

Please refer to the table below regarding the form completion and source document submission guidelines:

CRF Title	Baseline/Screening	Day 1 of Every Cycle	Day 15 of Every Cycle	Day 1 of Every 4 Cycles	Off-Study
Kidney Organ Response	X	X*		X	X
Med Hist Weight PS	X	X			
Neuropathy Response	X	X*		X	X
Hematologic Response	X	X	X	X	X
Other Lab	X	X		X	X
SPEP	X	X		X	X
Adverse Events	X	X	X	X	X
CxR	X				
Radiologic Assessment	X			X	
Urinalysis	X	X		X	
Serum Pregnancy	X	X			
Bone Marrow	X				
Consent Form	X				
CBC Lab	X	X	X	X	X
Liver Organ Response	X	X*		X	
Organ Assessment	X			X	X
Radiologic Assessment	X			X	X
Cardiac Assessment	X	X*		X	X
ECHO	X			X	X
EKG	X	X		X	X
Vital Signs		X	X		
Treatment		X			

* = If necessary, as deemed appropriate by an improvement demonstrating response or worsening demonstrating progression, compared to abnormal baseline results

1. At time of Central Registration, all redacted source documentation for tests required at Baseline/Screening is required to be submitted.
2. For all CRF time points after registration, all redacted source documentation is required within 30 days of visit date.

12.2 Record Maintenance and Retention

The investigator-sponsor will maintain records in accordance with Good Clinical Practice guidelines; to include:

- IRB correspondence (including approval notifications) related to the clinical protocol; including copies of adverse event reports and annual or interim reports
- Current and past versions of the IRB-approved clinical protocol and corresponding IRB-approved consent form(s) and, if applicable, subject recruitment advertisements
- Signed FDA Form 1572 Statements of Investigator listing all sub-investigators involved in the treatment and/or evaluation of research subjects
- Financial disclosure information (for the investigator-sponsor and all sub-investigators involved in the treatment and/or evaluation of research subjects)
- Curriculum vitae (investigator-sponsor and clinical protocol sub-investigators)
- Certificates of required training (e.g., human subject protections, Good Clinical Practice, etc.) for investigator-sponsor and listed sub-investigators
- Listing of printed names/signatures of investigator-sponsor and listed sub-investigators
- Normal value(s)/range(s) for medical/laboratory/technical procedures or tests included in the clinical protocol
- Laboratory certification information
- Signed informed consent forms
- Completed Case Report Forms; signed and dated by investigator-sponsor
- Source Documents or certified copies of Source Documents
- Monitoring visit reports
- Copies of investigator-sponsor correspondence to sub-investigators, including notifications of safety information
- Subject screening and enrollment logs
- Subject identification code list
- Investigational drug accountability records, including documentation of drug disposal.
- Retained biological specimen log
- Interim data analysis report(s)
- Final clinical study report

Subject identity on study records will be indicated by a case number rather than by name, and the information linking the case numbers with the subject's identity will be kept separate from the research records. All records related to this research study will be stored in a locked file cabinet.

The investigator-sponsor will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug.

13 ETHICS

13.1 Institutional Review Board (IRB) Approval

The investigator-sponsor will obtain, from Columbia University Medical Center Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.
The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the investigator-sponsor will promptly notify the CUMC IRB of the deviation.

The CUMC IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).

13.2 Ethical and Scientific Conduct of the Clinical Study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol; ICH Guidelines on GCP; and relevant policies, requirements, and regulations of the Columbia University Medical Center New York State Laws and applicable federal agencies.

13.3 Subject Informed Consent

The investigator-sponsor will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The investigator-sponsor, or a sub-investigator(s) designated by the investigator-sponsor, will obtain the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The date and time that the subject, or the subject's authorized representative, signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The investigator-sponsor will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.
The investigator-sponsor will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the investigator-sponsor will obtain the informed consent of enrolled subjects for continued participation in the clinical study.

REFERENCES

1. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 1989;10:1-10.
2. Falk RH, Comenzo RL, Skinner M. The systemic amyloidoses. N Engl J Med. 1997;337:898-909.
3. Kyle RA. Amyloidosis: a convoluted story. Br J Haematol. 2001;114:529-538.
4. Kyle RA. Diagnosis of multiple myeloma. Semin Oncol. 2002;29:2-4.
5. Kyle RA, Gertz MA, Witzig TE, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc. 2003;78:21-33.
6. Merlini G, Stone MJ. Dangerous small B-cell clones. Blood. 2006;108:2520-2530.
7. Comenzo RL. Managing systemic light-chain amyloidosis. J Natl Compr Canc Netw. 2007;5:179-187.
8. Gertz MA, Lacy MQ, Dispenzieri A, Hayman SR, Kumar S. Transplantation for amyloidosis. Curr Opin Oncol. 2007;19:136-141.
9. Merlini G, Westermark P. The systemic amyloidoses: clearer understanding of the molecular mechanisms offers hope for more effective therapies. J Intern Med. 2004;255:159-178.
10. Gertz MA, Lacy MQ, Dispenzieri A, et al. Effect of hematologic response on outcome of patients undergoing transplantation for primary amyloidosis: importance of achieving a complete response. Haematologica. 2007;92:1415-1418.
11. Sanchorawala V, Seldin DC. An overview of high-dose melphalan and stem cell transplantation in the treatment of AL amyloidosis. Amyloid. 2007;14:261-269.
12. Comenzo RL, Gertz MA. Autologous stem cell transplantation for primary systemic amyloidosis. Blood. 2002;99:4276-4282.
13. Palladini G, Perfetti V, Obici L, et al. Association of melphalan and high-dose dexamethasone is effective and well tolerated in patients with AL (primary) amyloidosis who are ineligible for stem cell transplantation. Blood. 2004;103:2936-2938.
14. Lebovic D, Hoffman J, Levine BM, et al. Predictors of survival in patients with systemic light-chain amyloidosis and cardiac involvement initially ineligible for stem cell transplantation and treated with oral melphalan and dexamethasone. Br J Haematol. 2008;143:369-373.
15. Zonder JA, Sanchorawala V. Melphalan and Dexamethasone Plus Bortezomib Induces Hematologic and organ Responses in AL-Amyloidosis with Tolerable Neurotoxicity. Blood (ASH Annual Meeting Abstracts). 2009;114:abstract 746.
16. Dennie TW, Kolesar JM. Bendamustine for the treatment of chronic lymphocytic leukemia and rituximab-refractory, indolent B-cell non-Hodgkin lymphoma. Clin Ther. 2009;31 Pt 2:2290-2311.
17. Ponisch W, Mitrou PS, Merkle K, et al. Treatment of Bendamustine and Prednisone in patients with newly diagnosed multiple myeloma results in superior complete response rate, prolonged time to treatment failure and improved quality of life compared to treatment with Melphalan and Prednisone-a randomized phase III study of the East German Study Group of Hematology and Oncology (OSHO). J Cancer Res Clin Oncol. 2006;132:205-212.
18. Michael M, Bruns I, Bolke E, et al. Bendamustine in patients with relapsed or refractory multiple myeloma. Eur J Med Res;15:13-19.
19. Ponisch W, Rozanski M, Goldschmidt H, etal. Treatment with bendamustine, thalidomide, and prednisolone (BPT) in patients with refractory or relapsed multiple myeloma

after autologous stem cell transplantation or conventional chemotherapy: Results of a phase I clinical trial. Blood. 2006;108:abstract 3564.
20. Lentzsch S, O'Sullivan A, Lalo S, et al. A Phase I Study of Bendamustine Combined with Lenalidomide and Dexamethasone in Patients with Refractory or Relapsed Multiple Myeloma. Blood (ASH Annual Meeting Abstracts). 2009;114:abstract 1856.
21. Harrison CJ, Mazzullo H, Ross FM, et al. Translocations of 14q32 and deletions of 13q14 are common chromosomal abnormalities in systemic amyloidosis. Br J Haematol. 2002;117:427-435.
22. Hayman SR, Bailey RJ, Jalal SM, et al. Translocations involving the immunoglobulin heavy-chain locus are possible early genetic events in patients with primary systemic amyloidosis. Blood. 2001;98:2266-2268.
23. Zhou P, Hassoun H, Landau L, Comenzo R. Elevated Cyclin D1 (CCND1) expression in the plasma cells of patients with systemic AL Amyloidosis at diagnosis is associated with higher brain natriuretic peptide (BNP) levels, increased plasma cells and significantly poorer survival. Blood. 2009;abstract.
24. Zhou P, Teruya-Feldstein J, Lu P, Fleisher M, Olshen A, Comenzo RL. Calreticulin expression in the clonal plasma cells of patients with systemic light-chain (AL-) amyloidosis is associated with response to high-dose melphalan. Blood. 2008;111:549-557.
25. Thall PF, Simon RM, Estey EH. Bayesian sequential monitoring designs for single-arm clinical trials with multiple outcomes. Stat Med. 1995;14:357-379.
26. Durie et al B. Criteria for the classification of Monoclonal gammopathies, multiple myeloma, and related disorders: A report of the International Myeloma Working Group. 2003;121:749.
27. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649-655.
28. Hurst JW, Morris DC, Alexander RW. The use of the New York Heart Association's classification of cardiovascular disease as part of the patient's complete Problem List. Clin Cardiol. 1999;22:385-390.
29. J Clin Oncol. 2009 Sep 10;27(26):4378-84. Epub 2009 Aug 3. "Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia.
30. Knauf WU, Lissichkov T, Aldaoud A, Liberati A, Loscertales J, Herbrecht R, Juliusson G, Postner G, Gercheva L, Goranov S, Becker M, Fricke HJ, Huguet F, Del Giudice I, Klein P, Tremmel L, Merkle K, Montillo M." PMID: 19652068.
31. J Cancer Res Clin Oncol. 2006 Apr; 132(4):205-12. Epub 2006 Jan 10. "Treatment of bendamustine and prednisone in patients with newly diagnosed multiple myeloma results in superior complete response rate, prolonged time to treatment failure and improved quality of life compared to treatment with melphalan and prednisone--a randomized phase III study of the East German Study Group of Hematology and Oncology (OSHO)." Pönisch W, Mitrou PS, Merkle K, Herold M, Assmann M, Wilhelm G, Dachselt K, Richter P, Schirmer V, Schulze A, Subert R, Harksel B, Grobe N, Stelzer E, Schulze M, Bittrich A, Freund M, Pasold R, Friedrich T, Helbig W, Niederwieser D; East German Study Group of Hematology and Oncology (OSHO).
PMID: 16402269.
32. Eur J Med Res. 2010 Jan 29;15(1):13-9. "Bendamustine in patients with relapsed or refractory multiple myeloma." Michael M, Bruns I, Bölke E, Zohren F, Czibere A, Safaian NN, Neumann F, Haas R, Kobbe G, Fenk R. PMID: 20159666.
33. Cancer Chemother Pharmacol 2010 November; 66(6) 1039-1049).

34. Pönisch W, J Cancer Res Clin Oncol. 2013 Nov;139 (11):1937-46.
35. Palladini, G., et al. A staging system for renal outcome and early markers of renal response to chemotherapy in AL amyloidosis. Blood. 2014; 124.15: 2325-2332.

APPENDIX A. IMWG DEFINITIONS OF ASYMPTOMATIC AND SYMPTOMATIC MYELOMA[26]

Symptomatic Myeloma:

1. M-protein > 30 g/L
2. Bone marrow plasmacytosis > 10%
3. Myeloma related organ or tissue impairment as defined here:
    i. **C**alcium Elevation (Serum Calcium > 1 mg/dL above the IULN or > 11 mg/dL)
    ii. **R**enal Insufficiency (Serum Creatinine >2.0 mg/dL)
    iii. **A**nemia (hemoglobin < 10 g/dL or more than 2 g/dL below ILLN)
    iv. **B**one Disease (lytic bone lesions or osteoporosis with compression fractures)
    v. Other: symptomatic hyperviscosity, amyloidosis, >2 infections annually

Asymptomatic (Smouldering) Myeloma:

Meets criteria 1 and 2, but not 3 (*i.e.,* no disease-related organ or tissue impairment).

APPENDIX B. ECOG PERFORMANCE STATUS

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

* As published in American Journal of Clinical Oncology.[27]

APPENDIX C. NYHA FUNCTIONAL CLASSIFICATION FOR CONGESTIVE HEART FAILURE[28]

The New York Heart Association (NYHA) Functional Classification provides a simple way of classifying heart disease (originally cardiac failure), useful for preoperative assessment. It places patients in one of four categories, based on how much they are limited during physical activity:

Class I: patients with no limitation of activities; they suffer no symptoms from ordinary activities.

Class II: patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.

Class III: patients with marked limitation of activity; they are comfortable only at rest.

Class IV: patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

APPENDIX D. CREATININE CLEARANCE FORMULA

Creatinine clearance (CrCl) can be calculated using the Cockcroft-Gault equation as follows:

$$\text{CrCl (ml/min)} = \frac{(140\text{-age}) \text{ (actual wt in kg)}}{72 \times \text{serum creatinine (mg/dl)}}$$

For females use 85% of calculated CrCl value.

Note: In markedly obese patients, the Cockroft-Gault formula will tend to overestimate the creatinine clearance. (Adipose tissue tends to contribute little creatinine requiring renal clearance.)

APPENDIX E. KARNOFSKY PERFORMANCE STATUS

Karnofsky Performance Scale	
Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

Reference

Karnofsky DA, Burchenal JH: In Evolution of Chemotherapy Agents, Macleod CM (ed.). Columbia University Press, New York, 1949: pp 191-205