Methods

Patients

Eligible patients were ≥ 18 years with untreated or relapsed, biopsy proven AL and evidence of an underlying plasma cell dyscrasia, including clonal dominance of plasma cells in the bone marrow, and/or detection of a monoclonal gammopathy by immunofixation electrophoresis of serum and/or urine, and/or an abnormal serum free light chain ratio. Patients were required to have measurable disease, defined by an abnormal serum free light chain or monoclonal protein by immunofixation electrophoresis, proteinuria ≥ 0.5g/day, cardiac involvement with interventricular septal thickness ≥ 12 mm, and/or hepatomegaly >15 cm in the absence of congestive heart failure or alkaline phosphatase > 1.5 times upper limit of normal. Adequate organ function for inclusion was defined as absolute neutrophil count ≥ 1.0 x 10^9/L, platelet count ≥ 75 x 10^9/L, creatinine clearance ≥ 15 mL/minute, total bilirubin ≤ 2 times upper limits of normal, ECOG performance status of ≤ 3. Patients with amyloid cardiomyopathy and any degree of symptoms were enrolled, but after the first 15 patients the protocol was amended to include only patients with New York Heart Association (NYHA) class I or II symptoms. Cancer therapy must have been discontinued at least 4 weeks prior to treatment in this study. Patients had to be free of other malignancies (excluding MM) for ≥ 3 years with exception of treated skin basal cell or squamous cell carcinoma, or carcinoma “in situ” of the cervix or breast. All study participants were registered into the mandatory RevAssist® program. Females of childbearing potential were required to have a negative pregnancy test within 10 – 14 days prior to and within 24 hours of prescribing lenalidomide. Patients had to take aspirin, warfarin, or low molecular weight heparin as prophylactic anticoagulation. Patients were excluded if they had a history of hypersensitivity

to thalidomide or previous lenalidomide treatment. HIV positive patients were excluded. All patients provided written, informed consent.

Study Design

Patients were enrolled in this single center, single arm, open-label pilot study of lenalidomide, melphalan, and dexamethasone at the Stanford Amyloid Center in Stanford, CA between 2009 and 2012. The primary objective was to evaluate the safety of MDR in patients with AL, including those with advanced organ dysfunction. The secondary objectives were to evaluate the hematologic response rate, organ response rate, time to progression, event free survival, and overall survival. The study was carried out in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki, and was approved by the Stanford University Institutional Review Board. The trial was registered at www.clinicaltrials.gov (NCT00890552).

Patients received lenalidomide 10 mg/day orally on days 1-21, melphalan 0.18mg/kg orally on days 1-4, and dexamethasone 40 mg orally once weekly of a 28 day cycle. The melphalan dose was decreased by 20% for an estimated creatinine clearance of 15-40 ml/min. Patients received up to 9 cycles of treatment, with the option to continue on single agent lenalidomide if they responded to treatment. A new course of treatment began on the scheduled Day 1 of a cycle if the following criteria were met: ANC $\geq 1 \times 10^9$/L, platelet count $\geq 50 \times 10^9$/L, any other drug-related adverse events had resolved to $\leq$ grade 2 severity. A delay of 2 weeks was allowed without any dose modifications, after which point lenalidomide was decreased by a one-level dose reduction. Dose level 1 reduction of lenalidomide was defined as lenalidomide 5 mg PO on

days 1-21 every 28 days. Dose level 2 reduction was defined as lenalidomide 5 mg PO every other day on days 1-21 every 28 days. If patients could not tolerate dose level 2 reduction of lenalidomide they were removed from the study. The melphalan and dexamethasone doses could be decreased based on toxicity at the discretion of the investigators. Use of granulocyte colony-stimulating factor was permitted. Antimicrobial prophylaxis was not required.

Assessment

Baseline clinical assessments included a physical examination, evaluation of performance status, and determination of AL organ involvement. Baseline laboratory assessments included complete blood count with differential, complete metabolic panel, serum and urine immunofixation, free light chain analysis, and bone marrow aspirate and biopsy. Patients with cardiac involvement were evaluated pre and post-treatment with a transthoracic echocardiogram and electrocardiogram, but did not require Holter monitoring unless determined to be clinically indicated by an expert cardiologist. Patients were seen every 4 weeks during therapy for a follow-up visit including physical examination, laboratory assessments, and safety and adverse event assessment according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0. (26) Hematologic (HR) responses were defined by the updated guidelines from the 12th International Symposium on Amyloidosis. (27) Kidney and liver organ (OR) responses to treatment were defined by the 10th International Symposium on Amyloidosis (25). Cardiac OR and progression was defined by NT-proBNP changes that were both > 30% and > 300 ng/L, based on recent updates to the consensus criteria. (27) Hematologic and organ

assessments occurred at the end of every cycle, upon study completion, and every 3 months after completing treatment until progression or death.

Statistical Analysis

For the primary objective, we assessed the safety of MDR. The percentage of patients experiencing toxicities was compiled utilizing NCI CTCAE 3.0 guidelines. (26)

DOR was defined as the time from first response until the date of progression, death, or date of last follow-up in patients without progression in the subset of patients who responded to treatment. (22) Time to next treatment included all surviving patients (treatment responders and non-responders). Event free survival was defined as the time from the date of initiation of MDR until the date of next therapy, hematologic progression, or death by any cause. (21) Overall survival was calculated from the date of first dose of MDR until the date of death by any cause. Survival curves were plotted with the Kaplan-Meier method and compared by the use of the log-rank test.