Lenalidomide/melphalan/dexamethasone in newly diagnosed patients with immunoglobulin light chain amyloidosis: results of a prospective phase 2 study with long-term follow up

Ute Hegenbart,[1] Tilmann Bochtler,[1,2] Axel Benner,[3] Natalia Becker,[3] Christoph Kimmich,[1] Arnt V. Kristen,[4] Jörg Beimler,[5] Ernst Hund,[6] Markus Zorn,[7] Anja Freiberger,[8] Marianne Gawlik,[1] Hartmut Goldschmidt,[1] Dirk Hose,[1] Anna Jauch,[9] Anthony D. Ho[1] and Stefan O. Schönland[1,4]

[1]Department of Internal Medicine, Division of Hematology/Oncology, University of Heidelberg; [2]Clinical Cooperation Unit Molecular Hematology/Oncology, German Cancer Research Center (DKFZ) and Department of Internal Medicine V, University of Heidelberg; [3]Division of Biostatistics, German Cancer Research Center, Heidelberg; [4]Division of Cardiology, University of Heidelberg; [5]Division of Nephrology, University of Heidelberg; [6]Department of Neurology, University of Heidelberg; [7]Division of Clinical Chemistry, University of Heidelberg; [8]Coordination Center for Clinical Trials, KKS, Heidelberg and [9]Institute of Human Genetics, University Heidelberg, Germany

Haematologica 2017
Volume 102(8):1424-1431

Correspondence:
ute.hegenbart@med.uni-heidelberg.de

Received: December 24, 2016.
Accepted: May 9, 2017.
Pre-published: May 18, 2017.

doi:10.3324/haematol.2016.163246

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/102/8/1424

ABSTRACT

Chemotherapy in light chain amyloidosis aims to normalize the involved free light chain in serum, which leads to an improvement, or at least stabilization of organ function in most responding patients. We performed a prospective single center phase 2 trial with 50 untreated patients not eligible for high-dose treatment. The treatment schedule comprised 6 cycles of oral lenalidomide, melphalan and dexamethasone every 4 weeks. After 6 months, complete remission was achieved in 9 patients (18%), very good partial remission in 16 (32%) and partial response in 9 (18%). Overall, organ response was observed in 24 patients (48%). Hematologic and cardiac toxicities were predominant adverse events. Mortality at 3 months was low at 4% (n=2) despite the inclusion of 36% of patients (n=18) with cardiac stage Mayo 3. After a median follow-up of 50 months, median overall and event-free survival were 67.5 months and 25.1 months, respectively. We conclude that the treatment of lenalidomide, melphalan and dexamethasone is very effective in achieving a hematologic remission, organ response and, consecutively, a long survival in transplant ineligible patients with light chain amyloidosis. However, as toxicity and tolerability are the major problems of a 3-drug regimen, a strict surveillance program is necessary and sufficient to avoid severe toxicities. *clinicaltrials.gov Identifier: 00883623 (Eudract2008-001405-41).*

Introduction

Immunoglobulin light chain (AL) amyloidosis is a monoclonal plasma cell disorder characterized by the deposition of amyloid fibrils in different tissues. Although the burden of plasma cells in the bone marrow is generally low, the accumulation of amyloid protein leads to progressive and severe end organ failure and, eventually, death.[1]

The choice of upfront treatment depends on age, performance status and degree of amyloid-related organ dysfunction. High-dose chemotherapy (HDC) followed by autologous stem cell transplantation is very effective and has excellent long-term results, but is reserved for younger patients with nearly normal organ functions.[2] In patients not eligible for high-dose chemotherapy, melphalan-dexamethasone (M-Dex) is considered the standard treatment, and has shown good long-term results in patients without advanced cardiac involvement.[3] However, M-Dex is much less effective in patients with advanced cardiac disease when a dose reduction of Dex

40 mg (to Dex 20 mg or even less) is necessary.[3,4] To improve hematologic remission (HemR) rates, several combination therapies have recently been evaluated. Some of these combination protocols use M-Dex as a backbone and add either bortezomib or lenalidomide.[5-8] The combination of bortezomib with M-Dex (B-M-Dex) appears a particularly promising treatment option. A recent publication by Palladini *et al.* showed a complete remission (CR) rate for B-M-Dex of 42% (HemR 69%) as compared to a CR rate of 19% in a historical M-Dex group (HemR 51%).[8] However, the HemR rate was not different when the analysis was restricted to patients without severe cardiac involvement who were taking a full dose of Dex. This again underlines the huge impact of the Dex dosage on the results. Patient recruitment of a randomized clinical trial comparing B-M-Dex with M-Dex has been completed.[9] Other combination chemotherapies (lenalidomide plus cyclophosphamide/Dex) have also been evaluated as first-line therapy.[10-13]

We have performed a prospective phase 2 trial using the combination of lenalidomide, melphalan and dexamethasone (L-M-Dex). As compared to the 3 prior studies also testing this regimen,[5-7] the study herein includes the largest patient number with the longest follow up and provides further solid data to support this 3-drug therapy.

Methods

Patients

Fifty patients with untreated AL amyloidosis could be enrolled. They had to have measurable monoclonal gammopathy (M-spike and/or abnormal free light chain values) as well as symptomatic organ involvement. Diagnosis was made by a congo red positive biopsy, immunohistology to confirm AL type and exclusion of hereditary types when necessary. Patients were not eligible for HDC or autologous stem cell transplantation (center-specific criteria as published by Schönland *et al.*)[14] or refused to undergo it. WHO Performance Status had to be <3 and The New York Heart Association (NYHA) <stage 4. Patients with symptomatic multiple myeloma or a creatinine clearance <40 ml/min were excluded. The patients had to be able to visit the Amyloidosis Clinic once a month.

Study design

Patients were enrolled in this investigator initiated trial (IIT) phase 2, single center, open label study combining lenalidomide with M-Ddex (*clinicaltrials.gov Identifier: 00883623; Eudract2008-001405-41*) at the Amyloidosis Center Heidelberg, Germany between 04/2009 and 02/2012. Lenalidomide was supplied by Celgene (München, Germany). The database was closed in 09/2013. Long-term survival, HemR and organ response analysis were performed outside of the protocol in a retrospective fashion with a data cutoff on 01/07/2015.

Study treatment consisted of a total of 6 times 4 cycles of lenalidomide 10 mg/day for 21 days, melphalan 0.15 mg/kg/day and dexamethasone 20 mg/day for 4 days each. A prophylactic antibiotic treatment with ciprofloxacin was administered. Prophylactic anti-thrombotic treatment was aspirin 100 mg; low-molecular weight heparin was applied in patients with a history of deep vein thrombosis, pulmonary embolism or thrombophilic coagulation status. Toxicity was assessed using The National Cancer Institute (NCI) common toxicity criteria (version 3.0). Due to the well described "paradoxical" increase of N-terminal B-type natriuretic peptide (NT-BNP) during lenalidomide treatment this was not recorded as an adverse event or organ progression.[15]

The primary endpoint of the study was complete remission (CR) after 6 treatment cycles in patients who received at least 3 cycles of chemotherapy. HemR was defined in the protocol as CR or partial remission (PR).[16] Very good partial remission (VGPR) was evaluated but not as part of the protocol (it was not yet defined at the time of study initiation); VGPR was retrospectively analyzed in all 42 patients with a dFLC (difference between the involved and uninvolved free light chain) of >50 mg/l.[17] For better comparability with other studies we report HemR and CR+VGPR rates after treatment completion (5 or 6 cycles) as an "intention to treat" analysis (ITT, all patients). We defined early mortality as death due to any cause up until 3 months following the start of the treatment. Secondary endpoints were the rate of HemR after end of treatment (5 or 6 cycles) and organ response 6 months after end of treatment, and correlation of cytogenetic results with remission and survival.

A retrospective comparison with a historical control group (using the same inclusion and exclusion criteria) treated with M-Dex either at our center or by local hematologists was performed. Treatment with M-Dex consisted of melphalan 16 mg/m^2 day 1 intravenously[4] every 4 weeks and Dex 20-40 mg days 1-4 orally, as published previously.[4] Evaluation of HemR as well as organ response was performed at our outpatient amyloidosis clinic after every 3 cycles of M-Dex.

The study was performed in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki, and was approved by the Ethics Committee of the University of Heidelberg as well as the competent authority. The Data Monitoring Committee was informed every 6 months about safety data and occurrence of serious adverse events (SAE).

Assessment

Baseline assessments and procedures included physical examination, amyloid organ involvement, standard laboratory values as well as serum M-protein analysis, free light chains, NT-proBNP and cardiac troponin T (cTNT)/high-sensitive cTnT (hsTNT). Once a week blood evaluations were performed by the local physician (complete blood counts, creatinine, potassium, bilirubin, C–reactive protein[CRP]). The results were reviewed by the study physician and followed up by a personal phone call with the patient. In the case of any significant clinical problems lenalidomide was withheld until the next evaluation. Bone marrow aspiration and cytogenetic analysis with interphase fluorescence *in situ* hybridization (iFISH) after CD138$^+$ enrichment was carried out in all patients. Organ involvement and response to therapy were assessed according to the International Consensus Criteria.[16] Dose reduction due to hematological toxicity was performed sequentially (first melphalan, second lenalidomide). Dose reduction of dexamethasone was done by 25% in patients with fluid overload in the previous cycle.

Mayo cardiac staging was retrospectively applied in all patients using NT-ProBNP and troponin (cTNT or hsTNT).[18,19] In addition, organ response was also retrospectively analyzed using this new cardiac criteria.[15]

Statistical analysis

A one-sided exact binomial test was used to test the null hypothesis that the probability of achieving a CR after 6 cycles of L-Mel-Dex is not larger than 16%; this had been the CR rate of patients receiving at least 3 cycles of M-Dex in our institution. Overall survival (OS) was defined as time until death by any cause (failure time) or date of last follow up (censored time). For event-free survival (EFS) an event was defined as either death by any

Table 1. Patient characteristics.

Patient cohort	L-M-Dex
Age, years, median (range)	66.6 (47-75)
Sex	
Female	25
Male	25
Monoclonal light chain, number of patients	
κ	4
λ	46
Absolute involved free light chain concentration, mg/l, median (range):	
κ	121 (2-591)
λ	181 (19-3510)
dFLC	161 (0-3508)
Plasma cell content of the bone marrow, median % (range)	10 (1-31)
Number of organs involved, number of patients	
1 organ	9
2 organs	22
> 3 organs	19
Dominant organ, number of patients*	
Heart	26
Liver	2
Gut	1
Kidney	24
Peripheral Nerves	1
Other	6
NT-proBNP, ng/l, median (range)	2784 (54-15066)
hsTNT (pg/ml) median (range)	29.5 (0-263)
Cardiac involvement, number of patients	36
Mayo staging system,[16] number of patients:	
1	8
2	24
3	18
Creatinine clearance, ml/min, median (range)	71 (41-160)
Renal staging system,[23] number of patients**	
1	11
2	16
3	4

*patients might have several dominant organ involvements, therefore the sum of patients is larger than 50. **only given for patients with renal involvement. NT-proBNP: N-terminal prohormone of brain natriuretic; cTNT: cardiac troponin T; hsTNT: high-sensitive cTnT; dFLC: difference between the involved and uninvolved free light chain; L-M-Dex: lenalidomide, melphalan and dexamethasone.

cause, hematological relapse/progression or second-line chemotherapy.[16,17] All patients without an event were censored at the date of last contact, defined as date of last visit/response evaluation. Distributions of survival times were estimated by using the method of Kaplan & Meier. 95% confidence intervals (95% CI) were computed using Greenwood's formula for the variance of the Kaplan–Meier estimator. Comparisons of 2 survival curves were performed using the log-rank test. The distribution of follow up times were calculated by the reverse Kaplan-Meier estimate.[20] The prognostic impact of treatment in the study cohort and historical M-Dex cohort on EFS and OS was evaluated by the Cox proportional hazards regression model. Estimated hazard ratios (HR) and their corresponding 95% CI were used to express effect sizes.

Table 2. Distribution of the 132 adverse events (AEs) > grade 3 and serious adverse events (SAEs).

Type of AE	Grade 3	Grade 4	Grade 5	SAE
Cardiac	8		1	5
Hypotension/syncope	5			1
Thrombosis	1			
Neutropenia	12	1		
Lymphopenia	3			
Thrombocytopenia	4			
Infections	14	7	1	7
Anemia	1			
Kidney	2			1
Liver	6	5		
Gastrointestinal	6			
Other	51	4		2

The proportional hazards assumption was tested as proposed by Grambsch *et al.*[21] Multivariable Cox proportional hazards regression models were used to adjust effects for additional covariates (*Online Supplementary Table S1*). For dFLC log2-transformed data were used and HRs are reported according to a 2-fold increase of the original values. The influence of clinical covariates on the CR+VGPR rates was investigated using multivariable logistic regression (*Online Supplementary Table S1*). Fisher's exact tests were used for group comparisons of cytogenetic aberrations, response and distributions between the L-M-Dex and historical M-Dex cohorts. Tests are considered to be statistically significant if their corresponding *P*-value is ≤0.05. All analyses were performed with the statistical software environment R, version 3.1.1.[22]

Results

Patient characteristics

Fifty patients with newly diagnosed AL amyloidosis were included. Characteristics of the study cohort are shown in Table 1. Median age at start of L-M-Dex treatment was 66.6 years. Cardiac involvement was present in 36 patients (72%). The median NT-proBNP level was 2.784 ng/l, 6 patients (12%) had >8.500 ng/l. According to the cardiac Mayo 2004 staging system, 8 patients (16%) were stage 1, 24 patients (48%) were stage 2 and 18 patients (36%) were stage 3. The second most affected organ was the kidney (31 patients, 62%). Eleven of these patients were in renal stage 1, 16 patients in stage 2, and 4 patients in stage 3.[23]

Toxicity

All 50 patients were assessed for safety. Thirty-five patients received 6 cycles, and 6 patients completed their 6 cycles without any dose reduction. In total, 136 dose modifications for 43 patients were reported. Lenalidomide dose was reduced or paused in 1 or more cycles in 42 patients and subsequently reintroduced in the next cycle in all of these patients. Melphalan dose was reduced in 20 patients due to hematologic or renal toxicity and the reduced dosage was kept as such in the succeeding cycles. However, all patients received melphalan until the last treatment cycle. One-hundred thirty-two adverse events (AE) of grade >3, according to the Common Terminology

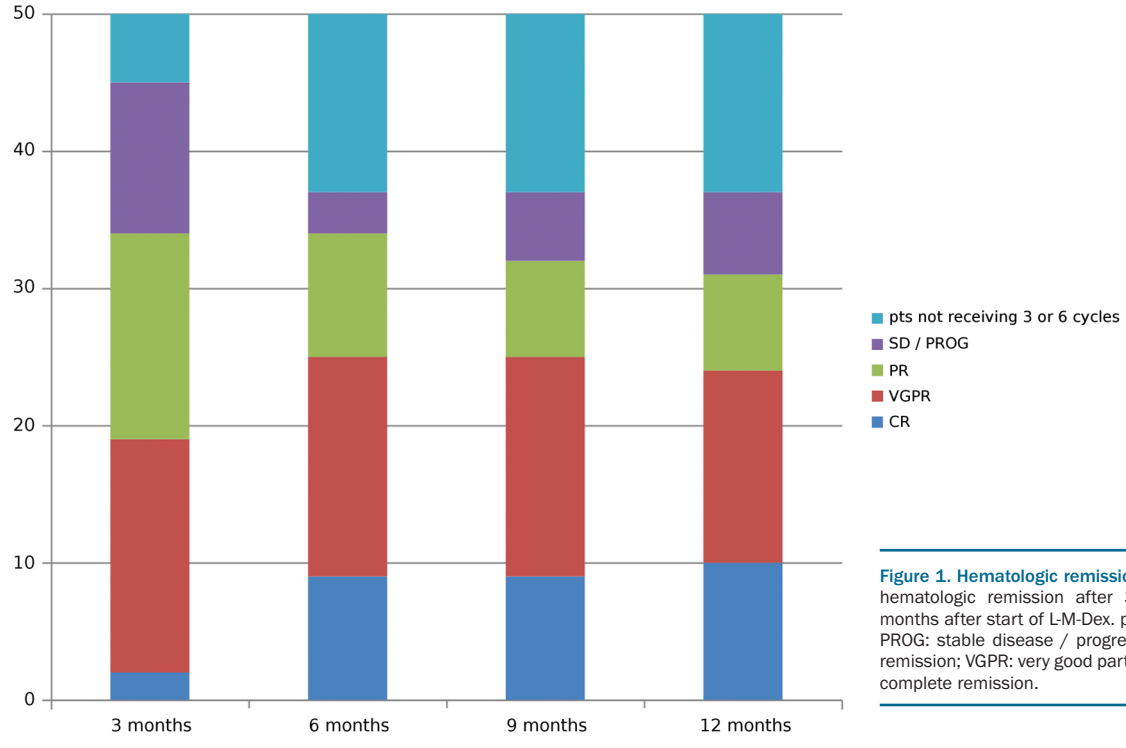

Figure 1. Hematologic remission. Distribution of hematologic remission after 3, 6, 9 and 12 months after start of L-M-Dex. pts: patients; SD / PROG: stable disease / progression; PR: partial remission; VGPR: very good partial remission; CR: complete remission.

Criteria for Adverse Events (CTCAE), were recorded in 50 patients including 16 severe AEs (Table 2; at least one AE grade 3 or 4 occurred in all study patients) and all were considered to be treatment-related, as it is often difficult to distinguish between side effects and amyloidosis-related events. Twenty-one hematologic AEs were observed; neutropenia 76%, CTCAE grade 4 in 1 patient and grade 3 in 12 patients, grade 3 thrombocytopenia was reported in 3 cases, anemia grade 3 (<8 g/dl) in 2 cases, and lymphopenia in 3 patients. The most common non-hematologic AE (14 patients) was worsening of cardiac function (e.g., worsening of cardiac failure, atrial fibrillation) or symptoms of autonomic neuropathy. As expected, the median NT-proBNP value increased from baseline (2.784 ng/l, n=50 patients) to 3 months (5.560 ng/l, n=47 patients) and in 38 patients more than 30% compared to baseline, whereas renal function was stable in most patients (baseline median creatinine value 1.0 mg/dl and at 3 months 1.1 mg/dl). The median creatinine level was equal at the start of cycle 2 and 3 (1.1 mg/dl, range 0.5-2.4 and 0.5-3.9 mg/dl, respectively). Eight patients suffered from an infection (1 patient died from sepsis in the first cycle, 4 patients had bronchitis or pneumonia, further AEs classified as infection were fever of unknown origin, gastroenteritis, erysipelas and CRP elevation in several cases (7 patients grade 4). One patient developed acute renal failure and 1 patient a deep vein thrombosis. These latter 2 patients remained within the study following adequate treatment and resolution of the AEs.

Feasibility, hematological remission and organ response

Forty-five patients (90%) received 3 cycles and 37 patients (74%) completed the treatment with 5 or 6 cycles. Overall, 253 cycles were administered. Therefore, 45 out of 50 patients were evaluable for the primary endpoint CR rate (1 patient died before the first cycle of L-M-Dex was completed, and 4 additional patients discontinued L-M-Dex after the first or second cycle due to the deterioration of their performance status mainly related to amyloidosis). After 3 cycles 2 patients achieved CR, 17 patients VGPR and 15 patients PR, respectively. After treatment completion 9 patients achieved CR, 16 VGPR and 9 PR. CR+VGPR rate improved from 38% to 50% from cycle 3 to cycle 6 (ITT analysis, n=50). Five patients received more than 3 but less than 6 cycles: 3 patients received 4 cycles, and 2 received 5 cycles. Two of those with 5 cycles achieved a remission (1 PR and 1 VGPR after 5 cycles) but chose to stop due to moderate toxicity. Interestingly, 3 additional patients achieved a negative immunofixation without further chemotherapy in the follow up at 7 months post start of therapy. With a CR rate of 20% (9/45) among patients with at least 3 cycles, the primary objective of the study was not achieved (P=0.29, one-sided exact binominal test). However, using the 7 month evaluation the primary objective would have been reached with a CR rate of 26.7% (12/45; P=0.05, one-sided exact binominal test). Organ response[16] was observed in 8 patients at 6 months after end of treatment. Using the new criteria for organ response,[17] 10 and 20 patients had an organ response after 12 and 24 months following start of therapy, respectively. Interestingly, another 4 patients developed an organ response later than 24 months without receiving new treatment (overall 24/50, 48%, 95% CI 34%-63%). Organ responses were observed in the heart (decrease of NT-BNP) and kidney in 10 patients (decrease of proteinuria; 1 patient had an organ response in both organs). Three other organ responses were seen in the soft tissue (normalization of factor X), autonomic nerve system and liver.

Survival and Progression

Median follow-up time from start of treatment was 50 months (range 1-72). The median duration of CR and VGPR was 39 months (range 1-72) and at 12 months the CR rate was still 20% (Figure 1). Fourteen patients relapsed or progressed with their underlying plasma cell dyscrasia after a median of 25.1 months from start of therapy (95% CI, 20.6–infinity). The duration of organ response in patients with CR/VGPR was 35 months (range 10-67). Twenty patients received second-line therapy (mostly proteasome inhibitors) because they did not respond to L-M-Dex or developed hematological or organ progression. The early death rate was low with 4% (1 death due to septicemia during the first treatment cycle and 1 cardiac death after 3 cycles). Overall, 20 deaths were observed, among them 19 due to progressive disease. The median OS was 67.5 months (95% CI, 49.6-infinity) and median EFS time was 25.1 months (95% CI, 20.6-infinity) (Figure 2). Cox univariate regression showed significant influence of cardiac Mayo 2004 score (3 *vs.* 1/2) on OS and EFS (P<0.001 in both analyses, Figure 3A,B).

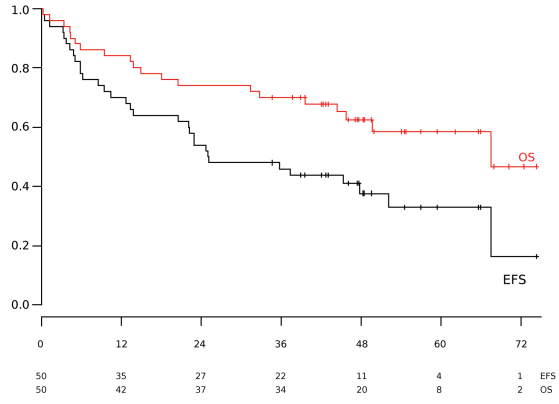

Figure 2. Survival of patients with lenalidomide, melphalan and dexamethasone. Estimated event-free survival (EFS) and overall survival (OS) in the L-M-Dex study group.

iFISH results

We were able to detect at least one cytogenetic abnormality in all 50 study patients. Twelve patients (24%) had gain of 1q21 and translocation t(11;14) was detected in 28 patients (56%). High-risk cytogenetic aberrations (t(4;14), t(14;16), del17p) were only seen in 3 patients and were not further analyzed due to this low number.

Gain of 1q21 had no negative influence on outcome. CR+VGPR rate after treatment completion was positively influenced by gain of 1q21 (10/12, 83% *vs.* 15/38, 39% of patients, P=0.02, Fisher's exact test). However, there was no significant survival benefit: the HR in the univariate Cox regression of gain 1q21 with no gain as a reference was 0.47 for EFS (95% CI: 0.18-21.24, P=0.13) and 0.66 for OS (95% CI: 0.22-2.01, P=0.47).

In patients with translocation t(11;14), the rate of CR+VGPR was significantly lower (32% as compared with 73%, P=0.01, Fisher's exact test). However, univariate Cox regression revealed no significant adverse influence of t(11;14) on EFS (P=0.09, HR 1.91 [95% CI:0.91-4.01]) or on OS (P=0.21, HR 1.83 [95% CI: 0.72-4.66]).

Comparison with historical M-Dex

We obtained a comparison cohort of 49 consecutive AL patients treated with M-Dex from 2004 to 2009 (median follow up was 87 months). Patient characteristics are shown in the *Online Supplementary Table S2*. The 2 cohorts were comparable regarding the main clinical characteristics. Thirty-eight patients (78%) completed at least 3 cycles and 22 patients (45%) completed at least 6 M-Dex cycles. Contrary to L-M-Dex, 13 patients received more than 6 cycles of M-Dex.

In the L-M-Dex study group, the CR+VGPR rate was higher (25/50, 50%) as compared to the M-Dex group (12/49, 24%, P=0.01, Fisher's exact test). There was also a longer EFS and OS in the L-M-Dex group (Figure 2) compared to the M-Dex study group (*Online Supplementary Figure S1*), with a median EFS of 25 *vs.* 16 months (P=0.005, log-rank test) and a median OS of 67.5 *vs.* 26.2 months, (P=0.02, log-rank test). In the multivariable regression analysis (*Online Supplementary Table S1*), L-M-Dex was again significantly associated with a higher CR+VGPR rate but lost its significance for EFS and OS, whereas the negative prognostic role of Mayo 2004 score 3 was confirmed for all endpoints. However, all these differences were mainly caused by the higher early mortality rate as only 2 L-M-Dex patients (2/50, 4%) died within 3 months following the start of chemotherapy compared to 10 patients (10/49, 20%) in the M-Dex-group (P=0.01, Fisher's exact test). As a result, the 3-month landmark analyses of EFS and OS no longer reached statistically significant differences (*data not shown*).

Discussion

We report on 50 patients with newly diagnosed AL amyloidosis who received lenalidomide, melphalan and dexamethasone. Poor cardiac function itself was not an exclusion criterion (36% had cardiac Mayo 2004 score 3), but kidney function had to be preserved (creatinine clearance >40 ml/min). To improve tolerability we used 10 mg of lenalidomide instead of the 15 mg proposed by the French phase 1 trial, and 20 mg dexamethasone.[5]

Treatment resulted in a high rate of CR+VGPR (50%, ITT analysis after therapy completion). Organ response[17] was observed in 10 patients 12 months after start of therapy and thereafter increased further in patients with long lasting remission by up to 48%. Sixteen SAEs and 132 AEs grade 3 or 4 occurred in 50 patients, including 1 death due to septicemia during the first treatment cycle and 1 cardiac death after 3 cycles (early mortality rate was only 4%). Most common toxicities were of a hematologic and cardiac type, leading to a (temporary) reduction of at least 1 of the study drugs in 88% of patients. Nevertheless, the targeted number of 6 cycles could be administered in 74% of the patients. Importantly, polyneuropathy neither occurred nor worsened in any of the patients. We imposed a very strict observation with a minimum of 4 weekly visits at our center, weekly evaluations of blood counts and phone calls. In case of symptoms or signs of infections or neutropenia less than 1.0/nl, lenalidomide was immediately stopped. After a median follow up of 50 months the median OS was 67.5 months. Estimated 2- and 4-year OS and EFS were remarkably good with 74/63% and 54/38%, respectively.

Table 3. Review of L-M-Dex treatment.

Reference	No. of pts % upfront	Single or multicenter recruitment Median follow up	Patient description	Median age (range)	NT-BNP (ng/l)	L-M-Dex dosage Number of cycles planned /median number of cycles received (n)	Toxicity CTCAE grade > 3, % of patients	HemR / CR (%)*	OS at 6 mo and 2 yrs
Moreau[5]	26 100	Multicenter 2008-09 19 months	Newly diagnosed Creatinine < 150 umol/l ECOG status < 2	57 yrs (27-70)	1100	4 cohorts L 5-20 mg Melphalan 0,18 mg/kg Dex 40 mg, days 1-4 9 / 7 cycles	38	58/23	82/82%
Sanchorawala[6]	16 69	Single center 2008-11 34 months	No end-stage renal failure SWOG PS < 2	70 yrs (57-84)	not reported	L 10 mg Melphalan 5 mg/m2 Dex 20-40 mg once a week 12 / 6 cycles	88	44/6	95/70%
Dinner[7]	25 92	Single center 2009-2012 6 months	No exclusion criteria	67 yrs (52-84)	2200	L 10 mg Melphalan 0,18 mg/kg Dex 40 mg once a week 9 / 3 cycles	100	58/8	58/58%
This study	50 100	Single center 2009-2012 50 months	Newly diagnosed Not eligible for HDC Creatinine clearance > 40 ml/min	67 yrs (47-75)	2900	L 10 mg Melphalan 0,15 mg/kg Dex: 20 mg 6 / 6	100	68/18	86/74%

A 28 days cycle was used in all L-M-Dex studies. Lenalidomide was given days 1-21; Melphalan was given on day 1 – 4. *HemR rate (PR or better) is reported as an intention-to-treat analysis. No data regarding VGPR were given in the 3 papers. Definitions of events for calculation of EFS were not uniform and are therefore not listed. Definition of OR has changed over time, therefore OR rates are also not listed. CR: complete remission; CTCAE: Common Terminology Criteria for Adverse Events; HDC: High-dose chemotherapy; HemR: hematological Response; L-M-Dex: lenalidomide, melphalan and dexamethasone; mo: months; yrs: years; OS: overall survival; pts: patients; NT-BNP: N-terminal B-type natriuretic peptide; ECOG: Eastern Cooperative Oncology Group; SWOG PS: Southwest Oncology Group performance status.

Comparison with other trials

Three other prospective clinical studies with fewer patients and shorter follow up have been published using the same combination. Results are summarized in Table 3 with the focus on inclusion criteria, drug dosages, toxicity, HemR (as an ITT analysis, although time points of evaluation could not be harmonized) and OS (although the 2 USA trials also included relapsed patients).

Moreau *et al*. performed a small phase 1/2 study in 26 patients.[5] Patients of all ages could be included. No dose-limiting toxicity was observed and the maximum tolerated dosage was defined as lenalidomide 15 mg (together with 40 mg of Dex). The short-term survival (median follow up 19 months) was very good with an estimated 2-year survival of 81%. Fifty-eight percent of patients achieved a HemR, 23% a CR and 50% an organ response, and the authors concluded that the dose escalation schedule tended to underestimate response rates. The phase 2 study of Sanchorawala *et al*. tested the tolerability, HemR and organ response rate in 16 patients (one-third of whom were not treated upfront).[6] It was stopped prior to the accrual goal due to toxicities and limited efficacy. The CR rate was lower compared to that reported by Moreau *et al*.[5] at 6%, and only 6 cycles, on average, of the planned 12 could be administered. After discontinuing and publishing the Boston trial, Palumbo and Cavallo advised against this combination treatment in AL patients.[24] Subsequently, Dinner *et al*. published the results of a third trial, and also concluded that this triple combination was toxic and rather ineffective.[7] Again, CR was low at 8%. This study included 25 patients, the oldest being 84 years old. The planned Dex dosage was 40 mg. On average, only 3 of the planned 9 cycles could be given, and the OS rate at 6 months was 58%. In our judgment the discrepant results are mostly due to large differences regarding inclusion criteria (age, severity of disease) and dosages of lenalidomide and Dex. The patient cohort of the French trial was younger, had a good performance status and kidney function; some of them were probably also eligible for HDC. Therefore, patients were able to receive a median of 7 of the 9 planned treatment cycles. In the 2 USA trials patients were older, had less strict exclusion criteria and also included patients with relapse or no response after previous chemotherapy, as a consequence of which tolerability and outcome were worse. In our experience, the triple combination chemotherapy was feasible and effective for non-HDC candidates not older than 75 years. Our rigorous surveillance strategy might have avoided a higher toxicity.

Data of patients with Cardiac Mayo 2004 stage 3

The outcome of patients with cardiac Mayo 2004 stage 3 is poor due to the high early mortality.[18] In a large retrospective European collaborative study the median OS for Mayo stage 3 patients was 7 months and longer depending on the NT-ProBNP level.[25] Patients who died within 3 months had a significantly higher NT-proBNP level (11.794 *vs.* 7.957 ng/l). Therefore, patients with very high NT-ProBNP levels (e.g., >8.500 ng/l) are mostly excluded from clinical trials. In a recently published multicenter study 60 patients with cardiac stage Mayo 2004 3 were treated with a combination of bortezomib, cyclophos-

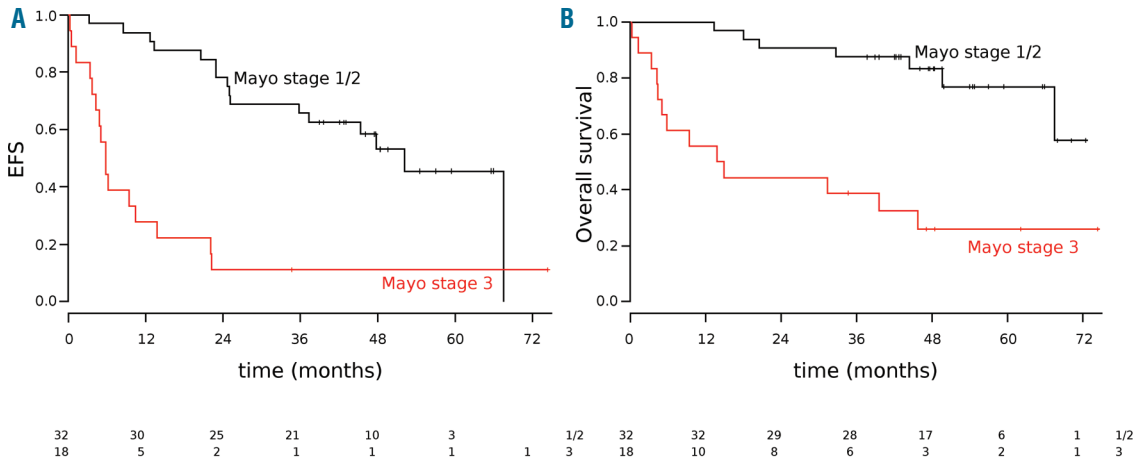

Figure 3. Survival in relation to the cardiac Mayo Stage. Estimated (A) event-free survival (EFS) and (B) overall survival (OS) in the L-M-Dex study group depending on cardiac Mayo stage 1/2 compared to Mayo stage 3.

phamide and dexamethasone.[26] Forty percent of patients died during therapy, the 2-year OS rate was about 50%. In our study, the 2-year OS rate was slightly better than 40% in stage 3 patients (Figure 3B). We summarize that treatment of stage 3 patients is still a challenge and a balancing act between the toxicity of chemotherapy and the goal of >VGPR achievement.

IFISH

The prognostic impact of iFISH results was not the same when compared to the patient cohort treated with M-Dex alone.[27] In the study herein, gain of 1q21 lost it's negative influence on remission and survival. This might be explained by the addition of lenalidomide. However, these results should be interpreted with caution as the patient number was smaller than in our previous analyses and thus did not allow for a meaningful multivariable analysis.[27,28] As of yet, no other published data regarding the influence of iFISH in L-M-Dex exists.[29]

Comparison with historical cohort of M-DEX

To further explore the role of lenalidomide in a triple therapy we used a historical M-Dex cohort. Although the relevant patient characteristics did not differ between L-M-Dex and M-Dex, the early mortality rate was higher in the M-Dex cohort (20% *vs.* 4%) leading to a higher CR+VGPR rate, and longer EFS and OS rates in the L-M-Dex study cohort. These differences diminished in the 3-month landmark analyses. We can only speculate about the causes of the higher early mortality; in our view this might be best explained by the rigid surveillance and the lower Dex dosage we used within the trial. Overall comparisons between the 2 cohorts should therefore be drawn cautiously given the retrospective nature of the M-Dex analysis and the fact that some of these patients were treated outside of our center.

Conclusion

We conclude that the combination treatment of L-M-Dex is effective in patients who are not eligible for HDC. In spite of concerns regarding toxicity raised by prior studies, the L-M-Dex regimen could be safely administered in our study. Definitively, a rigid surveillance is needed to immediately modify the dosage of lenalidomide in order to reduce toxicity and mortality. Therefore, this combination therapy is probably best performed by amyloidosis referral centers. Up to now, no direct comparison of Bortezomib-M-Dex *versus* L-M-Dex as upfront treatment has been performed, thus the best combination of standard chemotherapy with a novel agent remains elusive. In our opinion, principally those AL patients presenting with polyneuropathy, high dFLC levels and who are ineligible for HDM, should be considered for L-M-Dex.

Acknowledgments

We thank Celgene for their financial support for this investigator-initiated study, and also for supplying lenalidomide at no charge. We would like to acknowledge the excellent collaboration of hematologists from outside hospitals and private practices. We thank our patients for their participation in this trial.

We thank the three members of the Data Safety Monitoring Board: M. Kaufmann, MD, Stuttgart. E. Graf, PhD in statistics, and Freiburg, M. Hensel, MD, Mannheim, for their work and advice.

References

1. Merlini G, Seldin DC, Gertz MA. Amyloidosis: pathogenesis and new therapeutic options. J Clin Oncol. 2011; 29(14):1924-1933.
2. Cibeira MT, Sanchorawala V, Seldin DC, et al. Outcome of AL amyloidosis after high-dose melphalan and autologous stem cell transplantation: long-term results in a series of 421 patients. Blood. 2011;118(16):4346-4352.
3. Palladini G, Milani P, Foli A, et al. Oral melphalan and dexamethasone grants extended survival with minimal toxicity in AL amyloidosis: long-term results of a risk-adapted approach. Haematologica. 2014;99(4):743-750.
4. Dietrich S, Schonland SO, Benner A, et al. Treatment with intravenous melphalan and

dexamethasone is not able to overcome the poor prognosis of patients with newly diagnosed systemic light chain amyloidosis and severe cardiac involvement. Blood;116(4):522-528.
5. Moreau P, Jaccard A, Benboubker L, et al. Lenalidomide in combination with melphalan and dexamethasone in patients with newly diagnosed AL amyloidosis: a multicenter phase 1/2 dose-escalation study. Blood. 2010;116(23):4777-4782.
6. Sanchorawala V, Patel JM, Sloan JM. Melphalan, lenalidomide and dexamethasone for the treatment of immunoglobulin light chain amyloidosis: results of a phase II trial. Haematologica. 2013;98(5):789-792.
7. Dinner S, Witteles W, Afghahi A, et al. Lenalidomide, melphalan and dexamethasone in a population of patients with immunoglobulin light chain amyloidosis with high rates of advanced cardiac involvement. Haematologica. 2013;98(10):1593-1599.
8. Palladini G, Milani P, Foli A, et al. Melphalan and dexamethasone with or without bortezomib in newly diagnosed AL amyloidosis: a matched case-control study on 174 patients. Leukemia. 2014;28(12):2311-2316.
9. Kastritis E, Leleu X, Arnulf B, et al. A randomized phase III trial of melphalan and dexamethasone (MDex) versus bortezomib, melphalan and dexamethasone (BMDex) for untreated patients with AL amyloidosis. Blood 2016 128:646; abstr.
10. Palladini G, Russo P, Milani P, et al. A phase II trial of cyclophosphamide, lenalidomide and dexamethasone in previously treated patients with AL amyloidosis. Haematologica. 2013;98(3):433-436.
11. Cibeira MT, Oriol A, Lahuerta JJ, Mateos MV, et al. A phase II trial of lenalidomide, dexamethasone and cyclophosphamide for newly diagnosed patients with systemic immunoglobulin light chain amyloidosis. Br J Haematol 2015;170(6):804-813.
12. Kumar SK, Hayman SR, Buadi FK, et al. A. Lenalidomide, cyclophosphamide, and dexamethasone (CRd) for light-chain amyloidosis: long-term results from a phase 2 trial. Blood. 2012;119(21):4860-4867.
13. Kastritis E, Terpos E, Roussou M, et al. A phase 1/2 study of lenalidomide with low-dose oral cyclophosphamide and low-dose dexamethasone (RdC) in AL amyloidosis. Blood. 2012;119(23):5384-5390.
14. Schonland SO, Dreger P, de Witte T, Hegenbart U. Current status of hematopoietic cell transplantation in the treatment of systemic amyloid light-chain amyloidosis. Bone Marrow Transplant. 2012;47(7):895-905.
15. Tapan U, Seldin DC, Finn KT, et al. Increases in B-type natriuretic peptide (BNP) during treatment with lenalidomide in AL amyloidosis. Blood. 2010;116(23):5071-5072.
16. Gertz MA, Comenzo R, Falk RH, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. Am J Hematol 2005;79(4):319-328.
17. Palladini G, Dispenzieri A, Gertz MA, et al. New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers: impact on survival outcomes. J Clin Oncol 2012;30(36):4541-4549.
18. Dispenzieri A, Gertz MA, Kyle RA, et al. Serum cardiac troponins and N-terminal pro-brain natriuretic peptide: a staging system for primary systemic amyloidosis. J Clin Oncol 2004;22(18):3751-3757.
19. Kristen AV, Giannitsis E, Lehrke S, et al. Assessment of disease severity and outcome in patients with systemic light-chain amyloidosis by the high-sensitivity troponin T assay. Blood. 2010;116(14):2455-2461.
20. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials 1996;17(4):343-346.
21. Grambsch P, Therneau T. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika. 1994;81:515–526.
22. R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL http://www.R-project.org/.
23. Palladini G, Hegenbart U, Milani P, 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.
24. Palumbo A, Cavallo F. Lenalidomide in the treatment of plasma cell dyscrasia: state of the art and perspectives. Haematologica 2013;98(5):660-661.
25. Wechalekar AD, Schonland SO, Kastritis E, et al. A European collaborative study of treatment outcomes in 346 patients with cardiac stage III AL amyloidosis. Blood. 2013;121(17):3420-3427.
26. Jaccard A, Comenzo RL, Hari P, et al. Efficacy of bortezomib, cyclophosphamide and dexamethasone in treatment-naive patients with high-risk cardiac AL amyloidosis (Mayo Clinic stage III). Haematologica. 2014;99(9):1479-1485.
27. Bochtler T, Hegenbart U, Kunz C, et al. Gain of chromosome 1q21 is an independent adverse prognostic factor in light chain amyloidosis patients treated with melphalan/dexamethasone. Amyloid 2014;21(1):9-17.
28. Bochtler T, Hegenbart U, Kunz C, et al. Translocation t(11;14) is associated with adverse outcome in patients with newly diagnosed AL amyloidosis when treated with bortezomib-based regimens. J Clin Oncol 2015;33(12):1371-1378
29. Muchtar E, Dispenzieri A, Kumar SK, et al. Interphase fluorescence in-situ hybridization (iFISH) in untreated AL amyloidosis has an independent prognostic impact by abnormality type and treatment category. Leukemia. 2016. [Epub ahead of print].