A phase II trial of cyclophosphamide, lenalidomide and dexamethasone in previously treated patients with AL amyloidosis

Giovanni Palladini,[1] Paola Russo,[2] Paolo Milani,[1] Andrea Foli,[1] Francesca Lavatelli,[1] Mario Nuvolone,[1] Stefano Perlini,[1,3] and Giampaolo Merlini[1]

[1]Amyloidosis Research and Treatment Center, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy and Department of Molecular Medicine, University of Pavia, Pavia, Italy; [2]Transfusion Medicine Service, Melegnano Hospital, Vizzolo Predabissi, Italy, and Dept. of Computer Engineering and System Science, University of Pavia, Pavia, Italy; and [3]Department of Internal Medicine, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Italy

ABSTRACT

Immune-modulatory drugs are active in immunoglobulin light-chain amyloidosis and the addition of alkylating agents can potentiate their action. In this phase II prospective trial we used cyclophosphamide, lenalidomide and dexamethasone in the treatment of 21 patients who were refractory (n=13, 62%) or relapsed (n=8, 38%) after prior treatment including melphalan in all cases, bortezomib in 4 and thalidomide in 6. Median number of cycles administered was 4 (range 2-9 cycles). Severe adverse events were observed in 57% of patients, most common being neutropenia (29%). The hematologic response rate was 62%, with one complete response and 5 very good partial responses. Overall median survival was three years. The achievement of CR/VGPR was associated with a significant survival advantage. The combination of cyclophosphamide, lenalidomide and dexamethasone is an effective treatment for relapsed/refractory AL amyloidosis, and good quality hematologic response should be the aim of treatment in this setting.
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Introduction

Alkylating agents remain the mainstay of treatment of immunoglobulin light-chain (AL) amyloidosis, both for patients who are fit enough to undergo autologous stem cell transplant,[1] and for subjects who are not transplant candidates who commonly receive oral melphalan plus dexamethasone.[2,3] However, novel agents, such as proteasome inhibitors and immune-modulatory drugs, are radically changing the treatment approach to plasma cell dyscrasias, including AL amyloidosis.[4] Nevertheless, the best use of new agents and their combinations in first-line and rescue therapy still needs to be defined.[5]

The efficacy of lenalidomide in AL amyloidosis was first assessed in two parallel phase II trials that showed hematologic response rates ranging from 41% to 47%, and established the maximum tolerated dose at 15 mg/day.[6,7] We showed that treatment with lenalidomide and dexamethasone (LDex) can rescue patients refractory to bortezomib, alkylating agents and thalidomide.[8] Following reports on the combination of thalidomide and dexamethasone with alkylators,[9,10] several groups started independent trials to assess the efficacy of adding alkylating agents to LDex. The French group evaluated the combination of melphalan, lenalidomide and dexamethasone in newly-diagnosed patients, reporting a 58% hematologic response rate in a dose-escalation trial.[11] In two recently published studies that included both treatment-naïve and pre-treated patients, the Mayo Clinic and the Greek groups observed a 55-60% rate of hematologic response with the combination of cyclophosphamide, lenalidomide and dexamethasone (CLD).[12,13] In the present trial, we evaluated the safety and efficacy of CLD in previously treated patients with AL amyloidosis.

Design and Methods

This was a single-arm study. The protocol was approved by the "Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo", Pavia, Italy, Ethics Committee. All the patients gave their written informed consent, and the study was conducted according to the Declaration of Helsinki and Good Publication Practices. The trial is registered at *www.ClinicalTrials.gov as NCT00607581*.

The diagnosis of amyloidosis needed to be confirmed by biopsy and amyloid deposits were characterized as AL-type by immuno-electron microscopy.[14] Patients were required to have evidence of a monoclonal component at serum and urine immunofixation electrophoresis, circulating free light chain (FLC) above the reference limit, and an abnormal FLC κ/λ ratio.[15] Participants needed to be previously treated. Previous exposure to lenalidomide was not permitted. There was no limit to the number of previous treatments. Main exclusion criteria were bone marrow plasma cells over 30%, lytic bone lesions, cardiac troponin I over 0.1 ng/mL, complex ventricular arrhythmias at 24-h electrocardiogram,[16] and estimated glomerular filtration rate (eGFR) less than 30 mL/min per 1.73 m^2.

Organ involvement and response to treatment were assessed according to the consensus criteria of the International Society of Amyloidosis.[17,18] In particular, complete response (CR) required nega-

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Correspondence: gmerlini@unipv.it

tive serum and urine immunofixation and normal FLC κ/λ ratio, very good partial response (VGPR) was defined as a difference between involved (amyloidogenic) and uninvolved FLC (dFLC) less than 40 mg/L, partial response (PR) needed a dFLC decrease of over 50%.[18] Cardiac response or progression required a decrease or increase in N-terminal natriuretic peptide type-B (NT-proBNP) of more than 30% and more than 300 ng/L, respectively.[18,19]

Patients received 28-day cycles of cyclophosphamide 500 mg on Days 1, 8, 15; lenalidomide 15 mg daily for 21 days; dexamethasone 40 mg once weekly. The protocol was amended to allow administration of dexamethasone at 20 mg once weekly in patients presenting with fluid retention of over 3% of body weight despite optimal diuretic use. Aspirin 100 mg daily was used as thromboprophylaxis. The maximum allowed number of cycles was nine. Treatment was discontinued before completion of the ninth cycle in case of toxicity or in the event that a CR or PR/VGPR plus organ response was obtained after cycle 6.

Results and Discussion

Twenty-one patients were enrolled between April 2008 and July 2009. Their clinical characteristics are reported in Table 1. The median number of cycles administered was 4 (range 2-9 cycles).

Severe adverse events (SAE) were observed in 12 patients (57%): neutropenia in 6, fluid retention in 2, renal failure in 2, thrombocytopenia in one, and skin rash in one patient. There were no deaths during treatment. Two patients started dexamethasone at 20 mg and 19 at 40 mg. Of the 19 subjects who received dexamethasone 40 mg/day in cycle 1, 11 required a dose reduction to 20 mg/day in cycle 2 due to fluid retention that, however, met the criteria for an SAE only in 2 cases. The dose of lenalidomide was reduced to 10 mg/day in 13 subjects (62%) due to cytopenia and/or eGFR decrease. Two subjects (9%) progressed to end-stage renal failure (eGFR <15 mL/min per 1.73 m^2) during cycle 1. Baseline eGFR was 33 mL/min per 1.73 m^2 in one patient and 48 mL/min per 1.73 m^2 in the other. Overall, a median 138% increase in NT-proBNP was observed after cycle 1, and NT-proBNP remained elevated compared to baseline throughout lenalidomide administration. This was independent from changes in eGFR. The only baseline variable associated with NT-proBNP increase was heart involvement (median 256% *vs.* 97%, P=0.001). An increase in cTnI concentration was also observed, and this was affected by heart involvement (median 147% *vs.* 10%, P=0.001). No second primary malignancies occurred.

Hematologic response was achieved in 13 patients (62%) by the end of cycle 3, including one (5%) CR and 5 (24%) VGPRs. Seven patients responded after cycle 1. Three (19%) of the 16 subjects with renal involvement and one of the 4 patients with peripheral neuropathy achieved organ response. There were no cardiac responses. Of the 13 patients who attained hematologic response, 9 relapsed. Median duration of response was 13 months (range 6-44 months). Of the 9 relapsing patients, one died before additional treatment could be initiated and one refused further therapy, 2 received lenalidomide/dexamethasone off-study (1 PR, 1 NR), 4 were treated with bortezomib/dexamethasone (2 PR, 2 NR) and one, who had been previously exposed to bortezomib, received bendamustine/prednisone, achieving a PR. Of the 8 patients who were refractory to CLD, 3 died before initiation of salvage treatment, 3 received bortezomib/dexamethasone (1 CR, 2 NR), one melphalan/dexamethasone achieving CR, and one thalidomide/dexamethasone with no response.

Table 1. Patients' characteristics.

Variable	N (%)	Median (range)
Gender, male	7 (33)	–
Age, years	–	65 (49-79)
Previous treatment lines	–	1 (1-8)
2 or more prior treatments	10 (48)	–
Previous MDex	17 (81)	–
Previous thalidomide	6 (29)	–
Previous ASCT	5 (24)	–
Previous bortezomib	4 (19)	–
Refractory to prior therapy	13 (62)	–
Time from diagnosis, months	–	35 (6-131)
Kidney involvement	16 (76)	–
Heart involvement	13 (62)	–
Liver involvement	6 (29)	–
PNS involvement	4 (19)	–
Organs involved	–	2 (1-5)
2 or more organs involved	18 (86)	-
dFLC, mg/L	–	119 (51-1311)
dFLC >150 mg/L	9 (43)	–
BMPC, %	–	7 (3-15)
NT-proBNP >332 ng/L	13 (62)	–
NT-proBNP >1500 ng/L	6 (29)	–
mLVW thickness, mm	–	12.8 (9.5-17.7)
Ejection fraction, %	–	63 (52-70)
eGFR, mL/min per 1.73 m^2	–	55 (33-96)
Serum creatinine, mg/dL	–	1.33 (0.66-1.71)

ASCT: autologous stem cell transplantation; BMPC: bone marrow plasma cells; dFLC: difference between involved (amyloidogenic) and uninvolved free light chains; eGFR: estimated glomerular filtration rate; MDex: melphalan + dexamethasone; mLVW: mean left ventricular wall; NT-proBNP: N-terminal pro-natriuretic peptide type B; PNS: peripheral nervous system.

Twelve patients (57%) died, due to heart failure (n=8), sudden death (n=3) and liver failure (n=1). Median follow up of living patients was 38 months (range 36-47 months). Overall median survival was 36 months and progression-free survival was 13 months. Patients' outcome was significantly and independently affected by baseline dFLC and NT-proBNP concentrations (Table 2). Interestingly, there was no difference in survival between patients with NT-proBNP below and above 332 ng/L; however, a higher (1500 ng/L) NT-proBNP cut off was associated with a worse outcome. Achievement of CR or VGPR at three months improved survival (Figure 1).

In this trial, CLD induced an encouraging rate of hematologic response (62%), including VGPR/CR in 29% of cases, in pre-treated patients with AL amyloidosis, two-thirds of whom were refractory to prior therapy. The observed response rate is comparable to that reported with the same combination by Kumar *et al.*,[12] and by Kastritis and co-workers[13] in mixed populations including both pre-treated and treatment-naïve patients. Although median time to first response was one cycle, hematologic responses to CLD built up rather slowly, and at least the end of cycle 3 should be awaited before concluding that

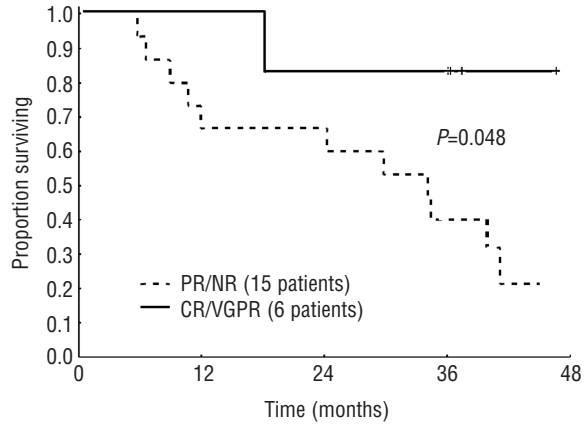

Figure 1. Survival according to hematologic response at 3 months.

Table 2. Baseline variables associated with survival (Cox's analysis).

Variable	HR (95% CI)	P
Univariable analysis		
Age	1.12 (1.01-1.23)	0.025
Time from diagnosis	1.00 (0.99-1.02)	0.490
dFLC >150 mg/L	3.22 (1.01-10.21)	0.048
ln(cTnI)	2.16 (1.03-4.51)	0.042
ln(NT-proBNP)	2.20 (1.10-4.39)	0.027
NT-proBNP >332 ng/L	3.06 (0.81-11.56)	0.102
NT-proBNP >1500 ng/L	9.96 (2.36-41.95)	0.002
mLVW thickness	1.20 (0.97-1.48)	0.087
eGFR	1.00 (0.98-1.02)	0.964
Proteinuria	0.96 (0.86-1.08)	0.528
Number of organs involved	1.41 (0.85-2.36)	0.188
Multivariable analysis		
NT-proBNP >1500 ng/L	11.85 (1.98-70.98)	0.007
dFLC >150 mg/L	6.75 (1.60-28.54)	0.010
Age	1.09 (0.96-1.23)	0.185

cTnI: cardiac troponin I; dFLC: difference between involved (amyloidogenic) and uninvolved free light chains; eGFR: estimated glomerular filtration rate; mLVW: mean left ventricular wall.

this treatment is ineffective. In the Mayo Clinic trial, both the rate and the quality of response improved with prolonged treatment, and 40% of patients achieved CR/VGPR across the entire trial.[12] However, Kumar *et al.* prolonged treatment up to two years. Conversely, our study was designed to administer the minimum amount of treatment needed to improve organ dysfunction or achieve CR, and no patient could be treated beyond cycle 9. The different study design could also have influenced the duration of hematologic response that was shorter in our study (13 *vs.* 28 months). Compared to the Greek study, progression-free survival was slightly shorter in our cohort (13 *vs.* 17 months). Nevertheless, approximately 50% of patients relapsing after CLD could be rescued with further lines of therapy, both in our series and in the study by Kastritis *et al.*[13] In multiple myeloma, prolonged treatment with lenalidomide improves progression-free survival, and it is likely that in the present study a prolonged exposure to lenalidomide in responding patients could have improved the quality and duration of response.[20,21] Further studies are warranted to establish the best end points for treatment with IMiDs in AL amyloidosis, as well as the role of maintenance therapy.

In our trial, organ responses were rather rare (19%), with no cardiac responses. Similar results were obtained by Kastritis *et al.* with a 22% organ response rate, mostly renal.[13] However, Kumar *et al.* report organ responses in 32% of cases, with cardiac improvement in 23% of subjects.[12] This might also be explained by the longer treatment duration. However, it should be kept in mind that in patients treated with IMiDs the assessment of organ response is hampered by the effect of therapy on markers of cardiac and renal function.[22-24] Importantly, and differently from other studies, we were able to demonstrate that major hematologic responses (CR or VGPR) to CLD translate into a significant overall survival benefit. Thus, good quality hematologic response should be the goal of treatment in this setting, particularly taking into account the difficulties in evaluating organ response in subjects exposed to lenalidomide.

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Authorship and Disclosures

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