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Bortezomib in a phase 1 trial for patients with relapsed AL amyloidosis: cardiac responses and overall effects

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Summary

Background: Bortezomib is approved for the treatment of multiple myeloma and a role has been suggested in the treatment of systemic AL amyloidosis (AL).

Methods: In this phase 1 dose-escalation portion of the first prospective study of single-agent bortezomib in AL, 31 patients with relapsed disease, including 14 (45%) with cardiac involvement, received bortezomib in seven dose cohorts on once-weekly (0.7, 1.0, 1.3, 1.6 mg/m^2) and twice-weekly (0.7, 1.0, 1.3 mg/m^2) schedules. Electrocardiographic, Holter and echocardiographic studies were evaluated in all patients to determine safety and response.

Results: During therapy (median treatment period 210 days), no patient developed significant ventricular or supraventricular rhythm disturbance on 24-h Holter monitoring; however, no patient satisfied study criteria for cardiac response using echocardiographic assessment or New York Heart Association classification. Seven patients (23%) had a $\geqslant$10% fall in left ventricular ejection fraction, but only one met criteria for cardiac deterioration. The predominant cardiac adverse events were peripheral edema (23%), orthostatic hypotension (13%) and hypotension (10%). Two patients developed grade 3 congestive heart failure, which resolved following

treatment interruption. In this Phase 1 portion, the maximum tolerated dose of bortezomib on either schedule was not reached. Hematologic responses occurred in 14 patients (45%), including seven (23%) complete responses. In non-responders mean left ventricular wall thickness increased during the course of treatment.

Conclusions: AL is frequently rapidly progressive; in these patients who had relapsed or progressed following previous conventional therapies, these results suggest that bortezomib may slow the progression of cardiac amyloid with limited toxicity.

Introduction

Primary systemic light-chain (AL) amyloidosis is a protein deposition disease caused by a clonal plasma cell dyscrasia. Immunogloblin light chains, produced by plasma cells, are deposited in an almost insoluble fibrillar matrix.[1–3] Amyloid cardiomyopathy carries a poor prognosis, with a median untreated survival of <6 months from the onset of symptoms.[4–6] Cardiac involvement[7–11] and the cardiac biomarkers N-terminal pro-brain natriuretic peptide (NT-proBNP) and serum cardiac troponin T and I are crucial prognostic features.[12–15] The current goal of treatment in AL is eradication of the responsible plasma cell clone,[1,2,16,17] largely based on regimens proven to be effective in multiple myeloma. Patients with advanced cardiac involvement derive limited benefit from standard oral therapies such as melphalan and dexamethasone (MDex) or MDex with thalidomide,[9,18] and are at high risk of treatment-related mortality when undergoing dose-intensive intravenous melphalan, followed by autologous stem cell transplantation.[8,10,19,20] Thus, cardiac amyloidosis represents the most important and common factor precluding access to aggressive treatment.

Recent success in the treatment of relapsed multiple myeloma[21] with bortezomib (VELCADE®), including its demonstrated superiority over dexamethasone,[22,23] a previous standard of care in relapsed myeloma, has prompted speculation that bortezomib may have a role in the treatment of patients with AL amyloidosis.[24] Bortezomib is a potent and specific dipeptide boronate inhibitor of the 26 S proteasome. The ubiquitin–proteasome pathway plays an essential regulatory role in the degradation of ubiquinated cellular proteins.[25] Through this mechanism of action, it has been suggested that amyloidogenic plasma cells may be particularly sensitive to bortezomib,[24] and promising efficacy has been seen in single-center patient series[26,27] and a multicenter analysis,[28] including reports of cardiac and other end-organ responses.[26,28,29] To date, activity in patients with cardiac AL amyloidosis has not been assessed prospectively.

The results of the Phase 1 dose-escalation portion of a phase 1/2 study, the first prospective study of single-agent bortezomib in patients with AL amyloidosis who had relapsed on conventional therapy, have recently been reported.[30] The primary aim of this portion of the study was to determine the maximum tolerated dose of bortezomib using once-weekly and twice-weekly dosing schedules, for evaluation in the subsequent Phase 2 portion of the study.[30] Here, we report detailed cardiac safety and response data for patients included in this Phase 1 portion.

Methods

Patients and study design

Patient eligibility criteria and study design details for the Phase 1 component of this Phase 1/2 study (ClinicalTrials.gov: NCT00298766) have been reported previously.[30] Briefly, 31 patients with biopsy-proven AL amyloidosis in association with a clonal plasma cell disorder were enrolled between 22 June 2005 and 18 September 2007 at six sites in Canada, France, Germany, Italy and USA. Amyloid-related cardiac involvement was defined according to the 2005 international consensus agreement:[31] presence of mean left ventricular wall thickness >12 mm on echocardiography, and/or a positive cardiac endomyocardial biopsy and clinical features (low electrocardiogram voltage, mean <0.5 mV in all limb and augmented leads) to suggest cardiac involvement.

Patients aged ⩾18 years who had been previously treated with at least one conventional therapy for systemic light-chain AL amyloidosis and required further treatment due to persistent clonal disease were eligible. In patients who had received stem cell transplantation as prior therapy, 6 months had to have passed since the procedure. Patients with clinically overt multiple myeloma or hereditary amyloid variants were excluded. Cardiac eligibility criteria included a requirement for echocardiographic left ventricular ejection fraction ⩾40% and New York Heart Association (NYHA) Class I or II. Patients were excluded if they had an enzyme-documented myocardial infarction within the previous 6 months, chronic atrial fibrillation,

grade 2/3 atrioventricular heart block, sustained or recurrent non-sustained ventricular tachycardia, a supine blood pressure <90 mmHg or symptomatic orthostatic hypotension. The study was approved by the Institutional Review Board/Independent Ethics Committee of all participating centers. Written informed consent was obtained from all participating patients.

Treatment

As previously reported,[30] patients were sequentially enrolled in seven cohorts to receive intravenous bortezomib on a once-weekly or twice-weekly schedule. Patients in cohorts 1–4 received bortezomib 0.7, 1.0, 1.3 and 1.6 mg/m^2, respectively, on a once-weekly schedule (days 1, 8, 15 and 22 of a 35-day cycle) and patients in cohorts 5–7 received bortezomib 0.7, 1.0 and 1.3 mg/m^2, respectively, on a twice-weekly schedule (days 1, 4, 8 and 11 of a 21-day cycle). A standard dose-escalation design was used to establish the maximum tolerated dose for each schedule, based on the occurrence of dose-limiting toxicity during cycle 1 of treatment,[30] which included any grade 4 thrombocytopenia or neutropenia, and any grade $\geqslant 3$ non-hematologic toxicity determined by the investigator to be related to bortezomib. Particular emphasis was placed on the occurrence of cardiac events, including life-threatening ventricular arrhythmias, atrial arrhythmias with hemodynamic instability, symptomatic congestive cardiac failure, hypotension or postural hypotension.

Treatment was scheduled to include up to eight cycles of bortezomib; a prolongation of therapy was permitted for patients showing benefit. Toxicities were recorded and graded during each cycle and dose reductions were permitted for specific adverse events. Patients were followed every 6 weeks until disease progression and then every 3 months until study completion.[30]

Cardiac investigations

All patients underwent baseline cardiac investigations including 12-lead resting electrocardiogram, 24-h Holter electrocardiogram, transthoracic echocardiography and measurement of the cardiac biomarkers brain natriuretic peptide (BNP) and NT-proBNP. All these investigations were repeated at each treatment cycle and at the end-of-treatment visit.

The baseline 12-lead resting electrocardiogram was analyzed for limb lead voltage, and heart rate, rhythm, PR, QRS and QT intervals were recorded. The QT interval was transformed to a rate-corrected QTc using the Bazett methodology. QTc was considered prolonged if >430 or >450 ms in male and female patients, respectively. Serial electrocardiograms were analyzed at each treatment cycle visit. Cardiac intervals (PR, QRS) and higher degrees of atrioventricular block were recorded; QTc changes during treatment are not reported as the study was not designed for the collection of such data, due to methodological limitations regarding timing of electrocardiogram relative to treatment administration and lack of electrolytes information at the time of electrocardiogram. Voltage in the limb leads was averaged for the baseline and end-of-treatment visits. Off-line electrocardiogram analyses were performed using Mac 1200 software, version 5.1, Milwaukee, USA.

On 24-h Holter electrocardiograms, complex ventricular arrhythmias were defined as ventricular ectopics that were multiform, paired (couplets) or triplet beats, based on previous reports by Falk *et al.*[32] and Palladini *et al.*,[33] and were classified according to the grading system of Lown and Graboys (Grade 1: <30 unifocal premature ventricular ectopics per hour; Grade 2: >30 unifocal premature ventricular ectopics per hour; Grade 3: multiform ventricular ectopic beats; Grade 4 a: ventricular couplets; Grade 4 b: ventricular tachycardia).[34] Supraventricular rhythm disturbance, including atrial fibrillation, was defined as runs of >5 consecutive beats at a rate of >100 beats/min.[35] Ventricular tachycardia was defined as $\geqslant 3$ consecutive beats. Analyses were performed using System VX3, century 3000 software, version 4.3, CA, USA.

All patients underwent standard transthoracic echocardiographic assessment. Measurements were averaged over three cardiac cycles and included interventricular septal thickness, left ventricular posterior wall thickness, right ventricular free wall thickness, left ventricular internal end diastolic diameter, derived left ventricular mass and left ventricular ejection fraction. Echocardiographic analyses were performed using the Digisonics cardiovascular image management and reporting system, version 3.6.2.11, Digisonics, Houston, TX, USA.

Hematologic and cardiac response assessments

Hematologic response was assessed as previously reported,[30] using serum and urine M-protein and free light chain analyses during the rest period of each treatment cycle, at the end-of-treatment visit, and every 6 weeks until disease progression. Responses were determined based upon established consensus criteria[31] but excluding confirmatory bone marrow assessment for complete response.

Hematologic response rates were updated from the previous report of this Phase 1 component,[30] based upon newly available data. Central laboratory assessments were used for efficacy parameters.

A cardiac response to therapy was defined as: a decrease in mean left ventricular wall thickness (mean of the sum of the interventricular septal and posterior wall thickness) by $\geqslant 2$ mm from baseline, a 20% improvement in left ventricular ejection fraction from baseline or an improvement in NYHA status by two classes without an increase in diuretic use and with no increase in wall thickness.[31] Cardiac disease progression was defined as an increase in mean left ventricular wall thickness by $\geqslant 2$ mm from baseline, and/or an increase in NYHA status by one class with a decrease in ejection fraction of $\geqslant 10\%$.[31] A central cardiology laboratory was used to evaluate cardiac data.

Statistical analysis

The safety population included all patients who received at least one dose of bortezomib. Electrocardiogram, echocardiogram and cardiac Doppler data were analyzed for the safety population and among patients with cardiac involvement at baseline using descriptive statistics. Paired data were analyzed using a two-tailed student's *t*-test with $P < 0.05$ regarded as significant.

Results

Patients

Baseline demographics and clinical status, including cardiac parameters, in the 31 patients are shown in Table 1. As previously reported,[30] 3, 3, 3, 6, 3, 6 and 7 patients were enrolled to cohorts 1–7, respectively; 13 patients [9 male, mean age 60 ± 10 years, median 61 years (range 45–74)] were thus treated at the maximum doses on the once-weekly (cohort 4) and twice-weekly (cohort 7) schedules. The maximum tolerated dose was not reached for either schedule, and so the maximum doses (1.6 mg/m^2 for the once-weekly schedule, 1.3 mg/m^2 for the twice-weekly schedule) were selected for use in the phase 2 component of the study.[30] By definition, 14 patients (45%) had cardiac amyloid involvement. Twelve patients had mean left ventricular wall thickness >12 mm and 17 had low voltage on electrocardiogram with seven satisfying both criteria.

Treatment exposure

Among the safety population ($n = 31$), 15 patients (48%) completed all eight cycles of treatment. Patients received a median of six cycles [range 1–33; mean ± standard deviation (SD) 7.4 ± 7.0]; the median period of treatment was 210 days (range 41–367; mean ± SD 211 ± 105) and the median cumulative dose was 22.4 mg/m^2 (range 3.9–128.1; mean ± SD 28.7 ± 24.9). Among 16 patients (52%) with early study termination, related adverse events were the cause in seven, including four in cohort 7, the highest twice-weekly dose cohort. One patient was withdrawn in cycle 1 due to dose-limiting toxicity, leaving 30 patients with follow-up data. Thirteen patients also received steroids, usually ($n = 9$) in the form of therapy prophylaxis during administration of bortezomib. Of these 13 patients, four received steroids at doses of $\geqslant 20$ mg/day of prednisone or equivalent for $\geqslant 4$ days (dexamethasone, $n = 3$; methylprednisolone, $n = 1$). The patients treated at the maximum doses ($n = 13$) received a median of four cycles of therapy (range 1–13, mean ± SD 4.8 ± 3.7). Median follow up for hematologic disease was 11.3 months.

Cardiac findings at baseline and during treatment

Seventeen patients (55%) had low voltage on baseline electrocardiogram. During treatment, three patients showed a rise in voltage to >0.5 mV, and in a further three patients voltage fell to <0.5 mV. Eleven patients (35%) exhibited a pseudo-infarction pattern on baseline electrocardiogram (10 in precordial leads, 1 in inferior leads); no patients developed this feature during treatment. Low voltage and a pseudo-infarction pattern were both present in 8 of 31 patients (26%) at baseline.

Electrocardiographic parameters are summarized in Table 2. At baseline the PR interval was >200 ms in five patients and a right bundle branch block was present in four. Three patients with first degree atrioventricular block at baseline developed further PR interval prolongation of between 26 and 34 ms during bortezomib therapy. No patient developed higher than first degree atrioventricular block during the study. The QRS interval duration did not change between baseline (mean ± SD, 96 ± 22 ms; median, 92 ms, range 70–156) and the last value on study (mean ± SD, 95 ± 22 ms; median, 88 ms, range 68–164). The QTc interval was prolonged on baseline electrocardiogram in 13 (36%) patients, including 10 male patients with QTc $\geqslant 430$ ms and three female patients with QTc $\geqslant 450$ ms. Of these 10 male patients, three had complete right bundle branch block and a further four had either partial right or partial left bundle branch block. Neither of the two female patients had evidence of interventricular conduction delay.

Table 1 Baseline demographics and disease characteristics, prior therapies and electrocardiographic features

Characteristics	*N* = 31
Age, years: mean (SD)/median (range)	60 (10)/59 (38–77)
Male/female (*n*)	19/12
Time from initial diagnosis, months: mean (SD)/median (range)	37 (25.4)/32 (5–95)
1/2/ ⩾3 lines of prior therapy (*n*)	14/12/5
Prior therapies for amyloidosis, *n* (%)	
Dexamethasone	26 (84)
Melphalan/bendamustine	28 (90)/1 (3)
Thalidomide/lenalidomide	13 (42)/2 (6)
Cyclophosphamide	5 (16)
Doxorubicin	4 (13)
Vincristine	3 (10)
Autologous stem cell transplantation	19 (61)
Organ involvement, *n* (%)	
Heart	14 (45)
Kidney	21 (68)
Peripheral nervous system	4 (13)
Liver	4 (13)
Systolic blood pressure (mmHg): mean (SD)/median	118 (15)/119
Diastolic blood pressure (mmHg): mean (SD)/median	69 (10)/70
Electrocardiogram evidence of cardiac involvement, *n* (%)	
Low voltage (mean limb lead <0.5 mV)	17 (55)
Pseudo-infarction pattern	11 (35)
Both of the above	8 (26)
BNP (pg/ml): mean (SD)/median (range)	243 (383)/109 (13.8–1560)
NT-proBNP (pg/ml): mean (SD)/median (range)	1988 (5298)/384 (126–18 771)

Normal levels: BNP <100 pg/ml (29 pmol/l); NT-proBNP <400 pg/ml (47 pmol/l). Raised levels: BNP 100–400 pg/ml (29–116 pmol/l); NT-proBNP 400–2000 pg/ml (47–236 pmol/l).[36]

On serial 24-h Holter electrocardiograms, performed within each treatment cycle, unifocal ventricular ectopic activity at a low frequency (Grade 1) was seen in 22 of 30 evaluable patients (73%). A further three patients exhibited frequent (Grade 2) unifocal ventricular ectopics. Multiform ventricular ectopics (Grade 3) or ventricular couplets (Grade 4 a) were seen in 20/30 (67%) and 13/30 (43%) patients, respectively. Ventricular tachycardia (⩾3 beats) was present in 7/30 patients (23%). No case of ventricular tachycardia was sustained, with the longest occurrence being an isolated four-beat run in one patient. Supraventricular tachycardia (>5 beats at >100 beats/min) was recorded in 9/30 patients (30%). In four of these patients the heart rate exceeded 150 beats/min. In five patients the number of consecutive beats exceeded 10 beats, with only one patient exceeding a run of 20 beats (275 beats at 116/min). In total, 3/31 patients received antiarrhythmic agents (all amiodarone) during treatment.

Cardiac responses and measurements

No patient satisfied echocardiographic study criteria for a cardiac response to therapy, nor did any patient achieve an improvement in NYHA status by two classes, without an increase in diuretic use and no increase in wall thickness. Echocardiogram findings are summarized in Table 2. One patient met the criteria for a deterioration, experiencing a fall in ejection fraction of >10% (from 77% down to 65%; remaining within normal clinical limits) and an increase in NYHA class from I to II. This patient had an initial complete hematologic response, stable diuretic use and a modest increase in electrocardiogram voltage over the eight cycles of bortezomib therapy.

Overall, mean left ventricular wall thickness increased from 12.3 mm at baseline to 12.6 mm at the end of treatment/last visit ($P = 0.09$); among patients with cardiac involvement, the increase was from 15.2 to 15.4 mm. Changes in mean left ventricular wall thickness by dose cohort and by hematologic response are shown in Figure 1; additionally, mean change from baseline by hematologic response is shown in Supplementary Figure 1A. Overall, 18 of 29 evaluable patients (62%) showed some degree of increase in wall thickness over the study period and 10/29 patients (34%) had some degree of decrease. In the remaining patient, wall

Table 2 NYHA class, electrocardiographic findings and echocardiographic data at baseline and end of treatment for all evaluable patients ($n = 31$) and among evaluable patients with cardiac involvement ($n = 13$)

	All patients, $n = 31$		Patients with cardiac involvement, $n = 13$	
	Baseline	End of treatment/ last value on study	Baseline	End of treatment/ last value on study
NYHA class I/II (n[a])	22/9	21/9	8/5	7/6
Electrocardiographic parameter, mean (SD)/median (range)				
Limb lead voltage (mV[b])	0.55 (0.241)	0.51 (0.214)	0.48 (0.241)	0.46 (0.232)
	0.48 (0.2–1.1)	0.49 (0.1–1.0)	0.48 (0.2–1.1)	0.43 (0.1–1.0)
PR interval duration (ms)	173 (34)	179 (34)[a]	172 (36)	178 (30)
	164 (126–256)	176 (116–260)	172 (134–256)	170 (144–252)
QRS interval duration (ms)	96 (22)	95 (22)	108 (27)	106 (29)
	92 (70–156)	88 (68–164)	100 (70–156)	100 (68–164)
Echocardiographic parameter, mean (SD)/median (range)				
LV IVS wall thickness (mm)	12.0 (3.4)	12.1 (3.7)	15.4 (4.5)	15.3 (4.7)
	11 (8–19)	11 (7–20)	15.1 (9.4–19.4)	15.0 (10.6–20.3)
LV PW thickness (mm)	12.6 (3.2)	13.0 (3.1)	15.3 (2.6)	15.5 (2.9)
	11 (9–21)	12 (9–20)	15.2 (10.6–20.6)	14.9 (9.0–19.6)
Mean LV wall thickness (mm)	12.3 (3.2)	12.6 (3.3)	15.2 (2.7)	15.4 (2.8)
	11.1 (8.3–19.2)	11.7 (8.6–19.6)	15.6 (10.0–19.6)	15.0 (9.8–19.6)
LV ejection fraction (%)	66.1 (7.9)	63.5 (6.9)	64.1 (7.1)	61.9 (7.3)
	66.4 (50.1–81.2)	63.8 (49.0–78.0)[a]	65.3 (53.0–77.1)	61.3 (54.8–78)
LV end diastolic diameter (mm)	46.9 (5.8)	48.7 (7.2)	45.9 (7.1)	47.3 (7.1)
	47 (39–60)	48 (40–65)	46.9 (39–60)	50 (40–65)
RV free wall thickness (mm)	6.7 (2.7)	6.6 (1.8)	8.3 (3.0)	7.7 (3.0)
	6 (4–15)	6 (4–13)	8.3 (4.7–14.8)	7.2 (4.6–12.9)

[a]One patient not evaluable for NYHA class post-baseline; patients without heart involvement were recorded as NYHA class I. Wall thickness values and derived values are for 29 patients and ejection fraction values for 30 patients. [b]Changes in mean limb lead voltage from baseline by hematologic response are shown in Supplementary Figure 1C. IVS, interventricular septum; LV, left ventricular; PW, posterior wall; RV, right ventricular.

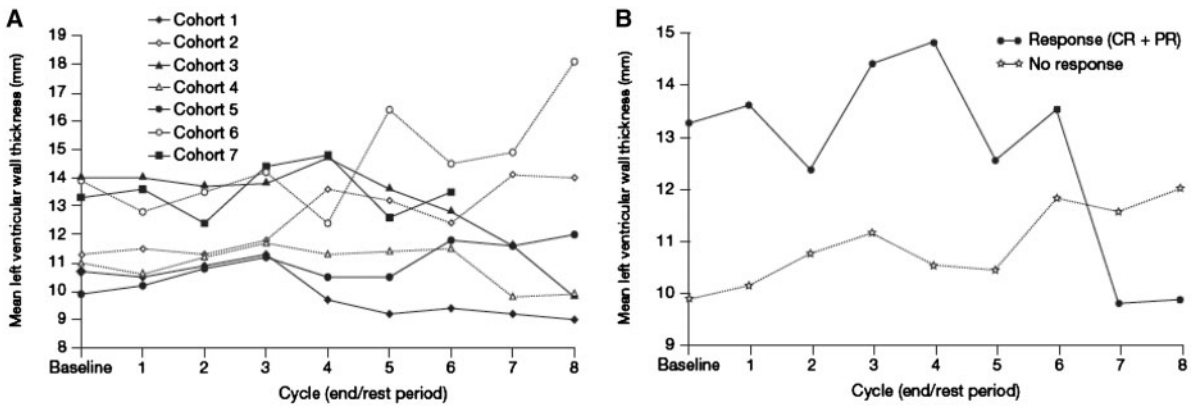

Figure 1. Mean left ventricular wall thickness (**A**) by dose cohort and (**B**) by hematologic response during treatment with bortezomib. Response (CR + PR), patients responding with either a complete (CR) or partial (PR) hematologic response.

thickness had increased by 1.21 mm by the end-of-treatment visit, and further increased to 2.5 mm above baseline at the final follow-up visit. At this time point, the patient had progressed from stable to progressive hematologic disease.

Among 13 evaluable patients with cardiac involvement, seven (54%) had some degree of wall thickness increase, including three with >1 mm increases, four (31%) had some degree of decrease and wall thickness did not change in two patients.

Overall, mean left ventricular ejection fraction fell from 66.1% to 63.5%, and among patients with cardiac involvement, the decrease was from 64.1% to 61.9%. Despite a mean overall decrease in ejection fraction, 10 of 30 evaluable patients (33%) showed an increase in ejection fraction over the study period, including by ⩾10% in two patients. In the remaining 20 patients (67%), ejection fraction decreased, including by ⩾10% in seven patients. No changes of ⩾20% were seen. Mean change from baseline by hematologic response is shown in Supplementary Figure 1B. Among 13 patients with cardiac involvement who were evaluable for changes in ejection fraction, 5 (38%) had ejection fraction increases, including the 1 patient with ⩾10% increase, and decreases were seen in the remaining 8 (62%) patients, including by ⩾10% in 3 patients.

No change in NYHA status was seen in 23 of 30 evaluable patients (77%) overall, including in 8 of 13 patients (62%) with cardiac involvement. NYHA status fell by at least one class in 3/30 patients (10%) overall, including 2/13 (15%) with cardiac involvement and increased by at least one class in 4/30 patients (13%) overall, three of whom had cardiac involvement. No change (or <50% alteration) in diuretic use over the course of treatment was seen in 25 of 30 patients (83%) overall, including 9 of 13 patients (69%) with cardiac involvement. Two of these patients did not require diuretics at any time. A reduction or an escalation by ⩾50% in diuretic therapy was seen in 2 (7%) and 3 (10%) of 30 patients overall, respectively, three of whom (one with reduction, two with escalation) had cardiac involvement. Cardiac parameters in patients treated at the maximum doses in cohorts 4 and 7 are summarized in Table 3.

Table 4 presents mean BNP and NT-proBNP levels during treatment for patients in whom data were available according to cardiac involvement. As shown, these values fluctuate during phases of the treatment protocol, with levels of both markers appearing generally lower among patients without cardiac involvement, as would be expected.

Hematologic responses

By an intention-to-treat analysis, a hematologic response was achieved in 14 of 31 patients (45%), including 7 (23%) complete responses. Excluding the patient who was not evaluated due to withdrawing for dose-limiting toxicity in cycle 1, the response rate was 47%. Hematologic responses were confirmed in 13 patients. Among these confirmed responses, mean time to first response was 1.6 months (median, 1.2 months, range 0.6–4.8), mean time to complete response was 1.3 months (median, 1.2 months, range 0.8–2.1) and median duration of response was not reached; 83% of responders remained in response for 1 year or longer. At study completion, 7 (23%) patients had hematologic disease progression, including three of the patients with confirmed responses. Among patients treated at the maximum doses, 7 of 12 evaluable patients (58%) had a hematologic response, including five

Table 3 Cardiac parameters for patients treated at the maximum doses in cohorts 4 and 7

	Baseline	End of treatment/ last value on study
NYHA class I/II (*n*[a])	7/5	10/2
Electrocardiographic parameter, mean (SD)/median (range)		
Limb lead electrocardiogram voltage (mV)	0.52 (0.216)	0.53 (0.216)
	0.50 (0.2–1.1)	0.52 (0.1–1.0)
PR interval (ms)	174 (28)	175 (34)
	172 (122–230)	178 (116–252)
QRS duration (ms)	99 (21)	95 (20)
	94 (74–142)	88 (70–148)
Echocardiographic parameter, mean (SD)/median (range)		
Left ventricular wall thickness (mm)	12.33 (2.89)	12.83 (3.35)
	11.1 (9.2–16.6)	11.41 (9.0–18.8)
Right ventricular wall thickness (mm)	6.45 (2.5)	7.5 (2.6)
	5.8 (4.0–11.9)	6.9 (4.6–14.8)
Left ventricular ejection fraction (%)	64.8 (6.72)	62.9 (6.7)
	67.4 (53.0–74.2)	63.3 (54.8–74.8)

[a]Data exclude patient withdrawn in cycle 1 of treatment.

Table 4 Mean values of cardiac biomarkers BNP and NT-proBNP over the course of treatment, according to AL cardiac involvement

Parameter/time point	Patients with cardiac involvement		Patients without cardiac involvement	
	N	Mean (SD)	*N*	Mean (SD)
BNP, ng/l				
Baseline	13	455.63 (512.138)	17	79.61 (67.301)
Cycle 2, day 1	12	573.05 (845.820)	16	110.50 (104.695)
Cycle 2 rest period	10	221.36 (261.317)	16	78.54 (67.597)
Cycle 3 rest period	9	256.58 (297.830)	14	75.12 (63.082)
Cycle 4 rest period	8	336.96 (341.983)	12	82.62 (88.584)
Cycle 5 rest period	8	290.08 (368.883)	12	60.59 (69.897)
Cycle 6 rest period	8	392.49 (463.988)	10	58.79 (69.056)
End of treatment	10	466.57 (875.986)	14	108.92 (95.084)
NT-proBNP, pg/ml				
Baseline	6	3561.10 (7454.525)	6	414.95 (492.929)
Cycle 2, day 1	6	5639.92 (12687.184)	5	575.24 (633.358)
Cycle 2 rest period	4	574.50 (463.916)	6	559.62 (805.014)
Cycle 3 rest period	5	787.40 (759.317)	4	678.23 (931.763)
Cycle 4 rest period	4	757.75 (438.448)	4	987.48 (1196.129)
Cycle 5 rest period	4	752.00 (279.887)	4	260.98 (141.147)
Cycle 6 rest period	4	1167.50 (689.151)	3	175.10 (105.160)
Cycle 7 rest period	–	–	4	242.15 (96.699)
End of treatment	6	1010.17 (677.817)	6	585.58 (769.899)

Normal levels: BNP < 100 pg/ml (29 pmol/l); NT-proBNP < 400 pg/ml (47 pmol/l). Raised levels: BNP 100–400 pg/ml (29–116 pmol/l); NT-proBNP 400–2000 pg/ml (47–236 pmol/l).[36]

complete responses and two partial responses (one unconfirmed). Four patients had stable disease and one patient had progressive disease. At study completion, three of these 12 patients had hematologic disease progression.

Adverse events

As reported previously, the most common adverse events of any grade were gastrointestinal events ($n = 26$, 84%), fatigue/asthenia ($n = 23$, 74%), infections ($n = 20$, 65%) and nervous system disorders ($n = 22$, 71%).[30] Adverse events related to the cardiovascular system are shown in Table 5. A total of 16 patients (52%) experienced grade 3/4 adverse events, predominantly in the highest dose cohorts, and nine patients experienced serious adverse events (Table 6). As reported previously,[30] two patients experienced dose-limiting toxicity. One patient in cohort 4 had grade 3 restrictive cardiomyopathy, which was also reported as a serious adverse event; the event was considered treatment-related and resulted in discontinuation. One patient in cohort 6 had grade 3 worsening congestive heart failure, which resolved following an interruption to bortezomib therapy; treatment was subsequently recommenced at a reduced dose. At data cut-off, seven patients had died, due to AL progressive disease in four (based on a 2.4 mm increase in interventricular septal thickness plus a clinically significant increase in NT-proBNP compared to baseline in one patient; no cardiac associations in the other three patients), progression of prostate cancer in one, renal failure (with graft-vs.-host disease and gastrointestinal bleeding post-allogeneic transplant) in one and interstitial lung disease considered possibly related to treatment in one patient. Only the latter death occurred within 30 days after the last dose of bortezomib.

Discussion

Bortezomib represents a new class of therapy in the treatment of AL amyloidosis. It is the first proteasome inhibitor to be approved for use, being approved for the treatment of previously untreated and relapsed multiple myeloma and the treatment of mantle cell lymphoma following at least one prior therapy.[37] In the previous report of this phase 1 dose-escalation component of our phase 1/2 study, it was demonstrated that the maximum tolerated dose was not reached;[30] the maximum planned doses of bortezomib, of 1.6 mg/m^2 on a

once-weekly schedule and 1.3 mg/m^2 on a twice-weekly schedule, were investigated in the phase 2 component. Here, we focused on cardiac safety and efficacy parameters among the 31 patients enrolled in the phase 1 component, as well as specifically in the 14 patients with cardiac amyloid involvement at baseline/first on-study evaluation. Our findings indicate that bortezomib might slow the progression of cardiac amyloid with limited toxicity.

Low voltage on electrocardiogram is indicative of the presence of amyloid in the heart and predictive of survival in patients with AL amyloid heart disease.[38] Our electrocardiographic studies demonstrated that there was a clinically insignificant fall in mean voltage from baseline (0.48 mV) to the end-of-treatment visit (0.46 mV) in patients with cardiac involvement. In addition, there was no change in the total overall number of patients with low voltage on electrocardiogram. Similarly, no significant changes were seen on 24-h Holter electrocardiogram, with seven patients (23%) showing non-sustained ventricular tachycardia (maximum run of 4 beats) and nine patients (30%) demonstrating supraventricular tachycardia, with only one patient having a maximum run of >20 beats. Thus there appears to be no association between bortezomib treatment and any excess of sustained or serious ventricular rhythm disturbance; however, it should be noted that patients with significant rhythm disturbances were screened out as ineligible.

The results of our study showed that overall, based on these preliminary findings, bortezomib is well tolerated in AL amyloidosis, although the side-effect profile is not insignificant and is dominated by gastrointestinal events;[30] concomitant infection and fatigue were also frequent.[30] This safety profile is similar to that characterized in relapsed multiple myeloma.[22,39,40] The predominant cardiac adverse events reported were peripheral edema and hypotension; both peripheral edema/fluid retention and postural/orthostatic hypotension were also reported in other studies of bortezomib in patients with AL amyloidosis.[26,27] We do not report serious issues with regard to blood pressure and rhythm disturbance that would not have occurred in the absence of therapy. Two of our patients survived a dose-limiting adverse event of restrictive cardiomyopathy or congestive heart failure, the former

Table 5 Cardiovascular adverse events during treatment

Cardiovascular adverse events, *n* (%)	*N* = 31
Dizziness	9 (29)
Peripheral edema	7 (23)
Dyspnea	7 (23)
Edema	6 (19)
Palpitations	5 (16)
Orthostatic hypotension	4 (13)
Hypotension	3 (10)
Congestive heart failure	2 (6)
Chest pain	2 (6)
Chest discomfort	1 (3)
Orthopnea	1 (3)
Falls	1 (3)
Syncope	1 (3)
Hypertension	1 (3)

Table 6 Serious adverse events by dose cohort

Dose cohort	Dose, mg/m^2	Patients with events, *n*	Serious adverse events
Once-weekly			
1	0.7	2	Lobar pneumonia, dyspnea
2	1.0	0	–
3	1.3	1	Bronchitis, staphylococcal bacteremia and renal failure[a]
4	1.6	4	Biventricular heart failure (*n* = 1)[b] Escherichia coli bacteremia (*n* = 1) Pneumonia (*n* = 1) Upper respiratory tract infection (*n* = 1) Cerebral ischemia (*n* = 1)
Twice-weekly			
5	0.7	0	–
6	1.0	0	–
7	1.3	2	Congestive cardiac failure (*n* = 1) Interstitial lung disease (*n* = 1)[a] Nausea/vomiting (*n* = 1)

[a]Patient died; patient in cohort 3 had prior diagnosis of prostate cancer. [b]Dose-limiting toxicity.

resulting in treatment discontinuation, and the latter resolving following a temporary suspension of bortezomib therapy. Seven patients have died; none were reported to be directly due to a cardiac cause [in one patient with death due to progressive disease (PD), PD was based on changes in cardiac parameters].

A substantial proportion of patients (45%) in these dose-escalation cohorts achieved a hematologic response, with a complete response occurring in almost a quarter of patients (23%). Moreover, the time taken to achieve these responses was generally short; median time to first response was 1.2 months and to complete response was also 1.2 months. This is crucial in AL amyloidosis, which frequently exhibits rapid deterioration, particularly in patients with cardiac involvement. Our data compare favorably with median times to first hematologic response of 3.4–6.4 months reported for some other non-stem cell transplant therapies.[41–47]

Among both the total population and the patients with cardiac involvement, no clinically relevant changes were detected in echocardiographic data between baseline and the end-of-treatment visit. Specifically, left ventricular wall thickness did not progress, which is relevant in the context of progression of left ventricular wall thickening at rates of up to 1.45–2.16 mm/month in patients with cardiac amyloid involvement having been described.[48] Satisfying the criteria for an improvement in left ventricular ejection fraction proved difficult, as the majority of patients had ejection fraction values within the normal range at baseline; the mean value at baseline was 66%, with only two patients having a value <55%. Study eligibility criteria also limited the ability to show any significant improvement in NYHA class with treatment, as patients with class III or IV heart failure were ineligible.

While no patient satisfied the criteria for a cardiac response, it might reasonably be argued that the treatment period (median 210 days) and follow-up period (median 11.3 months) were not long enough for an organ such as the heart to show a response; organ responses may occur up to 12–24 months following achievement of a hematologic response. Furthermore, it should be noted that cardiac response criteria are based upon electrocardiographic and echocardiogram data, which may take years to change following a hematologic response; in addition, the majority of patients in this phase 1 portion were treated at sub-optimal doses of bortezomib. Cardiac AL has been shown to be responsive to bortezomib; a recent case report of a patient treated with eight cycles of bortezomib at a dose of 1.3 mg/m^2 demonstrated progressive resolution of microvoltage on follow-up electrocardiograms at 14 and 24 months, plus significant regression of myocardial amyloid deposition, decreased interventricular septum and posterior wall thickness, decreased left atrial diameter and improvement in left ventricular ejection fraction from 35% to 55% on follow-up echocardiography.[29] Our paired analyses of changes in mean ventricular wall thickness, left ventricular ejection fraction and limb lead voltage from baseline by hematologic response, while only demonstrating small changes over the limited treatment period, may nevertheless be suggestive of the association between hematologic response and subsequent cardiac improvement, indicating some slight positive differences in these cardiac parameters in responding vs. non-responding patients over the course of treatment. Importantly in the present study, none of the 14 patients with cardiac involvement at baseline met the criteria for progression of heart involvement. Inhibition of the ubiquitin–proteasome pathway using bortezomib affects multiple signaling pathways[25] and, as such, there was a theoretical possibility that proteasome inhibition might lead to an accumulation of amyloidogenic material and potentially a progression of the disease. This study does not support any suggestion of an acceleration of what is often a rapidly progressive disease process.[8,48]

Supporting the findings of the present analysis, the potential for combination therapy with bortezomib and dexamethasone, including in patients with cardiac involvement, has been demonstrated in a number of reports.[26–28] The patient characteristics and findings from a multicenter retrospective analysis of 94 patients with AL amyloidosis treated with bortezomib (primarily at a dose of 1.3 mg/m^2 on a twice-weekly schedule) with/without dexamethasone are shown in comparison with those from the present study in Table 7. In this multicenter series, which included 18 previously untreated patients and 51 patients with refractory disease, as well as 59 with NYHA Class $\geqslant$ II at baseline, the hematologic response rate was 72%, including 25% complete responses.[28] The median time to cardiac response was just 2 months. Among patients with a cardiac response, 15/20 (75%) had an improvement in NYHA status by 2 classes (without an increase in wall thickness or increase in diuretic use) and the other 5 (25%) had a decrease in wall thickness.[28]

Explanations for the higher proportion achieving a hematologic response in the study by Kastritis *et al.*,[28] compared to our study, are multifold. Fewer patients in the Kastritis study had refractory disease (69 vs. 100%), and 19% of patients in this study were newly diagnosed and received bortezomib as initial therapy; the rate of hematologic

Table 7 Comparison of patient characteristics, treatment, and outcomes between the present study[30] and a previously published retrospective multicenter analysis of bortezomib in AL amyloidosis[28]

	Present study[30]	Kastritis *et al.*[28]
Enrolled/evaluable, *n*	31/30	94/93
Previously untreated, *n* (%)	0	18 (19)
Relapsed or refractory to prior therapy, *n* (%)	31 (100)	51 (69)
Median age, years (range)	59 (38–77)	62 (40–82)
Cardiac involvement at baseline, *n* (%)	14 (45)	69 (73)
NYHA class at baseline, *n*	22 Class I; 9 Class II	59 Class $\geqslant$ II
Treatment	Single-agent bortezomib	Single-agent bortezomib, $n=10$; bortezomib + dexamethasone, $n=84$
Bortezomib dose	0.7–1.6 mg/m^2 (d 1, 8, 15, 22, 35-d cycles)/ 0.7–1.3 mg/m^2 (d 1, 4, 8, 11, 21-d cycles)	1.3 mg/m^2 (d 1, 4, 8, 11, 21-d cycles), $n=74$; 0.7/1.0 mg/m^2 twice-weekly, $n=9$; 1.0/1.3 mg/m^2 once-weekly, $n=11$
Median number of cycles (range)	6 (1–33)	4 (1–8)
Hematologic responses, *n*/*N* (%)	14/30 (47)	67/93 (72)
Complete hematologic responses, *n*/*N* (%)	7/30 (23)	23/93 (25)
Mean (median) time to first response, months	1.6 (1.2)	NR (1.7; 0.9 in previously untreated, 2.0 in treated patients)
Cardiac responses, *n*/N (%)	None	20/69 (29)
Common AEs (%)	GI events (84), fatigue/asthenia (74), nervous system disorders (71), infections (65)	Peripheral neuropathy (40), orthostatic hypotension (36), edema (33), diarrhea (21), constipation (18), neuropathic pain (14)
Grade 3/4 AEs (%)	Fatigue (23), congestive cardiac failure (6), thrombocytopenia (6), vomiting (6)	Peripheral neuropathy (30, grade 2–4), edema (15), orthostatic hypotension (13), neuropathic pain (9, grade 2–4), fever/infection (8), diarrhea (6)
Dose reductions, *n* (%)	4 (13)	NR
On-study deaths, *n* (%)	1 (3)	3 (3) deaths within 2 months; all multiorgan involvement with symptomatic CHF

AE, adverse event; CHF, congestive heart failure; d, day; GI, gastrointestinal; NR, not reported.

response was higher in these previously untreated patients compared with in previously treated patients (81 vs. 68%).[28] Moreover, patients in the study by Kastritis *et al.* received bortezomib at a higher dose-intensity overall compared with in our study and 89% received bortezomib in combination with dexamethasone, whereas all patients in our study received single-agent bortezomib therapy.

The greater hematologic response rate in the study by Kastritis *et al.* might also explain the difference in cardiac response rate between these two studies (29% vs. none); Kastritis *et al.* described cardiac response as being associated with hematologic response.[28] In addition, more patients in the Kastritis study had cardiac involvement at baseline (73 vs. 45% in our study) and the majority of cardiac responses described (75%) were due to improvements in functional class,[28] which, due to entry criteria, could not be achieved in our study.

The most common non-hematologic toxicity in the study by Kastritis *et al.* was peripheral sensory neuropathy, which was reported in 40% of patients (and at grade 2–4 in 30% of patients);[28] the rate was lower in the present study, possibly due to the lower doses/dose intensities of bortezomib received by the majority of patients.[30] In addition, 36% of patients in multicenter series reported orthostatic hypotension, including 13% grade 3/4,[28] whereas only three patients in our study developed hypotension and four had postural hypotension.

In conclusion, considering the acknowledged aggressive nature of AL amyloidosis and the fact that our 31 patients had relapsed or progressed following previous conventional therapies, our results are encouraging. This is the first study to suggest a lack of significant progression of cardiac amyloid disease following bortezomib treatment alone. Our preliminary findings from this phase 1 dose-escalation portion of this study suggest that bortezomib may be of benefit in patients with cardiac AL amyloidosis, and this will be explored further in the expanded phase 2 portion using the maximum planned doses of bortezomib on each schedule. Toxicity was limited; adverse events were similar to those previously reported in studies of bortezomib in AL amyloidosis, and cardiovascular adverse events, while present, were mostly manageable using dose adjustments. Bortezomib may thus prove to be an efficacious addition to the armamentarium for fighting amyloid heart disease.

Supplementary Data

Supplementary Data are available at *QJMED* Online.

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