A prospective observational study of 915 patients with systemic AL amyloidosis treated with upfront bortezomib

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- Upfront bortezomib results in deep, durable haematological responses in AL amyloidosis.
- A stringent dFLC response (dFLC<10mg/L) confers superior outcomes.

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Abstract

Bortezomib is a standard therapy in AL amyloidosis (AL), but little is known about response duration. A difference in involved amyloidogenic and uninvolved serum free light chains (dFLC) less than 10mg/L (low-dFLC response) post-treatment predicts survival in AL patients with low presenting dFLC (20-50mg/L).

We report outcomes in the largest AL cohort treated with upfront bortezomib and explore impact of post-treatment dFLC<10mg/L (a 'stringent dFLC response') in all patients.

915 newly diagnosed AL patients treated with bortezomib in the UK and assessed at our centre were included. Haematologic responses, 6 month dFLC, organ responses, overall survival (OS) and time-to-next-treatment (TNT) were evaluated. Analysis of TNT excluded patients that died without starting second-line treatment.

Overall response rate (intent-to-treat) was 65%, with 49% complete response (CR)/very good partial response/low-dFLC response. The proportion of patients with a stringent dFLC response, dFLC 10-40mg/L and >40mg/L was 30%, 22% and 48%, respectively. Median OS was 72 months. 289 patients died without progressing to second-line treatment. Of the remaining patients, median TNT was not reached and 55% had not progressed to further treatment at 7 years. Patients with stringent dFLC responses had significantly better OS and TNT than those with lesser responses. 72% of CR patients did not progress to further treatment at 3 years, compared to 84% with stringent dFLC responses. Cardiac responses were better in those with stringent dFLC responses (61%) compared to lesser responses (45%), (p=0.005).

Upfront bortezomib confers durable haematologic responses. A stringent dFLC response predicts prolonged TNT and impressive organ responses.

This manuscript was presented as an abstract (poster) at the American Society of Haematology conference, 2018.

Introduction

Systemic AL amyloidosis (AL) is characterised by fibrillary deposition of monoclonal immunoglobulin light chains within organs. Cardiac involvement determines outcome. Treatment centres upon rapid reduction of amyloidogenic light chains, and the magnitude of reduction predicts organ response and survival. The main upfront treatment choice is between a high dose melphalan autologous stem cell transplant (ASCT) or combination chemotherapy. Most newly diagnosed AL patients are not eligible for upfront ASCT due to advanced cardiac involvement or poor performance status.

Bortezomib-based regimens have become standard therapy in AL. Early studies (largely in relapsed AL) reported good haematologic responses with bortezomib, with an overall response rate (ORR) of 60-94% and complete haematologic response (CR) rate of 23-71%.[1-4] Disappointingly, a multicentre retrospective European study concluded that upfront bortezomib-cyclophosphamide-dexamethasone (CyBorD) was unable to overcome poor outcomes in Mayo Stage III disease, with median survival of 4.6 months and 17% CR rate.[5] Previous studies have not reported in detail about time-to-next treatment (TNT) and long-term organ responses in AL with bortezomib.

Haematologic responses in AL are defined by international amyloidosis consensus criteria (ICC) published in 2012.[6] These utilise the difference in involved and uninvolved light chains (dFLC) in response assessment, as this parameter is more predictive of outcomes in AL than M-protein response.[7] The ICC defined that dFLC<40mg/L (very good partial response (VGPR)) is the goal of therapy, whilst >50% reduction in dFLC (partial response (PR)) is inadequate. Presenting dFLC≥50mg/L is regarded as 'measurable' for response assessment, but this excludes 20% of patients from response assessment. Lately 'low-dFLC' response criteria have been reported[8,9], whereby a reduction in dFLC after treatment to <10mg/L predicted favourable overall and renal survival in patients with presenting dFLC of 20-50mg/L.

Given data on low-dFLC response and the emerging prognostic value of minimal residual disease testing, we posited that a target of dFLC<10mg/L (termed a 'stringent dFLC response') may be important in all AL patients, irrespective of baseline light chain levels. We therefore report outcomes in the largest cohort of AL patients treated with upfront bortezomib and explore the impact of dFLC<10mg/L on outcomes.

Methods

All patients from a prospective observational study of newly diagnosed AL (ALchemy) treated with upfront bortezomib-based regimens from February 2010-August 2017 were included. Patients were treated at their local centres as per nationally agreed protocols. All were seen at the UK National Amyloidosis Centre at three months post treatment-initiation and then at least six monthly for comprehensive assessment. All investigations were done at the UK National Amyloidosis Centre, where data was collected and analysed. Dose modifications and number of treatment cycles were at the discretion of the locally treating physician. Patients were treated with intravenous bortezomib until 2013, and subcutaneous bortezomib thereafter. Steroid doses were at the discretion of the locally treating physician. All decisions for any change in treatment were taken by a multidisciplinary team at the UK National Amyloidosis Centre and took into account the patients' dFLC response (or progression), depth of organ damage, improvement in organ function and functional status.

Diagnosis of AL was confirmed with biopsy immunohistochemistry and/or proteomic analysis. All patients underwent serial biochemical tests for organ function, serum free light chains, serum and urine protein electrophoresis and immunofixation, cardiac biomarkers, echocardiography and/or cardiac MRI (unless contraindicated). Organ involvement and responses were defined by ICC.[10,11] Organ responses were assessed at 12 and 24 months from the time of treatment initiation. The European modification of Mayo 2004 staging was

used, with Stage III stratified into IIIa (NT-proBNP <8500ng/L) and IIIb (NT-proBNP ≥8500ng/L).[12]

Haematologic responses at 6 months were assessed by the international consensus criteria in patients with presenting dFLC>50mg/L[6]. CR was defined as negative serum and urine immunofixation, and normal serum free light chain ratio (0.26-1.65). VGPR was defined as dFLC<40mg/L, and PR as >50% dFLC reduction.

Patients with presenting dFLC 20-50mg/L were regarded as achieving a low-dFLC PR if dFLC<10mg/L was reached after treatment. Patients with dFLC<20mg/L at presentation were included in survival analysis but excluded from response assessment (20mg/L is the lowest dFLC analysed in recent low-dFLC studies[8,9]). Absolute dFLC at 6 months was assessed in all patients. Given recent reports of low-dFLC response[8,9], we assessed absolute dFLC at 6 months in all patients and termed 6 month dFLC<10mg/L as a 'stringent dFLC response'. Outcomes were assessed in those with stringent dFLC responses and those without. Outcomes were also assessed in patients who achieved dFLC>10 mg/L but <40mg/L (i.e. VGPR patients without a stringent dFLC response). Survival outcomes were analysed using the Kaplan-Meier method with comparisons done using the log-rank test.

TNT was defined as time from first-line therapy to beginning of second-line therapy. Patients that died without having progressed to second-line treatment were excluded from analysis of TNT. Haematological progression was an indication for second-line treatment. While some criteria require a substantial increase in FLC to define progression, patients cannot wait until this threshold is reached. The novel criteria of high-risk progression defined by the Italian amyloidosis group are critically important[13] but not universally adopted – we are incorporating these in our decision-making algorithms but they were not routinely used for the duration of this study. Conversely, in some patients, second-line treatment was deferred after multidisciplinary discussions taking into account all factors in the patients' disease status including organ function, performance status, light chain burden, frailty and co-morbidities.

Since chemotherapy does not directly impact end-organ damage or its improvement, it is challenging to capture the true benefit of treatment when a patient dies of amyloid-related organ dysfunction. Hence, we have used TNT in a real world attempt to capture the true impact of benefit or loss of benefit from front-line treatment. All p-values were two-sided with a significance level of <0.05; median values were used to dichotomise continuous variables. Factors that were significant on univariate analysis were further assessed in multivariate modelling by Cox's regression analysis. Statistical analysis was performed using SPSS version 24. Approval for analysis and publication was obtained from NHS institutional review board; written consent was obtained from all patients in accordance with the Declaration of Helsinki.

Results

Patients

915 patients underwent bortezomib-based therapy from February 2010-August 2017 (Figure 1). Table 1 demonstrates annual patient recruitment. Baseline characteristics are described in Table 2. The proportion of patients with cardiac, renal, liver, peripheral nerve, autonomic and gastrointestinal involvement was: 71.4%, 68.1%, 13.5%, 6.2%, 5.8% and 3.0%. Mayo (2004) Stage I, II and III disease was found in 15.7%, 33% and 51.3% patients, respectively. The median NT-proBNP and dFLC were 2228ng/L (range 29-93776) and 180mg/L (0-15898), respectively.

All were treated with bortezomib: CyBorD 94.9%, bortezomib-dexamethasone 2.9%, bortezomib-thalidomide-dexamethasone 1.3%, bortezomib-melphalan-prednisolone 0.4%, bortezomib-melphalan-dexamethasone (BMDex) 0.2%, bortezomib-lenalidomide-dexamethasone 0.2% and bortezomib-melphalan-thalidomide-dexamethasone 0.1%. The median number of chemotherapy cycles was 5 (1-9). Patients found not to be in a

haematological response at three months continued bortezomib with addition of either cyclophosphamide or thalidomide.

Haematologic responses

Of 915 total patients, response analysis excluded 43 patients due to presenting dFLC<20 mg/L (the lowest dFLC analysed in recently reported 'low-dFLC' studies[8,9]) and 53 patients due to missing response data. The latter group did not attend for follow-up at our centre at 12 months, but survival data for these patients is available and has been incorporated into survival analysis. 819 patients were therefore included in haematologic response intent-to-treat (ITT) analysis. Evaluable response analysis included 612 patients (this excluded the patients who died before response assessment). Haematologic responses are shown in Figure 2A. Haematologic responses (ITT) were: CR 25%, VGPR 20%, PR 16%, low-dFLC PR 4%, non-response 35% (including 207 deaths (25%)). Evaluable haematologic responses (n=612) were: CR 35%, VGPR 27%, PR 20%, low-dfLC PR 5% and non-response 13%. Of 421 patients with Mayo Stage III disease, haematologic responses (ITT) were: CR 23%, VGPR 19%, PR 11%, low-dFLC PR 1%, non-response 46% (including deaths 39%)). Evaluable haematologic responses (i.e. deaths excluded, n=256) in Stage III patients were: CR 38%, VGPR 32%, PR 19%, low-dFLC PR 1% and non-response 10%. ITT haematological responses in Stage IIIB disease were: CR 13%, VGPR 17%, PR 8%, low dFLC-PR 1% and non-response 61% (including deaths 59%). Evaluable haematological responses (deaths excluded) in Stage IIIB patients included: CR 33%, VGPR 41%, PR 18%, low-dFLC PR 2% and non-response 6%.

The proportion of patients in a CR with presenting dFLC 50-200mg/L, 201-600mg/L and >600mg/L was 58%, 28% and 14%, respectively.

Overall survival and time-to-next-treatment

Median follow-up for all patients and living patients was 23 and 32 months, respectively. Median OS (ITT, n=915) was 72 months (Figure 2B). 289 patients (31.6%) died without progressing to second-line treatment. Of the remaining 626 patients, the median TNT was not reached and 55% had not progressed to further treatment at 7 years. Median OS in Mayo Stage I, II, IIIa and IIIb was: not reached, 80, 36 and 4 months ($p<0.0001$) (Figure 2C). Median TNT was not reached in Mayo Stage I, II, IIIa and was 38 months in Stage IIIb.

Median OS was not reached in patients in CR or low-dFLC PR; median OS was 71 and 39 months in patients in PR and non-response, respectively. Median TNT in patients in CR, VGPR and low-dFLC response was not reached; it was 17 and 13 months in those in a PR and non-response, respectively (Figure 2D). At one, five and seven years, 98%, 77% and 60% of CR patients did not require further treatment, respectively.

Of patients considered transplant-eligible upfront (i.e. age<70 years, NT-proBNP<5000ng/L, cardiac troponin T<60ng/L, serum creatinine<150umol/L, and organ involvement<3) but were treated with upfront bortezomib instead, the median OS and TNT were not reached. At five years, 78% were still alive and 71% had not progressed to next treatment.

Assessing impact of achieving a stringent dFLC response (dFLC<10mg/L) at 6 months

Absolute 6 month dFLC responses (ITT, n=819) were: dFLC<10 mg/L 30%; dFLC 10-20mg/L 11%; dFLC 20-30mg/L 6%; dFLC 30-40mg/L 5%, dFLC 40-50mg/L 3%, dFLC>50mg/L 20% and deaths 25% (Table 3). Presenting dFLC levels did not impact upon achievement of stringent dFLC responses. Presenting dFLC for all 246 patients reaching a stringent dFLC response were: <50mg/L 22%, 50-200mg/L 45% and >200mg/L 33%. Of patients in a stringent dFLC response, 13% had an abnormal serum free light chain ratio.

Median OS and TNT were significantly better for patients achieving a stringent dFLC response compared to lesser responses (even if they achieved a VGPR by ICC). Median OS was not

reached in patients achieving a stringent dFLC response or dFLC 10-40mg/L, compared to 53 months in patients with dFLC≥40mg/L (log-rank p<0.0001) (Figure 3A). At one, three and five years, 98%, 92% and 86% of patients in a stringent dFLC response were alive. In the dFLC 10-40mg/L group, 97%, 84% and 66% of patients were alive at these time periods. Median TNT in patients with 6 month dFLC<10mg/L, 10-40mg/L and dFLC>40mg/L was not reached, 38 and 13 months (log-rank p<0.0001) (Figure 3B).

There was a significant difference in OS between CR patients with stringent dFLC responses (n=145, median not reached) compared to CR patients without stringent dFLC responses (n=67, median not reached) (p=0.033) (Figure 4A). The median TNT in CR patients with stringent dFLC response was not reached, compared to 38 months in CR patients without a stringent dFLC response (p<0.001) (Figure 4B).

OS was significantly better in patients with a stringent dFLC response (without CR) compared to dFLC 10-40mg/L (without CR), although the median was not reached in both groups (p=0.043) (Figure 4C). The median TNT in VGPR patients with and without a stringent dFLC response was not reached and 40 months, respectively (p<0.001) (Figure 4D). The median OS was not reached in patients with a CR and those with a stringent dFLC response without CR, although OS appeared to be better in the latter (p=0.089).

In a multivariate analysis, 6 month dFLC was predictive of TNT, independent of Mayo disease stage and presenting serum free light chains (Table 4).

22.9% of patients in a dFLC response had amyloidogenic light chains of kappa isotype. These patients had a better OS than those with lambda light chain isotype (p=0.033). The median was not reached in both groups; 100% alive in 5 years with kappa light chains and 83% alive at 5 years with lambda light chains. Of patients with a stringent dFLC response, patients with lambda light chain preponderance were more likely to have cardiac involvement (72.5%) than kappa (54.5%), (p=0.026). In the overall cohort, 61% of patients with kappa light chain preponderance had cardiac involvement, compared to 68% with lambda light chain

preponderance (p=0.073). There was no significant difference in median OS or TNT between these two groups in the overall cohort. There was no difference in TNT between the two light chain isotypes. The proportion of patients in a stringent dFLC response with reduced creatinine clearance (<30ml/min) was 44/246 (17.9%). Of these 44 patients, 7 (15.9%) had kappa amyloidogenic light chains. There was no difference in median OS and TNT in patients with severe renal dysfunction (eGFR < 30 ml/min) who had a kappa or lambda light chain preponderance.

Organ responses

Organ responses were assessed at 12 and 24 months. Table 5 outlines organ responses at 12 months according to 6 month haematological response. On an ITT basis, 517 patients with cardiac involvement were assessable. At 12 months, 32.5% achieved cardiac responses, 21.7% did not, and 45.8% had died. By 24 months, 88 patients had progressed to next treatment (including 41 who had previously been in a cardiac response). Of the remaining patients, cardiac responses at 24 months were as follows: 28.6% in a cardiac response, 11.6% were not and 59.8% had died. dFLC responses were assessed in patients who were alive at 6 months. 12 month cardiac responses were achieved in 76/125 (61%) of those with stringent dFLC responses at 6 months, compared to 90/199 (45%) of patients with lesser responses (p=0.005) (Figure 5).

On an ITT basis, 611 patients with renal involvement were assessable for renal response: 15.4% achieved renal responses, 59.9% did not and 24.7% had died. At 24 months, 148 patients had progressed to next treatment (including five that had achieved a renal response at 12 months). Of the remaining patients, renal responses were as follows: renal response 26%, non-response 32% and deaths 42%. 12 month renal responses were achieved in 55/204

(27%) in patients with stringent 6 month dFLC responses, compared to 38/267 (14%) with lesser responses (p=0.001).

On an ITT basis, 95 patients with liver involvement were assessable for liver response, 30% achieved liver responses, 45.3% did not and 25.3% had died. A stringent dFLC response did not affect liver responses (39.2 % vs. 29.2% respectively (p=0.31)).

Toxicity

Table 6 details reported toxicity, the commonest of which was Grade 1-2 lethargy, constipation, fluid overload and sensory neuropathy.

Discussion

In this study, we report outcomes in the largest unselected cohort of AL patients treated with upfront bortezomib (mainly CyBorD) therapy. This cohort captures nearly all AL patients seen at our centre, two-thirds of whom presented with cardiac involvement and half with Mayo Stage III disease. Haematologic response rates were high (ORR 65%) with half achieving VGPR/better in an ITT analysis. TNT was excellent, with median TNT not reached for responders at 7 years – data that rivals outcomes reported with upfront ASCT. However, we remain unable to abrogate the high incidence of early mortality in AL, with 40% of Stage III patients dying within six months of diagnosis – data that is not captured in prospective clinical trials due to selection bias. Lastly, achieving a stringent dFLC response resulted in significantly better TNT, and translated into two-thirds achieving cardiac responses. Achievement of a stringent dFLC response may therefore be a new potential therapy goal in AL.

Treatment in AL has evolved, and the Mayo group reported that the glass ceiling of poor outcomes has been overcome.[14] The pro-apoptotic effect of proteosomal inhibition is a desirable mechanism of treatment since AL plasma cells are substantially more sensitive to

proteasome inhibition due to added burden of proteotoxicity.[15,16] While ASCT is associated with excellent haematologic responses and OS[17], only 20% of patients are ASCT-eligible and there are concerns about treatment-related toxicity outside of experienced centres. Bortezomib-based therapy makes up the mainstay of treatment for most newly diagnosed AL patients at our centre.

Table 7 summarises outcomes with upfront bortezomib in previous AL studies. We reported a matched comparison of CyBorD compared to cyclophosphamide-thalidomide-dexamethasone with ORR of 71% and CR of 40.6% in the former group.[18] A multicentre study of 60 treatment-naïve Mayo Stage III AL patients treated with CyBorD revealed an ORR of 68%, with a poorer CR rate (17%).[5] A European multicentre retrospective study of 230 AL patients treated upfront with CyBorD demonstrated an ORR of 60% and CR of 21%, with inferior outcomes in Stage III patients.[4] An international randomised phase III study of melphalan-dexamethasone compared to BMDex revealed significantly better ORR in the latter group (81%) than the former (56%).[19] The heterogeneous outcomes in these studies likely reflect their differing sample sizes and proportions of patients with severe cardiac involvement. Haematologic and organ responses in our study were similar to the European collaboration study of CyBorD[4] and the multicentre study of CyBorD in Stage III patients[5], reflecting the large proportions of Stage III patients in these studies.

There is a paucity of data on duration of response with upfront bortezomib in AL. Our European multicentre study of CyBorD reported that 55% of patients were alive at five years, and median progression-free survival (PFS) was 13 months.[4] Four-year OS in AL reported by the Mayo group was 31% in 2000-2004, 54% in 2005-2009 and 60% in 2010-2014.[14] The current cohort shows that the contemporary OS of unselected AL patients was 72 months, confirming a progressive improvement in OS in AL over the last decade at our centre[20] and others.

PFS and TNT after chemotherapy (particularly bortezomib-based regimes) remain less robustly studied compared to those reported after ASCT. The median PFS in the Mayo study

was 6, 11 and 16 months in 2000-2004, 2005-2009 and 2010-2014, respectively[14]. The Mayo study reported a median PFS of 53 and 8 months in the ASCT and non-ASCT group treated from 2010-2014, respectively. However, most patients were not treated with upfront bortezomib. Outcomes with ASCT in AL have dramatically improved in recent years, with median OS of 7-10 years;[17,21] the median PFS has been reported as 2.6-4 years in two large retrospective analyses of patients treated with ASCT.[21,22] In this study, patients that would be regarded as eligible for upfront ASCT but were treated with upfront bortezomib instead had excellent outcomes – with 78% still alive and 71% having not progressed to further treatment at five years.

The median TNT was not reached and 55% did not progress to next treatment at 7 years. Of note, 98% of patients in a CR did not require further treatment at one year, and 77% and 60% at five and seven years, respectively. Two key messages are apparent. Firstly, bortezomib cannot overcome the high early mortality rates that plague AL. Secondly, patients achieving haematologic responses, especially deep responses, have a prolonged TNT almost rivalling those achieved with upfront ASCT. Most patients in this study were treated with CyBorD. Perhaps, given remarkable early data with BMDex in the recently concluded phase III study[19], the latter may induce more durable responses.

Light chains drive AL and their reduction has been key to improving outcomes. Current ICC derive from the pivotal international series reported in 2012[6]. Most patients in the latter series were not treated with an upfront bortezomib-based regime and deep responses were less frequent. Hence, a dFLC of <40mg/L (VGPR) was considered as 'adequate' and the goal of therapy. Low-dFLC criteria (for patients not assessable by standard criteria due to baseline dFLC<50mg/L) have subsequently been identified, whereby a low-dFLC response is defined as dFLC<10mg/L in patients with presenting dFLC 20-50mg/L, predictive of overall and renal survival. The importance of light chain burden has also been demonstrated in a recent study by the Mayo group, whereby an involved free light chain level (iFLC) of <20mg/L translated in

better organ responses, PFS and OS. In the latter study, normalisation of the serum free light chain ratio was not associated with better outcomes.[23]

We demonstrate here that low-dFLC response criteria can be extended to all AL patients irrespective of presenting dFLC. Patients achieving a stringent dFLC response have excellent outcomes. Due to already excellent survival in patients with a deep response, at this time, there is no significant apparent difference in OS using dFLC response criteria; although this may become apparent on longer follow up. However, the TNT in the group achieving absolute dFLC<10mg/L is markedly better than those with lesser responses (even if dFLC was below the threshold of a VGPR (<40mg/L)). Even in patients who had achieved a conventional CR by ICC, 84% with stringent dFLC responses did not progress to further treatment at three years, compared to 72% of patients in a CR without stringent dFLC responses. More importantly, stringent dFLC responses translated into strikingly high cardiac responses, with 61% of patients with stringent dFLC responses achieving cardiac responses, compared to 32% in the entire cohort.

This study's strength is its ability to capture a large 'real-world' AL cohort treated in multiple centres. However, it only includes patients seen at the UK National Amyloidosis Centre. While most UK patients are seen here for assessment, there is a referral bias in that the sickest patients are unable to travel to our centre. Due to its observational nature, exact treatment regimens and doses were at the discretion of the locally treating physician. Whilst there was an attempt to capture toxicity, this was reported at the discretion of the local physicians. Due to lack of source data verification, it is likely that toxicity data has been missed and likely to be under-reported. This remains a significant limitation. The proportion of patients in a CR with and without stringent dFLC responses was small, limiting comparison of outcomes between a stringent dFLC response and CR. Importantly, we have evaluated the importance of a stringent dFLC response in patients treated with only bortezomib-based therapy. The impact of stringent dFLC response remains to be ascertained in patients treated with other therapies.

In conclusion, this study confirms the substantial impact of bortezomib in improving long term outcomes in AL. We show that patients responding to therapy have a long TNT perhaps approaching that seen with ASCT. High early mortality, unfortunately, remains a feature of AL and this glass ceiling has not yet been breached. Finally, this is the first study to apply the new 'low-dFLC' response criteria to an unselected AL population. Achieving absolute dFLC<10mg (a stringent dFLC response) predicts prolonged overall survival, TNT and led to impressive organ responses. If these findings are validated in an independent international collaborative cohort, a stringent dFLC response may potentially become the new goal of therapy in AL.

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Authorship contributions

RM and ADW designed, collected data, analysed data and composed the manuscript. OC collected data and reviewed the manuscript. FS, SM, SS, DF, HJL, CQ, MF, JDG, CW and PNH reviewed the manuscript and provided critical input.

Disclosure of conflict of interests

None of the authors have any conflicts of interest that are relevant to the content in this manuscript.

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Table 1. Patients recruited to the study per year.

Year	Total patients treated	Number of patients with absent response data
2010	7	0
2011	46	0
2012	83	3
2013	113	5
2014	170	11
2015	188	10
2016	191	16
2017	117	8

Table 2: Baseline characteristics.

n=915	Median (range) / n(%)
Median age (years)	66 (29-89)
Male:Female	540 (59%): 375 (41%)
NYHA class 1 2 3 4 Unrecorded	 223 (24.4%) 446 (48.7%) 108 (11.8%) 4 (0.1%) 134 (15%)
ECOG 0 1 2 3 4 Unrecorded	 205 (22.4%) 363 (40.0%) 259 (28.3%) 49 (5.3%) 0 39 (4.0%)
Cardiac involvement	653 (71.4%)
Median NT-proBNP (ng/l)	2228 (29-93776)
Median high-sensitivity cardiac troponin T (ng/l)	54 (1-458)
Mayo Stage I II III, NT-proBNP ⩽8500ng/l) III, NT-proBNP>8500ng/l	 144 (15.7%) 302 (33%) 344 (37.6%) 125 (13.7%)
Median systolic blood pressure (mmHg)	118 (63-198)
Median LV wall thickness (mm)	13 (6-23)
Median LV ejection fraction (%)	58 (11-80)
Renal involvement	623 (68.1%)
Median serum creatinine (umol/l)	97.5 (26-1124)
Median GFR (ml/min)	64 (3-100)
Median proteinuria (g/24h)	3.14 (0.08-36.05)
Liver involvement	124 (13.5%)
Median serum bilirubin (umol/l)	6 (1-449)
Median ALP (units/l)	90 (26-2142)
Soft tissue involvement	124 (13.5%)
Peripheral nerve involvement	57 (6.2%)
Autonomic nerve involvement	53 (5.8%)
GI involvement	28 (3.0%)
Median number of involved organs	2 (1-5)
Involved light chains: Kappa	 186 (20.3%)

Lambda No monoclonal light chain excess	680 (74.3%) 49 (5.4%)
Median dFLC (mg/l)	180 (0-15898)
IgG/IgA/ IgM/IgD/light chain/no detectable serum paraprotein	239 (26.1%) / 93 (10.2%) / 26 (2.8%) / 4(0.04%) / 61 (6.7%) / 492 (53.8%)
Median serum monoclonal protein (g/l)	4
Median duration between diagnosis and treatment initiation (days)	27 (0-98)

NYHA: New York Heart Association
ECOG: Eastern Cooperative Oncology Group
NT-proBNP: N-terminal pro-brain natriuretic peptide
LV: left ventricular
GFR: glomerular filtration rate
ALP: alkaline phosphatase
GI: gastrointestinal
dFLC: difference in involved amyloidogenic and uninvolved light chains

Table 3. Absolute 6 month dFLC (difference in uninvolved and involved amyloidogenic free light chains) response (ITT=819).

6 month dFLC	% response in each category	Cumulative total number of patient in a response
<10mg/L	30%	30%
10-20mg/L	11%	41%
20-30mg/L	6%	47%
30-40mg/L	5%	52%
40-50mg/L	3%	55%
>50mg/L	20%	75%

Table 4. Multivariate model incorporating Mayo disease stage, presenting difference in amyloidogenic involved and uninvolved light chains (dFLC) and 6 month absolute dFLC values.

	Hazard ratio	p-value	95% confidence interval
OS			
Mayo disease stage			
1	2.05	0.06	0.96-4.34
2	3.7	0.01	1.74-7.86
3	6.49	<0.001	2.96-14.21
Presenting dFLC<180mg/L	1.3	0.04	1.01-1.68
ECOG			
1	1.25	0.29	0.83-1.9
2	2.12	<0.001	1.39-3.22
3	2.3	0.004	1.3-4.08
TNT			
Mayo disease stage			
1	1.01	0.96	0.70-1.47
2	0.84	0.40	0.56-1.26
3	0.91	0.73	0.52-1.58
Presenting dFLC<180mg/L	0.94	0.70	0.71-1.26
6 month dFLC			
<10mg/L			
10-20mg/L	2.76	<0.001	1.72-4.43
20-30mg/L	2.78	<0.001	1.74-4.46
30-40mg/L	3.27	<0.001	1.82-5.87
40-50mg/L	3.81	<0.001	2.32-6.28
>50mg/L			

Table 5. Organ responses 12 months after treatment according to 6 month haematological response.

6 month haematological response	12 month cardiac response	12 month renal response	12 month liver response
Complete response	67/113 (59.3%)	32/148 (21.6%)	9/20 (45%)
CR, dFLC<10mg/L	43/72 (59.7%)	28/100 (28%)	6/16 (37.5%)
CR, dFLC≥10mg/L	24/41 (58.5%)	4/48 (8.3%)	3/4 (75%)
Very good partial response	55/119 (46.2%)	23/122 (18.9%)	9/21 (42.9%)
Partial response	21/59 (35.6%)	12/78 (15.4%)	6/17 (35.3%)
Non-response	5/25 (20%)	6/56 (10.7%)	2/7 (28.6%)

Table 6. Grade 1-2 and Grade 3-4 toxicity experienced by patients included in this cohort.

	Grade 1-2	Grade 3-4
Lethargy	55.7%	6.5%
Fluid overload	24.1%	4.8%
Non-neutropenic infection	6.2%	4%
Neutropenia	2.5%	0.2%
Febrile neutropenia	0.04%	0.001%
Hypotension	12.3%	2%
Sensory neuropathy	21.1%	1.3%
Motor neuropathy	0.8%	0.07%
Diarrhoea	13.1%	1.3%
Constipation	25.5%	0.03%
Rash	7.4%	0.03%

Table 7. Summary of studies with upfront bortezomib-based therapy in systemic AL amyloidosis.

Bortezomib-containing regime	Number of newly diagnosed patients treated with bortezomib	Stage III patients	Median follow-up (months)	ITT ORR	ITT CR	Organ response	Median OS	Median PFS or TNT
Current cohort (mainly CyBorD)	915	51.3%	23 months in all patients (32 months in living patients)	65%	15%	ITT: Cardiac 32.5% Renal 15.4% Liver 30%	72 months	Median TNT not reached (55% had not progressed to next treatment at 7 years)
CyBorD [5]	60	100%	11.8	68%	17%	Cardiac 32% Renal 23% Liver 25%	1 year OS 57%	Not documented
CyBorD [18]	69	58%	12.7	71%	40.6%	Evaluable responses: Cardiac 35% Renal 43% Liver 53%	1 year OS 65.2%	Median PFS 28 months
Bortezomib, dexamethasone [24]	49 treated with bortezomib-dexamethasone (26 with twice weekly bortezomib, 23 with once weekly bortezomib)	28% (twice weekly bortezomib), 48% (once weekly bortezomib)	57	77%	39%	Cardiac 45% Renal 53%	1 year OS 67%; 4 year OS 43%	1 year PFS 58%; 4 year PFS 26%
CyBorD[4]	230	49%	25 (living patients)	62%	21%	Cardiac 17% Renal 25% Liver 32%	5 year OS 55%	Median PFS 13 months
Bortezomib, melphalan, dexamethasone[19]	53	15%	25 (living patients)	81%	CR/VGPR 64%	Cardiac 38% Renal 48%	1 year OS 80%	Not documented

Figure 1. Flow diagram of patient recruitment and those included in response assessment.

Figure 2. A) Haematologic responses in the cohort. (ITT: intent-to-treat; CR: completed haematologic response, VGPR: very good partial response; PR: partial response). B) Median overall survival (OS) and time-to-next-treatment (TNT). C) Median overall survival (OS) by Mayo cardiac staging. D) Median time-to-next-treatment (TNT) in patients by haematologic response. (CR: complete haematologic response; VGPR: very good partial response; PR: partial response; low-dFLC (difference in involved and uninvolved light chains) response defined by post-treatment dFLC<10mg/L in patients presenting with dFLC 20-50mg/L).

Figure 3. A) Median overall survival (OS) by absolute 6 month difference in involved and uninvolved light chains (dFLC) value. B) Median time-to-next-treatment (TNT) by absolute 6 month dFLC value.

Figure 4. Survival outcomes in patients with a complete response (CR) with and without a stringent dFLC response. A) Median overall survival (OS) was not reached in CR patients with and without a CR, although appeared better in the former group (p=0.033). B) Time-to-next-treatment (TNT) in CR patients with and without a stringent dFLC response. Median TNT was not reached in the former and 38 months in the latter (p=0.001). C) OS was significantly better in patients with a stringent dFLC response (without CR) compared to dFLC 10-40mg/L (without CR), although the median was not reached in both groups (p=0.043). D) Median time-to-next treatment (TNT) was not reached and 40 months, respectively (p<0.001).

Figure 5. Cardiac responses according to absolute dFLC values at 6 months.