Clonal plasma cell features in light chain amyloidosis are associated with depth and timing of cardiac response independent of hematologic response

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Abstract

Cardiac response is associated with survival in AL amyloidosis, but substantial variation exists in response kinetics. We investigated variables associated with deep cardiac responses to characterize the factors that govern organ recovery. We retrospectively studied newly diagnosed AL amyloidosis patients (N=401) diagnosed between 2010-2022 in whom cardiac response could be assessed. Cardiac responses were recorded at 6, 12 and 24 months and the best cardiac response. A deep cardiac response was defined as a cardiac very good partial response or better (>60% reduction in baseline N-terminal pro-B-type natriuretic peptide or ≤350 pg/mL). High-risk cytogenetic abnormalities (HRCA) included del(17p), t (4;14), t(14;16), and t(14;20). Logistic and competing-risk regression analyses (treating death as a competing event) were used to examine variables associated with cardiac response. The median age of the patients was 65 years, and their median follow-up was 5.5 years. At the 6-, 12- and 24-month and best overall response landmarks, 12%, 24%, 33% and 45%, respectively, of patients had obtained a cardiac very good partial response or better. Having baseline bone marrow plasma cells (BMPC) ≥20%, obtaining a hematologic very good partial response or better within 6 months, having a κ isotype, having HRCA and undergoing autologous stem cell transplantation (ASCT) were significantly associated with deep cardiac response on logistic regression and competing-risk analysis. In line with their impact on cardiac response, ASCT, κ isotype and deep hematologic response within 6 months were associated with improved overall survival on multivariable Cox proportional hazards modeling. Conversely, high BMPC burden and the presence of HRCA had no association with overall survival on adjusted analysis. As this cohort was retrospectively selected for cardiac response assessment, these results need to be interpreted accordingly. Nonetheless, the association between a '*myeloma phenotype*' and cardiac response kinetics, endorses the role of direct light chain toxicity and suggests that clonal plasma cell features significantly influence organ response in AL amyloidosis.

Introduction

Light chain (AL) amyloidosis is a plasma cell neoplasm characterized by the production of aberrant immunoglobulin light chains, which misfold and accumulate in tissues to cause progressive organ damage.[1,2] The degree of organ dysfunction determines a patient's prognosis,[2,3] with the severity of cardiac involvement being the most important predictor of short- and long-term overall survival (OS).[4,5] While recent therapeutic advances have improved survival, the proportion of patients dying within 6 to 12 months after diagnosis remains unacceptably high at 30 to 45%.[6-8] Anti-plasma cell therapies which curtail amyloidogenic light chain production are the cornerstone of the treatment of AL amyloidosis and they are assessed according to hematologic response criteria.[1-3] However, hematologic response is an imperfect predictor of cardiac response in AL amyloidosis, as anti-plasma cell therapies halt amyloid production without removing existing deposits. The cardiac response subsequently lags after the hematologic response and is subject to the influence of other factors which are poorly described.[9,10] Indeed, investigation of organ response kinetics has shown that the median time to best organ response varies between 24 to 36 months according to the

organ involved.[10] Despite the critical importance of cardiac response to patients' outcomes and the high early mortality of this disease, substantial variation exists in response kinetics. Here, we investigated variables associated with timely and deep cardiac responses to better characterize the factors which govern organ recovery from amyloid.

Methods

Study design

The study was approved by the institutional review board of Mayo Clinic. A retrospective review was conducted of all newly diagnosed AL amyloidosis patients seen at Mayo Clinic within 90 days of diagnosis between 2010-2022. Patients were eligible if they had: (i) cardiac involvement with consistent cardiac imaging; (ii) were assessable for cardiac response defined as a baseline N-terminal pro-B-type natriuretic peptide (NT-proBNP) >650 pg/mL or B-type natriuretic peptide (BNP) >150 pg/mL; and (iii) had cardiac response measurements, at least twice annually in the first year from treatment and annually afterward. Serum free-light chains (FLC) were measured at our institution using the FreeLite assay.

Definitions

Hematologic responses were defined according to the survival-validated consensus response criteria.[9] Cardiac responses were defined according to the new graded cardiac response criteria.[11] Cardiac progression was defined according to the criteria proposed by Palladini *et al*.[9] Cardiac stage was defined according to the European modification of the Mayo 2004 model.[12] A conversion tool for the various cardiac biomarkers was applied, as previously reported.[13]
Cardiac responses were recorded from the time of first-line treatment initiation at 6, 12, and 24 months and at the time-point of the best cardiac response. In the fixed time-point analyses and in the time-covariate analyses, the best response observed by the landmark time was used. When data on cardiac response at fixed time-points were missing, we imputed the best response observed before the fixed time-point so long as the patient remained alive without organ progression. Patients who had not achieved a deep cardiac response and died before a time-point assessment were considered non-responders for all subsequent milestone assessments. Imputation of cardiac response was done for 61 (15%), 39 (10%), and 51 (13%) patients at 6, 12, and 24 months.

Endpoints and statistical analysis

Univariate logistic regression was performed on pre-specified variables hypothesized to be associated with cardiac response. Due to the gradual nature of organ response, covariates were selected for multivariable logistic regression models of any cardiac response (cardiac partial response or better, i.e., >30% reduction in NT-proBNP or BNP from baseline) at 6 months and deep cardiac response (cardiac very good partial response, VGPR [>60% reduction in NT-proBNP or BNP from baseline] or a cardiac complete response, CR [NT-proBNP≤350 or BNP≤80 pg/mL]) at 12 and 24 months or best response using best subset selection followed by a stepwise backward elimination process.
The time to deep cardiac response was defined as the time from treatment initiation to cardiac VGPR or cardiac CR. Cumulative incidence rates for deep cardiac response were calculated using competing risk methods, treating death as a competing event. A proportional subdistribution hazards (Fine-Gray) model for competing risk was used to model the effect of covariates on deep cardiac response. Factors examined in the Fine-Gray model for their association with the incidence of deep cardiac response included those identified on univariate logistic regression analysis, as well as cardiac stage because of previous evidence of its association with organ response.[11]
Finally, OS was defined as the time from treatment initiation to death, censored on the date of last follow-up. A multivariable Cox proportional hazards model was used to evaluate the influence of covariates on OS. The influence of obtaining a deep cardiac response on OS was modeled as a time-dependent covariate using the Simon-Makuch method and with landmark analyses. Further methodological details are provided in the *Online Supplementary Material*.

Results

Baseline characteristics

A total of 401 patients were included in this study. A diagram illustrating how this population of patients was obtained from the total population of patients treated at Mayo Clinic is provided in Figure 1. Compared to patients who were eligible for cardiac response evaluation but who did not have sufficient follow-up for response evaluation (N=670), the study cohort (N=401) was younger (median age 64 *vs*. 67 years; $P<0.001$), resided closer to our center (median distance 236 *vs*. 286 miles; $P=0.005$), and had a lower proportion of stage IIIB patients (9.7% *vs*. 30%; $P<0.001$). There was no difference in the sex distribution ($P=0.84$) or light chain isotype ($P=0.4$) between these two groups.
The patients' demographics, disease characteristics, cardiac stage, treatment history, and hematologic response are summarized in Table 1. The rates of deep hematologic response (hematologic VGPR or better) at 3 and 6 months after starting treatment were 53% and 69%, respectively. Sixty-four patients (16%) had an Eastern Cooperative Oncology Group performance status of 2 or more at the time of diagnosis. Ninety-five patients (24%) had baseline bone marrow plasma cells (BMPC) ≥20%. The median baseline difference between involved and uninvolved free light chains (dFLC) was significantly higher in κ compared

to λ isotype (59 vs. 24 mg/dL, *P*<0.001) and among those with BMPC ≥20% compared to those with BMPC <20% (50 *vs*. 25 mg/dL; *P*<0.001). Patients with high-risk cytogenetic abnormalities (HRCA) had no significant difference in the median baseline dFLC compared to patients without HRCA (38 *vs*. 30 mg/dL; *P*=0.4). Patients with BMPC ≥20% had comparable 6-month hematologic response rates to those of patients with BMPC <20% (≥hematologic VGPR 64% *vs*. 71%; *P*=0.5). Patients with HRCA trended toward better 6-month hematologic responses than patients without HRCA (≥hematologic VGPR 88% *vs*. 67%; *P*=0.08). First-line treatment regimens received by patients with and without the t(11;14) translocation were comparable (*P*=0.7).

Baseline NT-proBNP levels were available for 389 patients and baseline BNP levels were available for 29 patients, with 17 patients having data for both pre-treatment BNP and NT-proBNP levels. The median baseline NT-proBNP and BNP levels were 2,988 pg/mL (interquartile range [IQR], 1,696-5,732) and 372 pg/mL (IQR, 233-744), respectively. The median time from diagnosis to treatment initiation was 34 days (IQR, 15-65).

Treatment and cardiac response at landmarks

Autologous stem cell transplantation (ASCT) was used in a total of 141 patients (35%): 82 patients (20%) received ASCT as part of first-line therapy with induction treatment, 37 patients (9%) received ASCT as first-line therapy without preceding induction and 22 patients (5%) received ASCT at their second or later-line of therapy. A complete list of first- and second-line treatments received is provided in *Online Supplementary Table S1*. In the first year following diagnosis, 125 patients (31%) received more than one therapy and 23 patients (6%) received more than two therapies.

At best cardiac response, 151 patients (38%) had no cardiac response, 74 (18%) had obtained a cardiac partial response, 97 (24%) had obtained a cardiac VGPR, and 82 (20%) had obtained a cardiac CR. At the 6-, 12- and 24-month response landmarks, 12%, 24%, and 33%, respectively, of patients had obtained a deep cardiac response (≥cardiac VGPR). A summary of cardiac responses at landmark time-points is provided in *Online Supplementary Table S2*. The median time to cardiac CR was not reached, and the median time to cardiac VGPR was 37.5 months (95% confidence interval [95% CI]: 30.3-50.7).

Factors associated with the ability to achieve a cardiac response

Achieving any cardiac response or a deep cardiac response

Variables significantly and positively associated with obtaining a deep cardiac response at best response on univariate logistic regression included age <60 years, κ

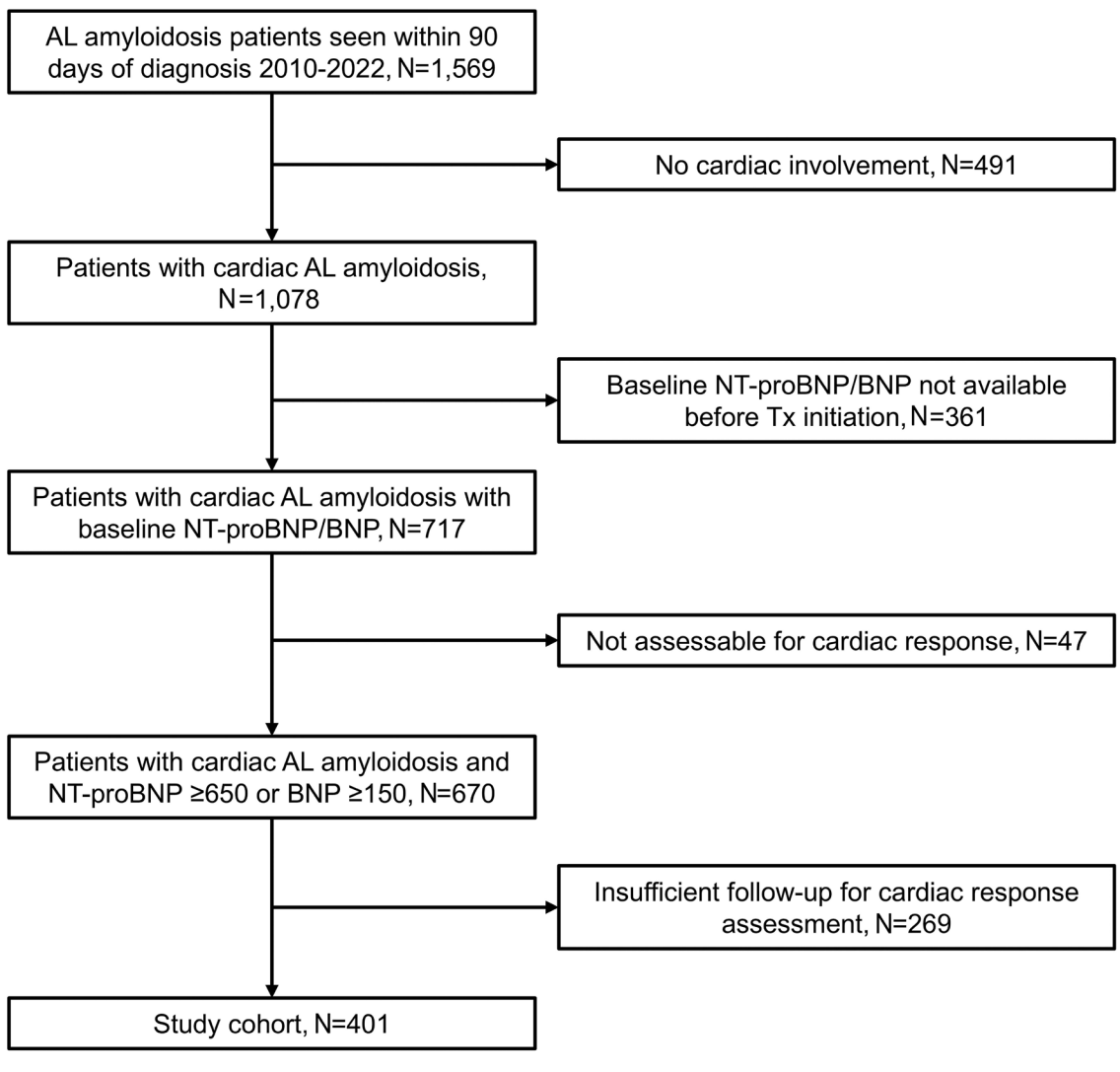

Figure 1. Cohort selection from the total number of newly diagnosed AL amyloidosis patients seen at the Mayo Clinic over the period from 2010-2022. NT-proBNP: N-terminal pro-B-type natriuretic peptide; BNP: B-type natriuretic peptide; Tx: treatment.

light chain isotype, BMPC ≥20%, dFLC ≥60 mg/dL, the presence of HRCA, absence of t(11;14), cardiac stage II, obtaining a deep hematologic response within 6 months and receiving front-line ASCT. A dFLC cutoff of 60 mg/dL was selected as the 75th percentile of dFLC levels. The complete results of univariate logistic regression at landmarks are shown in *Online Supplementary Table S3*. Multivariable logistic regression models for any cardiac response at 6 months and a deep cardiac response at 12 and 24 months after treatment initiation and best response are shown in Table 2. Across these four landmarks, BMPC ≥20%, κ isotype, and obtaining a deep hematologic response within 6 months were consistent predictors of obtaining a favorable cardiac response, while ASCT and HRCA were positive predictors of deep cardiac response at later time-points. We also examined dFLC ≥60 mg/dL as a predictor on multivariable analysis in place of BMPC burden (*Online Supplementary Table S4*). Due to the significant amount of missing data for chromosome 1q copy number abnormalities, it was included as a HRCA in a separate multivariable logistic regression and competing risk analysis (*Online Supplementary Tables S5* and *S6*). The results of this analysis were consistent with those of the main analysis, albeit at reduced power with lower odds ratios.

Factors associated with the cumulative incidence of ob-

Table 1. Patients' demographics, disease characteristics, treatment history and hematologic response stratified according to the attainment of a deep cardiac response (cardiac very good partial response or better) at best response.

Characteristic	Overall N=401	Deep CarR N=179	No deep CarR N=222	*P**
Age, years, median (IQR)	65 (58-71)	63 (56-69)	66 (59-72)	<0.001
Female, N (%)	143 (36)	74 (41)	69 (31)	0.033
≥6 months from first medical encounter to diagnosis, N (%)	248 (63)	102 (58)	146 (67)	0.056
Renal involvement, N (%)	160 (40)	67 (37)	93 (42)	0.4
Liver involvement, N (%)	40 (10)	11 (6)	29 (13)	0.022
Lambda isotype, N (%)	295 (74)	121 (68)	174 (78)	0.015
Baseline dFLC, mg/dL, median (IQR)	29 (14-61)	32 (14-78)	27 (13-55)	0.040
Baseline BMPC ≥20%, N (%)	95 (24)	54 (30)	41 (18)	0.006
FISH successfully performed, N (%) t(11;14) HRCA [del17p, t(4;14), t(14;16), t(14;20)] del17p t(4;14) t(14;16) t(14;20)	291 (73) 140 (48) 32 (11) 9 (3) 8 (3) 12 (3) 4 (1)	140 (78) 56 (40) 23 (16) 6 (4) 8 (6) 9 (6) 0	151 (68) 84 (56) 9 (6) 3 (2) 0 3 (2) 4 (3)	0.023 0.008 0.004 0.5 0.003 0.057 0.052
First-line treatment, N (%) PI-based Daratumumab-based ASCT without induction Chemotherapy IMID-based	 270 (67) 54 (13) 36 (9) 31 (8) 10 (2)	 116 (65) 28 (16) 18 (10) 10 (6) 7 (4)	 154 (69) 26 (12) 18 (8) 21 (10) 3 (1)	0.2
Cardiac stage - Mayo 2004 with modification, N (%) Stage II Stage IIIA Stage IIIB	 210 (52) 142 (35) 49 (12)	 102 (57) 63 (35) 14 (8)	 108 (49) 79 (36) 35 (16)	0.040
Best hematologic response within 6 months, N (%) No response Partial response VGPR or dFLC-PR Complete response	 29 (7) 95 (24) 168 (42) 109 (27)	 4 (2) 30 (17) 85 (47) 60 (34)	 25 (11) 65 (29) 83 (37) 49 (22)	<0.001

*Wilcoxon rank sum test, Pearson χ^2 test or Fisher exact test. CarR: cardiac response; IQR: interquartile range; dFLC: difference between involved and uninvolved free light chains; BMPC: bone marrow plasma cells; FISH: fluorescence *in-situ* hybridization; HRCA: high-risk cytogenetic abnormalities; PI: proteasome inhibitor; ASCT: autologous stem cell transplant; IMID, immunomodulatory; VGPR: very good partial response; dFLC-PR: difference of free light chains partial response.

taining a deep cardiac response while accounting for the competing risk of death are shown in Figure 2. Patients with a high baseline BMPC percentage, κ light chain isotype, and HRCA and patients who lacked a t(11;14) translocation all had a higher likelihood of deep cardiac response on competing risk regression analysis. For instance, the median time to deep cardiac response for patients with a HRCA was 13 months, compared to 36 months for those without a HRCA. Obtaining a deep hematologic response within 3 or 6 months produced a significant increase in the likelihood of a deep cardiac response and reduction in the competing risk of death on competing risk regression analysis (*P*<0.001) (*Online Supplementary Figure S1*). Patients with cardiac stage IIIB were significantly less likely to obtain a cardiac VGPR (*P*=0.049) on competing risk regression analysis (*Online Supplementary Figure S2*). The multivariable proportional subdistribution hazards (Fine-Gray) model of factors associated with deep cardiac response, treating death as a competing risk, are shown in Table 3. κ isotype, the attainment of a deep hematologic response within 6 months and front-line ASCT had a positive influence on deep cardiac responses.

The presence of t(11;14) was a significant negative predictor of obtaining a deep cardiac response on univariate competing risk regression, but not on adjusted analysis ($aHR_{CarVGPR}$=0.85, 95% CI: 0.58-1.23; *P*=0.4). A possible explanation for this is collinearity, associated with the reduced rate of deep hematologic responses within 6 months in patients with t(11;14) compared to patients without t(11;14) in our cohort (62% *vs*. 75%; *P*=0.038).

Achieving a cardiac complete response

Patients with cardiac stage II disease were significantly more likely to achieve a cardiac CR than were patients with stage IIIA or IIIB disease (26% *vs*. 18% *vs*. 4%; *P*=0.003); the cumulative incidence function of death and obtaining a cardiac CR according to cardiac stage is shown in *Online Supplementary Figure S3*. Multivariable logistic regression models of variables associated with cardiac CR at 24 months and as best cardiac response are shown in Table 4.

The impact of cardiac response on overall survival

The median OS was 8.7 years, with a median follow-up of 5.5 years (95% CI: 5.1-6.1). The median OS for patients with stage II, IIIA and IIIB disease was 10.1, 6.8 and 3.9 years, respectively (*P*<0.001). Patients diagnosed between 2017-2022 did not have a significantly different OS compared to that of patients diagnosed in 2010-2016 (HR=0.83, 95% CI: 0.58-1.17; *P*=0.28).

A Cox proportional hazards model of variables associated with OS is shown in *Online Supplementary Table S7*. Cardiac stage, hematologic response, receipt of front-line ASCT and light chain isotype were all significantly associated

Table 2. Multivariable logistic regression model of covariates associated with any cardiac response (cardiac partial response or better) at 6 months and deep cardiac response (cardiac very good partial response or better) at 12 months, 24 months, or best cardiac response achieved.

Characteristic	6-month any CarR (≥CarPR)			12-month deep CarR (≥CarVGPR)			24-month deep CarR (≥CarVGPR)			Deep CarR at best response (≥CarVGPR)		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
BMPC ≥20%	3.06	1.73-5.48	<0.001	3.61	1.94-6.85	<0.001	1.98	1.10-3.58	0.023	2.20	1.24-3.95	0.007
Lambda isotype	0.52	0.29-0.93	0.029	0.48	0.25-0.92	0.027	0.46	0.25-0.83	0.010	0.46	0.25-0.83	0.009
HRCA [del17p, t(4;14), t(14;16), t(14;20)]	1.56	0.71-3.43	0.3	3.43	1.51-7.89	0.003	3.88	1.71-9.28	0.001	2.79	1.21-6.90	0.015
≥HemVGPR within 6 months	3.20	1.74-6.14	<0.001	3.46	1.68-7.68	<0.001	3.41	1.82-6.66	<0.001	2.33	1.33-4.17	0.003
≥6 months from first medical encounter to Dx	0.61	0.36-1.04	0.067	0.48	0.26-0.87	0.015	-	Not included	-	0.66	0.39-1.11	0.12
Age ≥60 years	-	Not included	-	0.55	0.30-1.01	0.055	-	Not included	-	-	Not included	-
Front-line ASCT	-	Not included	-	-	Not included	-	2.41	1.32-4.44	0.004	2.48	1.42-4.39	0.001
Cardiac stage - Mayo 2004 with modification	-	Not included	-	-	Not included	-	-	-	0.032	-	Not included	-
Stage II	-	-	-	-	-	-	Ref	-	-	-	-	-
Stage IIIA	-	-	-	-	-	-	2.20	1.21-4.07	-	-	-	-
Stage IIIB	-	-	-	-	-	-	1.24	0.46-3.18	-	-	-	-

CarR: cardiac response; CarPR: cardiac partial response; CarVGPR: cardiac very good partial response; OR: odds ratio; 95% CI: 95% confidence interval; CarR: cardiac response; BMPC: bone marrow plasma cells; HRCA: high-risk cytogenetic abnormalities; HemVGPR: hematologic very good partial response; Dx: diagnosis; ASCT: autologous stem cell transplant.

with OS. The presence of HRCA had no association with OS on univariate (HR_{OS}=0.94, 95% CI: 0.50-1.76; *P*=0.85) or adjusted Cox proportional hazard modeling (aHR_{OS}=1.09, 95% CI: 0.58-2.07; *P*=0.80). Likewise, BMPC ≥20% had no significant association with OS (aHR_{OS}=0.86, 95% CI: 0.58-1.28; *P*=0.50).

As BMPC ≥20% and the presence of HRCA improved the likelihood of obtaining a deep cardiac response without an associated improvement in OS, we examined the impact of these factors on therapy utilization. There was a trend towards patients with BMPC ≥20% receiving more therapies in the first year of diagnosis: 40% received two or more therapies compared to 29% for patients with BMPC <20% (χ^2 test, *P*=0.056). Similarly, patients with BMPC ≥20% more frequently changed therapy secondary to hematologic progression or inadequate treatment response

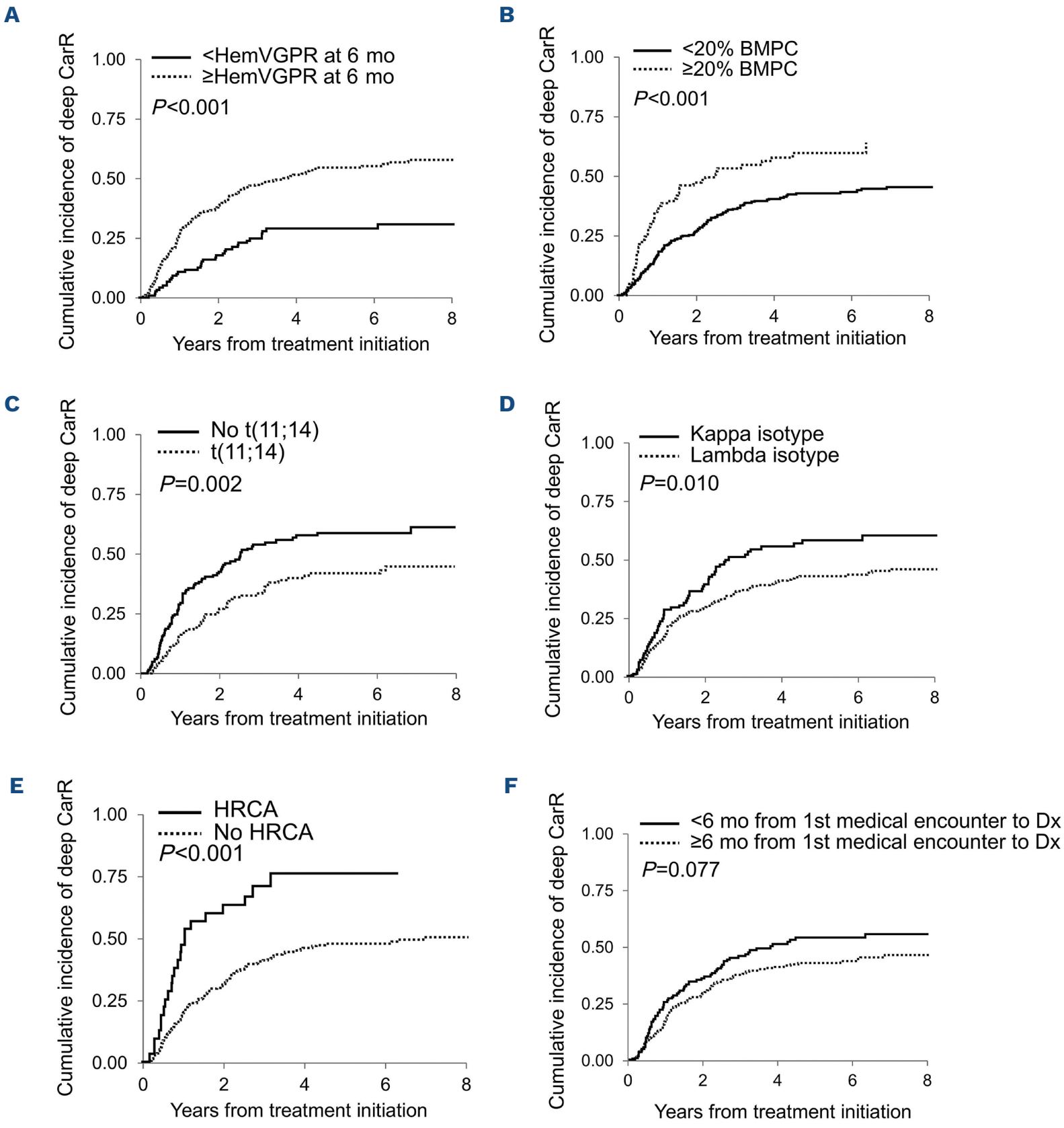

Figure 2. Cumulative incidence of deep cardiac response (cardiac very good partial response or better) with death as a competing event. (A-F) Cumulative incidence of deep cardiac response (i.e., cardiac very good partial response or better) according to (A) hematologic response at 6 months, (B) bone marrow plasma cell percentage, (C) the presence of t(11;14), (D) light chain isotype, (E) the presence of high-risk cytogenetic abnormalities [del(17p), t(4;14), t(14;16), t(14;20)], and (F) time from first medical encounter to amyloid diagnosis. CarR: cardiac response; HemVGPR: hematologic very good partial response; mo: months; BMPC: bone marrow plasma cells; HRCA: high-risk cytogenetic abnormalities; Dx: diagnosis.

compared to patients with BMPC <20% (59% *vs*. 47%, χ^2 test, *P*=0.063). This occurred despite patients with BMPC ≥20% more frequently receiving front-line ASCT (35% *vs*. 24%, χ^2 test, *P*=0.24) and maintenance post-transplant (35% *vs*. 14%, χ^2 test, *P*=0.01). Patients with a high plasma cell burden, compared to patients with BMPC <20%, were also significantly more likely to have cardiac stage IIIA (46% *vs*. 32%, respectively) or IIIB (17% *vs*. 11%, respectively) (χ^2 test, *P*=0.0024), which may have offset the survival benefit of obtaining a deep cardiac response. The presence of HRCA compared to the absence of HRCA had no association with the number of therapies received in the first year of diagnosis (χ^2 test, *P*=0.90), the rate of next-line therapy utilization (χ^2 test, *P*=0.99), the use of front-line ASCT (34% *vs*. 29%, χ^2 test, *P*=0.70) or cardiac stage (χ^2 test, *P*=0.91). However, patients with HRCA were significantly more likely to receive maintenance therapy after ASCT compared to patients without HRCA (50% *vs*. 17%, χ^2 test, *P*=0.02). Last, we examined the hematologic response of patients who received first-line ASCT in more detail (*Online Supplementary Table S8*).

Since organ response is a time-dependent variable and subject to survivorship bias, we examined the influence of cardiac response on survival several ways. First, we constructed a Simon-Makuch plot of OS for patients who did and did not obtain a deep cardiac response as best response (Figure 3). Second, Cox proportional hazard regression treating the attainment of a deep cardiac response as a time-dependent covariate identified that patients who obtained a deep cardiac response had significantly improved OS compared to those who did not (HR=0.22, 95% CI: 0.13-0.36; *P*<0.001). Last, we examined the influence of cardiac response on survival at the 6-month, 1-year and 2-year landmark analyses. At each landmark time-point, patients who obtained a cardiac response or deep cardiac response had significantly superior OS compared to those who did not (Figure 4).

Discussion

Despite improvements in survival, the early mortality rate of patients with cardiac amyloidosis remains exceedingly high.[14,15] While more effective plasma cell therapies have improved our ability to clear circulating amyloidogenic proteins, reaching early and deep cardiac response is key to reducing early deaths.[11,16-18] To better inform strategies to improve organ response in AL amyloidosis, we investigated the factors that govern deep cardiac response. We have identified that features of the plasma cell clone, namely

Table 3. Multivariable proportional subdistribution hazards (Fine-Gray) model of factors associated with obtaining a deep cardiac response (cardiac very good partial response or better), treating death as a competing risk.

Characteristic	HR for deep CarR (≥CarVGPR)	95% CI	*P*
Age ≥60 years	0.83	0.58-1.17	0.3
BMPC ≥20%	1.64	1.14-2.38	0.008
Lambda isotype	0.59	0.41-0.84	0.003
HRCA [del17p, t(4;14), t(14;16), t(14;20)]	2.50	1.59-3.93	<0.001
≥HemVGPR within 6 months	2.00	1.34-2.97	<0.001
Cardiac stage - Mayo 2004 with modification	-	-	0.11
Stage II	Ref	-	-
Stage IIIA	1.48	0.99-2.21	-
Stage IIIB	0.88	0.46-1.70	-
Front-line ASCT	1.78	1.22-2.59	0.003

HR: hazard ratio; CarR: cardiac response; CarVGPR, cardiac very good partial response; 95% CI: 95% confidence interval; BMPC: bone marrow plasma cells; HRCA: high-risk cytogenetic abnormalities; HemVGPR, hematologic very good partial response; ASCT: autologous stem cell transplantation..

Table 4. Multivariable logistic regression model of covariates associated with cardiac complete response at 24 months and best response.

Characteristic	24-month cardiac CR			Cardiac CR at best response		
	OR	95% CI	*P*	OR	95% CI	*P*
BMPC ≥20%	2.30	1.12-4.66	0.025	2.73	1.49-5.03	0.001
Cardiac stage - Mayo 2004 with modification	-	-	0.003	-	-	0.003
Stage II	Ref	-	-	Ref	-	-
Stage IIIA	0.76	0.36-1.52	-	0.70	0.38-1.25	-
Stage IIIB	0.00	0.00-1,000+	-	0.12	0.02-0.46	-
≥HemVGPR within 6 months	2.63	1.18-6.75	0.017	3.82	1.93-8.18	<0.001
Front-line ASCT	2.39	1.23-4.67	0.011	3.22	1.86-5.59	<0.001
Lambda isotype	-	Not included	-	0.60	0.33-1.10	0.10

CR: complete response; OR: odds ratio; 95% CI: 95% confidence interval; BMPC: bone marrow plasma cells; HemVGPR: hematologic very good partial response; ASCT: autologous stem cell transplantation.

light chain isotype, BMPC burden and HRCA, influence the likelihood of deep cardiac response, independently of hematologic response and ASCT, endorsing the role of light chain toxicity in organ dysfunction and response.

κ light chain isotype, high BMPC and HRCA, which were predictive of deeper cardiac responses, are more commonly seen in AL patients with co-existing multiple myeloma than in those with AL alone.[19] In our view, the association observed between a myeloma-like phenotype and a higher rate of deep cardiac responses supports the role of light chain toxicity in organ dysfunction in AL amyloidosis. Beside tissue damage resulting from amyloid deposition, the concept of direct light chain precursor cellular toxicity has been shown in several *in vitro* models.[20-26] In animal models, direct cardiotoxicity occurs in a dose-dependent manner in response to circulating soluble FLC which are internalized to negatively impact cellular function through oxidative stress, and MAPK signaling.[21,22,24,25] We hypothesize that patients with a myeloma-like phenotype have a higher reliance on light chain toxicity compared to amyloid deposition-driven organ dysfunction because of the more proliferative nature of their underlying plasma cell clone. In these patients, the level of the circulating light chains rises faster and reaches a threshold at which light chain toxicity occurs. These patients are expected to experience more rapid recovery of cardiac function with successful plasma cell therapy once the level of the amyloidogenic light chains is decreased by treatment. Several observations support this hypothesis. First, κ light chain isotype, BMPC >20% and HRCA are associated with higher baseline dFLC, as shown in this work. A high dFLC suggests a higher amyloidogenic threshold, that is the level of involved FLC required to misfold, deposit and produce organ dysfunction may be greater, thus allowing for light chain toxicity to play a greater role. Second, given the non-clonal nature of the FLC assay, reducing the FLC levels to the near-normal/normal range with therapy better secures a deep hematologic response among those with high baseline dFLC (i.e., deep hematologic response) than among those with only mild elevation in baseline dFLC, enabling greater chances for improvement in cardiac function.[27] For the latter, monoclonal FLC may persist even with normalization of the FLC ratio. It is also conceivable that hitherto unrecognized differences exist in the biology of indolent and aggressive plasma cell clones which contribute to the mechanism and reversibility of organ damage.

Historically, a high plasma cell burden and dFLC have been associated with poor OS, with higher dFLC levels being observed in patients with cardiac involvement.[16,17,19,28,29] In our cohort, we observed a neutral effect on survival in these subgroups despite a higher rate of deep cardiac responses, possibly because this effect was offset by increased treatment burden and more advanced cardiac stage.[28] Additionally, the introduction of more effective plasma cell therapies, such as bortezomib, daratumumab and venetoclax, may more effectively address the underlying plasma cell clone in these patients, thereby mitigating the negative prognostic effect seen in previous series.[30,31] Furthermore, as HRCA and elevated BMPC required more plasma cell therapy, data on the duration of organ response in patients with these features, may also explain the absence of a survival advantage. While high plasma cell burden correlates with the occurrence of HRCA,[19] both appeared to predict for deep cardiac response independently. In this analysis,

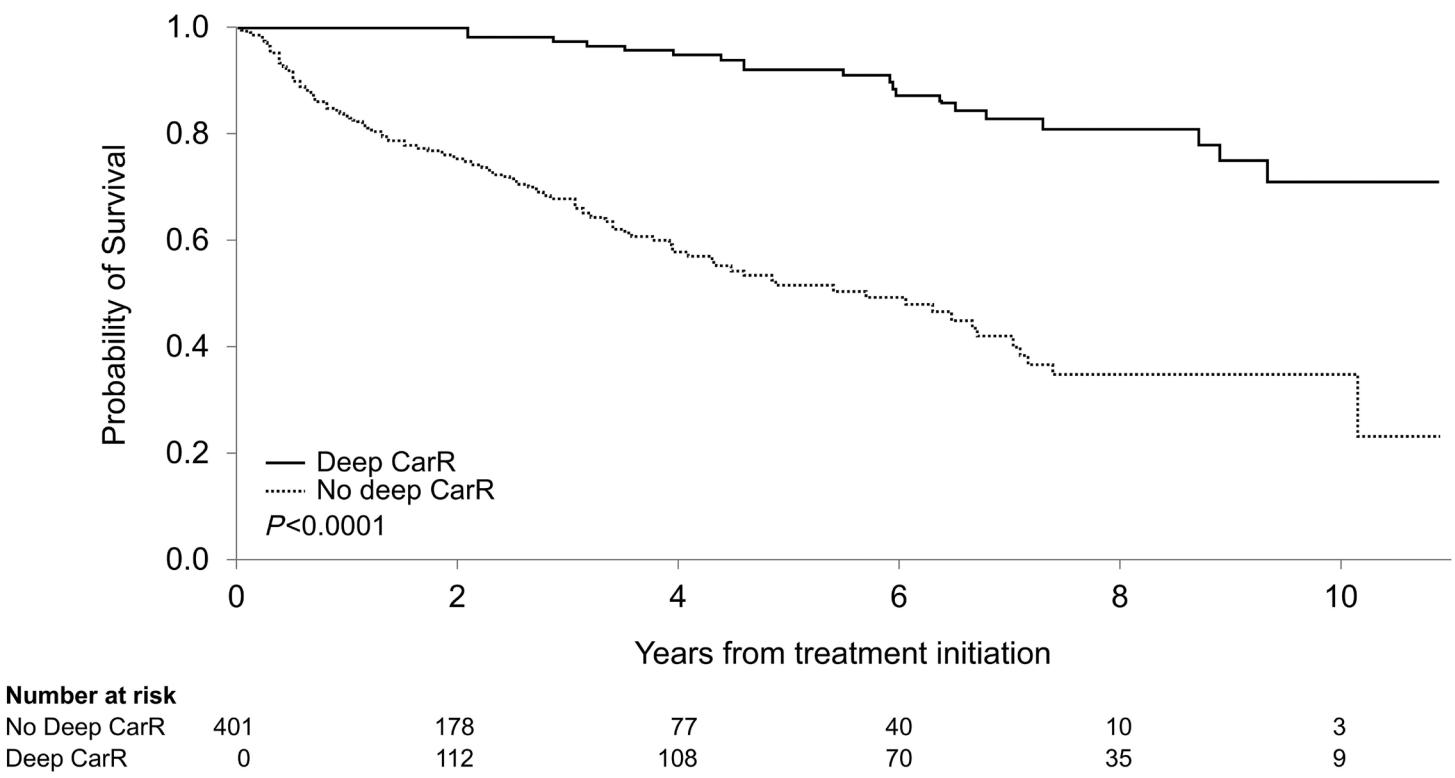

Figure 3. Simon-Makuch plot of overall survival of patients stratified according to whether they did or did not obtain a deep cardiac response (cardiac very good partial response or better). CarR: cardiac response.

four distinct cytogenetic lesions were grouped in line with their association with multiple myeloma and to allow for a meaningful but limited sample size. However, it is plausible that the impact of each of these lesions on cardiac response is distinct, consistent with known differences in their biology.[32]

A shorter period between reporting symptoms and diagnosis was associated with a higher rate of cardiac response in the first 12 months following treatment. This is consistent with the kinetics of amyloid protein aggregation, which is substantially enhanced after the formation of a fibrillar nuclei, as the structural repetitiveness of the amyloid fiber provides a seed for amyloid replication.[33] In addition, this observation may also support the light chain toxicity hypothesis. Patients with shorter duration of time from symptom onset to diagnosis may have more rapid worsening of symptoms, potentially reflecting higher plasma cell proliferation and organ dysfunction from light chain toxicity. However, we do not have laboratory proof to support this hypothesis. A study from Boston University found a longer period from symptom onset to diagnosis was a negative prognostic marker consistent with the lower cardiac response rates observed in our study, particularly at an earlier stage of treatment.[34] Commensurate with this, we observed that patients with advanced cardiac involvement who survived long-term follow-up seldom achieved a cardiac CR, likely secondary to irreversible functional and structural changes resulting from amyloid deposits. Serial cardiac imaging of amyloid patients has identified improvements in cardiac strain and deceleration time in responding patients, however, it is uncommon for the interventricular septal thickness or left ventricular mass index to decrease.[10,27]

AL patients with κ light chain isotype undergoing ASCT have a superior survival compared to that of patients with the λ isotype, even when adjusting for other known prognostic markers.[35] Besides variations in organ involvement which may be attributable to differences in the light chain variable region,[36] it has been recognized that the depth of hematologic response has a more significant impact on survival in λ-restricted patients. In one series, patients with λ light

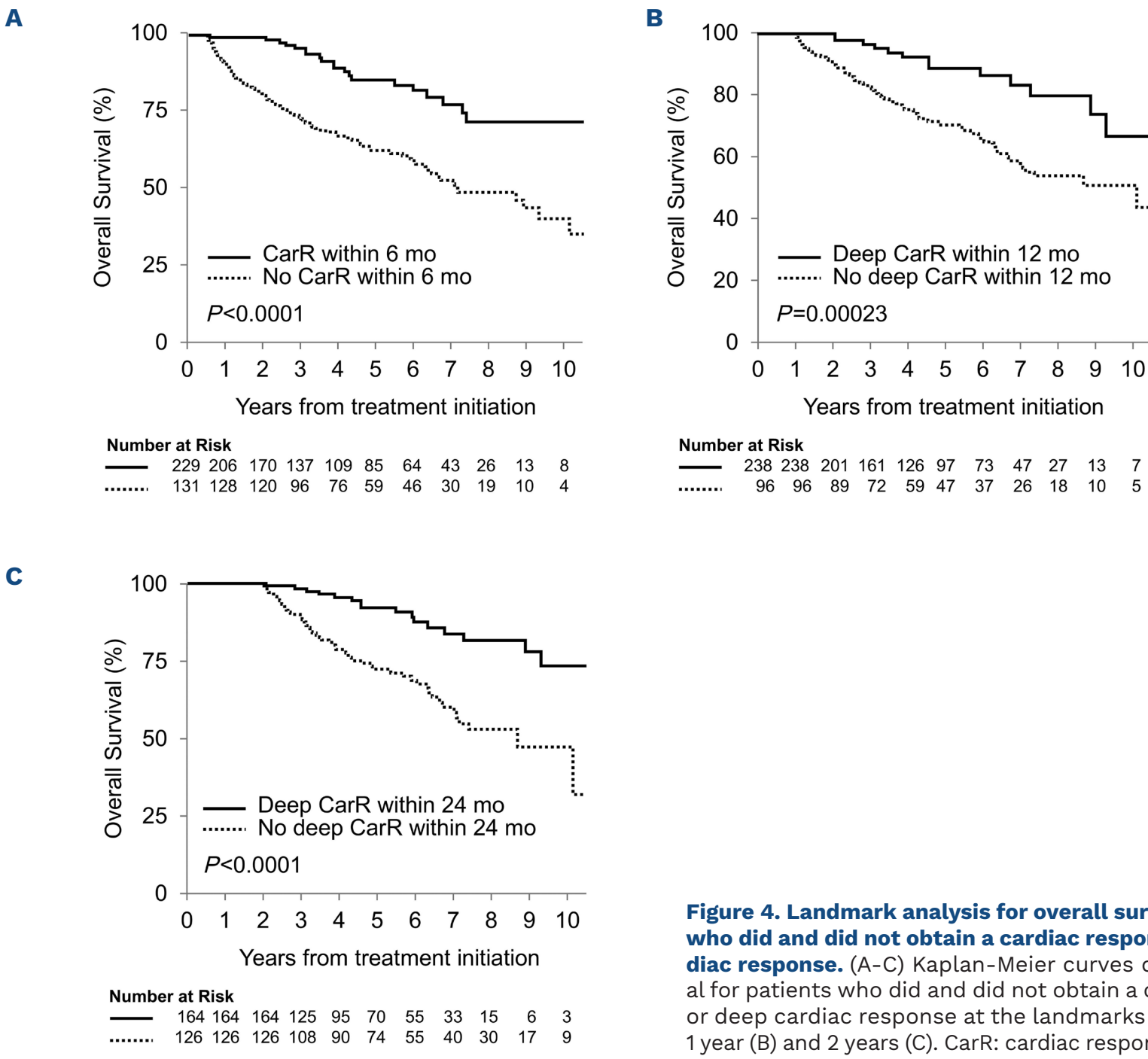

Figure 4. Landmark analysis for overall survival of patients who did and did not obtain a cardiac response or deep cardiac response. (A-C) Kaplan-Meier curves of overall survival for patients who did and did not obtain a cardiac response or deep cardiac response at the landmarks of 6 months (A), 1 year (B) and 2 years (C). CarR: cardiac response; mo: months.

chain disease who only achieved a hematologic partial response after ASCT experienced significantly worse OS compared to that of κ light chain patients with the same response. While inconclusive, this difference suggests a disparity in the amyloidogenicity of the different light chain isotypes, possibly affected by the different baseline levels of the light chains in each of these isotypes.[35] A recent study from Boston also found that λ isotype was associated with a lower likelihood of cardiac response independently of hematologic response, supporting the notion of different amyloidogenicity or toxicity between light chain isotypes.[37] The association observed between ASCT and cardiac response independent of hematologic response is likely secondary to patient selection, and possibly the achievement of a deeper level of disease control. With regard to the latter, patients who received first-line ASCT more often achieved a hematologic CR at best response, in support of this. It has also become clear that not all CR in AL amyloidosis are equal, and patients who obtain complete eradication of monoclonal FLC experience superior survival as well as higher organ response rates.[38] While we do not have any laboratory data to support this in our cohort, ASCT has a long history of deepening hematologic response rates across plasma cell disorders and this nicely fits with our observations.

This study was designed to explore predictors of cardiac response, looking both at the time cardiac response was achieved as well as its depth, a new concept in organ response assessment in AL amyloidosis which enables this study and its findings.[10] We were able to show that patients who achieved cardiac VGPR fared better than patients with a lower level of cardiac response, suggesting that depth of cardiac response is a crucial element for long-term survival. The study period spans over 13 years, and therefore only a minority of patients (13% of the study cohort) included received contemporary front-line treatment, i.e. daratumumab-based therapy. Daratumumab-based front-line therapy significantly improves depth of hematologic response and produces a higher 6-month cardiac response rate over bortezomib-based therapy.[30] Whether this type of therapy will affect the predictors of deep cardiac response observed in this work will require future studies with sufficient power and follow-up time to ascertain that outcome discrimination was adequately addressed.

This study is subject to all the limitations inherent to a single-center retrospective review. Survival in our study exceeded that observed in contemporary cardiac amyloidosis cohorts. Since regular follow up for reassessment of hematologic and organ response was a requisite for patients' eligibility for this study, our cohort was enriched for healthier patients. Patients eligible for cardiac response assessment at baseline who were not included in this study were older, lived farther from the clinic, and were more likely to be in stage IIIB. Therefore, both access to the clinic and disease-related factors influenced the selection of patients in this study. As such, caution should be exercised when extrapolating our data to the general AL amyloidosis population. Second, missing cardiac response at landmarks required imputation in 10-15% of patients based on nearby measurements. Lastly, treatment practices and supportive care varied over the period of this study, introducing heterogeneity to our cohort which may have influenced cardiac response rates.

The concept of direct light chain toxicity was first proposed to explain the rapid improvement in cardiac function which occurs with normalization of FLC levels in the absence of a reduction in amyloid burden.[9,27] Here we identify clonal features, namely light chain isotype, BMPC burden and HR-CA, which are associated with deeper cardiac response and support the role of light chain toxicity. Understanding these differences, which predict cardiac response independently of hematologic response, will allow clinicians to better prognosticate a patient's likelihood of achieving cardiac response and, therefore, survival. Furthermore, the biological differences that underlie these factors may help to refine our understanding of organ dysfunction and response.

Disclosures

MAG has received honoraria from Astra Zeneca, Alnylym, Ionis/Akcea, Medscape, Dava Oncology and Alexion; has received personal fees from AbbVie, for Data Safety Monitoring board participation, and from Sanofi, Janssen, Prothena and Johnson & Johnson. AD has received research funding from Pfizer, Janssen, Alnylam and HaemaloiX and has provided consultancy services for and received research funding from BMS, Alexion and Takeda. DD has received research funding from K36 Therapeutics; has acted as a consultant for and received honoraria from Sorrento, Sanofi, BMS, Janssen, Novartis, MSD, Regeneron and Alexion; has provided consultancy services for Genentech; and has received honoraria and research funding from and acted as a consultant for Apellis. PK has received research funding from Karyopharm, Ichnos, Loxo Pharmaceuticals, Bristol Myers Squibb, Regeneron and Amgen; has acted as a consultant for Keosys and CVS Caremark; has been or is a member of the Board of Directors or advisory committees for Mustang Bio, Pharmacyclics, X4 Pharmaceuticals, Kite, Oncopeptides and Angitia Bio; and has received research funding from and been a member of the Board of Directors or advisory committees for Sanofi, AbbVie, GlaxoSmithKline and Beigene. NL currently holds stock options in Checkpoint Therapeutics and AbbVie. TK has received research funding from Pfizer and Novartis. JK held $600 of stocks in Geron Corp for 1 week before selling them without profit. SK has received research funding from Sanofi, Roche, Novartis and Merck; has received research funding from and been a member of the Board of Directors or advisory committees for Kite, AbbVie, Celgene, Adaptive, Janssen, Takeda and MedImmune/AstraZeneca and has participated in an independent review committee for Oncopeptides. EM has received a consultation

fee from Protego. The remaining authors have no conflicts of interest to disclose.

Contributions

MJR and EM conceived and designed the study. MJR, EM, BY, NT and SAA-Y collected and assembled data. MJR, EM and SG analyzed and interpreted data. All authors provided study material or patients and approved the final version of the manuscript.

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Data-sharing statement

Original data will be shared upon reasonable request, addressed to muchtar.eli@mayo.edu.

References

1. Muchtar E, Dispenzieri A, Gertz MA, et al. Treatment of AL amyloidosis: Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) consensus statement 2020 update. Mayo Clin Proc. 2021;96(6):1546-1577.
2. Palladini G, Milani P, Merlini G. Management of AL amyloidosis in 2020. Blood. 2020;136(23):2620-2627.
3. Al Hamed R, Bazarbachi AH, Bazarbachi A, Malard F, Harousseau JL, Mohty M. Comprehensive review of AL amyloidosis: some practical recommendations. Blood Cancer J. 2021;11(5):97.
4. Muchtar E, Gertz MA, Lacy MQ, et al. Ten-year survivors in AL amyloidosis: characteristics and treatment pattern. Br J Haematol. 2019;187(5):588-594.
5. Muchtar E, Gertz MA, Kumar SK, et al. Improved outcomes for newly diagnosed AL amyloidosis between 2000 and 2014: cracking the glass ceiling of early death. Blood. 2017;129(15):2111-2119.
6. Kumar SK, Gertz MA, Lacy MQ, et al. Recent improvements in survival in primary systemic amyloidosis and the importance of an early mortality risk score. Mayo Clin Proc. 2011;86(1):12-18.
7. Wechalekar AD, Gillmore JD, Hawkins PN. Systemic amyloidosis. Lancet. 2016;387(10038):2641-2654.
8. Ravichandran S, Lachmann HJ, Wechalekar AD. Epidemiologic and survival trends in amyloidosis, 1987-2019. N Engl J Med. 2020;382(16):1567-1568.
9. Palladini G, Dispenzieri A, Gertz MA, et al. New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers: impact on survival outcomes. J Clin Oncol. 2012;30(36):4541-4549.
10. Muchtar E, Dispenzieri A, Leung N, et al. Depth of organ response in AL amyloidosis is associated with improved survival: grading the organ response criteria. Leukemia. 2018;32(10):2240-2249.
11. Muchtar E, Dispenzieri A, Wisniowski B, et al. Graded cardiac response criteria for patients with systemic light chain amyloidosis. J Clin Oncol. 2023;41(7):1393-1403.
12. Palladini G, Sachchithanantham S, Milani P, et al. A European collaborative study of cyclophosphamide, bortezomib, and dexamethasone in upfront treatment of systemic AL amyloidosis. Blood. 2015;126(5):612-615.
13. Muchtar E, Kumar SK, Gertz MA, et al. Staging systems use for risk stratification of systemic amyloidosis in the era of high-sensitivity troponin T assay. Blood. 2019;133(7):763-766.
14. Palladini G, Schönland S, Merlini G, et al. Systemic light chain amyloidosis across Europe: key outcomes from a retrospective study of 4500 patients. Blood. 2021;138(Supplement 1):153.
15. Vaxman I, Kumar SK, Buadi F, et al. Outcomes among newly diagnosed AL amyloidosis patients with a very high NT-proBNP: implications for trial design. Leukemia. 2021;35(12):3604-3607.
16. Gustine JN, Staron A, Mendelson L, et al. Predictors of treatment response and survival outcomes in patients with advanced cardiac AL amyloidosis. Blood Adv. 2023;7(20):6080-6091.
17. Basset M, Milani P, Foli A, et al. Early cardiac response is possible in stage IIIb cardiac AL amyloidosis and is associated with prolonged survival. Blood. 2022;140(18):1964-1971.
18. Kaufman GP, Dispenzieri A, Gertz MA, et al. Kinetics of organ response and survival following normalization of the serum free light chain ratio in AL amyloidosis. Am J Hematol. 2015;90(3):181-186.
19. Muchtar E, Gertz MA, Kourelis TV, et al. Bone marrow plasma cells 20% or greater discriminate presentation, response, and survival in AL amyloidosis. Leukemia. 2020;34(4):1135-1143.
20. Sikkink LA, Ramirez-Alvarado M. Cytotoxicity of amyloidogenic immunoglobulin light chains in cell culture. Cell Death Dis. 2010;1(11):e98.
21. Mishra S, Guan J, Plovie E, et al. Human amyloidogenic light chain proteins result in cardiac dysfunction, cell death, and early mortality in zebrafish. Am J Physiol Heart Circ Physiol. 2013;305(1):H95-103.
22. Diomede L, Rognoni P, Lavatelli F, et al. A Caenorhabditis elegans-based assay recognizes immunoglobulin light chains causing heart amyloidosis. Blood. 2014;123(23):3543-3552.
23. Brenner DA, Jain M, Pimentel DR, et al. Human amyloidogenic light chains directly impair cardiomyocyte function through an increase in cellular oxidant stress. Circ Res. 2004;94(8):1008-1010.
24. Guan J, Mishra S, Shi J, et al. Stanniocalcin1 is a key mediator of amyloidogenic light chain induced cardiotoxicity. Basic Res Cardiol. 2013;108(5):378.
25. Shi J, Guan J, Jiang B, et al. Amyloidogenic light chains induce cardiomyocyte contractile dysfunction and apoptosis via a non-canonical p38alpha MAPK pathway. Proc Natl Acad Sci U S A. 2010;107(9):4188-4193.
26. Marin-Argany M, Lin Y, Misra P, et al. Cell damage in light chain amyloidosis: fibril internalization, toxicity and cell-mediated seeding. J Biol Chem. 2016;291(38):19813-19825.
27. Palladini G, Lavatelli F, Russo P, et al. Circulating amyloidogenic free light chains and serum N-terminal natriuretic peptide type B decrease simultaneously in association with improvement of survival in AL. Blood. 2006;107(10):3854-3858.
28. Kourelis TV, Kumar SK, Gertz MA, et al. Coexistent multiple myeloma or increased bone marrow plasma cells define equally high-risk populations in patients with immunoglobulin light chain amyloidosis. J Clin Oncol. 2013;31(34):4319-4324.
29. Kumar S, Dispenzieri A, Lacy MQ, et al. Revised prognostic staging system for light chain amyloidosis incorporating cardiac biomarkers and serum free light chain measurements. J Clin

Oncol. 2012;30(9):989-995.
30. Kastritis E, Palladini G, Minnema Monique C, et al. Daratumumab-based treatment for immunoglobulin light-chain amyloidosis. N Engl J Med. 2021;385(1):46-58.
31. Roussel M, Pirotte M, Rizzo O, et al. Venetoclax-targeted therapy in t(11;14) AL amyloidosis patients after frontline daratumumab and Cybord: a retrospective analysis from the French Amyloidosis Network. Blood. 2022;140(Supplement 1):10086-10087.
32. Rees MJ, Kumar S. High-risk multiple myeloma: redefining genetic, clinical, and functional high-risk disease in the era of molecular medicine and immunotherapy. Am J Hematol. 2024;99(8):1560-1575.
33. Westermark GT, Fändrich M, Lundmark K, Westermark P. Noncerebral amyloidoses: aspects on seeding, cross-seeding, and transmission. Cold Spring Harb Perspect Med. 2018;8(1):a024323.
34. Schulman A, Connors LH, Weinberg J, et al. Patient outcomes in light chain (AL) amyloidosis: the clock is ticking from symptoms to diagnosis. Eur J Haematol. 2020;105(4):495-501.
35. Sidiqi MH, Aljama MA, Muchtar E, et al. Light chain type predicts organ involvement and survival in AL amyloidosis patients receiving stem cell transplantation. Blood Adv. 2018;2(7):769-776.
36. Abraham RS, Geyer SM, Price-Troska TL, et al. Immunoglobulin light chain variable (V) region genes influence clinical presentation and outcome in light chain–associated amyloidosis (AL). Blood. 2003;101(10):3801-3807.
37. Staron A, Mendelson LM, Joshi T, Burke N, Sanchorawala V. Factors impeding organ recovery despite a deep haematological response in patients with systemic AL amyloidosis. Br J Haematol. 2024;205(6):2268-2272.
38. Bomsztyk J, Ravichandran S, Giles HV, et al. Complete responses in AL amyloidosis are unequal: the impact of free light chain mass spectrometry in AL amyloidosis. Blood. 2024;143(13):1259-1268.