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Hepatic response criteria in light chain amyloidosis: a multicenter validation study

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Keywords*:* survival, alkaline phosphatase, bilirubin, amyloid, organ response

Abstract

Liver involvement in light chain amyloidosis (AL) is seen in 10-20% of patients and is associated with poor prognosis. The goal of this study was to assess the prognostic impact of the hepatic response criteria. AL patients diagnosed between 2010 and 2015 with liver involvement [serum alkaline phosphatase (AP) >1.5 upper reference limit (URL)] who achieved hematological response were included. Hepatic response was defined as >50% reduction (or normalization) of AP from baseline. Hepatic response was assessed at 6, 12, and 24-months after therapy initiation and at best response. Overall survival (OS) was assessed from time of therapy initiation. Hepatic response was evaluated in 116 patients. The median baseline serum AP was X2.6 URL. Hematological very good partial response (VGPR) or better was achieved in 69% of patients. AP decreased with time, with a median reduction of 22%, 34%, and 53% at 6-, 12-, and 24-months, respectively, and a median AP reduction of 56% at the time of best response. The median time to hepatic response was 13.3 months and was longer for patients undergoing autologous stem cell transplantation. Achievement of hepatic response, particularly as early as 12 months, and at best response, was associated with improved survival, independent of other prognostic factors. Predictors of hepatic response include higher baseline AP level, lower total bilirubin, hematological ≥VGPR, and cardiac and renal responses, when applicable. Hepatic response measured by the change in alkaline phosphatase is a prognostic factor in patients with AL amyloidosis.

Introduction

Liver involvement in AL amyloidosis has been reported in up to 20% of the patients. (1, 2) It is typically encountered as part of multi-organ involvement and is rarely the sole involved organ. (3) Therefore, large series focusing on hepatic AL amyloidosis are uncommon. Although the prognosis of hepatic AL amyloidosis is poor, it is governed by hepatic and non-hepatic factors . (4-6) The survival of hepatic AL amyloidosis is poor and has not changed over the past four decades in two single-center studies that reported the outcomes of patients diagnosed from 1975 to 1997 and 2004 to 2019, respectively. (5, 6) However, in more contemporary series, survival was better for those attaining hematological response. As survival in AL amyloidosis has improved significantly in the past years, particularly in referral centers,(7-9) with better hematological responses achieved with modern therapies,(10-12), improvement in outcomes in hepatic AL amyloidosis is expected as well.

The International Society of Amyloidosis (ISA) published hepatic response criteria in 2005. (13) Hepatic response was defined as >50% decrease in abnormal alkaline phosphatase levels or a decrease in liver size by at least 2 cm radiographically. The prognostic value of these criteria was evaluated only in a small series of AL patients with liver involvement who underwent ASCT. (14) Therefore, a broader assessment of their prognostic utility and their independence from other factors is required. More recently, Mayo Clinic investigators proposed 4-level graded hepatic response criteria in a single-center study (15), which has not yet been externally validated. This multicenter study aimed to determine the prognostic significance of hepatic response criteria in AL amyloidosis and to establish optimal response definitions.

Methods

The study was approved by the institutional review board of the participating centers. Patients provided their informed consent for the use of their medical records for medical research. Patients with biopsy-proven AL amyloidosis diagnosed between January 2010 and December 2015 were included if met the following criteria: (1) liver involvement with serum alkaline phosphatase (AP) >1.5 times the institutional upper reference limit (URL) attributed to liver involvement; (2) achievement of at least a hematological partial response (PR) to therapy within 12 months of diagnosis; and (3) serum AP measurements recorded at least twice annually in the first three years and annually thereafter. Hepatic response, assessed at fixed time points from treatment initiation (6, 12, and 24-months) and at the best hepatic response, was defined as >50% reduction in AP, consistent with the ISA criteria. (13) Receiver operating characteristic (ROC) analysis also identified a >50% reduction in AP at 12 months as the optimal cut-off point for 5-year survival discrimination (area under the curve, 0.67). Additionally, patients who had normalization of AP were considered to achieve a hepatic response, regardless of their ability to achieve a >50% reduction in AP. Radiographic assessment of liver span was not utilized due to its infrequent use in routine practice. Alternatively, the Mayo Clinic proposed 4-graded hepatic response criteria were also tested, (15) based on the reduction in serum AP with cut-off points at 30%, 60%, and absolute AP ≤90 U/L for hepatic PR, hepatic very good partial response (VGPR), and hepatic complete response (CR), respectively (**Table 1**). Owing to the limited number of patients to adequately power a four-level analysis of hepatic response, and considering the comparable survival outcomes observed between hepatic VGPR

and hepatic CR, we combined these two categories into a collective hepatic CR/VGPR group for all survival analyses.

Given the gradual nature of organ response, missing hepatic response data at fixed time points were imputed using adjacent time-point data, affecting 15%, 23%, and 22% of patients at the 6-, 12-, and 24-month intervals, respectively. Serum AP values were standardized as folds of URLs to account for inter-institutional variability (**Supplementary Table 1**). Cardiac and renal staging were assigned based on the established criteria. (16, 17) Graded cardiac and renal responses were used, as previously reported. (18, 19)

Pearson $\chi 2$ and Kruskal–Wallis tests were used to compare nominal and continuous variables, respectively. Overall survival (OS) was analyzed using the Kaplan-Meier method and compared via log-rank tests. To mitigate survivorship bias, landmark analyses excluded patients who experienced an event or were lost to follow-up before the assessed time point (**Supplementary Figure 1**). To evaluate the influence of dynamic hepatic response on OS, we utilized Cox regression models in which hepatic response was treated as a time-dependent covariate. These serial measurements included those at fixed time intervals and at the time of best hepatic response. A multivariable Cox proportional hazards model was used to determine the independent prognostic factors for OS. P-values <0.05 were considered significant. Statistical analyses were conducted using the JMP software (SAS, Cary, NC, USA), SAS (version 9.4.1; SAS Institute), and R (version 4.2.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics

One hundred and sixteen (n=116) patients are included in this study. The median age was 60 (range 37-83) (**Table 2**). The median serum AP level was 2.6 folds of URL (IQR, 1.9-4.4; median absolute value 336 U/L, IQR 240-577) with a median total bilirubin (TB) of 0.6 mg/dL (IQR 0.4-0.9). Fourteen patients (13.2% of those with available data, n=106) had a TB at diagnosis of >1.2 mg/dL. Concomitant kidney involvement was observed in 63.8% of patients, whereas coexisting heart involvement was observed in 56% of patients. Renal and cardiac staging are provided in Table 1, with an early cardiac stage (stage I/II) documented in 64.6% of the patients. The first-line therapy was bortezomib-based therapy in 66.4% of the patients, followed by ASCT in 19.8% of patients (all high-dose melphalan; 15 patients received full-dose melphalan 200 mg/m^2, 8 patients attenuated dose 100-140 mg/m^2). Patients who received full-dose melphalan exhibited a higher baseline AP then those receiving attenuated-dose (median 3.5 vs 2.0 folds of ULN, p=0.008) The best hematological responses were CR, VGPR, and PR in 42.3%, 26.7%, and 31.0% of patients, respectively. The differences in baseline characteristics between ASCT and non-ASCT patients are presented in **Supplementary Table 2**. With a median follow-up of 104 months (95% confidence interval 99-110 months), 42 deaths were reported (36.2% of the study cohort). The 2-, 5- and 10-year OS rates were 82%, 68%, and 61%, respectively. The median survival was not reached.

Association between liver function abnormalities and disease presentation

Patients with TB >1.2 mg/dL had higher baseline serum AP than those with TB ≤1.2 mg/dL, but this difference was not statistically significant (mean fold of URL: 4.5 vs 2.5, respectively; p=0.12). In addition, patients with serum AP >2.5 folds of URL had a lower serum albumin on than their counterparts (median 3.0 vs 3.7 g/dL; p=0.02). Serum AP levels did not correlate with any other baseline characteristics listed in **Table 2**. Specifically, there was no correlation between the AP folds of the URL and heart (p=0.15) or renal (p=0.41) involvement. Patients with TB >0.4 mg/dL had a higher proportion of concomitant cardiac involvement (62.2% vs 43.8%, p=0.07) and a lower proportion of renal involvement (54% vs 84.4%, p=0.001) than those with TB ≤0.4 mg/dL. Overall, patients with TB >0.4 mg/dL were more likely to have cardiac stage IIIA/IIIB than their counterparts, reaching borderline statistical significance (43.7% vs 25%, p=0.06).

Hepatic response at fixed time points

The hepatic response deepened over time, with median serum AP folds of URL of 2.0, 1.8, and 1.3, respectively, at 6, 12, and 24 months from the time of therapy initiation, representing 22%, 34%, and 53% reductions, respectively, from the baseline value. In addition, 2, 1, and 2 patients at the 6, 12, and 24-month time points, respectively, reached AP values within the reference limit of normal but with less than 50% reduction in AP (range: 40-48% reduction in AP). Overall, of the evaluable patients for response at these time points, 17% (19/112), 38% (39/104), and 55% (52/94) of patients, respectively, achieved hepatic response (>50% reduction in serum AP and/or AP reaching the normal reference value) by these landmark timepoints. The proportion

of patients achieving alternative graded hepatic responses by these three landmark points is shown in **Figure 1A**.

Best hepatic response

Most patients (75/116 = 65%) achieved a hepatic response at any time point, where 95% of these patients (n=71) had a response based on >50% reduction in AP, while four patients achieved AP normalization without >50% reduction in AP (40-46% reduction in AP). In patients who achieved any hepatic response, the median time to hepatic response was 13.3 months (IQR, 8.6-25). At hepatic response, the median AP level was 1.1-fold of the URL (IQR 0.9-1.8), representing a 56% reduction in serum AP from the baseline value (IQR 52-64%). Patients undergoing ASCT as their primary therapy had a longer time to hepatic response than those receiving non-ASCT therapies (medians: 23.8 vs. 12.2 months, respectively; P=0.09). A larger proportion of ASCT patients achieved hepatic response beyond 24 months than non-ASCT patients (47.1% vs 12.1%, P=0.003). In contrast, the rate of hepatic response between the groups was numerically higher in the ASCT cohort, but not statistically different (73.9% vs 62.4%, p=0.29). Analysis of hepatic response stratified by the depth of hematological response following ASCT, though limited by small numbers, suggested a trend toward superior hepatic outcomes in those achieving deeper hematological responses. Of the patients who achieved hemCR (n=19), 73.7% had a subsequent hepatic response. Furthermore, all three patients who achieved hemVGPR following ASCT also achieved a hepatic response (100%). In contrast, the single patient who achieved hemPR following ASCT did not achieve a hepatic response (0%;

p=0.1). When examining graded hepatic response categories hemCR patients achieved hepCR (31.6%), hepVGPR (26.3%), hepPR (36.8%), and hepNR (5.3%). HemVGPR patients after ASCT attained hepVGPR (66.7%) and hepPR (33.3%), while the one hemPR patient was hepNR (100%, p=0.19).

Of the hepatic responders (n=75), bilirubin at the time of response was available for 80% of patients (n=60). Their median TB was 0.5 mg/dL (IQR, 0.3-0.5) with 6 patients (10%) having TB above 1.2 mg/dL at the time of hepatic response (range 1.4-4.0). Four of these patients had elevated bilirubin levels at the time of diagnosis, which eventually normalized. In 2 patients, TB was within normal limits at diagnosis, and bilirubin was transiently increased at the time of reaching hepatic response.

A graphical representation of the proportion of patients who achieved alternative graded hepatic responses is provided in **Figure 1B**. Using these criteria, 83% of patients achieved a hepatic response, either as hepatic PR (31-60% reduction in AP; 33% of patients), hepatic VGPR (>60% reduction in AP; 28% of patients), or hepatic CR (AP≤ 90 U/L; 22% of patients).

Factors associated with hepatic response

Hepatic responders had higher baseline AP and lower TB than hepatic non-responders (**Table 2**). Patients with serum AP greater than 3 folds of the URL had a higher hepatic response rate than patients with AP ≤X3 folds of the URL (78% vs 54.6%, P=0.007). A hepatic response was more likely to occur in patients who achieved a deep hematological response. Hepatic

responses were achieved in 71.4%, 74.2%, and 47.2% of patients who achieved hematological CR, VGPR, and PR, respectively (P=0.03, **Table 2**).

Among patients with concomitant liver and heart involvement (n=65), hepatic responders were more likely to achieve any degree of cardiac response (CarPR or better, defined as >30% reduction in NT-proBNP/BNP from baseline) than hepatic non-responders (88.6% vs 33.3%, P<0.001). In the cardiac response category, the CarCR (NT-proBNP/BNP <350/80 pg/mL) , CarVGPR (>60% reduction in NT-proBNP/BNP from baseline not meeting CarCR definition), and CarPR (>30% but ≤60% reduction in NT-proBNP/BNP from baseline) rates were 31.8%, 34.1%, and 22.7% vs 0%, 14.3%, and 19.0%, respectively. Among patients with coexisting liver and renal involvement (n=74), hepatic responders were more likely to achieve a renal response (RenPR or better, defined as >30% reduction in 24-h urine protein) than those who did not achieve hepatic response (86.3% vs 60.8%, P=0.01). By depth of renal response, RenCR (24-h urine protein <200 mg), RenVGPR (>60% reduction in 24-h urine protein not meeting RenCR), and RenPR (>30% but ≤60% reduction in 24-h urine protein) rates were 43.1%, 29.4%, and 13.7% vs 21.7%, 30.4%, and 8.7%, respectively.

Hepatic response rates, stratified by the best hematological response achieved, were 71.4% for patients hemCR, 74.2% for those with hemVGPR, and 47.2% for those hemPR (p=0.03).

Impact of baseline hepatic variables on survival

Survival was not affected by the baseline serum AP level at any cutoff. ROC analysis for survival at 5 years detected TB at a 0.4 mg/dL cut point as the best threshold for survival discrimination (area under the curve 0.737). Patients with TB greater than 0.4 mg/dL had shorter survival

compared to patients with TB ≤0.4 mg/dL (5-year OS 52% vs 97%, P<0.001; **Figure 2A**). Survival could not be discriminated against using TB at the 1.2 mg/dL cut point (**Figure 2B**).

Impact of hepatic response on survival

Kaplan-Meier curves were used to graphically evaluate the influence of hepatic response at 6, 12, and 24 months from treatment initiation and at best hepatic response on survival. While hepatic response at 6 months was not associated with survival (p=0.42), patients who achieved hepatic response at 12 months (p=0.006) had a longer survival than those who did not achieve hepatic response by that time point (**Figures 3A-B**); whereas hepatic response by 24 months tended to correspond with improved survival compared to non-responders, although this difference was not statistically significant (p=0.097; **Figure 3C**). Similarly, the best all-time hepatic response was associated with longer survival compared with those unable to achieve a hepatic response (**Figure 3D**). Hepatic responders maintained a survival advantage over hepatic non-responders in subgroups based on the status of cardiac stage or renal involvement (Figure 4).

Figure 5 depicts survival curves using the alternative graded hepatic responses at landmark time points and at the best response. Patients who collectively achieved best hepatic CR/VGPR had a longer survival than those who achieved hepatic PR or hepatic NR.

Univariate and multivariate analyses were performed to assess the independent effects of the hepatic response and other factors on survival. The results of these analyses are presented in

Table 3. Overall, five variables were found to be significantly associated with OS in the univariate setting: age ≥65 years, ASCT use, cardiac stage, best hematological response, and best hepatic response. In a multivariable model incorporating hepatic response as a time-dependent covariate, four variables remained significantly associated with survival, with hepatic response being the most influential parameter on survival (hepatic response vs hepatic non-response HR 0.4, P<0.001). Results from a similar multivariate model using the 3-level graded hepatic response as a time-dependent covariate demonstrate that hepatic VGPR/CR was the most prognostic factor for survival. The results of Cox regression models with hepatic response (using binary or 3-level hepatic response criteria) as a time-dependent covariate in landmark analyses for the different time points are presented in **Supplementary Tables 3-8**.

Discussion

This study, representing one of the largest series of hepatic AL amyloidosis, aimed to determine the prognostic utility of hepatic response criteria for OS. We have shown that hepatic response based on the ISA 2005 binary hepatic response criteria is significantly associated with improved survival independent of other known prognostic factors. Further grading using 4-level response classification did not enhanced risk-stratification for survival. Although hepatic AL amyloidosis is often present alongside involvement of other organs, liver involvement can be the predominant organ in some patients, underscoring the need for distinct hepatic response criteria. Notably, hepatic response emerged as the strongest independent prognostic factor for survival in multivariate analyses, emphasizing its importance, even in the context of multi-organ disease. Nonetheless, achieving a deep hematologic response, namely

hematological CR/VGPR, remains essential, as it directly facilitates hepatic response and independently predicts superior survival.

This study highlights the challenges of accurately assessing hepatic response in AL amyloidosis, as the primary measure, serum AP levels can be affected by factors other than liver amyloid infiltration. Notably, concomitant heart involvement can cause passive liver congestion, further elevating AP levels. Therefore, a reduction in AP may reflect not only hepatic response but also cardiac response. This may explain the exceptionally low hazard ratio for survival for hepatic response in univariate and multivariate analyses, emphasizing the critical role of cardiac response on survival. The hepatic responders in our study were more likely to have renal and cardiac responses, when applicable. While this was expected, hepatic non-responders had far lower concomitant cardiac response rates than concomitant renal response rates (36.3% vs 64%), whereas the rate of cardiac or renal response among hepatic responders was similar (88.4% and 85.7%). This observation implies the possible role of the cardiac response in lowering AP levels, leading to a hepatic response. While the ideal method to isolate true hepatic recovery is a comprehensive subgroup analysis of patients without cardiac involvement, the limited sample size of only 116 patients restricted us from conducting the ideal, dedicated analysis of patients without cardiac involvement, as this cohort is significantly underpowered. We observed a delayed hepatic response in patients treated with ASCT, with a median time to hepatic response of 23.8 months vs 12.2 months in the non-ASCT group. A previous single-center study on organ response among ASCT AL patients also reported a prolonged time to hepatic response in ASCT patients, rising from 16% at 12 months to 32% at 24 months,(14) using the same criteria as in this study. A prior study from the same center showed a high

hepatic response rate at 12 and 24 months (57% and 63%, respectively) but used reduction in hepatomegaly by physical examination as an additional criterion for hepatic response.(20) This delay in response possibly reflects treatment-related toxicity, as has been reported for heart and kidney involvement in patients undergoing ASCT(21-24); however, this remains speculative. It is noted that the hematological response was superior in the ASCT cohort than in the non-ASCT cohort, supporting toxicity as a cause of delayed response rather than inadequate hematological response to therapy. As ASCT patients typically have a favorable prognosis, a longer observation period for a hepatic response is feasible before considering interventions to improve it. In contrast, for non-ASCT patients who do not achieve hepatic response within 12-18 months of therapy, initiating or changing anti-plasma cell therapy should be considered, particularly if the underlying clone is not adequately controlled. Ideally, this should be tested in a well-designed clinical trial of patients with persistent organ dysfunction at landmark time points and evidence of clonal disease below the threshold of hematological progression. Alternatively, therapies against amyloid deposits, if proven effective in clinical trials, may offer an alternative avenue to achieve hepatic response.

The results of our analysis highlight the limitations and complexities of the current hepatic response criteria. Therefore, alternative methods for liver response assessment, particularly imaging-based approached are needed. Magnetic resonance elastography (MRE), for example, has been assessed in patients with hepatic AL amyloidosis at the time of diagnosis and revealed different patterns of liver involvement, including diffuse, heterogeneous, and focal lesions. (25) Our finding that elevated AP levels may predict a higher chance of hepatic response presents a complex issue. While higher AP may simply correlate with a greater extent of amyloid

deposition, the relationship between the pattern of liver involvement and the likelihood of response remains uncertain. In such cases, imaging-based modalities, such as MRE, offer a more sensitive and nuanced method for response assessment, providing critical information on the pattern and burden of liver involvement Other imaging modalities of interest include FibroScan and administration of amyloid-reactive peptide radiotracer, followed by positron emission/computed tomography (PET/CT). (26)

The main goal of this study was to assess the prognostic value of Mayo Clinic's proposed 4-level graded hepatic response criteria. (15) These criteria, which were developed in a single-center study, did not provide additional prognostic discrimination over the established binary International Society of Amyloidosis (ISA) 2005 definition. We attribute this outcome primarily to the significantly smaller sample size available for the liver response analysis compared to our prior studies on graded cardiac and renal responses. Given the infrequent prevalence and inherent heterogeneity of hepatic AL amyloidosis, this small sample size likely lacked the statistical power required to robustly resolve subtle prognostic differences between the four response grades. Furthermore, the limitations of the current liver response biomarker, alkaline phosphatase, may contribute to this observation. AP is a less dynamic organ biomarker compared to NT-proBNP or proteinuria, which exhibit rapid and profound changes in response to treatment. The limited dynamic range of AP may inherently restrict the ability of any graded criteria to distinguish between outcomes, irrespective of sample size. Another explanation for the failure to show the prognostic utility of 4-level hepatic response criteria is the effect of cardiac response on hepatic response, as discussed above. This effect may increase the unpredictability of the impact of hepatic response on survival.

Several studies have indicated that elevated bilirubin levels in patients with hepatic AL amyloidosis, using a cutoff of 2 mg/dL, have prognostic significance. (5, 6) We could not confirm this finding in our study, because only a minority of the patients had TB above the normal value, likely due to our study's selection criteria. In addition, this study included patients undergoing modern therapies who were more likely to respond to therapy and overcome negative prognostic factors at baseline. However, we did observe that patients with baseline bilirubin greater than 0.4 mg/dL had inferior survival compared with patients with baseline TB of 0.4 mg/dL or less. This finding that a much lower TB cut-off is prognostically significant is novel and likely reflects a difference in patient selection and survivorship bias in our cohort. The traditional >2 mg/dL threshold identifies patients with overt, end-stage liver failure, a group typically not surviving long. Conversely, the TB >0.4 mg/dL cut-off likely captures subclinical hepatic dysfunction or higher residual amyloid burden that remains prognostically relevant in a healthier, long-surviving population who achieved a hematologic response. This inferior survival may also explain the lower rate of hepatic response in the former group, due to survivorship bias towards hepatic responders (however, a lower hepatic response seen in this subgroup may lead to the observed inferior survival). The reason for this poorer prognosis in patients with bilirubin levels within the normal range warrants further investigation. Interestingly, this group had a lower rate of renal (but not cardiac) involvement than those with a lower baseline TB. Thus, the inferior survival in patients with TB >0.4 mg/dL may be partly related to a less favorable overall disease presentation. Notably, an inverse correlation between proteinuria and TB has been reported in both diabetic and non-diabetic patients with proteinuria, (27, 28) and a similar effect may occur in AL amyloidosis. The mechanism for this is unclear, but it has been

hypothesized that the antioxidant properties of TB may offer renal protection in patients with proteinuria.

Our study has several limitations, including its retrospective nature, which introduces a potential selection bias, as the assessment of hepatic response and its prognostic value was restricted solely to patients who achieved a prior hematologic response. Consequently, the findings are specific to this healthier, selected cohort and may not be generalizable to the entire, unselected treated population, including non-responders who represent a clinically relevant group with the poorest prognosis. Secondthis retrospective study is constrained by the absence of consistent imaging data (such as detailed ultrasound or MRI findings) to directly correlate with biochemical changes in AP. Third, the study's power is limited for a definitive comparison between 2-level and 4-level response systems; however, this is the first large multicenter study to demonstrate the prognostic significance of hepatic response. Finally, we acknowledge the potential for survivorship bias in the assessment of the best hepatic response. To address this, we evaluated the response at fixed time points, which supports the prognostic utility of alkaline phosphatase in assessing hepatic response. In addition, we performed survival analyses that accommodated hepatic response as a time-dependent covariate in the survival models.

In conclusion, we confirmed the prognostic impact of the ISA 2005 hepatic response criteria as early as 12 months after therapy initiation. These criteria are independent of other known prognostic markers and may therefore have clinical implications for patient management.

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Table 1. Hepatic response criteria	
Hepatic response eligibility	Serum AP >1.5 times the institutional upper limit of normal and attributed to liver involvement
2005 ISA binary response criteria	
Hepatic response	>50% decrease in AP from baseline or normalization of AP
No hepatic response	≤50% decrease in AP from baseline
Graded hepatic response criteria	
Hepatic complete response (HepCR)	AP ≤90 U/L
Hepatic very good partial response (HepVGPR)	>60% decrease in AP from baseline level not meeting HepCR criterion
Hepatic partial response (HepPR)	31–60% decrease in AP from baseline
Hepatic no response (HepNR)	≤30% decrease in AP from baseline

Abbreviation: AP, Alkaline phosphates

Table 2. Baseline characteristics and treatment across the whole study cohort and by hepatic response category

	Whole cohort (n=116)	Hepatic responders (n=75)	Hepatic non-responders (n=41)	P-value
Age, years, Median (range)	60 (37-83)	58 (37-82)	62 (40-83)	0.2
Male sex, N (%)	66 (56.9%)	43 (57.3%)	23 (56.1%)	0.89
Lambda restricted, N (%)	69 (59.5%)	41 (55.7%)	28 (68.3%)	0.15
Intact immunoglobulin isotype, N (%) IgG IgA IgM Light chain only	 35 (30.2%) 6 (5.2%) 7 (6%) 68 (58.6%)	 24 (32.0%) 2 (2.7%) 4 (5.3%) 45 (60.0%)	 11 (26.8%) 4 (9.8%) 3 (7.3%) 23 (56.1%)	0.40
dFLC, mg/L, median (IQR)	171 (62-509)	155 (63-494)	174 (47-637)	0.89
BMPCs %, median (IQR)	10 (6-15)	10 (5-13)	14(8-20)	0.06
Serum alkaline phosphatase, U/L, median (IQR)	336 (240-567)	390 (252-603)	278 (224-402)	**0.01**
Serum alkaline phosphates, X the upper limit of institutional normal, median (IQR)	2.6 (1.9-4.4)	3.0 (2.0-4.7)	2.2 (1.8-3.3)	**0.02**
Serum total bilirubin, mg/dL, median (QR)	0.6 (0.4-0.9)	0.5 (0.4-0.7)	0.8 (0.5-1.2)	**0.0009**
Serum albumin, g/dL, median (IQR)	3.4 (2.6-4.0)	3.1 (2.6-4.1)	3.7 (2.7-4.0)	0.28
Concomitant heart involvement, N (%)	65 (56.0%)	44 (58.7%)	21 (51.2%)	0.44

Concomitant kidney involvement, N (%)	74 (63.8%)	51 (68.0%)	23 (56.1%)	0.20
Cardiac stage, % I / II / IIIA/ IIIB	25.7/38.9/24.8/10.6	28.9/38.4/21.9/11.0	20/40/30/10	0.68
Renal stage, % I/II/III	47.3/37.3/15.4	45.8/34.7/19.4	50.0/42.1/7.9	0.24
Number of lines of therapy in the first 12 months, N (%)				
1	87 (75%)	58 (77.3%)	29 (70.7%)	0.76
2	25 (21.6%)	15 (20.0%)	10 (24.4%)	
>2	4(3.4%)	2 (2.7%)	2 (4.9%)	
First-line therapy, N (%)				
Bortezomib-based	77 (66.4%)	51 (68.0%)	26 (63.4%)	
ASCT	23 (19.8%)	17 (22.7%)	6 (14.6%)	0.27
Alkylator-based	9 (7.8%)	4 (5.3%)	5 (12.2%)	
IMiD-based	7 (6.0%)	3 (4.0%)	4 (9.8%)	
Best hematological response, N (%)				
CR	49 (42.3%)	35 (46.7%)	14 (34.2%)	**0.03**
VGPR	31 (26.7%)	23 (30.7%)	8 (19.5%)	
PR	36 (31.0%)	17 (22.6%)	19 (46.3%)	

Abbreviations: AL, light chain amyloidosis; ASCT, autologous stem-cell transplantation; BMPC, Bone marrow plasma cells; CR, complete response; dFLC, difference between involved and uninvolved light chains; HR, hazard ratio; IMiD, immunomodulatory drug; IQR, Interquartile range; NR, no response; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)

Table 3. Univariate and multivariable Cox regression models for overall survival using hepatic response as a time-dependent covariate

	Univariate analysis		**Multivariate analysis with 2-level hepatic response**		**Multivariate analysis with 3-level hepatic response**	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
Age≥65 years (vs age <65)	2.9 (1.6-5.4)	**<0.001**	2.1 (1.1-39)	**0.027**	2.1 (1.1 - 4.0)	**0.027**
ASCT as primary therapy	0.3 (0.1-0.9)	**0.03**	0.6 (0.2-2.0)	0.43	0.6 (0.2 - 2.0)	0.44
dFLC ≥ 180 mg/L	1.5 (0.8-2.8)	0.17	n\a		n\a	
Cardiac stage						
Stage IIIA + IIIB (vs Stage I + II)	2.1 (1.1-4.0)	**0.017**	2.0 (1.04-3.9)	**0.038**	1.9 (1.0 - 3.7)	0.055
Best hematological response						
Hematological CR/VGPR (vs hematological PR)	0.3 (0.2-0.6)	**<0.001**	0.4 (0.2-0.8)	**0.011**	0.5 (0.2 - 0.9)	**0.027**
Binary hepatic response						
Response (vs non-response)	0.4 (0.2-0.9)	**0.021**	0.4 (0.2-0.8)	**0.009**		
Graded hepatic response						
HepNR	Reference				Reference	
HepPR	0.8 (0.4 - 1.8)	0.66			0.8 (0.3 - 1.8)	0.59
HepVGPR/CR	0.35 (0.15 - 0.9)	**0.021**			0.3 (0.1 - 0.9)	**0.025**

Abbreviations: AL, light chain amyloidosis; ASCT, autologous stem-cell transplantation; BMPC, Bone marrow plasma cells; CR, complete response; dFLC, difference between involved and uninvolved light chains; HepNR, Hepatic no response; HepPR, Hepatic

partial response; HepVGPR/CR, Hepatic very good partial response/complete response; HR, hazard ratio; IMiD, immunomodulatory drug; IQR, Interquartile range; NR, no response; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)

Figure legends:

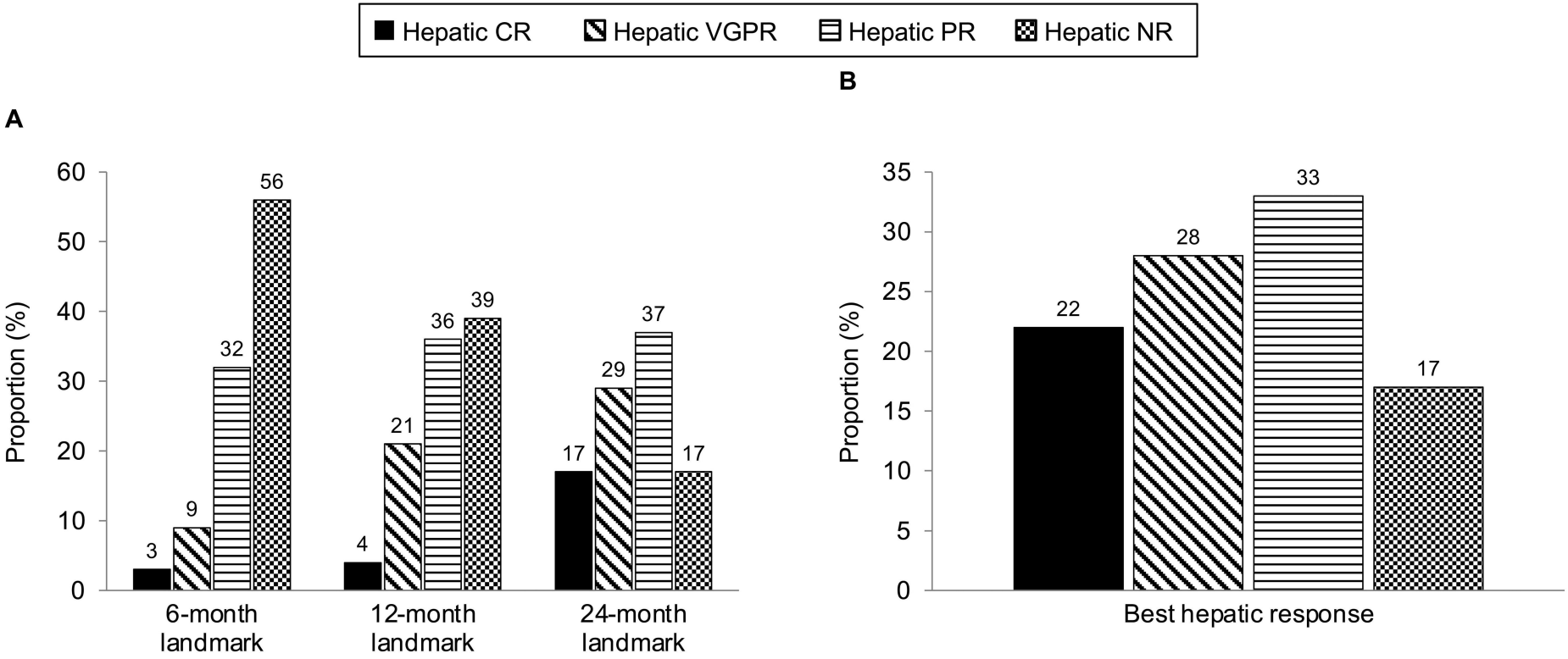
Figure 1: Distribution of graded hepatic response criteria. A. landmark time points. B. Best hepatic response

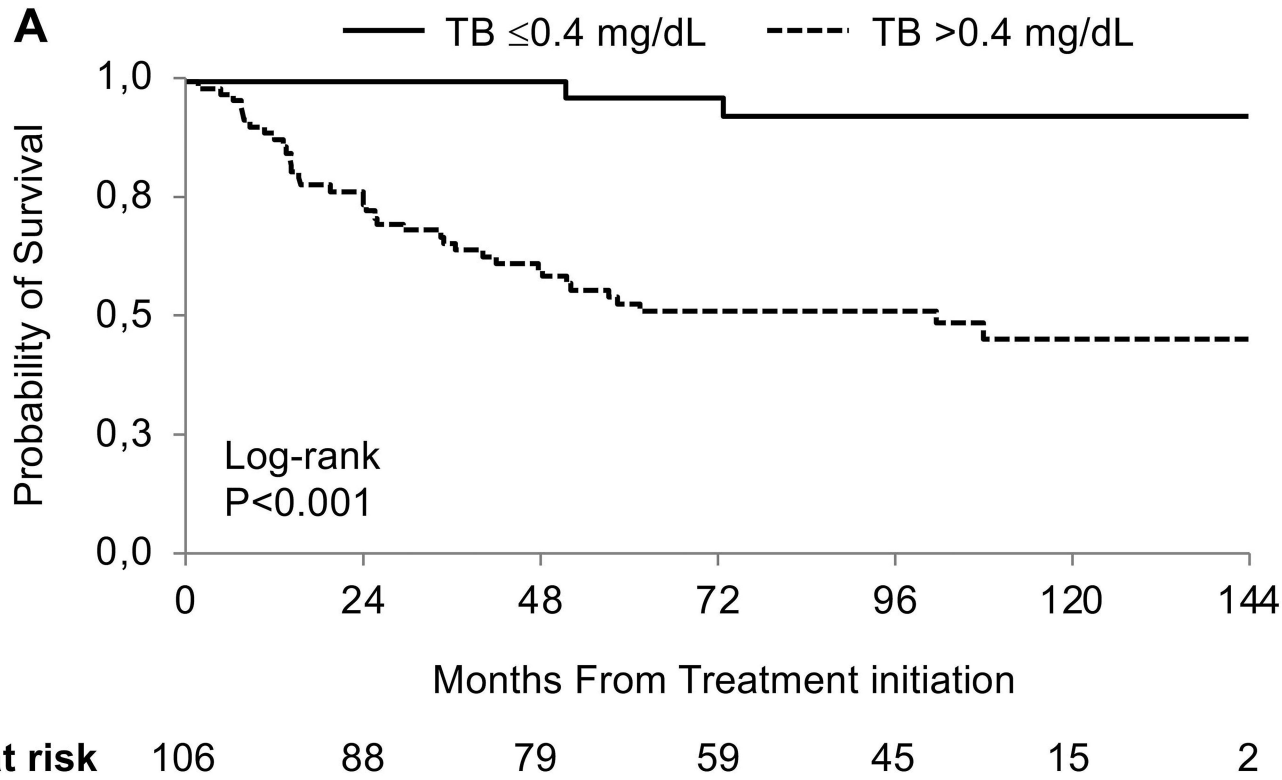
Figure 2: Overall survival by total bilirubin level at baseline. A. at 0.4 mg/dL cut point (by the best cut point by ROC analysis for 5-year OS). B. at 1.2 mg/dL cut point (by upper limit of normal)

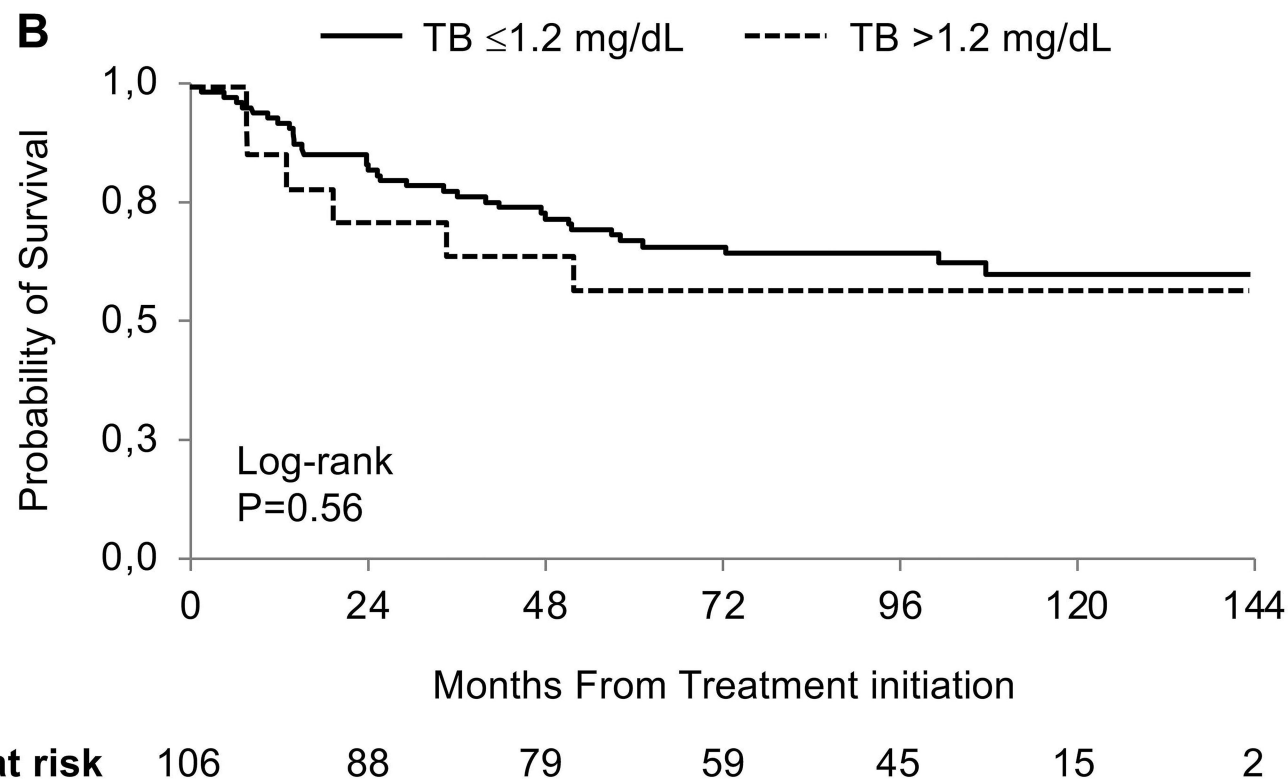
Figure 3: Overall survival stratified by the binary hepatic response status. A. 6 months from treatment initiation landmark. B. 12 months from treatment initiation landmark. C. 24 months from treatment initiation landmark. D. Best hepatic response.

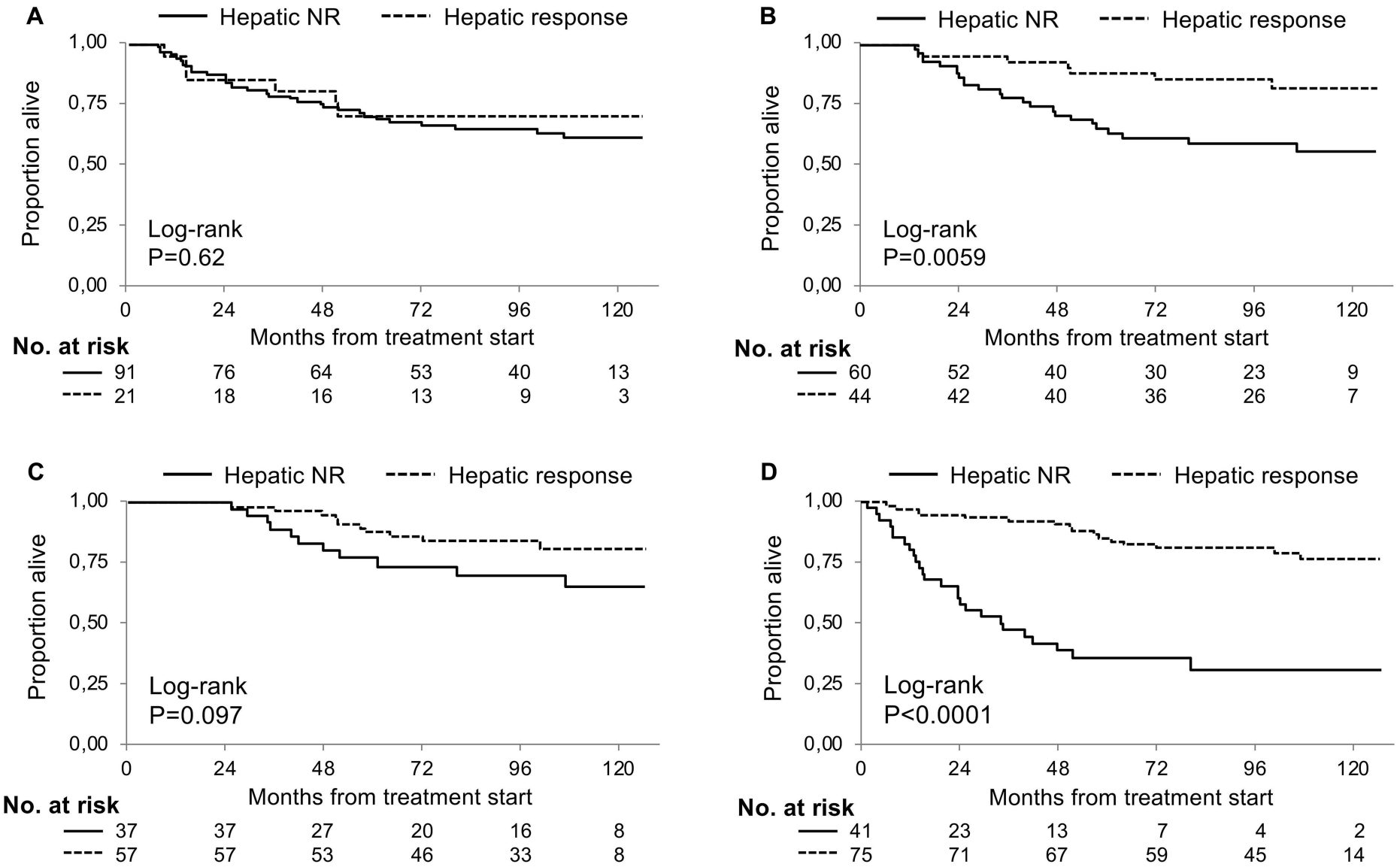
Figure 4: Overall survival stratified by best hepatic response and concurrent heart and renal involvement status: A. Patients with concurrent heart involvement (n=65). B. Patients without concurrent heart involvement (n=51). C. Patients with concurrent renal involvement (n=74). D. Patients without concurrent and renal involvement (n=42).

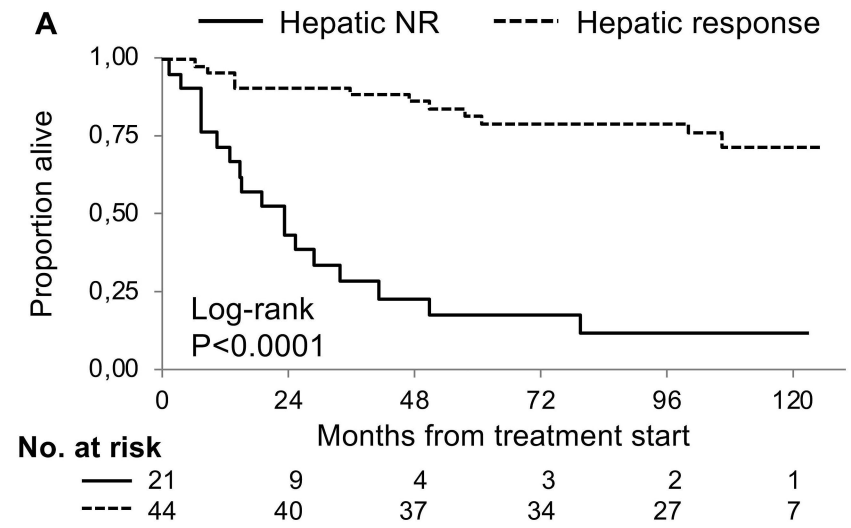
Figure 5: Overall survival stratified by the graded hepatic response. A. 6 months from treatment initiation landmark. B. 12 months from treatment initiation landmark. C. 24 months from treatment initiation landmark. D. Best hepatic response.

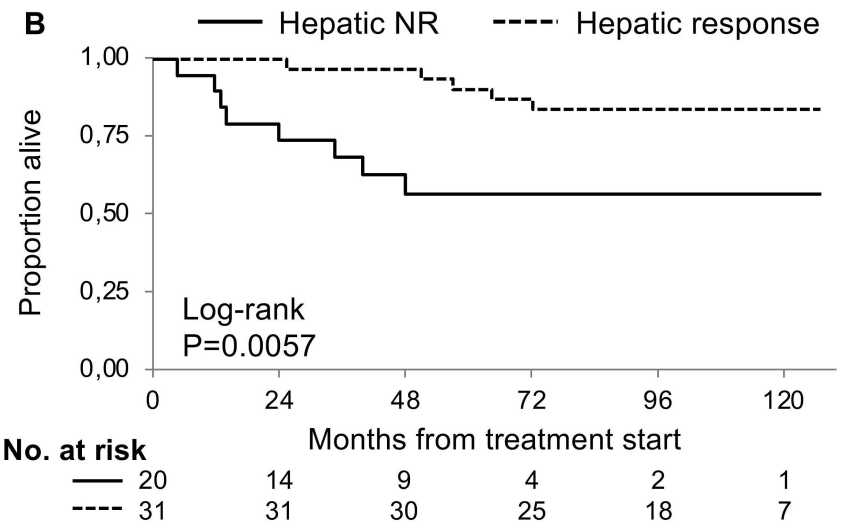

Hepatic CR
Hepatic VGPR
Hepatic PR
Hepatic NR
A
Proportion (%)
60
50
40
30
20
10
0
3
9
32
56
4
21
36
39
17
29
37
17
6-month landmark
12-month landmark
24-month landmark
B
Proportion (%)
35
30
25
20
15
10
5
0
22
28
33
17
Best hepatic response

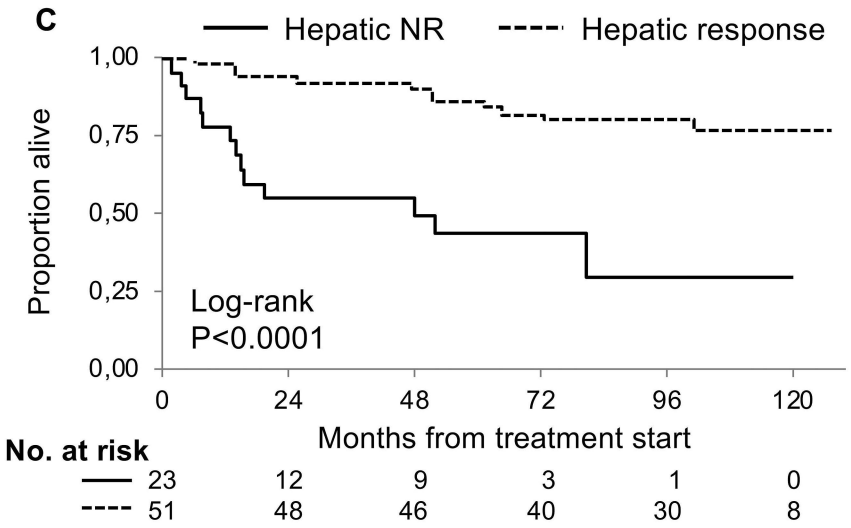

A
TB ≤0.4 mg/dL
TB >0.4 mg/dL
Probability of Survival
1,0
0,8
0,5
0,3
0,0
Log-rank
P<0.001
0
24
48
72
96
120
144
Months From Treatment initiation
Pt. at risk
106
88
79
59
45
15
2

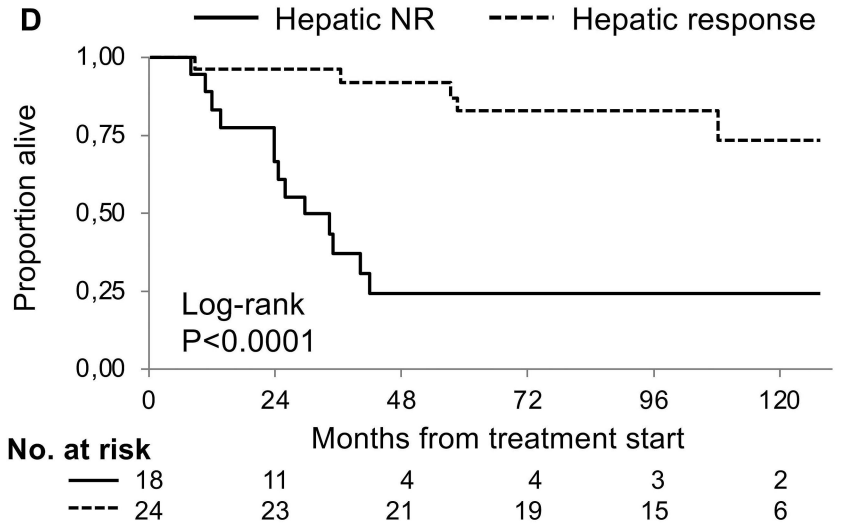

B
TB ≤1.2 mg/dL
TB >1.2 mg/dL
Probability of Survival
1,0
0,8
0,5
0,3
0,0
Log-rank
P=0.56
0
24
48
72
96
120
144
Months From Treatment initiation
Pt. at risk
106
88
79
59
45
15
2

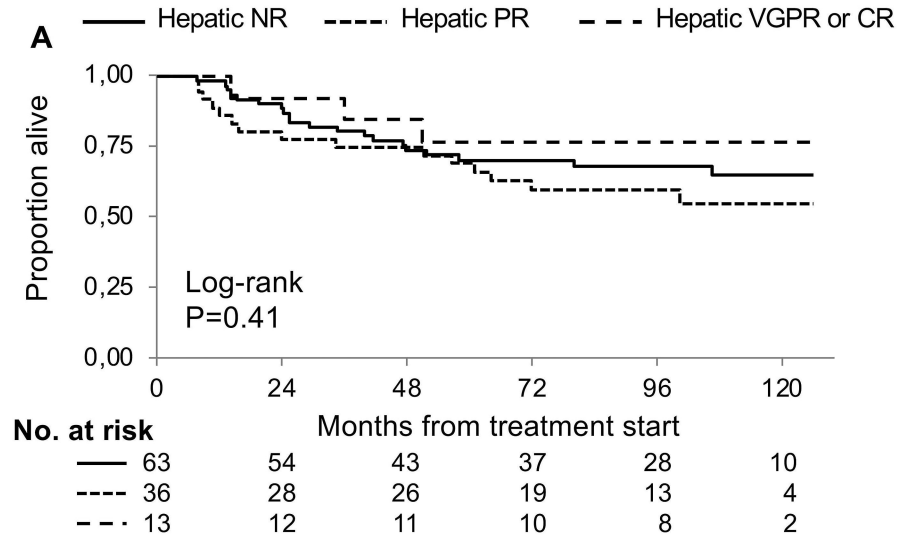


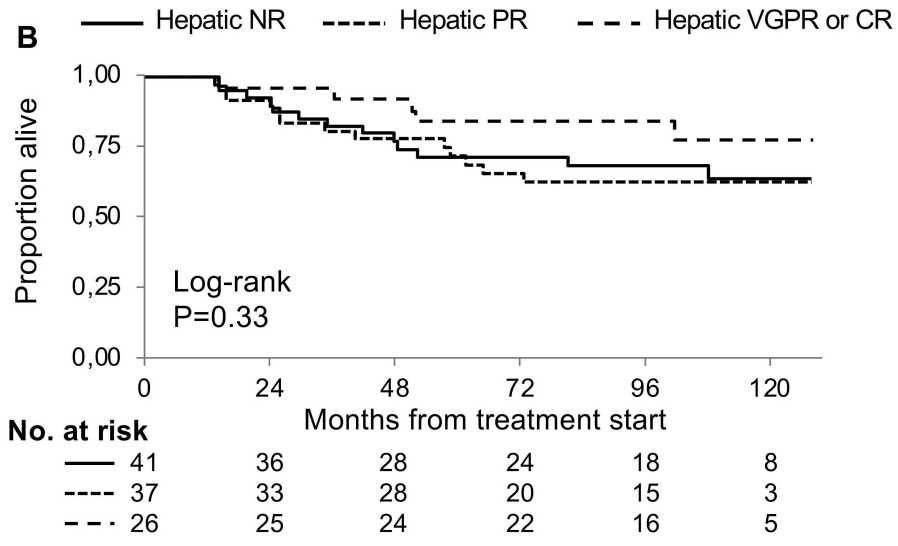


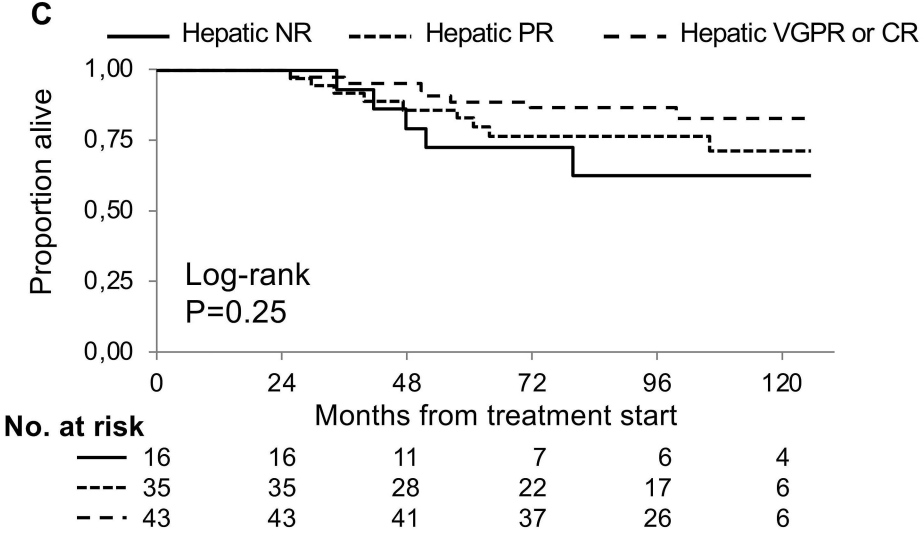


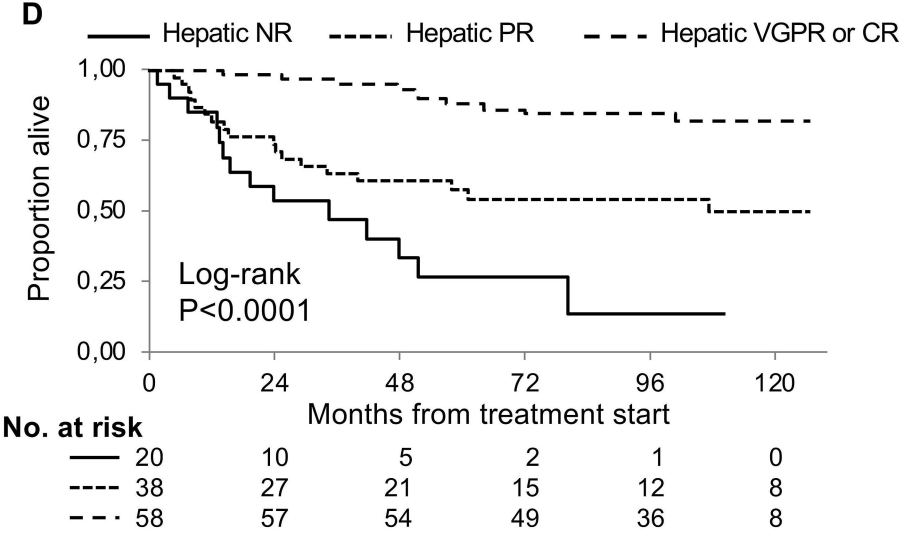












Supplemental material to:

Hepatic response criteria in light chain amyloidosis: A multicenter validation study. Muchtar E,

Supplementary Table 1. Alkaline phosphates normal reference values in the participating centers	
Center	**Reference**
Mayo Clinic, Rochester, MN	Men: 40-129 U/L Female: 35-104 U/L
Pavia, Italy	46-150 U/L
Heidelberg, Germany	40-130 U/L
National amyloidosis center, London, UK	40-129 U/L
Athens, Greece	40-124 U/L
Columbia, New York, NY	40-129 U/L
Boston University, Boston, MA	25-100 U/L
Memorial Sloan Kettering, New York, NY	45-129 U/L
Stanford University, Stanford, CA	35-105 U/L
Barcelona, Spain	46-116 U/L

Supplementary Table 2. Comparison of Baseline characteristics between ASCT and non-ASCT patients			
	ASCT (n=23)	Non-ASCT (n=93)	P-value
Age, years, Median (range)	58 (37-82)	62 (40-83)	0.08
Male sex, N (%)	14 (60.9%)	52 (57.8%)	0.66
Lambda restricted, N (%)	12 (52.2%)	57 (61.3%)	0.42
Intact immunoglobulin isotype, N (%) IgG IgA IgM Light chain only	 6 (26.1%) 2 (8.7%) 1 (4.3%) 14 (60.9%)	 29 (31.2%) 4 (4.3%) 6 (6.4%) 54 (58.1%)	0.81
dFLC, mg/L, median (IQR)	170 (17-494)	193 (93-539)	**0.02**
BMPCs %, median (IQR)	10 (5-17)	10 (7-15)	0.98
Serum alkaline phosphatase, U/L, median (IQR)	357 (247-593)	307 (238-548)	0.55
Serum alkaline phosphates, folds of the upper limit of institutional normal, median (IQR)	3.2 (2.0-4.9)	2.4 (1.8-4.3)	0.17
Serum total bilirubin, mg/dL, median (IQR)	0.6 (0.4-1.0)	0.6 (0.4-0.9)	0.63
Serum albumin, g/dL, median (IQR)	3.5 (3.0-4.1)	3.3 (2.4-4.0)	0.18
Concomitant heart involvement, N (%)	6 (26.1%)	59 (63.4%)	**0.0011**
Concomitant kidney involvement, N (%)	15 (65.2%)	59 (63.4%)	0.87

Cardiac stage, % I / II / IIIA/ IIIB	65.2/34.8/0/0	15.6/40.0/31.1/13.3	**<0.001**
Renal stage, % I/II/III	68.2/27.3/4.5	42.0/39.8/18.2	0.05
Best hematological response CR VGPR PR	 19 (82.6%) 3 (13.0%) 1 (4.4%)	 30 (32.3%) 28 (30.1%) 35 (37.6%)	**<0.001**

Abbreviations: AL, light chain amyloidosis; ASCT, autologous stem-cell transplantation; BMPC, Bone marrow plasma cells; CR, complete response; dFLC, difference between involved and uninvolved light chains; HR, hazard ratio; IQR, Interquartile range; NR, no response; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)

Supplementary Table 3. 6-month landmark univariate and multivariate analysis for overall survival using binary 6-month hepatic response

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
Age≥65 years (vs age <65)	2.75 (1.45 to 5.22	**0.002**	1.8 (0.9 – 3.6)	0.1
ASCT as primary therapy	0.36 (0.13 to 1.01)	**0.053**	0.7 (0.2 - 2.1)	0.5
dFLC ≥ 180 mg/L	1.58 (0.83 to 3.02)	0.16	n\a	
Cardiac stage Stage IIIA + IIIB (vs Stage I + II)	1.72 (0.89 to 3.35)	**0.11**	1.5 (0.7 - 3.0)	0.28
Best hematological response Hematological CR/VGPR (vs hematological PR)	0.32 (0.17 to 0.62)	**<0.001**	0.4 (0.2 - 0.8)	**0.011**
Hepatic response Response (vs non-response)	0.8 (0.3 - 1.9)	0.62	0.6 (0.2 – 1.6)	0.34

Abbreviations: ASCT, autologous stem-cell transplantation; CI, Confidence interval; CR, complete response; dFLC, difference between involved and uninvolved light chains; HR, hazard ratio; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)

Supplementary Table 4. 12-month landmark univariate and multivariate analysis for overall survival using binary 12-month hepatic response

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
Age≥65 years (vs age <65)	2.67 (1.31 to 5.43)	**0.007**	1.8 (0.8 – 3.9)	0.14
ASCT as primary therapy	0.43 (0.15 to 1.24)	0.12	0.6 (0.2 – 1.9)	0.37
dFLC ≥ 180 mg/L	1.43 (0.70 to 2.90)	0.32	n\a	
Cardiac stage Stage IIIA + IIIB (vs Stage I + II)	1.52 (0.72 to 3.23)	**0.27**	0.9 (0.4 – 2.2)	0.81
Best hematological response Hematological CR/VGPR (vs hematological PR)	0.25 (0.12 to 0.51)	**<0.001**	0.2 (0.1 - 0.5)	**<0.001**
Hepatic response Response (vs non-response)	0.3 (0.1 – 0.75)	**0.009**	0.2 (0.1 – 0.5)	**<0.001**

Abbreviations: ASCT, autologous stem-cell transplantation; CI, Confidence interval; CR, complete response; dFLC, difference between involved and uninvolved light chains; HR, hazard ratio; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)

Supplementary Table 5. 24-month landmark univariate and multivariate analysis for overall survival using 24-month binary hepatic response				
	Univariate analysis		**Multivariate analysis**	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
Age≥65 years (vs age <65)	2.65 (1.11 to 6.30)	**0.028**	1.6 (0.6 – 4.2)	0.34
ASCT as primary therapy	0.65 (0.22 to 1.95)	0.45	0.7 (0.2 – 2.7)	0.63
dFLC ≥ 180 mg/L	1.31 (0.55 to 3.08)	0.54	n\a	
Cardiac stage Stage IIIA + IIIB (vs Stage I + II)	1.20 (0.45 to 3.16)	0.72	1.0 (0.3 – 2.9)	0.95
Best hematological response Hematological CR/VGPR (vs hematological PR)	0.31 (0.13 to 0.73)	**0.008**	0.2 (0.1 - 0.7)	**0.01**
Hepatic response Response (vs non-response)	0.5 (0.2 – 1.2)	0.1	0.25 (0.1 – 0.7)	**0.007**

Abbreviations: ASCT, autologous stem-cell transplantation; CI, Confidence interval; CR, complete response; dFLC, difference between involved and uninvolved light chains; HR, hazard ratio; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)

Supplementary Table 6. 6-month landmark univariate and multivariate analysis for overall survival using the best graded (3-level) hepatic response criteria by 6 months				
	Univariate analysis		**Multivariate analysis**	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
Age≥65 years (vs age <65)	2.75 (1.45 to 5.22	**0.002**	1.8 (0.9 – 3.7)	0.089
ASCT as primary therapy	0.36 (0.13 to 1.01)	0.053	0.7 (0.2 - 2.2)	0.55
dFLC ≥ 180 mg/L	1.58 (0.83 to 3.02)	0.16	n\a	
Cardiac stage Stage IIIA + IIIB (vs Stage I + II)	1.72 (0.89 to 3.35)	0.11	1.35 (0.7 - 2.7)	0.4
Best hematological response Hematological CR/VGPR (vs hematological PR)	0.32 (0.17 to 0.62)	**<0.001**	0.4 (0.2 - 0.8)	**0.009**
Graded hepatic response				
HepNR	Reference		Reference	
HepPR	1.4 (0.7 – 2.7)	0.34	1.6 (0.8 – 3.2)	0.1
HepVGPR/CR	0.7 (0.2 – 2.2)	0.5	0.6 (0.1 – 2.5)	0.45

Abbreviations: ASCT, autologous stem-cell transplantation; CI, Confidence interval; CR, complete response; dFLC, difference between involved and uninvolved light chains; Hep, hepatic; HR, hazard ratio; NR, No response; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)

Supplementary Table 7. 12-month landmark univariate and multivariate analysis for overall survival using best graded (3-level) hepatic response criteria by 12-months				
	Univariate analysis		**Multivariate analysis**	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
Age≥65 years (vs age <65)	2.67 (1.31 to 5.43)	**0.007**	1.5 (0.7 – 3.4)	0.33
ASCT as primary therapy	0.43 (0.15 to 1.24)	0.12	0.8 (0.2 – 2.6)	0.67
dFLC ≥ 180 mg/L	1.43 (0.70 to 2.90)	0.32	n\a	
Cardiac stage Stage IIIA + IIIB (vs Stage I + II)	1.52 (0.72 to 3.23)	0.27	1.1 (0.5 – 2.6)	0.76
Best hematological response Hematological CR/VGPR (vs hematological PR)	0.25 (0.12 to 0.51)	**<0.001**	0.25 (0.1 - 0.6)	**0.001**
Graded hepatic response				
HepNR	Reference		Reference	
HepPR	1.1 (0.5 – 2.4)	0.81	0.8 (0.3 – 1.8)	0.56
HepVGPR/CR	0.5 (0.2 – 1.5)	0.21	0.3 (0.1 – 1.01)	0.051

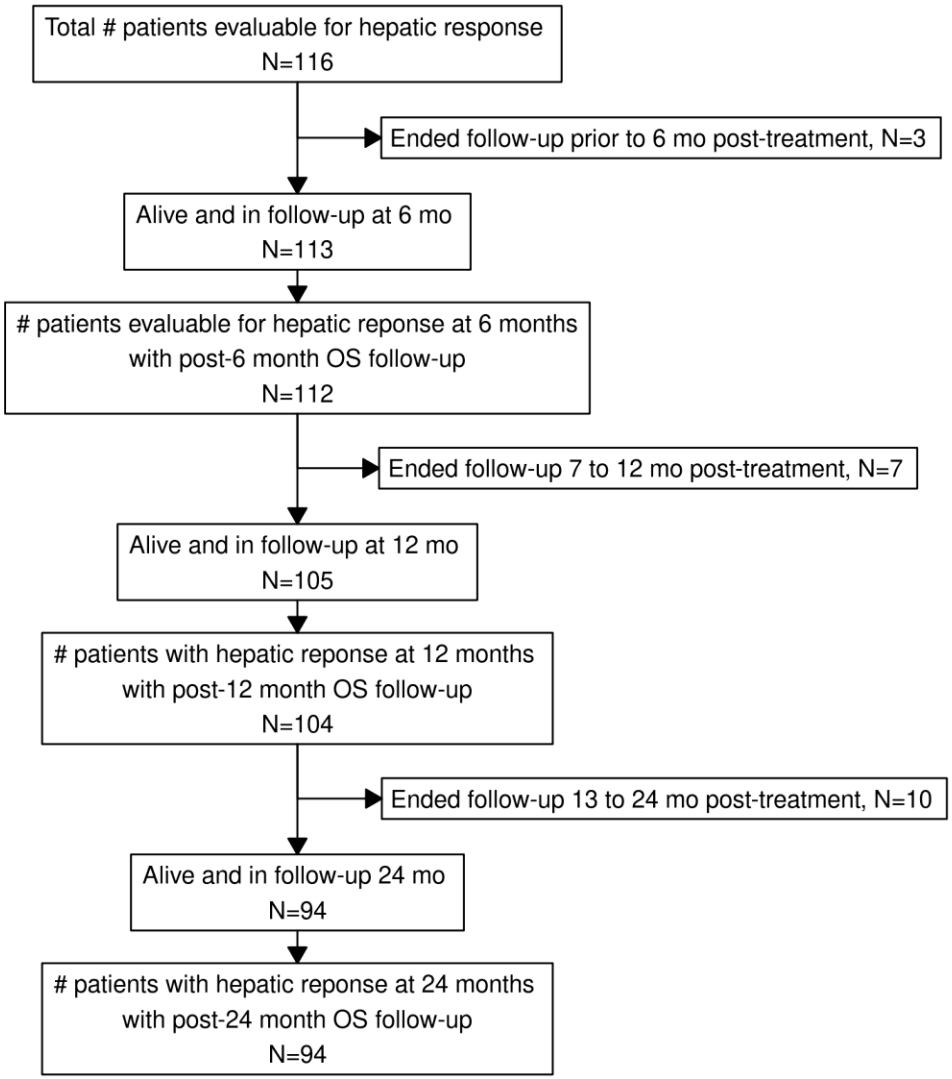
Abbreviations: ASCT, autologous stem-cell transplantation; CI, Confidence interval; CR, complete response; dFLC, difference between involved and uninvolved light chains; Hep, hepatic; HR, hazard ratio; NR, No response; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)

Supplementary Table 8. 24-month landmark univariate and multivariate analysis for overall survival using best graded (3-level) hepatic response criteria by 24-months				
	Univariate analysis		**Multivariate analysis**	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
Age≥65 years (vs age <65)	2.65 (1.11 to 6.30)	**0.028**	1.4 (0.5 –3.7)	0.54
ASCT as primary therapy	0.65 (0.22 to 1.95)	0.45	1.0 (0.3 – 3.5)	0.95
dFLC ≥ 180 mg/L	1.31 (0.55 to 3.08)	0.54	n\a	
Cardiac stage				
Stage IIIA + IIIB (vs Stage I + II)	1.20 (0.45 to 3.16)	0.72	1.1 (0.4 – 3.2)	0.89
Best hematological response				
Hematological CR/VGPR (vs hematological PR)	0.31 (0.13 to 0.73)	**0.008**	0.3 (0.1 - 0.8)	**0.016**
Graded hepatic response				
HepNR	Reference		Reference	
HepPR	0.7 (0.2 – 2.1)	0.54	0.4 (0.1 – 1.4)	0.16
HepVGPR/CR	0.4 (0.1 – 1.3)	0.12	0.2 (0.05 – 0.7)	**0.014**

Abbreviations: ASCT, autologous stem-cell transplantation; CI, Confidence interval; CR, complete response; dFLC, difference between involved and uninvolved light chains; Hep, hepatic; HR, hazard ratio; NR, No response; PR, partial response; VGPR, very good partial response

Bold signifies statistical significance (P-value <0.05)



Supplementary Figure 1: Flow diagram for landmark analysis cohorts