Haematologic responses and survival do not significantly decrease with subsequent lines of therapy in systemic AL amyloidosis: Results from an analysis of real-world longitudinal data

Short title: Natural history of AL amyloidosis

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Abstract

Systemic AL amyloidosis is an incurable disorder, and natural history is incompletely understood. In this study, we describe its natural history based on an analysis of real-world longitudinal data.

All patients seen at the National amyloidosis Centre, UK, between Feb- 2010 and Aug-2019 and treated with upfront Bortezomib are included. 1276 patients received the 1st line treatment. 259, 85, & 32 patients received 2, 3, and 4 treatment lines, respectively. 77.2% of patients requiring further treatment after the 1st line started the 2nd line within two years of the 1st line. 50.5%, 50.6%, 40.1% and 40.6% of patients had achieved ≥ VGPR after the 1st, 2nd,3rd, and 4th treatment lines. Median OS from 1st, 2nd, 3rd, and 4th lines was 45 months, 56 months, 37 months and not reached, respectively (p=0.109).

In summary, although relapses occur in AL amyloidosis, the outcomes and responses do not worsen with each subsequent relapse making it attractive to design therapeutics with curative intent.

Introduction

Systemic immunoglobulin light chain amyloidosis (AL) is a multi-system disorder associated with an underlying monoclonal B-cell or plasma cell dyscrasia. Proteasome inhibitors (Bortezomib) are the established standard of care for newly diagnosed AL amyloidosis, and it has vastly improved the outlook for these patients. (1, 2) Several other agents are also available for the treatment of AL amyloidosis. (3-6) Despite the remarkable improvement in the treatment of AL amyloidosis, it is an incurable disease, and it is believed that all patients will inevitably relapse. The true natural history of the condition, particularly concerning the longitudinal follow-up of individual patients through the disease course and progression through the different treatment lines, remains poorly studied. While therapy goals in newly diagnosed AL amyloidosis are well understood, in the relapsed setting, these are less clearly validated.

Several prospective and retrospective studies have reported outcomes of individual chemotherapy agents (or regimes) in relapsed/refractory AL amyloidosis. However, very few studies have systematically assessed patients at a specific line of treatment. Three large amyloidosis groups have published the outcomes following 2nd line treatment in their respective cohorts. (7-9) They reported varying outcomes- median OS (from 2nd line) 38.8- 66.8 months and the median time to next treatment (TNT) (from 1st line) 16.2-49 months. Only one study reported TNT from 2nd line- 31 months and OS from 3rd line-32.1 months. (9) Two of these studies found that the type of treatment at 1st relapse had no impact on OS. (7, 9) All the studies found that organ progression at the time of relapse conferred a poorer prognosis. In patients with multiple myeloma, the outcomes are progressively worse at each subsequent relapse – it is not clear that in AL amyloidosis, where the clone has less malignant potential, the same holds.

This study describes the natural history of a large cohort of AL patients

treated with upfront Bortezomib following them throughout their treatment course to last follow up or death.

Patients and methods

Patients

The ALchemy study is an ongoing, prospective, observational study of newly diagnosed AL amyloidosis seen at the National Amyloidosis Centre (NAC), UK, from April 2009. All patients in the ALchemy database treated with upfront Bortezomib are included in this analysis. All baseline and follow up investigations were performed at the National Amyloidosis Centre. Diagnosis of AL was confirmed with biopsy demonstrating amyloid deposition by Congo red staining and fibril typing confirming AL type by immunohistochemistry and/or proteomic analysis.

Investigations at baseline and follow up included serum-free light chains (sFLC), serum/urine protein electrophoresis and immunofixation, biochemical tests for organ function and cardiac biomarkers (N-terminal pro B-type natriuretic peptide (NT-proBNP) and high sensitivity cardiac Troponin T (TropT)). Echocardiography was performed on all patients at baseline and then serially. Organ involvement was defined by ICC. (10, 11) The cardiac disease stage is reported using the European modification of the Mayo 2004 staging and sub-classifying Mayo stage 3 patients into 3A (NT-proBNP <8500ng/L) and 3B (NT-proBNP ≥8500ng/L). (12) We also collected the dates of death or last known follow up and the dates, reasons, and type of any subsequent lines of therapy.

Responses were assessed at six months after each line of treatment according to the validated response criteria published by the international society of amyloidosis (ISA) - complete response (CR), very good partial response (VGPR), partial response (PR) and no response (NR), respectively. (13, 14) All response assessment was performed on an intent-to-treat basis.

Overall survival (OS) from 1st line, 2nd line, 3rd line and 4th line treatments were defined as the period from the start of cycle 1 of each line to the date of death or last known follow up, respectively. Patients who had not died were censored at their last known follow-up date.

While the ISA has defined criteria for clonal and organ progression, a consensus is lacking on definitions of progression. In practice, most patients in the UK are treated based on increasing sFLC, worsening organ function or a combination as agreed by a multidisciplinary team at the NAC.(7, 15). Therefore, we use the time to next treatment (TNT) as a surrogate for progression. We define progression free survival (PFS) from each line of therapy as the time from day 1, cycle 1 of the line to day 1, cycle 1 of subsequent therapy (TNT), death or last known follow up. We define TNT as the period from day 1, cycle 1 of previous treatment to day 1, cycle 1 of subsequent therapy, or last known follow up (deaths were censored). Patients alive without subsequent therapy were censored on the date of their last follow up.

The study is approved by the relevant institutional review board, and all patients provided informed written consent per the declaration of Helsinki.

Statistical Analysis

We performed all statistical analysis using SPSS version 26 (IBM Inc, USA). The method of Kaplan & Meier was used to generate survival curves. The two-sided log-rank test was used to assess statistical significance between survivals. All reported p values are two-sided with the conventional significance of <0.05. All analyses are on an intent to treat basis unless specified otherwise. We also performed landmark analysis at 12 months, 2 years and 5 years.

Results

Baseline

Two thousand and eleven patients were enrolled in the ALchemy study from its inception until August 2019. 1276/2011 (63.5%) patients were treated with upfront Bortezomib. The 1276 patients treated with upfront Bortezomib are included in this analysis. In the UK, Bortezomib was widely adopted as the frontline therapy for AL since 2010. The percentage of patients treated with bortezomib upfront was: 2010 – 7%; 2011 – 23%; 2012 – 46%; 2013-57% and 2014 onwards ~80% (Table SA1, Supplementary Appendix). There is no significant difference in the case-mix of the patients over time. Table I lists the baseline characteristics of the patients treated with upfront Bortezomib. The median age at diagnosis was 67 years (29-89 years). 63.4% and 68.8% of patients had cardiac and renal involvement, respectively. 15.4% of patients had advanced cardiac involvement (Mayo stage IIIB). The median NT-proBNP, urine protein, alkaline phosphatase (ALP) and difference between involved and uninvolved light chains (dFLC) was 1284.50 ng/l (range 4- 93602 ng/l), 3 gm/24 hours (range 0-36 gm/24 hours), 89.5 u/l (16-2389 u/l) and 188.45 mg/l (0-15898 mg/l), respectively.

The median follow-up of the cohort was 45 months (95% CI 42.3-47.7). 328 (25.7%) patients had died within the 1st year, 416 (32.6%) by the end of the 2nd year, and 541 (42.4%) by the end of the 5th year after the start of treatment. Figure 1A shows the number of patients alive each year during their follow up. 629 and 192 patients remained at risk at the end of 2 and 5 years, respectively. (Figure 1A).

Relapses and subsequent treatment

At the time of this analysis, 376 (29.5%) patients had received more than 1 line of treatment, 424 (33.2%) patients had died without subsequent therapy, and 476

(37.3%) patients were alive without subsequent treatment. The median follow up of the patients dying without subsequent therapy was 4 months (range 0-89 months), alive without subsequent therapy was 27.5 months (range 0-120 months) and patients who received more than 1 line was 42.5 months (range 3-121 months). The number of patients needing second or more lines of treatment are: ≥ 2 lines - 376 (29.5%); ≥ 3 lines - 117 (9.2%); ≥ 4 lines - 32 (2.5%); ≥ 5 lines - 8 (0.6%) and six or more - 2 (0.2%) (Figure 1B). Of these, the patients who received *only* two lines of treatment were 259, received three lines of treatment were 85 and received four lines were 32, respectively. 77.2% of patients receiving 2 or more treatment lines had received the 2nd line within two years of the 1st line therapy (Figure 1B). Table II lists the number of patients in each Mayo stage who received 2, 3 and 4 lines of treatment, respectively. Due to early deaths, a significantly fewer proportion of patients with advanced Mayo stage (IIIb) received salvage therapy- 10.2% of Mayo IIIb received 2 lines vs 21.4% of Mayo I; 1% of Mayo IIIb received 4 lines vs 4.5% of Mayo I.

The median dFLC at the start of the 2nd, 3rd, and 4th lines of treatment was 91.5 mg/l (48.6% of baseline dFLC), 96.5 mg/l (51.2% of baseline dFLC) and 136.4 mg/l (72.4% of baseline dFLC). The other baseline characteristics at the start of the 2nd, 3rd and 4th lines of treatment are available in the supplementary appendix (Table SA2).

Lenalidomide was the commonest agent used in 2nd (46.5%) and 3rd (42.6%) lines, followed by Daratumumab (13.3 and 22.2%). Daratumumab was the commonest agent used in the 4th line (43.8%). A detailed breakdown of the agents used is available in the supplementary appendix (Table SA3).

Haematologic or organ progression was the commonest reason for initiation of therapy in all lines (64.6%, 51.2% & 65.6% in 2nd, 3^{rd,} and 4th lines), followed by an inadequate response (30.1%, 40.2%, & 31.3% in 2nd, 3rd, and 4th lines)

(Supplementary Appendix Table SA4).

The proportion of patient who experienced organ progression at starting of 2nd, 3rd and 4th line treatments were: 125/259 (48.3%), 32/85 (37.6%) and 16/32 (50%), respectively. The proportion of patients who died within 12 months of starting of 2nd, 3rd and 4th line treatments were: 68/259 (26.25%), 18/85 (21.2%) and 5/32 (15.6%), respectively. Of these early deaths, the patients who had organ progression after 2nd, 3rd, 4th line treatment respectively were: 40/68 (58.8%), 14/18 (77.8%) and 5/5 (100%), respectively.

Haematologic response

Table III describes the haematologic response after the 1st, 2nd, 3rd, and 4th lines of treatment. The overall response rate (CR+VGPR+PR) was 67.5%, 59.6%, 52.1% and 56.2% following 1st, 2nd, 3rd, and 4th lines of treatment. The proportion of patients achieving ≥ VGPR was 50.5%, 50.6%, 40.1% and 40.6% after the 1st, 2nd,3rd, and 4th lines of treatment. The proportion of patients with no response was higher in the 2nd/3rd/4th lines than after the 1st line- 37.2%/41%/37.5% vs 27.8%. Using logistic regression, the time to next treatment from 1st line did not impact the depth of haematologic response (≥ VGPR vs < VGPR) at 2nd line (HR 0.966, 95% CI 0.951-0.983, $p < 0.005$).

Survival

The median overall survival of the entire cohort was 56 months (95% CI 47.57-64.42 months) (Figure 2A). The median PFS of the entire cohort was 20 months (95% CI 17.39-22.60 months) (Figure 2B). In the ITT cohort, due to the high burden of early deaths (<12 months), the survival of patients who received more than one line of treatment was significantly better- median OS 78 months (95% CI 64.45-91.54 months) vs 45 months (95% CI 35.36-54.64 months) for patients who only received one line of treatment (this included those who died before receiving a

subsequent line of treatment) (P < 0.005) (Figure 2C). In a landmark analysis at 12 months (to overcome the bias from early deaths), there was no significant difference in survival between these two groups of patients - median OS 87 months (95% CI 71.40-102.59 months) vs 89 months (p=0.135) (Figure 2D).

In the intent to treat cohort, the probability of ≥ 1-year survival was 74%, ≥ 5-year survival was 48%, and ≥ 10-year survival was 29%. A landmark analysis was conducted for the probability of survival of patients alive at 2 and 5 years from diagnosis. In a landmark analysis of patients alive at 2 years from diagnosis, the probability ≥ 5-year survival was 74%, and ≥ 10-year survival was 45%. In a landmark analysis of patients alive at five years from diagnosis, the probability of surviving beyond ten years was 61%.

We analysed the overall survival from each line of treatment. There was no significant difference in overall survival from the start of the 1st, 2nd, 3rd and 4th line of treatment. Median OS from 1st line was 45 months (95% CI 35.36-56.64 months), from 2nd line was 56 months, from 3rd line was 37 months (95% CI 23.80-50.19 months) and not reached from 4th line (p=0.109) (Figure 3).

The cohort included 184/1276 (14.4%) patients with an FLC ratio of ≥ 100 at presentation. The median overall survival of patients with a ratio ≥100 was 32 months compared to 58 months for those with an FLC ratio < 100. In both cohorts, due to early deaths, the outcomes were better for patients who received >1 line of treatment (p < 0.005, Figures SA1 & SA2 in Supplementary Appendix). However, in the patients with an FLC ratio ≥ 100 early deaths were markedly greater (37% died ≤ 12 months) compared to those with an FLC ratio <100 (24.9 % died ≤12 months). Therefore, we performed the same analysis in the 12-month landmark cohort. In the 12-month landmark cohort, there was no significant difference in survival based on lines of treatment (> 1 line vs 1 line only) between the two FLC groups (p= 0.070 for FLC ratio < 100 and p= 0.638 for FLC ratio ≥ 100 (Figures

SA3 & SA4 in Supplementary Appendix).

We also compared the survival of patients who received more than 1 line of treatment with those who did not receive any subsequent therapy based on their Mayo stage (European modification) at diagnosis. In the ITT cohort, 220, 435, 425, and 196 patients were in Mayo stage I, II, IIIa & IIIB at diagnosis. For patients receiving 1 line of treatment vs > 1 line of treatment, the outcomes for Mayo stage II, IIIa and IIIb were similar to the ITT cohort previously described, but there was no significant difference in survival in the Mayo stage 1 patients based on the lines of treatment (p=0.089) (Figures SA5-8 in Supplementary Appendix). To overcome the bias of early deaths, we repeated the analysis in a 12-month landmark analysis - 190, 333, 270, and 72 patients were in Mayo stage I, II, IIIa & IIIb, respectively). Similar to the previous analysis, there was no difference in survival in Mayo stage II, IIIa and IIIb patients receiving > 1 line vs only 1 line of treatment. However, in the landmark cohort, patients with Mayo stage I disease receiving >1 line of treatment had significantly poorer outcomes compared to those who receive only 1 line of treatment (p=0.0001)(Figures SA9-12 in Supplementary Appendix).

Impact of haematologic response (after 2nd/ 3rd line) on outcomes.

The depth haematologic response after 2nd line treatment was predictive of both survival from the start of 2nd line treatment and TNT after 2nd line treatment (Figure 4A & 4B). Patients reaching a haematologic CR or VGPR had significantly better survival than those with a PR or NR- median OS not reached / 57 months vs 47 months (95% CI 18.4-75.59 months) / 19 months (95% CI 11.86-26.13 months) ($p < 0.005$). Patients with CR had significantly better survival when compared to VGPR ($p = 0.019$) (Figure 4A). Patients with CR or VGPR had a significantly longer TNT than those with a PR or NR- median TNT not reached / 49 months vs 30 months (95% CI 11.46-48.54 months) / 19 months (95% CI 9.86-23.13 months) ($p < 0.005$). There was no difference in TNT between CR and VGPR ($p = 0.469$) (Figure 4B).

Haematologic response after 3rd line treatment was also predictive of survival from the start of 3rd line therapy. Patients with CR or VGPR had significantly better survival than those with a PR or NR- median OS not reached / non reached vs 31 months (95% CI 15.52-46.47 months) / 19 months (95% CI 16.85-21.14 months) ($p < 0.005$). There was no difference in survival between CR and VGPR ($p = 0.596$) (Figure SA13 in Supplementary Appendix). Patients with CR or VGPR after the 3rd line also had a significantly longer TNT than those with PR/NR- median TNT 32 months (24.46-39.53) / 44 months vs 36 months / 13 months (95% CI 5.11-20.88 months) ($p=0.008$). There was no difference in TNT between CR and VGPR ($p = 0.436$) (Figure SA14 in Supplementary Appendix).

Too few patients received 4th line treatment to conclude the impact of haematologic response on survival or TNT.

Discussion

This study maps the life treatment journey of a large cohort of AL patients. The data shows that the majority of patients with AL need further treatment and three-quarters of all patients needing 2nd line treatment appear to do so with the initial two years of therapy. Each subsequent treatment line still achieves deep and durable responses, strikingly, with little difference in outcomes for those needing the 2nd line and beyond. Deeper responses at each line are associated with better overall survival and prolonging time to the next treatment.

AL Amyloidosis is an incurable disorder with a relapsing-remitting course. Other groups and we have previously shown that the depth of haematologic response after the 1st line is a predictor for the length of treatment-free survival. (7, 8, 16) 37.3% patients in this cohort have not required subsequent treatment after a median follow up of 27.5 months from frontline Bortezomib, showing that durable responses are possible. A substantial number of patients who needed further treatment needed

this treatment for an inadequate response rather than a true relapse. The efficacy of the frontline regimes will impact this metric. When more widely adopted, the recently licensed combination, daratumumab-VCD, may reduce the requirement for moving to second-line treatment due to inadequate response. We found that patients with an sFLC ratio ≥ 100 at diagnosis had significantly poorer outcomes in the initial 12 months due to a very high proportion of early deaths, as previously reported by the Mayo group (higher plasma cell burden equals greater cardiac involvement). (17) However, beyond the initial 12 months, in the landmark analysis, the sFLC ratio (≥ 100 or <100) did not directly impact outcomes suggesting that the behaviour of the clone still follows a more benign pattern than true symptomatic myeloma. However, the numbers are small, and we do not have the baseline bone marrow plasma cell percentages – which remains a limitation in interpreting these results. This data may have enabled us to understand the clonal biology that facilitates such a durable response to proteosome inhibitor-based treatments and allow for appropriate treatment selection.

The present study shows that depth of the haematologic response criteria based on the criteria published by the ISA for newly diagnosed AL amyloidosis remain useful in predicting both survival and TNT following 2nd and 3rd line therapy. Patient achieving ≥ VGPR had the best long term outcomes even in salvage therapy and should remain the ideal goal of therapy even in relapsed disease.

In patients with relapsed refractory myeloma, both survival and length of response consistently decrease with each further line of treatment. (18, 19) The survival of relapsed/refractory multiple myeloma is poor, with a reported OS of 13-27 months after the 3rd line and less than 12 months for quad or Penta-refractory patients. (20, 21) In contrast, a key observation from this study is that there is no significant difference in the overall response rate or survival from 1st, 2nd, 3^{rd,} and 4th lines of therapy in patients with AL amyloidosis who do not succumb to early cardiac deaths.

This counter-intuitive finding in AL amyloidosis is due to a cardinal feature of disease – two separate processes are going on in AL – the relapse of the plasma cell clone (which itself does not cause any problems) and organ damage from the amyloidosis (which causes symptoms and deaths). The latter (especially cardiac involvement) leads to very deaths in the early months following diagnosis, which is very different from symptomatic multiple myeloma, where progressive clonal evolution and treatment refractoriness leads to a higher proportion of deaths in multiply relapsed patients. A patient with AL amyloidosis is closer to death at presentation than a myeloma patient who is nearer death at 4^{th} or subsequent line of treatment. In AL, there are deaths following each line of therapy. We also show that any patients with organ progression at relapse were more likely to die in year following treatment for relapse than those treated before organ progression. These data confirm a previous report from the Mayo group. This is an important clinical message to consider initiation of treatment for relapse before organ progression has ensued.

Real-world studies in multiple myeloma have shown that 61%, 38%,15% and 1% of patients receive 2, 3, 4 and 5 lines of treatment. (18) The corresponding number of patients receiving 2, 3, 4 and 5 lines of treatment in the current study was 29.5%, 9.2%, 2.5% and 0.6%. The possible reasons for the lower number of patients receiving subsequent therapies in AL include high early mortality and long duration of remission in patients achieving a deep response. Whilst the median follow-up of this cohort is over 4 years, this is not long enough to capture the full natural history of AL amyloidosis due to prolonged remissions and TNT after each line of therapy.

The results of the present study have direct implications for clinical practice. The median dFLC at the start of 2^{nd}, 3^{rd} and 4^{th} lines in this cohort was ≥ 90 mg/L and ≥ 50% of the baseline dFLC; leading to organ progression in half of all patients with significant early mortality (~20-25%) at the start of each subsequent line of

treatment, primarily due to organ progression. These results strongly suggest that it is important to initiate salvage treatment early and before any organ progression. Secondly, outcomes of patients with deeper responses at each line were better, showing a need to select treatment regimens more likely to achieve a deep clonal response.

We acknowledge the limitations of the present study. This study is a retrospective analysis, and we do not have baseline bone marrow findings. Most patients in the UK are re-treated based on increasing sFLC, worsening organ function or a combination after discussion in a multidisciplinary meeting rather than on reaching a fixed FLC threshold. Due to lack of clarity on the definition of "progression free survival", we have used the time to next treatment (TNT) as a surrogate for progression. Since Troponin T is not routinely measured in the UK following initial diagnosis, we lack the data to analyse the impact of the cardiac stage on outcomes in the setting of treatment for relapse.

In summary, this study shows that although relapses occur in AL amyloidosis, responses are durable even in relapsed disease. Depth of response remains predictive of outcomes after each line of treatment, and achieving deep responses should remain a focus in the development of novel therapeutics. Patients with organ progression at relapse have poorer outcomes highlighting the importance of early detection of relapse and initiation of treatment. Further study of clonal and disease characteristics is needed to understand factors predicting long term remission in AL to allow the design of curative treatment strategies.

Authorship declaration

SR collected/analysed the data and wrote the manuscript.

OC, SL, DF, MF, AMN, CW, JDG, HJL, SS, SM and PNH reviewed and approved the final manuscript.

ADW supervised the study, reviewed, and approved the final manuscript.

Conflict of Interest declaration

ADW has received honoraria from Janssen, GSK, Celgene, and Takeda. The other authors do not have any conflict of interest to disclose.

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Table I: Baseline characteristics (n=1276)

Characteristics	**n (%) or Median (Range)**
Age	67 years (29-89 years)
Gender (Male/Female)	756 (59.2%) / 520 (40.8%)
Organ involvement	
Cardiac	809 (63.4%)
Renal	878 (68.8%)
Liver	154 (12.1%)
Gastrointestinal (GI)	49 (3.8%)
Peripheral nervous system	88 (6.9%)
Autonomic nervous system	85 (6.7%)
Soft tissue	190 (14.9%)
Performance status	
ECOG 0-2	1196 (94)
ECOG>2	80 (6)
Mayo stage (European modification(12))	
Stage I	220 (17.2%)
Stage II	435 (34.1%)
Stage IIIa	425 (33.3%)
Stage IIIb	196 (15.4%)
NT-proBNP	1284.50 ng/l (4-93602 ng/l)
High sensitivity cardiac troponin T	54 ng/l (1-742 ng/l)
Creatinine	97 µmol/l (26-1124 µmol/l)
Proteinuria	3 g/24 hours (0-36 gm/24 hours)
ALP	89.5 u/l (16-2389 u/l)
Left ventricular septum	13 mm (6-24 mm)
dFLC	188.45 mg/l (0*-15898 mg/l)
Monoclonal protein serum	8 gm/l (1-45 gm/l)
Involved light chain isotype	
Kappa	267 (20.9%)
Lambda	1009 (79.1%)
Serum Immunofixation	
IgA	176 (13.8%)
IgD	9 (0.7%)
IgG	429 (33.6%)
IgM	43 (3.4%)
Light chain only	312 (24.5%)
None	307 (31.1%)

NYHA, New York Heart Association; NT-proBNP, N-terminal pro B-type natriuretic peptide; GFR, glomerular filtration rate; dFLC, difference between involved and uninvolved light chains * Two patients with dFLC < 20 mg/l had same involved and uninvolved light chain level

Table II: No of patients receiving 2, 3 & 4 lines of treatment in each Mayo stage

Mayo stage	2 lines n(%)	3 lines n(%)	4 lines n(%)
Mayo stage I n=220	47 (21.4)	18 (8.2)	10 (4.5)
Mayo stage II n=435	111 (25.5)	37 (8.5)	12 (2.8)
Mayo stage III n= 425	81 (19)	25 (5.9)	8 (1.9)
Mayo stage IIIb n=196	20 (10.2)	5 (2.6)	2 (1)

Table III: Haematologic response after 1st, 2nd, 3rd and 4th lines of treatment

	CR n (%)	VGPR n (%)	PR n (%)	NR n (%)	NA n (%)
1st line	326 (25.6)	319 (25)	217 (17)	355 (27.8)	59 (4.6)
2nd line	66 (17.6)	124 (33)	34 (9)	140 (37.2)	12 (3.2)
3rd line	21 (18)	26 (22.2)	14 (12)	48 (41)	8 (6.8)
4th line	5 (15.6)	8 (25)	5 (15.6)	12 (37.5)	2 (6.3)

CR, complete response; VGPR, very good partial response; PR, partial response; NR, no response; NA, not available

Figure Legends

Figure 1A: This shows the number at risk, deaths, and number without any treatment after 1st line. 328 (26%) patients had died at the end of 1 year from 1st line treatment. 692 and 192 patients were at risk at the end of 2 and 5 years, respectively.

Figure 1B: The distribution of 2nd line treatment and beyond in AL amyloidosis. 376, 117, 32, 8 and 2 patients received 2, 3, 4, 5 and 6 lines of treatment. 77.2% patients received the 2nd line within two years of their 1st line treatment.

Figure 2A: Kaplan-Meier curve showing the OS of the entire cohort. The median OS of the cohort was 56 months (95% CI 47.57-64.42 months).

Figure 2B: Kaplan-Meier curve showing the PFS of the entire cohort. The median PFS of the cohort was 20 months (95% CI 17.39-22.60 months).

Figure 2C: Kaplan-Meier curve showing the OS of the ITT cohort based on the lines of treatment received (> 1 line vs only 1 line). Patients receiving > 1 line of treatment had a significantly better survival than those who did not receive any therapy after their 1st line- median OS 78 months (95% CI 64.45-91.54 months) vs 45 months (95% CI 35.36-54.64 months) ($P < 0.005$).

Figure 2D: Kaplan-Meier curve showing the OS of the 12-month landmark cohort based on the lines of treatment received (> 1 line vs only 1 line). There was no significant difference in survival between the two groups- median OS 87 months (95% CI 71.40-102.59 months) vs 89 months (p=0.135) (Figure 2D).

Figure 3: Kaplan-Meier curve showing the OS from 1st, 2nd, 3rd, and 4th lines of treatment. There was no significant difference in survival from 1st, 2nd, 3rd, and 4th lines of treatment. Median OS from 1st line was 45 months (95% CI 35.36-56.64 months), from 2nd line was 56

months, from 3rd line was 37 months (95% CI 23.80-50.19 months) and not reached from 4th line (p=0.109).

Figure 4A: Kaplan-Meier curve showing the impact of haematologic response after 2nd line on OS after 2nd line treatment. Patients with CR or VGPR had a significantly better survival than those with a PR or NR- median OS not reached / 57 months vs. 47 months (95% CI 18.4-75.59 months) / 19 months (95% CI 11.86-26.13 months) ($p < 0.005$). Patients with CR had a significantly better survival when compared to VGPR ($p = 0.019$).

Figure 4B: Kaplan-Meier curve showing the impact of haematologic response after 2nd line on TNT after 2nd line treatment. Patients with CR or VGPR had a significantly longer TNT than those with a PR or NR- median TNT not reached / 49 months vs. 30 months (95% CI 11.46-48.54 months) / 19 months (95% CI 9.86-23.13 months) ($p < 0.005$). There was no difference in TNT between CR and VGPR ($p = 0.469$).

Figure 1A

Temporal profile of number at risk, deaths and number without any treatment after 1st line

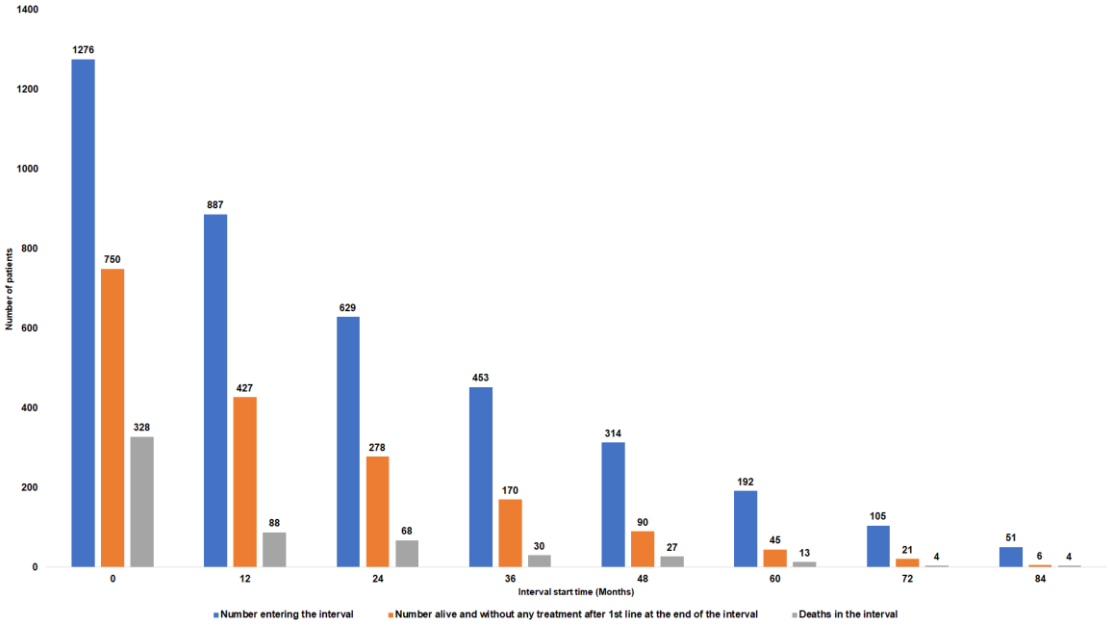


Figure 1B

Temporal distribution of 2nd line treatment and beyond in AL amyloidosis

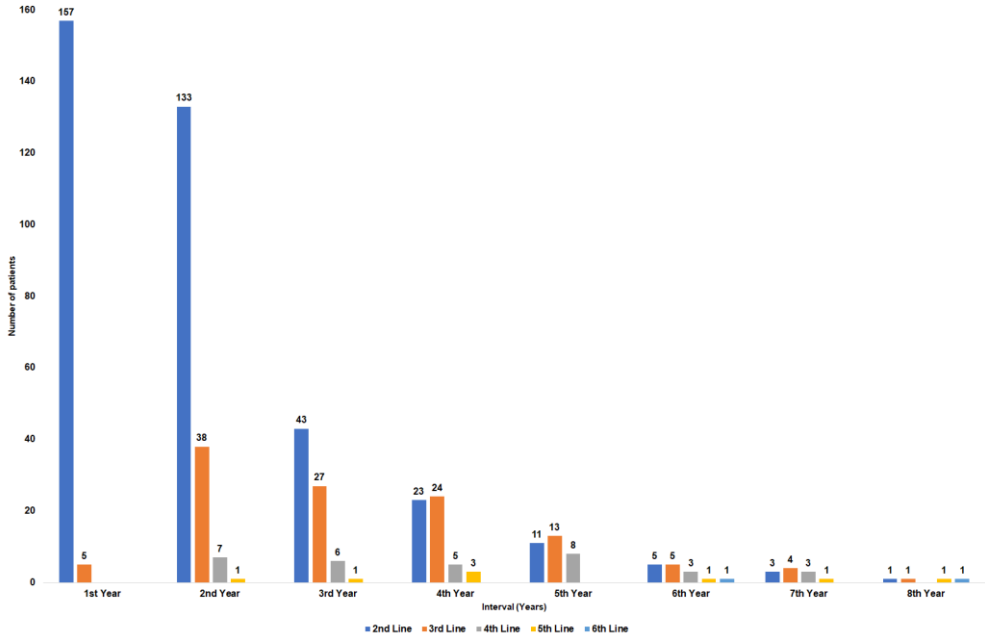


Figure 2A

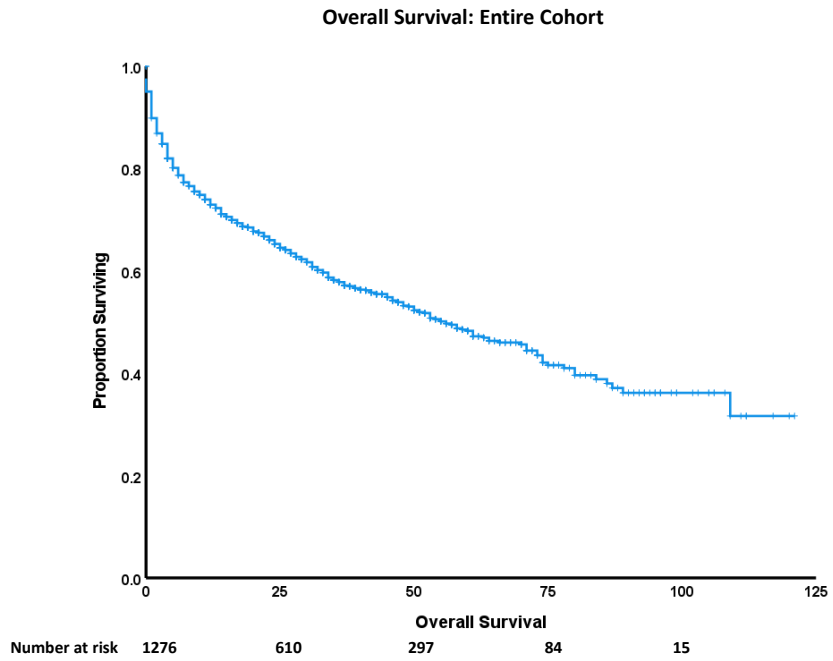


Figure 2B

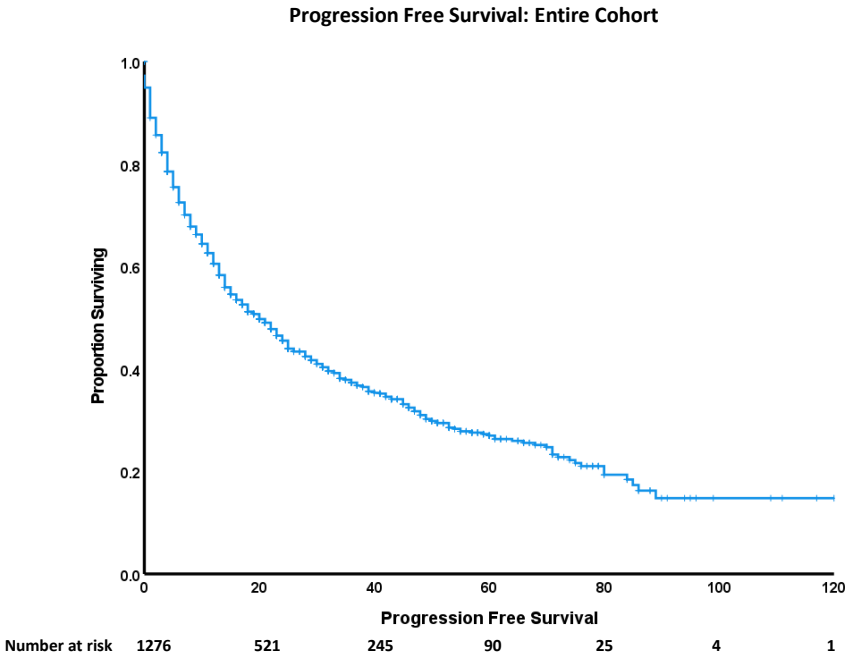
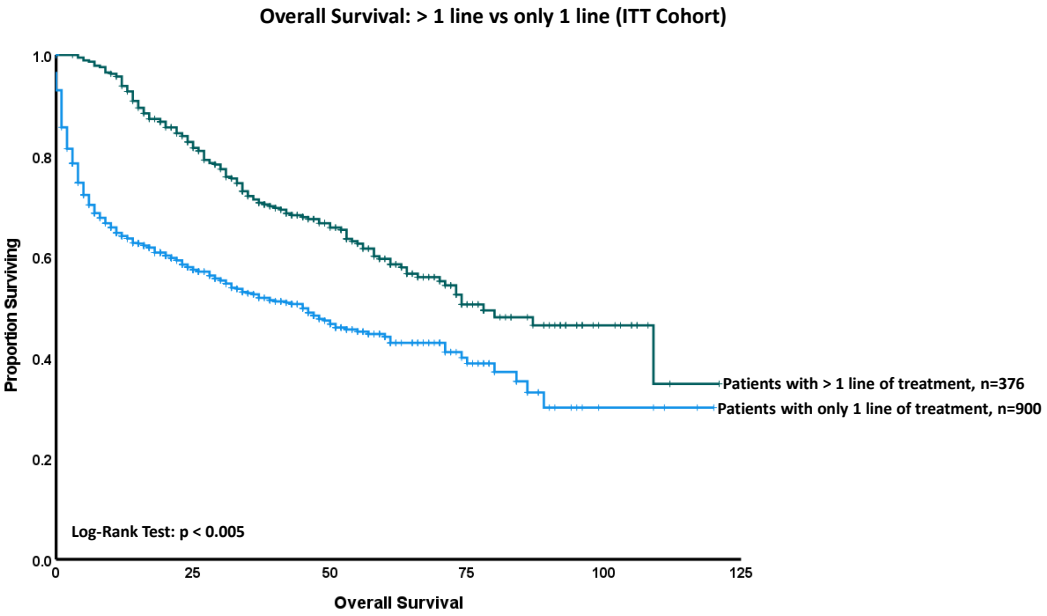
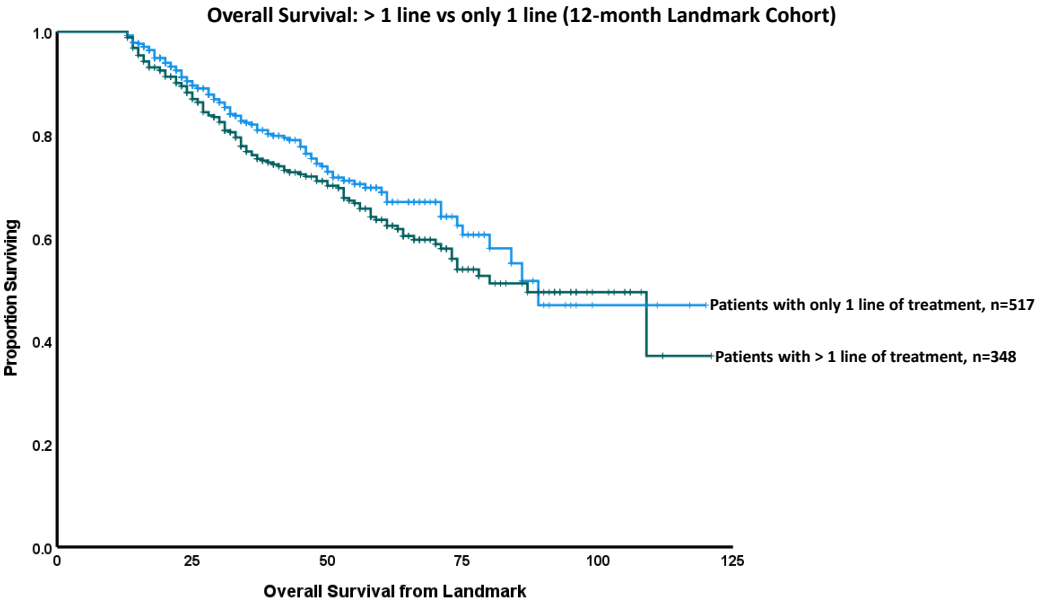


Figure 2C



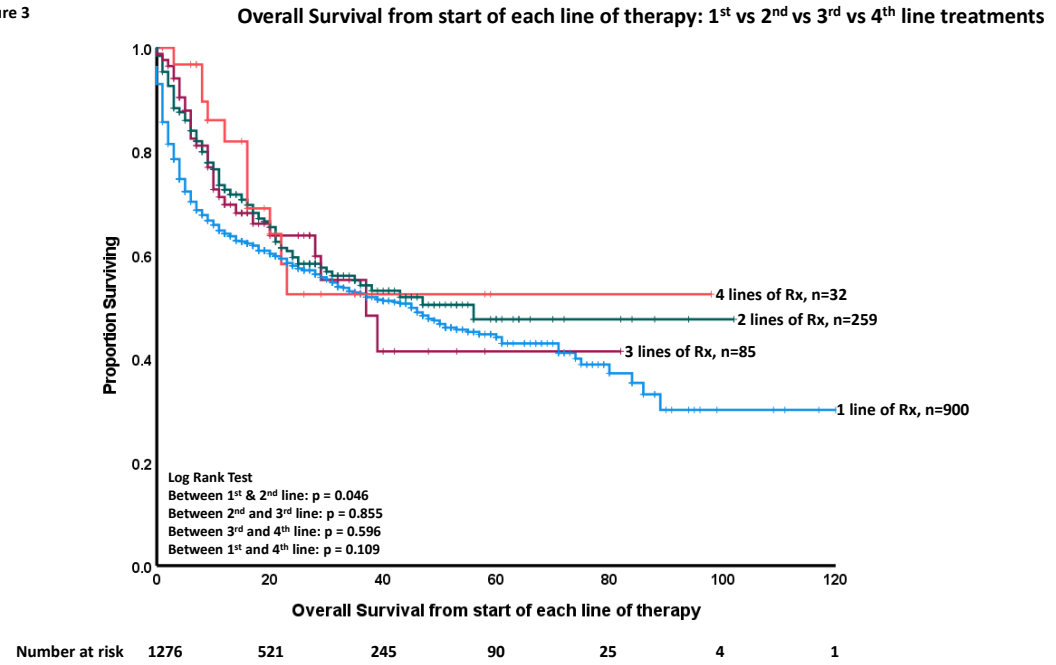
Number at risk	1276	610	297	84	15

Figure 2D



Number at risk	1276	865	610	297	84	15

Figure 3



Number at risk	1276	521	245	90	25	4	1

Figure 4A

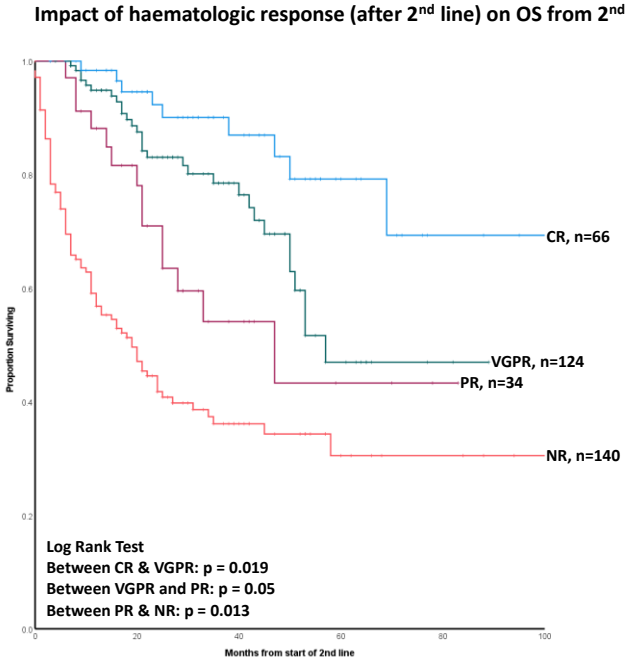


Figure 4B

Impact of haematologic response (after 2nd line) on TNT from 2nd line