Title

Complete responses in AL amyloidosis are unequal – the impact of free light chain mass spectrometry in AL amyloidosis

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Running title

FLC-MS in AL Amyloidosis

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Key Points

- FLC-MS can detect persistent light chains in a significant proportion of patients in a conventional haematological complete response
- Patients with no detectable FLC by FLC-MS have significantly better overall survival and organ response irrespective of conventional haematological response

Abstract

Amyloidogenic serum free light chains (sFLC) drive disease progression in AL amyloidosis. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry-based free light chain assay (FLC-MS) has greater sensitivity than conventional sFLC assays allowing for the detection of serological residual disease. We report the utility of FLC-MS in a large series of patients with AL amyloidosis assessing the impact of FLC-MS negativity after treatment on overall survival and organ response rates.

Serum samples were analysed using FLC-MS at diagnosis and 6-, and 12-months post-treatment. The impact of FLC-MS negativity over standard haematological responses on survival and organ response was assessed.

487 patients were included; 290 (59%) & 349 (71.5%) had cardiac and renal involvement, respectively. There was 100% concordance between the light chain (LC) fibril type and LC-isotype identified by FLC-MS.

At 6- and 12-months, 81 (16.6%) and 101 (20.7%) were FLC-MS negative. Of those achieving a conventional haematological complete response (CR) at 6- and 12-months, 42 (26.4%) and 64 (39%) were FLC-MS negative. At 12 months, median overall survival for CR+FLC-MS negative was not reached vs 108 months in CR+FLC-MS Positive (p=0.024). At 12 months, 70% of FLC-MS negative (vs. 50% FLC-MS positive) patients achieved a cardiac response (p=0.015). In a multivariate analysis, FLC-MS negativity at 12 months was an independent predictor of better outcomes.

FLC-MS can detect persistent monoclonal light chains in a significant proportion of patients in a conventional haematological CR. FLC-MS assessment promises to be a new standard for response assessment in AL amyloidosis.

Introduction

AL amyloidosis is a condition whereby abnormal light chains, produced by an underlying clonal plasma cell/B-cell disorder, misfold and form insoluble amyloid fibrils. Deposition and accumulation of amyloid fibrils in multiple organs results in progressive organ dysfunction[1]. The abnormal amyloidogenic free light chains (FLC) drive the disease in AL amyloidosis. In the absence of therapies targeting the removal of amyloid fibrils, treatment is directed towards the amyloid-producing clonal disorder with the aim of reducing or eliminating FLC production, reducing proteotoxicity and allowing for natural macrophage-led amyloid regression[2]. The depth of hematological response directly correlates with outcomes in AL amyloidosis[3]. Patients who achieve a complete response (CR) have best outcomes and, lately, minimal residual disease (MRD) negativity on bone marrow has been correlated with even better outcomes[4].

The greatest step change in the diagnosis and monitoring of AL amyloidosis was the development of assay(s) to measure serum FLC. This continues to be the backbone of response assessment in AL[3]. The main limitation of all FLC assays is the lack of ability to directly measure the monoclonal FLC; this is inferred indirectly by a skew in the kappa:lambda ratio. The interpretation of FLC levels is further limited by the impact of a loss of linearity at low concentrations and the impact of renal dysfunction,[5] which causes polyclonal retention of FLC, a common problem in AL amyloidosis. With the availability of agents that achieve very deep responses, these flaws become critically limiting in AL amyloidosis. A persistent low level monoclonal FLC, undetectable by current FLC assays, have the potential to cause significant proteotoxicity and lead to ongoing amyloid deposition with progressive organ dysfunction –despite the response being assessed as a conventional haematological CR.

Recently, detection of monoclonal proteins by matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry (MALDI-TOF MS) has been a major advance in monoclonal protein detection and monitoring in plasma cell disorders. MALDI-TOF MS was adopted by the International Myeloma Working Group as a potential surrogate for immunofixation electrophoresis (IFE)[6-9]. The standard intact light chain MALDI-TOF MS assays that have been developed (MASS-FIX and EXENT) detect *total* light chains (i.e. light chains bound to intact immunoglobulins as well as the FLC together), which will have reduced sensitivity compared to FLC specific assays[9,10]. Therefore, these assays may have limited utility in AL amyloidosis where the FLC is the disease driver.

We have previously reported a small series of patients analysed by MALDI-TOF MS using FLC specific reagents (FLC-MS) for detecting the presence of a monoclonal FLC[11-13]. The Mayo clinic group found that residual disease was detectable in 2/33 (6.1%) and 4/33 (12.1%) patients in haematological CR by MASS-FIX (an assay that does not include FLC specific reagents) and liquid-chromatography mass spectrometry (LC-MS) respectively. These studies reported patients, in CR by standard criteria, still showed evidence of a monoclonal light chain by mass spectrometry (MS), highlighting persistent residual disease[11,14]. The impact of this low-level persistent disease on survival and organ response was not assessed although the presence of residual disease by MS was associated with a poorer time to progression at 50 months of 75% vs. 13% (p=0.003)[14].

We report here the utility of FLC-MS in serial assessments for a large series of patients with systemic AL amyloidosis with long follow up showing the impact of achieving FLC-MS negativity after treatment on overall survival (OS) and organ-specific response rates.

Methods

All patients from a prospective observational study of newly diagnosed AL amyloidosis (ALCHEMY) seen at the UK National Amyloidosis Centre (NAC) who had serum samples stored at baseline, 6 months and 12 months post diagnosis were included. The diagnosis of AL amyloidosis was confirmed by central review of histological material. Amyloid subtype was identified by immunohistochemistry with specific antibodies, or by laser capture microdissection and tandem MS, as appropriate. All patients underwent a protocolised serial assessment including assessment of organ function, clonal parameters and serial echocardiography. Serum FLC were analysed with the Freelite™ assay (The Binding-Site, Birmingham, UK) at diagnoses, 6- and 12-months. Amyloidotic organ involvement, haematologic responses to chemotherapy, and organ responses were defined as per the International Society of Amyloidosis (ISA) consensus criteria [3,15-17]. A CR was defined as the absence of a monoclonal protein in serum/urine by protein electrophoresis/immunofixation and the normalisation of the FLC ratio or where the uninvolved FLC was greater than the involved FLC (iFLC). A further analysis based on the CR definition used in the Andromeda trial was also undertaken. This was defined as an iFLC level less than the upper limit of the normal range with negative serum and urine immunofixation.[18] All patients were treated with a Bortezomib-based chemotherapy regimen (none with daratumumab upfront).

FLC-MS

Serum samples were analysed by FLC-MS at baseline, 6- and 12-months post diagnosis. Commercially available paramagnetic microparticles were covalently coated with polyclonal sheep antibodies specific for human kappa FLCs (anti-free κ)

and lambda FLCs (anti-free λ) (The Binding-Site, Birmingham, UK). The microparticles were incubated with patient sera, washed and then eluted with acetic acid (5% v/v), containing tris(2-carboxyethyl)phosphine (TCEP) (20 mM). Mass spectra were acquired on a Microflex LT/SH smart MALDI-TOF mass spectrometer (Bruker, GmbH) over a mass range of 5000-32000 Da. Spectra were visually inspected in the mass-to-charge (*m/z*) region for the doubly charged light chain (*m/*z 11000-13000) and singly charged light chain (*m/*z 22000-26000). Reviewers were blinded to the results of the assessments using standard techniques at the time of analysing the mass spectra. The presence of N-linked glycosylation was identified by the detection of polytypic peaks with mass differences between the peaks consistent with N-linked glycans. Confirmation that these polytypic peaks represented glycosylated FLC was also obtained by incubating samples from five of the patients with PNGase F, which is an enzyme which cleaves N-linked glycans[19]. Patients with evidence of a monoclonal spike were denoted as 'FLC-MS positive' and those without a monoclonal spike were denoted as 'FLC-MS negative'.

Statistical analysis

Statistical analysis was performed using SPSS version 27. Approval for analysis and publication was obtained from the National Health Service institutional review board; written consent was obtained from all patients in accordance with the Declaration of Helsinki. The Kaplan-Meier method was used to analyse survival outcomes. The analysis in this series is automatically a landmark analysis since only patients with baseline and two follow up samples at 6 and 12 months were included. Multivariate modelling by Cox regression analysis was performed on factors found to significantly impact survival on univariate analysis. All factors that were significant in univariate modelling were studies in various multivariate models. Mayo staging

system (European modification of 2004 and Mayo 2012) were studied in separate models. Two tailed unpaired t-tests were used to compare continuous variables whilst analysis of variance (ANOVA) was used when >2 variables were included. All p values were 2-sided with a significance level of <0.05.

Data Sharing Statement

For original data, please email a.wechalekar@ucl.ac.uk

Results

Baseline characteristics

A total of 487 patients were included in this study. The baseline characteristics are outline in **Table 1**. 291 (59.6%) patients were male with median age at diagnosis of 67 (range 36 – 88 years). There was cardiac and renal involvement in 290 (59.4%) and 349 (71.5%) patients, respectively, with 256 (52.4%) having 2 or more organs involved. Cardiac disease stage (by European Modification of Mayo 2004 staging[20]) was 1, 2, 3a and 3b in 101 (20.7%), 177 (36.5%), 168 (34.5%) and 39 (8%) patients respectively. The ECOG performance status was ≥ 2 in108 (22.2%). Median iFLC at diagnosis was 197mg/l (range 11.6 – 15900mg/l) and median difference between the involved and uninvolved FLC (dFLC) was 177.7mg/l (range 0–15898mg/l).

Patients who were female and of a Kappa isotype were more likely to become FLC-MS negative at any point following therapy. Patients with a higher iFLC or dFLC at baseline were less likely to become FLC-MS negative, possibly reflecting a higher disease burden at baseline, although not statistically significant. There was no difference in disease severity based on Mayo staging between FLC-MS positive and negative patients.

Concordance between FLC-MS and FLC by standard assays

483 (99.2%) patients had a monoclonal FLC peak identified using FLC-MS. 4 patients had no clear monoclonal FLC isotype identified by FLC-MS including 2 patients who had an atypical spectrum, 1 had no detectable clone in serum by FLC-MS and Freelite assay but identified as AL Lambda type on a renal biopsy by laser capture MS and 1 patient had an insufficient volume for diagnostic testing. In the assessable patients, FLC isotype detected by FLC-MS was 100% concordant with

amyloid fibril type on tissue biopsy (4 patients as above were not assessable). 26 (5.3%) patients had a dual Kappa and Lambda isotype identified by FLC-MS. The presence of N-linked glycosylation was identified in 73 (15%) patients of which 39/90 (37.8%) were Kappa and 34/396 (8.6%) were Lambda.

Haematologic response and FLC – MS

At the 6- and 12-month, by standard ISA criteria, a haematological CR was seen in 162 (33.2%) and 164 (33.7%) patients, a very good partial response (VGPR) in 165 (33.9%) and 169 (34.7%) and partial response (PR) or less in 160 (32.8%) and 154 (31.6%) respectively. By FLC-MS, 81 (16.6%) and 101 (20.7%) at 6- and 12-month were FLC-MS negative. Of those achieving a CR, at 6- and 12-months, 45 (27.7%) and 64 (39%) were FLC-MS negative (**Fig 1**), respectively. At 12 months only 87/164 (53.0%) patients were in a CR by Andromeda criteria and by ISA criteria of which 40/87 (46.0%) were FLC-MS negative. At 6- and 12-months respectively, FLC-MS negativity was noted in 31 (18.8%) and 26 (15.3%) for those in VGPR and in 5 (3.1%) and 11 (7.1%) for those achieving PR or who were non-assessable by standard FLC.

Of the patients in a VGPR and FLC-MS negative, at 6- and 12-month time points, 2 (5.8%) and 2 (7.7%) had a dFLC >40 mg/l;18 (52.9%) and 17 (65.4%) had dFLC >10 but <40 mg/L; 14 (41.2%) and 7 (26.9%) had dFLC <10 mg/L. At 6 and 12 months 14 (41.2%) and 16 (61.5%) had the presence of an intact paraprotein detected by immunofixation and 1 (2.94%) and 3 (11.5%) had a BJP detected. At 6 and 12 months, 16 (47.1%) and 9 (34.6%) of patients had no evidence of BJP or paraprotein on IFE but were considered a VGPR due to an abnormal FLC ratio. Data for patients classed as PR by ISA criteria appear in the supplementary material.

A total of 199 and 167 patients, at 6- and 12-months, achieved a dFLC <10mg/l of which 50 (25.1%) and 48 (28.7%) were FLC-MS negative. Similarly, an iFLC <20mg/l was seen in 154 and 101 patients at 6- and 12-months with FLC-MS negativity in 45 (29.2%) and 38 (37.6%), respectively.

Organ response

290 (59.5%) patients had cardiac involvement at diagnosis with 261 and 237 assessable for cardiac response at 6 and 12 months, respectively. Overall, at 6- and 12-months, 71/261 (27.2%) and 128/237 (54%) had a cardiac response. In patients reaching FLC-MS negativity, 15/39 (38%) and 31/44 (70%) had a cardiac response at 6- and 12-months respectively (p=0.08 and p=0.015 vs. FLC-MS positive). In accordance with the new graded cardiac response criteria[21], a cardiac-CR in FLC-MS negative patients (vs. positive) at 12 months, was achieved in 19.6% (vs. 9.0%), respectively. By logistic regression, being FLC-MS negative (vs. positive) 12 months had an HR 2.7302 (95% CI 1.2035 – 6.1932, p=0.0162) for achieving a cardiac response.

Those in an Andromeda CR, 57/87 (65.5%) had cardiac involvement at diagnosis and 40 (70.2%) had a cardiac response at 12 months. A cardiac response was seen in 17/23 (73.9%) who were FLC-MS negative vs. 23/34 (67.6%) (P=0.681) who were FLC-MS positive. Of those in an ISA CR but not Andromeda CR, a cardiac response was seen in 5/9 (55.5%) vs 12/30 (40%) (P=0.409).

349 (71.1%) patients had renal involvement at diagnosis with 303 and 313 patients, respectively, assessable for a renal response at 6 and 12 months. Overall, at 6- and 12-months, 67/302 (22.2%) and 82/313 (26.2%) had a renal organ response. Of the patients reaching FLC-MS negativity, 15/58 (26%) and 25/66 (38%) had a renal

response at 6- and 12-months respectively (p=0.45 and p=0.015 compared to those with FLC-MS positivity).

Survival

The median OS of the cohort was 72 months and for patients achieving CR, VGPR and PR was 110, 66 and 59 months, respectively. Median OS of patients who were FLC-MS negative vs. positive (at both 6- and 12-months) was not reached (NR) vs. 63 months (p=<0.001), respectively (**Fig 2a**). At 3 and 5 years, 89% (vs. 67%) and 73% (vs. 45%) patients who were FLC-MS negative (vs. positive) were alive.

The median OS in patients who were FLC-MS negative + ISA CR vs. FLC-MS positive + ISA CR, at 6 months, was not reached vs. 80 months (p=0.026) and, 12 months, was NR vs. 108 months (p=0.024) (**Fig 2b**). In the Andromeda CR cohort, the median OS was better in those who did achieve FLC-MS negativity at 12 months (NR vs. 110 months) (p=0.381) although not significant. In those who achieved an ISA CR (without Andromeda CR) a median OS was NR vs. 56 months (FLC-MS negative vs. positive) (p=0.116) **(SFig 1,2)**

For patients in a VGPR, median OS at 6 months was 85 vs. 60 months (p=0.055) and, at 12 months, was 78 vs 63 months (p=0.725), respectively **(Fig 2c,2d)**. Patients who had dFLC <10mg/l or iFLC <20mg/l and were FLC-MS negative also had a significant improvement in survival compared to those who were FLC-MS positive **(SFig 3,4).**

The univariate analysis of the factors impacting survival is shown in **Table 2**. A multivariate model using cardiac disease stage and depth of response showed advanced cardiac disease stage and achieving FLC-MS negativity at 12 months were independently predictive of better outcomes. The hazard ratio incrementally increased

with depth of response as graded by standard ISA criteria plus FLC-MS negative/positivity (CR+FLC-MS Negative - reference, CR-FLC-MS Positive – HR 3.6, VGPR-FLC-MS Negative – HR 5.08, VGPR-FLC-MS Positive – HR 6.07 and PR or worse HR – 9.51) **(Table 3**).

Discussion

This study reports the largest cohort of patients with AL amyloidosis using FLC-MS to identify the presence of a monoclonal FLC at diagnosis and assess the impact of FLC-MS response at 6- and 12-months following treatment. The baseline light chain isotype identified by FLC-MS shows complete concordance with standard FLC assays and the amyloid fibril type on tissue biopsy. This study shows that FLC-MS negativity identifies a potential new category of deep response in AL amyloidosis which translates into significantly higher organ responses and marked survival benefit across all disease stages.

Patients who remained FLC-MS positive were more likely to have higher starting iFLC/dFLC and lambda isotype at diagnosis. Since fewer patients with higher presenting FLC achieve FLC-MS negativity, it may be important to evaluate whether treatment regimens for such patients should be tailored differently with longer regimes, earlier consideration of transplantation and maintenance therapy. All of these points need to be addressed in future studies.

The use of MS to detect the presence of a monoclonal protein has evolved over the last 5 years with MS being considered a replacement for IFE for patients with multiple myeloma[6]. We have previously reported FLC-MS in a small cohort of patients with AL amyloidosis using the technique described in the current study with FLC immobilization with immunomagnetic beads and subsequent detection of the FLC by MALDI-TOF MS[8,11,14]. It showed complete concordance with serum FLC by standard assay and amyloid fibril for light chain presence and isotype. In this cohort of 17 patients, two patients with normal FLC by standard assay following treatment but MRD positive on bone marrow using next generation flow cytometry (NGF) demonstrated

persistent FLC-MS peaks. The Mayo clinic group demonstrated in 33 bone marrow MRD negative patients (in haematological CR) with AL amyloidosis, that 12% had residual detectable FLC by LC-MS and MALDI-TOF MS and it was associated with a poorer time to progression (at 50 months - 75% versus 13%, $p = 0.003$)[14]. Our study validates, in a large cohort, both the previous findings – the complete concordance of fibril light chain isotype with detected monoclonal FLC by FLC-MS and the persistence of abnormal FLC by FLC-MS in a significant proportion of patients with CR defined by standard ISA criteria. Here, only 20.7% of the total cohort became FLC-MS negative at 12 months and 39% of patients in a haematological CR by standard ISA criteria became FLC-MS negative.

Depth of response is directly linked to outcomes in AL amyloidosis especially for patients with advanced stage disease[22]. Presently, organ response is governed by the reduction in FLC following therapy and those who achieve at least a VGPR have better organ response[23-25]. Furthermore, patients with AL amyloidosis, who are bone marrow MRD negative by NGF, have significantly better survival and organ responses[26,27]. A European collaboration demonstrated that a renal response was seen in 92% vs. 57% and a cardiac response was demonstrated in 95% vs. 71% respectively in those who were MRD negative vs. positive[4]. The use of FLC-MS to assess depth of response or even as a surrogate of MRD is an attractive prospect as it can be performed on a serological sample without the need for an invasive procedure. The potential benefit of FLC-MS is that it can provide a further stratum of response without the limitations associated with the current Freelite assay such as the impact of the increased background polyclonal light chain levels as seen in renal dysfunction.

The present study is the first to report the impact of FLC-MS negativity on outcomes in AL amyloidosis. It was striking that the benefit of FLC-MS negativity translated to better survival even for patients in CR and those achieving a dFLC <10 mg/L (both currently considered as the “ideal” goals of treatment in AL amyloidosis). We note the current differences in CR definition used by the ISA and that used in the Andromeda trial. Despite the increased stringency of the ISA criteria less than half of the patients were FLC-MS at 12 months. Although not reaching statistical significance, the OS and organ response even in this cohort favoured those who were FLC-MS negative. Interestingly, the difference in outcome was more apparent in those who did not fulfil Andromeda criteria but were in an ISA CR, in most cases due to increased polyclonal FLC in the context of renal dysfunction where 59/77 (76.6%) patients had an eGFR of <60ml/min/1.73m^2. It is in this cohort where the utility of FLC-MS may be more apparent in determining potential ongoing amyloidotic disease progression.

At present, the FLC-MS assay is qualitative, but research is ongoing to determine whether FLC-MS can be used quantitatively. We hypothesize, that in the context of an Andromeda CR the level of the residual monoclonal FLC detected by FLC-MS may be lower than those detected in an ISA CR, where the ratio is normal but the iFLC may be raised.

Whilst 15% of patients in standard VGPR were FLC-MS negative at 12 months, there was no statistical survival advantage compared those who remained FLC-MS positive although due to the small numbers of patients who attained a VGPR and were FLC-MS negative any conclusion is limited. However, it may suggest that a persistent intact M protein or very low level persistent amyloidotic FLC below threshold of FLC-MS methods of detection may be contributory. The presence of FLC-MS negative patients who only achieve a VGPR can appear puzzling, however this can occur for a

number of different reasons. Patients who do not have a normalized FLC ratio, a criterion for a CR, but a dFLC<40mg/l are considered to be in a VGPR. The lack of FLC ratio normalization is not solely due an excess of amyloidotic light chain and can be attributed to changes in the polyclonal background. In contrast, an intact monoclonal protein in the serum is not detected by the FLC-MS assay and patients with essentially normal light chains but with a trace of intact monoclonal protein in the serum/urine would be classed as VGPR. The limitation of this study is that we did not do an additional MS assay for the intact M-protein. The only patients who require further investigation and do not have a clear explanation were 4 patients where a Bence Jones Protein in the urine was identified on immunofixation but no abnormal FLC-MS spike detected on serology. Unfortunately, due to the retrospective nature of the study, we are unable to repeat the urine tests to confirm the accuracy of IF positivity. At present, the FLC-MS has not been optimized to detect FLC in urine although this a potential avenue of further research.

The FLC-MS assay is currently only available in a research capacity undertaken by the Binding Site/Thermo Fisher Scientific. Work is underway to validate the assay for it to become an additional component to the currently available EXENT assay. Critically, the EXENT assay measures individual immunoglobulins and light chains but only “total” light chains which include immunoglobulin bound light chains. The FLC-MS assay uses FLC beads to specifically isolate the FLC (similar to the actual Freelite assay) and hence measure only the FLC. The total light chains measured by the EXENT technique are not sensitive enough to detect low level FLC below the polyclonal background and should not be used interchangeably with FLC-MS. The FLC-MS assay currently requires manual analysis of the spectra for each patient but there is scope for this to be automated in a similar way to the EXENT and MASS-FIX

assays. This process requires development of a machine learning algorithm which would be refined with multiple generations of training data as has been performed for the EXENT assay. Over the last four years protocols for the processing of the samples using automated liquid handlers to perform the immune precipitation and spot the samples onto MALDI target platelets have been developed and could easily be adapted for high throughput testing. MALDI-TOF MS is a high throughput MS technique taking less than 30 seconds for each patient's sample to be analysed. We appreciate a major limitation to our study is lack of associated EXENT measurements and a further study is underway looking at a combination of EXENT and FLC-MS assays.

We acknowledge other limitations of the current study. This study only included patients with samples available at baseline, 6- and 12-months thus by definition a landmark analysis of 12-month survivors. The utility of FLC-MS assessment for early response and mortality remains unclear as does its utility in prognosis in an unselected intent to treat population. However, the role of FLC-MS may lie in identifying long term therapy needs that may correlate with long term organ response rather than a parameter to impact early therapy changes. There were no parallel bone marrow samples to assess comparative bone marrow MRD assessment in patients with FLC-MS negativity. All patients in the current cohort were treated with bortezomib-based regimens (commonly CyBorD) but none with upfront daratumumab. The results of the Andromeda study showed improved haematological CR rates with Daratumumab-CyBorD compared to CyBorD and therefore higher rates of FLC-MS negativity are likely as Daratumumab-CyBorD becomes available as standard first line treatment[18].

In summary, this study is the first large cohort study of patients with AL amyloidosis using FLC detection by MS using a novel assay that detects monoclonal

FLC based on the unique *m/z* value of the FLC and demonstrates that FLC-MS is a reliable method of detecting a monoclonal amyloidogenic FLC. Patients achieving FLC-MS negativity have significantly superior organ responses. Patients with no detectable residual monoclonal FLC by FLC-MS have significantly better OS and FLC-MS negativity is an independent predictor of better survival in AL irrespective of the cardiac disease stage. FLC-MS is potentially a key serological MRD marker in AL amyloidosis. FLC-MS assessment should be validated in an independent cohort and, if findings confirmed, has the potential to be a new definition of response in AL amyloidosis and an end point of trials with novel agents in AL amyloidosis.

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Authorship

Contribution: J.B, S.R, A.W. designed the study, performed research, analyzed the data, and wrote the manuscript; H.V.G, N.W, O.B, S.H, G.P, J.G, designed the study, performed research and wrote the manuscript; J.K, S.H, B.W, O.C, D.F, M.U.R, N.S, A.M.N, L.V., C.W, M.F, P.N.H, J.D.G, H.L, performed research and wrote the manuscript and all authors approved the final version of the manuscript.

Conflict of interest: H.V.G received research funding from the Binding Site. M.F is consulting income from Intellia, Novo-Nordisk, Pfizer, Eidos, Prothena, Akcea, Alnylam, Caleum, Alexion, Janssen, Ionis and Astra-Zeneca. C.W reports honoraria from Akcea, Alnylam, Novartis, and Pfizer. J.D.G has consulting income from Ionis, Eidos, Intellia, Alnylam and Pfizer. G.P received an honorarium for advisory boards and educational support from BMS, Janssen, Binding Site, Sanofi, Takeda, GSK. A.W has consulting income from Alexia, Astra-Zeneca, Janssen, Attralus and Prothena. The other authors declare no competing financial interests.

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References

1. Wechalekar AD, Gillmore JD, Hawkins PN: Systemic amyloidosis. Lancet 387:2641-2654, 2016
2. Gertz MA, Dispenzieri A: Systemic Amyloidosis Recognition, Prognosis, and Therapy: A Systematic Review. JAMA 324:79-89, 2020
3. 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 30:4541-9, 2012
4. Palladini G, Paiva B, Wechalekar A, et al: Minimal residual disease negativity by next-generation flow cytometry is associated with improved organ response in AL amyloidosis. Blood Cancer J 11:34, 2021
5. Long TE, Indridason OS, Palsson R, et al: Defining new reference intervals for serum free light chains in individuals with chronic kidney disease: Results of the iStopMM study. Blood Cancer J 12:133, 2022
6. Murray DL, Puig N, Kristinsson S, et al: Mass spectrometry for the evaluation of monoclonal proteins in multiple myeloma and related disorders: an International Myeloma Working Group Mass Spectrometry Committee Report. Blood Cancer J 11:24, 2021
7. Mills JR, Kohlhagen MC, Dasari S, et al: Comprehensive Assessment of M-Proteins Using Nanobody Enrichment Coupled to MALDI-TOF Mass Spectrometry. Clin Chem 62:1334-44, 2016
8. Milani P, Murray DL, Barnidge DR, et al: The utility of MASS-FIX to detect and monitor monoclonal proteins in the clinic. Am J Hematol 92:772-779, 2017
9. Giles HV, Wechalekar A, Pratt G: The potential role of mass spectrometry for the identification and monitoring of patients with plasma cell disorders: Where are we now and which questions remain unanswered? Br J Haematol, 2022
10. Giles HV: Free Light Chain-Specific Antisera Improves the Sensitivity of Matrix-assisted Laser Desorption/Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS) for the Detection of Residual Disease in Patients with Free Light Chain Only Multiple Myeloma. Presented at the BSH ASM 2022, 2022
11. Sharpley FA, Manwani R, Mahmood S, et al: A novel mass spectrometry method to identify the serum monoclonal light chain component in systemic light chain amyloidosis. Blood Cancer J 9:16, 2019
12. Barnidge DR, Dispenzieri A, Merlini G, et al: Monitoring free light chains in serum using mass spectrometry. Clin Chem Lab Med 54:1073-83, 2016
13. Moreau C, Lefevre CR, Decaux O: How to quantify monoclonal free light chains in plasma cell disorders: which mass spectrometry technology? Ann Transl Med 8:973, 2020
14. Dispenzieri A, Arendt B, Dasari S, et al: Blood mass spectrometry detects residual disease better than standard techniques in light-chain amyloidosis. Blood Cancer J 10:20, 2020
15. Gertz MA, Comenzo R, Falk RH, et al: Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. Am J Hematol 79:319-28, 2005
16. Comenzo RL, Reece D, Palladini G, et al: Consensus guidelines for the conduct and reporting of clinical trials in systemic light-chain amyloidosis. Leukemia 26:2317-25, 2012
17. Palladini G, Schonland SO, Sanchorawala V, et al: Clarification on the definition of complete haematologic response in light-chain (AL) amyloidosis. Amyloid 28:1-2, 2021
18. Kastritis E, Palladini G, Minnema MC, et al: Daratumumab-Based Treatment for Immunoglobulin Light-Chain Amyloidosis. N Engl J Med 385:46-58, 2021
19. Giles HV: On bead de-glycosylation coupled with MALDI-TOF mass spectrometry provides a simple method for confirming light chain glycosylation and provides a sensitive method for

residual disease detection Presented at the International Symposium on Amyloidosis XVIII, Heidelberg, 2022

20. Wechalekar AD, Schonland SO, Kastritis E, et al: A European collaborative study of treatment outcomes in 346 patients with cardiac stage III AL amyloidosis. Blood 121:3420-7, 2013

21. Muchtar E, Dispenzieri A, Wisniowski B, et al: Graded Cardiac Response Criteria for Patients With Systemic Light Chain Amyloidosis. J Clin Oncol 41:1393-1403, 2023

22. Palladini G, Schonland S, Merlini G, et al: The management of light chain (AL) amyloidosis in Europe: clinical characteristics, treatment patterns, and efficacy outcomes between 2004 and 2018. Blood Cancer J 13:19, 2023

23. Pinney JH, Lachmann HJ, Bansi L, et al: Outcome in renal Al amyloidosis after chemotherapy. J Clin Oncol 29:674-81, 2011

24. Rezk T, Lachmann HJ, Fontana M, et al: Prolonged renal survival in light chain amyloidosis: speed and magnitude of light chain reduction is the crucial factor. Kidney Int 92:1476-1483, 2017

25. Mahmood S, Palladini G, Sanchorawala V, et al: Update on treatment of light chain amyloidosis. Haematologica 99:209-21, 2014

26. Muchtar E, Dispenzieri A, Jevremovic D, et al: Survival impact of achieving minimal residual negativity by multi-parametric flow cytometry in AL amyloidosis. Amyloid 27:13-16, 2020

27. Staron A, Burks EJ, Lee JC, et al: Assessment of minimal residual disease using multiparametric flow cytometry in patients with AL amyloidosis. Blood Adv 4:880-884, 2020

Figure Legends

Figure 1 – Haematologic response at 6- and 12-month time points and FLC-MS status

Figure 2a – OS from diagnosis based on FLC-MS status at 12-month landmark analysis; Median OS NR vs. 63 months (p= <0.001) in those FLC-MS negative vs. positive.

Figure 2b – OS from diagnosis based on FLC-MS status at 12-month landmark analysis in patients who achieved a haematological CR; Median OS was NR vs. 108 months (p= 0.009) in those FLC-MS negative vs. positive.

Figure 2c – 6 Month analysis of OS from diagnosis by haematologic response combined with FLC – MS Status. CR – FLC MS Negative, median OS NR, CR – FLC MS Positive 80 months, VGPR – FLC MS Negative 85 months, VGPR – FLC MS positive 60 months.

Figure 2d – 12 Month analysis of OS from diagnosis by haematologic response combined with FLC – MS Status. CR – FLC MS Negative, median OS NR, CR – FLC MS Positive 108 months, VGPR – FLC MS Negative 78 months, VGPR – FLC MS positive 63 months.

Table 1 – Baseline characteristics (n= 487)

	Total cohort n = 487 (%)/median (range)	**FLC-MS negative n = 112**	**FLC-MS positive n = 375**	
Male	291 (59.6%)	55 (49%.1)	236 (62.9%)	**P=0.009**
Age (yrs)	67 (36 – 88)	65 (40 – 88)	67 (36 – 86)	P=0.62
Detectable M-protein by SPEP/IFE				
None	126 (26%)	44 (39.3%)	82 (21.9%)	
Immunofixation only	122 (25%)	19 (17.0%)	103 (27.5%)	
SPEP; median (range)(g/L)	239 (49.0%); 8g/l (1 – 45)	49 (43.8%) 8g/l (2 – 31)	190 (50.7%) 8g/l (1 – 45g/l)	P=0.907
M-Protein type				
A	70 (14.3%)	10 (8.9%)	60 (16%)	P=0.06
D	3 (0.6%)	0 (0%)	3 (0.8%)	
G	163 (33.4%)	41 (36.6%)	122 (32.5%)	P=0.422
M	13 (2.7%)	2 (1.8%)	11 (2.9%)	P= 0.509
LC	111 (22.7%)	15 (13.4%)	96 (25.6%)	**P=0.007**
Serum Light chain type				
Kappa	89 (18.2%)	35 (31.3%)	55 (14.6%)	
Lambda	396 (81.1%)	76 (67.9%)	319 (85.1%)	**P=<0.0001**
Involved light chain (mg/L)	197 (11.6 – 15,900)	145 (11.8 – 2211)	208 (11.6 – 15900)	P=0.065
dFLC (mg/L)	177.7 (0 – 15,898)	125 (0 – 2203)	186 (2.1 – 15898)	P=0.061
Organ involvement				
Renal	349 (71.5%)	83 (74.1%)	266 (70.9%)	P=0.513
Creatinine umol/l	94 (27 – 777)	104 (33 – 609)	91 (27 – 777)	P=0.243
eGFR mL/min/1.73m^2	67 (15 – >90)	61.5 (15 - >90)	69 (15 - >90)	P=0.017
24hr Urinary Protein (g/24h)	3.1 (0.1 – 31.6)	3.4 (0.1 – 19.6)	3.1 (0.1 – 31.6)	P=0.581
Heart	290 (59.4%)	61 (54.5%)	229 (61.1%)	P=0.212
NTProBNP pg/ml	1548 (12 – 44611)	1841 (12 – 34082)	1522 (34 – 44611)	P=0.121
Bilirubin mg/dL	5 (2 – 61)	5 (2 – 25)	6 (2 – 61)	P=0.246
Alkaline phosphatase U/L	85 (26 – 1035)	138 (39 – 734)	82 (26 – 1035)	P=0.195
LV Septum (mm)	13 (6 – 22)	12 (7 – 21)	13 (8 – 22)	**P=0.032**
LVEF %	60% (11 – 80)	59 (11 – 77)	60 (16 – 80)	P=0.278
Cardiac disease stage European Modification of Mayo 2004				
1	99 (20.3%)	29 (25.9%)	72 (19.2%)	P=0.125
2	178 (36.5%)	30 (26.8%)	146 (38.9%)	**P=0.019**
3A	167 (34.2%)	41 (36.6%)	126 (33.6%)	P=0.556
3B	39 (8%)	11 (9.8%)	28 (7.4%)	P=0.420
Missing	4 (0.8%)	1 (0.9%)	3 (0.8%)	P=0.924
Cardiac disease stage Mayo 2012				
1	71 (14.5%)	24 (21.4%)	47 (11.7%)	**P=0.019**
2	123 (25.2%)	26 (23.2%)	97 (25.8%)	P=0.571
3	138 (28.3%)	25 (22.3%)	113 (20.1%)	P=0.107
4	116 (23.8%)	26 (23.2%)	90 (24%)	P=0.864
Missing	39 (8.0%)	11 (9.8%)	28 (7.5%)	P=0.420
Other organs				
Liver	50 (10.2%)	19 (17.0%)	31 (8.3%)	**P=0.008**
Peripheral Neuropathy	29 (5.9%)	6 (5.4%)	23 (6.1%)	P=0761
Autonomic Neuropathy	29 (5.9%)	4 (3.6%)	25 (6.7%)	P=0.224
Soft tissue	80 (16.4%)	14 (12.5%)	66 (17.6%)	P=0.201
GI	15 (3.1%)	1 (0.8%)	14 (0.4%)	P=0.127
Other	4 (0.8%)	0	4 (0.01%)	P=272

Number of organs involved				
1	231 (47.3%)	56 (50%)	175 (46.7%)	P=0.535
2	170 (34.8%)	39 (34.8%)	131 (34.9%)	P=0.983
3	71 (14.5%)	15 (13.4%)	56 (14.9%)	P=0.685
4	15 (3.1%)	2 (1.7%)	13 (3.5%)	P=0.366

Table 2 – Univariate analysis of factors impacting overall survival

Univariate	**P - Value**	**HR (CI 95%)**
Sex (female)	0.067	0.726 (0.515 – 1.02)
Age	**<0.0001**	1.033 (1.016 – 1.051)
iFLC*	**0.009**	1.484 (1.111 – 1.981)
dFLC*	**0.029**	1.321 (1.028 – 1.697)
NTProBNP*	**<0.0001**	1.87 (1.447 – 2.416)
Creatinine*	**<0.0001**	3.294 (1.852 – 5.858)
Bilirubin	0.267	1.353 (0.793 – 2.309)
ALP	0.861	0.948 (0.522 – 1.721)
Mayo 2004 Staging with European Modification		
Mayo 2004 Stage 1	Ref	
Mayo 2004 Stage 2	0.378	1.274 (0.744 – 2.18)
Mayo 2004 Stage 3a	**<0.0001**	2.522 (1.529 – 4.161)
Mayo 2004 Stage 3b	**0.001**	2.916 (1.533 – 5.545)
Mayo 2012 Staging		
Mayo 2012 Stage 1	Ref	
Mayo 2012 Stage 2	0.194	1.592 (0.789 – 3.211)
Mayo 2012 Stage 3	**<0.0001**	3.646 (1.892 – 7.028)
Mayo 2012 Stage 4	**<0.0001**	3.654 (1.895 – 7.045)
Organ involvement		
Cardiac	**0.011**	1.575 (1.112 – 2.23)
Renal	0.352	1.188 (0.826 – 1.708)
Liver	0.753	0.925 (0.57 – 1.502)
Haematologic response at 12 months		
12 Month CR	**Ref**	
12 Month VGPR	**0.001**	2.343 (1.409 – 3.895)
12 Month PR	**<0.0001**	3.692 (2.196 – 6.208)
12 Month NR	**<0.0001**	4.219 (1.932 – 9.213)
FLC-MS Status		
FLC-MS Positive vs. FLC-MS Negative	**<0.0001**	3.098 (1.752 – 5.478
Combined haematologic response and FLC-MS status		
12M CR and FLC MS -	**Ref**	
12M CR and FLC MS +	**0.019**	3.37 (1.146 – 9.915)
12M VGPR and FLC MS -	**0.015**	4.801 (1.353 – 17.041)
12M VGPR and FLC MS +	**0.002**	6.054 (2.171 – 16.883)
12M PR/NR	**<0.0001**	10.15 (3.710 – 27.766)
Further response		
12M iFLC>20mg/l vs. <20mg/l	**<0.0001**	3.035 (1.845 – 4.992)
12M dFLC >10mg/l vs <10mg/l	**<0.0001**	2.108 (1.454 – 3.05)

Table 3 – Multivariate analysis of factors impacting overall survival

Multivariate (Model 1)	**P - Value**	**HR (CI 95%)**
Mayo 2004 Staging with European Modification		
Mayo 2004 Stage 1	Ref	
Mayo 2004 Stage 2	0.559	1.178 (0.68 – 2.038)
Mayo 2004 Stage 3a	**0.001**	2.302 (1.387 – 3.822)
Mayo 2004 Stage 3b	**0.011**	2.327 (1.217 – 4.449)
Combined haematologic response and FLC-MS status		
12M CR and FLC - MS -	Ref	
12M CR and FLC - MS +	**0.021**	3.576 (1.214 – 10.529)
12M VGPR and FLC- MS -	**0.012**	5.079 (1.421 – 18.155)
12M VGPR and FLC - MS +	**0.001**	6.068 (2.173 – 16.945)
12M PR/NR	**<0.0001**	9.507 (3.469 – 26.058)
Multivariate (Model 2)	**P - Value**	**HR (CI 95%)**
Mayo 2012 Staging		
Mayo 2012 Stage 1	Ref	
Mayo 2012 Stage 2	0.47	1.299 (0.639 – 2.639)
Mayo 2012 Stage 3	**0.02**	2.223 (1.131 – 4.367)
Mayo 2012 Stage 4	**0.006**	2.57 (1.31 – 5.044)
Creatinine*	**0.01**	2.263 (1.213 – 4.219)
Combined haematologic response and FLC-MS status		
12M CR and FLC - MS -	Ref	
12M CR and FLC - MS +	0.097	2.517 (0.847 – 7.481)
12M VGPR and FLC - MS -	0.125	2.823 (0.75 – 10.616)
12M VGPR and FLC - MS +	**0.005**	4.476 (1.586 – 12.632)
12M PR/NR	**<0.001**	6.356 (2.303 – 17.538)

Model 1 is based on Mayo Staging 2004 with European modification, dFLC at baseline, creatinine and the combined haematologic and FLC-MS response at 12 months. Creatinine and dFLC were found not to be significant on multivariate cox regression analysis. Model 2 is based on the Mayo 2012 staging, creatinine and the combined haematologic and FLC-MS response at 12 months.

*denotes Log10 transformation