# Systemic Amyloidosis: Moving into the spotlight

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**Abstract**

Systemic amyloidosis is a rare but increasingly recognised disease that is heterogeneous in presentation. Early diagnosis, whilst imperative, remains challenging but can improve prognosis and limit organ dysfunction. An increased repertoire of diagnostic imaging and histological techniques are becoming mainstream and promise to aid early diagnosis. Better risk stratification, via biomarkers and cytogenetics, has improved multi-disciplinary treatment decisions. The use of novel agents has improved treatment efficacy, which translates into survival benefit. Newer strategies targeting pre-deposited amyloidogenic protein are under investigation. The current paper reviews available data relating to the most recent advances in the field of systemic amyloidosis.

**Introduction**

Systemic amyloidosis is characterised by the misfolding of autologous proteins, which aggregate into an abnormal fibrillar form and deposit in organs leading to progressive dysfunction [1]. Whilst AL amyloidosis is still considered a rare disease, an epidemiological study in the United States reports a doubling in prevalence from 15.5 cases per million in 2007 to 40.5 cases per million in 2015 despite a stable incidence [2] reflecting key advances in detection, response assessment and the availability of novel agents. Whilst AL amyloidosis predominates, the recognition of wild type transthyretin amyloidosis (wtATTR) is rapidly increasing. In contrast, the declining prevalence of AA amyloidosis is indicative of key advances in the control of underlying inflammatory conditions (e.g. rheumatoid arthritis and inflammatory bowel disease). The key subtypes of amyloidosis are summarised in Table 1.

In this paper, we review the changing demographics of the disease and address the key issue of early recognition. We discuss the value of the latest available novel therapies and evaluate emerging treatments, which may become available in the future.

**Recognition of amyloidosis**

Amyloidosis is characterised by progressive dysfunction of the heart, liver and kidneys, which interplays with variable damage to nerves, soft tissue and the gastrointestinal tract. Early symptoms are non-specific (e.g. peripheral oedema, dyspnoea) leading to diagnostic difficulties and consequent delays. The patient pathway to diagnosis is varied – nephrology, haematology and cardiology dominate this pathway although most patients have seen a median of 4 specialists prior to diagnosis. One-third experience delays of ≥1 year from symptom onset [3]. Other common presenting features include a bleeding diathesis, paraesthesia, postural hypotension and macroglossia.

In each of the relevant specialities, there are specific clues to assist early recognition. In the haematology clinic, all patients with systemic AL amyloidosis have an underlying plasma cell (or B lymphoid) dyscrasia. Crucially, we know from a seminal study that a monoclonal immunoglobulin was

present in all samples taken ≤4 years before diagnosis [4] presenting a clear window of opportunity to identify amyloidosis early via vigilant assessment. In the cardiology clinic, the syndrome of heart failure with preserved ejection fraction (HFpEF), particularly if associated with renal impairment, carpal tunnel syndrome (CTS) or an unexpectedly high N-terminal pro-B type natriuretic peptide (NT-proBNP), should prompt investigation for amyloidosis. The triad of CTS, spinal stenosis and HFpEF is highly suggestive of wild type ATTR amyloidosis. Patients presenting with renal involvement often have the easiest diagnostic pathway as progressive renal impairment with proteinuria is identified early in primary care. The challenge here is to separate patients who need prompt referral to nephrology from a large population of older adults with renal impairment due to other co-morbidities. A careful family history in patients with neuropathic, cardiac (e.g. hereditary ATTR) or renal (e.g. fibrinogen alpha-chain amyloidosis) amyloidosis is imperative. In all settings, the presence of CTS is a seriously underappreciated clue to a future diagnosis of amyloidosis.

Despite an increased prevalence, systemic AL amyloidosis remains a rare disease. In patients with incidental asymptomatic amyloid deposits, the risk of developing systemic disease in the longer term remains unclear. Wild type ATTR amyloidosis, although still a rare disease, appears to be substantially under-diagnosed. A study of patients with HFpEF suggested that 13% may have underlying wtATTR amyloidosis [5]. Wild-type ATTR amyloidosis appears to be on course to emerge as a major public health issue in the elderly.

**Steps to a diagnosis of amyloidosis**

The diagnosis of amyloidosis is made by demonstration of amyloid deposition histologically or via a diagnostic imaging modality with high specificity for amyloidosis. The former method has the advantage of allowing for both confirmation of the diagnosis and, critically, typing amyloid deposits.

*Histological diagnosis of amyloidosis:*

Congo red staining, exhibiting characteristic birefringence under cross polarised light, remains the gold standard. The biopsy of an affected organ has the highest yield but carries a bleeding risk. Sampling of abdominal fat, via aspiration, is a preferred low risk alternative and detects amyloid deposition in over three quarters of cases of cardiac AL amyloidosis when undertaken and reported in experienced centres. This method is less sensitive for the detection of both hereditary (45% sensitivity) and wild-type (15% sensitivity) ATTR [6]. Laboratories must be vigilant for false positive and negative results, which may compromise diagnosis.

Amyloid referral centres routinely undertake typing of amyloid fibrils but cost and complexity are major deterrents for regional hospitals. Furthermore, the need for typing amyloid deposits in all cases remains a contentious issue. In cases of a clear free light chain excess, soft tissue amyloid (a pathognomonic feature of AL amyloidosis) and multi-organ involvement, one could conceivably omit typing. On the contrary, typing is critically important in cases of isolated renal or cardiac involvement to exclude non-AL amyloidosis. The use of laser microdissection and capture of Congo red positive tissue followed by protein identification by mass spectrometry and bioinformatics (LCMS), greatly improves sensitivity and specificity of amyloid fibril typing [7]. This approach is strongly recommended unless there is a well-established program of routine amyloid immunohistochemistry or immuno-electron microscopy to characterise the amyloidogenic protein. LCMS has led to the detection of multiple new amyloidogenic proteins in addition to providing greater diagnostic accuracy. A newer technique, independent of the need for Congo red staining, relies on detection of both the molecular weight and spatial distribution of biomolecules and the use of a novel peptide filter (MALDI-IMS MSI) to classify amyloid proteins in a less time and sample consuming manner [8].

*Imaging*

Echocardiography is a widely available first line method to identify patients who warrant further work up but is relatively non-specific. Two highly specific methods have changed the imaging approach to amyloidosis: cardiac magnetic resonance imaging (CMR) and bone scintigraphy tracer

imaging. Cardiac MRI is highly specific for the diagnosis and may have a role in monitoring serial changes via the hallmark pattern of late gadolinium enhancement [9]. Extracellular amyloid deposits lead to a marked increase in the myocardial extracellular volume, which can be measured by specific MRI sequences to provide a quantitative estimate of the myocardial amyloid burden. Extracellular volume (ECV), along with pre-contrast T1 mapping, appears to correlate with established markers of disease severity, such as the serum biomarkers, NT-proBNP and Troponin T (TnT), and predicts mortality [10]. Furthermore, myocardial amyloid regression can be accurately documented by a reduction in T1 and ECV – a novel modality to track the progress of a patient following treatment [11]. On CMR, T2 imaging is a marker of tissue oedema and can act as a potential myocardial "biomarker" of amyloid oligomer or light chain proteotoxicity [9]. Improvement in cardiac amyloid can be seen via these modalities as pictured in Figure 1A. Novel CMR methods are redefining our ability to track cardiac amyloid with clear prognostic value.

The use of radiolabelled bone seeking tracers such as $^{99m}$technetium-pyrophosphate or $^{99m}$-technetium-3,3-diphospono-1,2-propanodicarboxylic acid ([$^{99m}$Tc]-PYPY or DPD) has transformed imaging for cardiac amyloidosis. These methods are sensitive for cardiac involvement in transthyretin amyloidosis and, in the absence of a monoclonal protein in serum or urine, grade 2 or 3 myocardial radiotracer uptake is considered diagnostic for ATTR amyloidosis [12]. However, in AL amyloidosis sensitivity is lacking. Imaging is positive in just 51% of patients with biopsy-proven cardiac AL amyloidosis. [12] $^{99m}$-Tc-DPD uptake has also been reported in apolipoprotein A-I amyloidosis [13].

Imaging to quantify the amyloidogenic protein load is a valuable diagnostic tool and can be used to monitor progress. $^{123}$Iodine-labelled serum amyloid P component scintigraphy is in routine clinical use at the UK NAC and is able to image visceral amyloid deposits in the liver, spleen, kidneys, adrenal glands and bones [14]. This method can track regression over time in patients who have responded to treatment as seen in Figure 1B. However, this form of imaging is not useful for cardiac involvement and its availability is limited worldwide.

Positron emission tomography-based (PET) modalities have emerged as potentially useful tools to evaluate amyloid deposits using both 18F-florbetapir[15] (see Figure 2A) and 11C-PiB [16] as tracers. A trend towards greater PET avidity in newly diagnosed patients and poor treatment responders with cardiac amyloidosis is reported [15]. Imaging with 18F-florbetapir appears to be highly sensitive and further studies are required to validate this technique. A new radiotracer, designated p5+14, is a synthetic, basic polypeptide with 45 amino acids and forms an α-helix in the presence of highly sulfated glycosaminoglycan and amyloid fibrils, resulting in specific multivalent electrostatic interactions. The peptide binds a variety of amyloid fibrils and can be visualized on PET imaging when bound to $^{124}$I (see Figure 2B). It is currently under investigation in a first-in-man phase 1 study (NCT03678259).

**Figure 3** shows a suggested algorithm to make a definitive diagnosis of amyloidosis in suspected cases.

**Risk Stratification**

*Biomarkers: Initiation and Opportunity*

Cardiac involvement is the major determinant of prognosis in AL amyloidosis and consequently forms the basis of validated scoring systems e.g. Mayo 2010 based upon NT-proBNP, TnT and difference between involved and uninvolved light chains (dFLC) [17]. Although NT-proBNP has been validated as a marker of cardiac response following treatment [18], it is exquisitely sensitive to a large number of factors that affect fluid balance making serial monitoring challenging. Lately, it has been demonstrated that the depth of organ response for the heart, kidney and liver correlates with prolonged survival. This needs to be validated to update current organ response criteria in AL amyloidosis [19].

A number of biomarkers have been reported to have prognostic value as demonstrated in Figure 4 [20-27]. This increasing pool of biomarkers (growth differentiation factor-15, proadrenomedulin, osteopontin, hepatocyte growth factor, soluble suppression of tumorgenicity 2, von Willebrand factor

antigen, osteoprotegerin and immunoparesis) warrant further investigation in large case series' with a view to providing a more accurate assessment of individual risk. At present, these novel markers have not been incorporated into routine practice.

*Measurement of the underlying clone and clonal markers*

The monoclonal protein and serum free light chains (FLC) are the drivers of disease in AL amyloidosis but the underlying biology of the clonal plasma cells determines response to treatment, duration of response and outcomes at relapse.

*Advances in Light chain measurements:*

The development of assays to measure the total kappa and lambda free light chains (both monoclonal and normal polyclonal FLC) were transformative in the management of AL amyloidosis. A number of such assays are now available but two methods (Freelite$^{TM}$ by the Binding Site Group, Birmingham, UK and another immunoassay by Siemens Healthcare diagnostics, Germany) are most widely used. The assays use antibodies against hidden epitopes present on the light chain molecule. There are persisting challenges with antigen excess leading to non-linearity and resultant over or under estimation of the monoclonal protein. These assays continue to demonstrate a large coefficient of variance between centres [28]. However, the critical failing of the assays is their inability to distinguish between the monoclonal and polyclonal components making up the total reported measurement of the FLC, which limits utility of the FLC measurement in patients with low level disease.

Mass spectrometry can be applied in peripheral blood to identify the monoclonal component of the involved FLC (iFLC) as shown in Figure 5. The Mayo group pioneered a matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), a simple and sensitive method to detect serum monoclonal proteins, called MASS-FIX. This is a robust, reliable and highly automated technique that can be easily adopted for high throughput testing. The sensitivity is significantly greater than traditional electrophoresis and immunofixation. This method detected M-

proteins in 18 patients (out of 257) with no monoclonal protein detectable by standard serum/urine immunofixation techniques [29]. Our group have further developed this modality using FLC beads and a MALDI-TOF-MS method to characterise monoclonal light chains, which is highly sensitive and can detect disease in patients whose clone is only detectable in bone marrow by flow cytometry-based minimal residual disease (MRD) testing [30]. Whilst these methods are not yet widely available, their use may allow for more accurate monitoring of patients and formulation of early treatment decisions in the future.

*Assessment of the plasma cell clone*

The impact of clonal biology on patient management has been the focus of numerous recent studies. Coexistent hypercalcaemia, renal failure, anaemia or lytic bone lesions (CRAB criteria) and an increase in bone marrow plasma cells (>10%) define equally high risk populations in patients with AL amyloidosis [31]. Furthermore, the presence of circulating plasma cells by multi-parametric flow cytometry at diagnosis adversely impacts OS (although this is overcome by a good response to chemotherapy of very good partial response [VGPR] or better) [32].

Cytogenetic abnormalities further risk stratify patients with AL amyloidosis. The largest cohort from the Mayo group reported *t (11;14)* in 49%, monosomy *13/del (13q)* in 36% and trisomies in 26% by interphase fluorescence in situ hybridization (iFISH) in 692 patients with AL amyloidosis [33]. The presence of *t (11;14)* seems to be associated with a poorer response to bortezomib in patients with favourable disease and possibly immunomodulatory (IMiD) agent based treatment [33] but improved complete response (CR) rates following autologous stem cell transplantation (ASCT) [34]. The German amyloid group demonstrated that a gain of *Chr 1q21* is an adverse marker [35]. The biological basis of these findings remains unclear and needs further research to allow development of targeted therapies.

Whole exome sequencing has demonstrated 21 mutated genes in common between MM and AL amyloidosis whilst also identifying 4 recurrent mutations in AL amyloidosis patients: *PCMTD1*,

*C21orf33*, *NLRP12* and *NRAS* [36]. A second study of 10 patients identified that recurrent mutations in *ASB15*, *ASCC3* and *HIST1H1E* were associated with inferior OS[37]. A large genome-wide association study in 1229 patients identified single nucleotide polymorphisms (SNPs) at 10 loci with *rs9344* most significant; the cyclin D1 splice site, a promotor of *t(11;14)* [38]. Whilst there appears to be no unique genomic signature of AL amyloidosis *per se*, further genetic sequencing studies are needed to increase understanding of the drivers behind AL amyloidosis, provide prognostic information and identify targets for future therapies.

**Management**

A multidisciplinary approach with involvement from cardiologists, nephrologists, neurologists and haematologists, as required, is crucial in patients with systemic amyloidosis. Stringent supportive therapy is critical. In cases of renal or cardiac involvement, key elements include careful fluid balance and patient education in monitoring blood pressure and fluid status. In AL amyloidosis, chemotherapy remains the mainstay of treatment whilst in ATTR amyloidosis, the recent licencing of gene-silencing and protein stabilizing therapies are a landmark advancement in amyloid therapeutics. In the remainder of amyloid subtypes, management remains supportive.

*AL Amyloidosis*

*Aims of Treatment and response assessment*

Current treatment paradigms aim to suppress the plasma cell clone to reduce the production of light chain immunoglobulins thus halting amyloid deposition and allowing for a gradual organ response and improved survival. A rapid haematological response is associated with improved outcomes in patients with advanced disease [39]. The advantage of a particular choice of cytotoxic treatment must be balanced against the patient's baseline organ function, which may be significantly compromised as a result of amyloid deposition. Standardised assessment of disease status is key to inform treatment intensity and choice. Over the last decade, it has become apparent that deeper

responses improve outcomes. Reduction of the iFLC to <20 mg/L or dFLC to <10 mg/L translates to superior organ responses and OS, over and above a CR by traditional haematological response criteria[40, 41]. A small proportion of patients present with a dFLC of ≤50mg/L, which poses a challenge in terms of disease tracking.

Assessment of MRD, by flow cytometry or next generation sequencing (NGS), represents a more sensitive method to determine depth of response. Following treatment, the presence of ≥0.1% monoclonal plasma cells negatively impacts both progression free and overall survival [42]. In patients achieving a CR, the detection of bone marrow plasma cells by flow cytometry negatively impacts progression free survival (PFS) [43]. Patients who are MRD positive by flow cytometry have impaired organ recovery [44]. The role of both flow cytometry and NGS remains unclear and require further study before incorporating their use into routine treatment decisions.

*Plasma Cell Directed Therapy*

*Autologous Stem Cell Transplantation*

ASCT remains a standard of care in selected fit patients with AL amyloidosis. Key improvement in patient selection has reduced treatment related mortality (TRM), a major limitation of early studies (important exclusion criteria listed in Table 2). Lately, a US registry study reported a decrease in 100-day TRM from 20% to 5% in patients treated in 1995-2000 and 2007-2012 respectively [45]. Our group reported a PFS of 54 months with no TRM in 22 patients who were considered transplant-ineligible at presentation due to organ dysfunction, predominantly advanced cardiac involvement [46]. Whilst ASCT leads to durable remissions in patients achieving a CR or VGPR (OS of 7.6 years in series from Boston [47] and 11.6 years in UK series [46]), a CR is seen in only a third of all patients (34.8% in the Boston series [47]).

Two approaches have been considered to overcome this limitation of transplantation – induction chemotherapy and post-transplant consolidation. The use of bortezomib-based induction

chemotherapy prior to ASCT has demonstrated CR rates of 63% with median PFS and OS not reached at 36 months [48]. Furthermore, a good response to bortezomib-based induction chemotherapy may lead to an organ response and reversal of ASCT exclusion criteria such as high cardiac biomarkers and poor performance status. In this scenario, a PFS of 54 months is reported in 22 patients without TRM[49]. Autologous stem cell transplantation may still be of value for patients with primary refractory disease after bortezomib induction therapy (PR or worse), with one small study reporting a 42% CR rate in 12 refractory patients [50]. Conversely, bortezomib consolidation therapy for patients in a VGPR or worse following ASCT alone, led to one-third achieving a CR subsequently [51] The optimal timing, nature and duration of additional therapy around ASCT remains unclear and presents an ongoing dilemma.

*Standard chemotherapeutic approaches*

Bortezomib is established as the mainstay of upfront treatment for the majority of patients with AL amyloidosis (Treatment Combinations in Table 3). The benefit of bortezomib-melphalan-dexamethasone was clearly demonstrated in a recent randomised phase III trial of 100 newly diagnosed patients with AL amyloidosis; improving CR/VGPR rates from 28% to 53% [52]. The most widely used regimen is CyBorD (combination of cyclophosphamide, bortezomib and dexamethasone). A European collaborative study of 230 patients demonstrated efficacy of CyBorD, reporting haematological, renal and cardiac response rates of 60%, 25% and 17% respectively [53]. We have recently reported the outcomes of 915 patients with haematological, renal and cardiac response rates of 65%, 15.4% and 32.5% respectively[54]. A rapid response with bortezomib-based therapy can significantly improve outcomes even in advanced cardiac patients (median OS improving from 5m to 26m in patients achieving a CR/VGPR by end of one month [39]). Bortezomib is the key drug in newly diagnosed AL with a recent report from the Greek amyloid group questioning the additional benefit of cyclophosphamide within CyBorD as it does seem to not significantly improve efficacy or survival [55].

IMiDs are routinely used in relapsed AL amyloidosis. The efficacy of lenalidomide-dexamethasone was first reported over 10 years ago [56, 57]. The Greek amyloid group recently demonstrated 51% haematological, 22% renal, 7% liver and 3% cardiac response rates to lenalidomide as salvage therapy [58]. In combination with melphalan and dexamethasone, haematological response rate was similar (58%) but only 8% achieved an organ response. This combination was highly toxic with 40% of patients dying due to acute cardiac events within months of treatment and a median OS of 1.75 months for stage III patients [59]. However, a study by the German group of lenalidomide, melphalan and dexamethasone in untreated transplant ineligible patients yielded better outcomes with a 68% haematological response and 48% organ response. In this group, median OS was 67.5 months. There was just one cardiac death after 3 cycles of chemotherapy despite 18 patients (36%) having stage III disease [60]. However, stage III patients still had a PFS of <12 months and the proportion of patients with stage IIIb disease was not specified. In both studies, the lenalidomide dosing (10mg) and frequency was the same but the German group used a lower melphalan dose of 0.15mg/kg as opposed to 0.18mg/kg, which may have had some impact on the lesser toxicity reported.

Pomalidomide is rapidly acting (responses in approximately 1 month) and has shown a survival advantage as salvage therapy in heavily pre-treated patients [61-63]. A recent report demonstrated 66% haematological response with a median PFS of 15 months although no patients achieved a CR with pomalidomide alone [64]. Further evaluation of pomalidomide as part of combination chemotherapy is required to assess its efficacy in this setting although toxicity may be an issue in heavily pre-treated patients with our study reporting a 41.1% (7/19 evaluable patients) discontinuation rate due to adverse events in patients with a median of 4 prior lines of therapy.

The addition of clarithromycin to IMiD-based therapies has demonstrated some efficacy. One study examined 49 patients with either multiple myeloma (n=32) or AL amyloidosis (n=17) demonstrating a 94% haematological and 47% organ response rate in patients with AL amyloidosis

(35% haematological response prior to the addition of clarithromycin in the same cohort) [65]. However, the recent report of increased mortality when clarithromycin was added to lenalidomide-dexamethasone in multiple myeloma[66] suggests a need for caution when using this agent in AL amyloidosis.

*Novel chemotherapeutic agents*

*Proteasome Inhibitors*

Carfilzomib and Ixazomib are newer PIs with limited evidence for use in AL amyloidosis. Carfilzomib is associated with lesser neurotoxicity compared to bortezomib and has been examined as salvage therapy (given twice weekly) in a multi-centre phase I/II study demonstrating a haematological response in 63% and an organ response in 21% (5 patients: 3 renal, 1 gastrointestinal, 1 liver). However, toxicity was significant with 71% patients experiencing grade 3/4 toxicity, which was most commonly cardiac or pulmonary [67]. It appeared to be better tolerated with higher responses in a recently concluded phase I study of weekly carfilzomib with thalidomide-dexamethasone [68]. Further combinations of weekly carfilzomib with newer IMiDs or daratumumab need to be explored.

Ixazomib is an oral PI, which has also been examined in the relapsed/refractory setting. Sanchorawala *et al* (2017) reported a 52% haematological response and 56% organ response (50% cardiac, 50% renal) with a median PFS of 14.8 months [69]. However, a recent phase III clinical trial of ixazomib-dexamethasone compared to a regimen of physicians choice in relapsed AL amyloidosis did not meet the primary end point in a planned intern analysis. The results demonstrate improved PFS (11.2 v 7.4 months, p=0.043), time to next treatment (26.5 v 12.5 months, p=0.027) and prolonged time to vital organ deterioration (34.8 v 26.1 months, p=0.012) with Ixazomib-Dexamethasone compared to physician's choice [70]. These newer PIs have promising advantages in patients with neurotoxicity and those who would benefit from an oral agent to minimise visits to their

haematology centre but further work is required to fully characterise their efficacy and toxicity in larger groups of patients.

*Daratumumab – a transformative role in AL amyloidosis*

Daratumumab, an anti-CD38 monoclonal antibody, is showing remarkable promise in AL amyloidosis. In heavily pre-treated patients, inclusive of 72% with cardiac involvement, one study demonstrated a 76% haematological response rate (36% CR) with a median response time of 1 month [71]. A recent publication from the Mayo clinic reported impressive haematological response rates of 78% with daratumumab monotherapy and 88% with combination therapy (addition of bortezomib, lenalidomide or pomalidomide) [72]. Best cardiac response rate was similar in both groups but occurred earlier (8.3 v 14.6 months) in the monotherapy group. The treatment was well tolerated with 22% experiencing significant infusion reactions.

The ANDROMEDA trial (NCT03201965) is examining frontline daratumumab in combination with bortezomib, cyclophosphamide and dexamethasone. In a run-in cohort of 28 patients treated with CyBorD-Daratumumab, the overall haematological response rate was 96% (54% CR). At a median follow-up of 341 days, all patients in CR continued to respond to treatment [73]. This regimen used subcutaneous Daratumumab and grade 1 infusion related reactions were seen in 2 patients only. The provisional response rates are incredibly promising and suggest that the combination of daratumumab with CyBorD could represent a significant advance in the treatment of AL amyloidosis assuming results are confirmed in the ongoing phase III study.

*Venetoclax*

BCL-2 is an anti-apoptotic protein expressed at higher levels in patients with plasma cell dyscrasias harbouring the *t (11; 14)* translocation. Consequently, the inhibition by venetoclax, a BCL-2 inhibitor, to treat the condition is logical in AL amyloidosis where half of all patients harbour this translocation. Of 7 patients treated with venetoclax for AL amyloidosis (alone or in combinations

including bortezomib and lenalidomide), 2 achieved CR and 3 achieved VGPR. However, 2 patients discontinued therapy (1 cytopenia, 1 suboptimal response) and 4 patients suffered gastrointestinal side effects [74]. A further report of 2 heavily pre-treated patients, receiving venetoclax in combination with a PI, documented a CR in both patients. One patient stopped treatment due to pneumonia after cycle 2 whilst a second stopped treatment due to discontinuation of the BELLINI trial. The former has remained in CR, without further treatment, almost a year later [75]. Finally, a third series of venetoclax +/- bortezomib in relapsed-refractory cardiac AL amyloidosis presented evaluable outcomes in 4/7 patients (2 received 1 cycle only, 1 died of pneumonia after cycle 1) with a 50% response rate sustained at 76 and 713 days [76]. These early results are promising but a degree of caution is required given early toxicity data. A phase 1 trial aiming to enrol 25 patients to receive venetoclax is underway (NCT03000660).

*Amyloid fibril directed therapy and challenge of trial end points*

*AL Amyloidosis*

NEOD001, a drug that binds amyloidogenic light chains and promotes phagocytic clearance in vitro [77], failed to show efficacy in prospective trials and development has been discontinued. The PRONTO study used cardiac best response via NT-proBNP as a primary endpoint despite the variability of this biomarker. Furthermore, NT-proBNP increases after chemotherapy in 71% of patients at six months [78] thus early measurements can lead to false positive results. Whilst NT-proBNP does predict clinical outcome[18], these factors highlight the challenges associated with its use as a study endpoint. Analysis of the Phase 3 VITAL study of NEOD001 plus standard of care suggests a survival benefit in high-risk Mayo Stage IV patients' thus additional clinical studies of NEOD001 may be warranted in the future [79].

A phase 1 trial of (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC) also known as miridesap, with dezamizumab, a humanized monoclonal anti-SAP antibody, demonstrated hepatic and renal clearance with reduction of the splenic amyloid load

and improved hepatic function [80]. However, the trial assessing this combination (NCT03044353) in cardiac amyloidosis was stopped after a data review cited an unfavorable risk-benefit. The chimeric fibril-reactive monoclonal antibody, CAEL-101 (formally 11-1F4), has also been shown to be safe in a phase 1 setting with interim analysis reporting reduction in the amyloid burden with associated rapid improvement in organ function [81]. A randomized phase 2/3 trial further assessing CAEL-101 is planned in 2020.

Further work aiming to better evaluate the structure and pathogenesis of light chain protein misfolding is underway. Two studies have used cryo-electron microscopy mapping of tissue extracted fibrils from patients with AL amyloidosis to provide insight into the mechanism of protein misfolding. This work may lead to the development of novel ligands providing a foundation for future amyloid fibril-directed therapy [82, 83].

Doxycycline interferes with amyloid fibril formation in mouse models and, as such, a small retrospective study suggested that doxycycline added to standard therapy reduced early cardiac mortality but did not impact haematological response rate [84]. A further trial evaluating the addition of doxycycline to standard therapy in patients receiving bortezomib-based therapy for cardiac AL amyloidosis (NCT03474458) is underway. Publication of the DUAL study is awaited (NCT02207556) - a phase 2 study investigating the use of prolonged doxycycline used to upgrade response in AL amyloidosis.

*RNA inhibitors and protein stabilisers in ATTR and AL amyloidosis*

In hereditary ATTR (hATTR), liver transplantation was the historical standard of care[85] whilst wtATTR amyloidosis had no disease modifying treatment. Two strategies have transformed the therapeutic scenario in ATTR amyloidosis. Transthyretin stabilisers have been used as a means of slowing disease progression with some success. Diflunisal, a non-steroidal anti-inflammatory drug, and tafamidis, a thyroxine-like TTR-stabiliser, reduce neurological progression and improve quality of life scores in patients with hATTR[86, 87]. Tafamidis is licenced for this indication

in Europe. A phase III study demonstrated significant survival benefit for patients with cardiac ATTR treated with tafamidis which has led to the tafamidis being the first licensed treatment for this indication. Exciting gene-silencing therapies (patisaran[88]and inotersen[89]), selectively switching off transthyretin production, are now licenced for patients with neuropathic hATTR amyloidosis. Both agents have demonstrated highly significant improvements in neurological and quality of life scores. Patisiran also decreased mean left ventricular wall thickness, global longitudinal strain, NT-proBNP and adverse cardiac outcomes[90] suggesting an effect on patients with ATTR and associated cardiac involvement. Longer acting gene silencers (vutrisiran) and more potent transthyretin stabilisers (AG-10) are in clinical trials.

AL amyloidosis has trailed ATTR in these crucial therapeutic aspects. Recently, high-throughput screening and characterization identified several small molecules that kinetically stabilize free light chains by binding at the V-domain–V-domain interface in both kappa and lambda light chains providing the first step to a potential FLC stabilising approach [91]. Whilst pre-clinical work suggests potential in RNA inhibitors in reducing free light chains production [92], this remains challenging to translate into *in vivo* models.

*Conclusion and Future Directions*

Recent advances in the diagnosis and treatment of amyloidosis, hold promise. At present, there are 125 active trials relating to amyloidosis (clinicaltrials.gov) reinforcing the notion that systemic amyloidosis is truly moving into the spotlight. Early detection remains a critical barrier to improving outcomes. Early adoption of amyloid specific imaging has led to a marked increase in the detection of wtATTR amyloidosis. Use of new methods to detect monoclonal protein in the serum will help both diagnosis and monitoring during treatment. In AL amyloidosis, assessment of response and tracking of organ damage due to amyloid deposits continues to improve whilst new

MRD based methods may be used to detect early relapse and initiate next line therapy prior to the deposition of significant further amyloidogenic protein and associated organ dysfunction.

Rapid reduction in amyloidogenic light chains to preserve organ function in AL amyloidosis is critical. Risk stratification to direct therapy has improved outcomes in high risk AL patients. Both novel agents and new combinations of therapies show promise in achieving rapid responses and improving survival with a number of clinical trials underway investigating these agents. There have been significant therapeutic advanced in ATTR treatment, which may change the disease trajectory. Organ toxicity limits life expectancy in both AL and ATTR amyloidosis. The development of treatments that directly remove amyloidogenic protein from the circulation or accelerate clearance of tissue amyloid deposits, whist showing tantalizing promise, still remains a horizon to be reached.

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**Figure Legends**

Figure 1
A: Cardiac MRI Modalities demonstrating improvement following a complete response to chemotherapy. Image courtesy of Dr. Ana Martinez-Naharro and Dr. Marianna Fontana.
B: Serial SAP scintigraphy demonstrating regression of amyloid in the liver over a 5 year period

Figure 2
A : PET-CT imaging using 18F-Florbetapir as the tracer and demonstrating cardiac uptake in AL amyloidosis
B: PET-CT image demonstrating uptake of p5+14 labelled with iodine-124 by amyloid in the liver. Image courtesy of Dr. Jonathan Wall

Figure 3: Suggested algorithm of investigations to diagnose amyloidosis

Figure 4: Novel Biomarkers in AL Amyloidosis: [20-27]

Figure 5: Mass Spectrometry demonstrating 1) A monoclonal lambda light chain peak 2) A monoclonal and glycosylated kappa light chain peak

Table 1: Key Subtypes of Amyloidosis

Table 2: Exclusion Criteria for ASCT in AL amyloidosis

Table 3: Treatment regimens for patients with AL amyloidosis.