Novel investigations and treatment outcomes in systemic AL amyloidosis

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Declaration

I, Richa Manwani, confirm that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this

has been indicated in the thesis.

Abstract

Background

Systemic AL amyloidosis (AL) is a potentially fatal disorder characterised by fibrillary deposition of monoclonal immunoglobulin light chains within organs. Outcomes in AL have improved, but ~20% of patients die within six months of diagnosis.

Aims

Since upfront bortezomib-based therapy is the mainstay of contemporary AL management, I aim to assess outcomes with this therapy in a large UK cohort. Within this cohort, I shall explore the impact of achieving a 'stringent' light chain response. I also aim to explore outcomes in AL patients with advanced cardiac involvement, and patients who do not achieve early haematological responses to upfront therapy. Few AL patients are eligible for upfront autologous stem cell transplantation (ASCT) due to advanced cardiac involvement, and I shall explore the role of deferred ASCT in initially transplant-ineligible patients. I shall examine treatment outcomes with rituximab-bendamustine in IgM-associated AL. AL patients with severe neuropathic involvement have limited chemotherapeutic options, and I shall assess outcomes with the second-generation proteasome inhibitor, carfilzomib, in such patients. The final aim of this thesis is to perform a pilot study of ^{18}F-florbetapir, a novel imaging tracer, in cardiac AL.

Results and conclusion

Bortezomib-based therapy results in good overall survival and time-to-next-treatment in AL. A 'stringent' light chain response predicts prolonged time-to-next-treatment and excellent organ responses. Patients who do not achieve early haematological responses with bortezomib can still go on to achieve excellent haematological responses. AL patients with advanced cardiac involvement who achieve rapid, deep haematological responses have survival outcomes that are better than previously reported. Deferred ASCT is a potential treatment option in initially transplant-ineligible AL patients. Rituximab-bendamustine induces good haematological responses in IgM-associated AL. Carfilzomib is an effective treatment option in AL patients with significant neuropathic involvement. ^{18}F-florbetapir PET is associated with cardiac uptake in AL patients with cardiac involvement.

Impact statement

In this thesis, I focus on outcomes in AL amyloidosis with standard upfront therapy, as well as studying outcomes in complex AL amyloidosis subgroups. I also explore the role of a novel imaging tracer, ^{18}F-florbetapir, in AL. The work described here has resulted in a number of publications and international presentations.

AL is a rare, potentially fatal plasma cell disorder. Outcomes in AL have improved, but cardiac involvement still determines prognosis. Due to disease rarity and the poor clinical condition of many patients at diagnosis, there are few prospective randomised studies examining treatment options in AL.

Bortezomib-based therapy is a standard upfront therapy in AL, as most patients are ineligible for upfront ASCT. This thesis confirms that bortezomib-based therapy induces good haematological responses and durable survival outcomes. Patients with excellent haematological responses had outcomes that are akin to those with ASCT. If these findings are corroborated in further studies, selected patients could potentially be spared the toxicity of ASCT.

I also define a 'stringent dFLC response' as a post-treatment dFLC<10mg/L. This is associated with excellent overall survival, prolonged time-to-next-treatment and impressive organ responses. Outcomes with a stringent dFLC

response are better than those seen with a complete response. A deep light chain response should therefore be the therapy goal in this disease.

AL patients with advanced cardiac involvement are excluded from most clinical trials and have historically had dismal survival outcomes. We report that patients with advanced cardiac involvement who achieve excellent haematological responses have survival outcomes that are better than previously reported in this subgroup. These findings provide some confidence that patients with advanced cardiac involvement should be considered for treatment, and that they should be included in clinical trials.

This thesis explores other complex AL subgroups. Deferred ASCT is shown here to be an effective, safe treatment option in patients who are initially transplant-ineligible due to extent of cardiac involvement, but go on to achieve organ responses with induction chemotherapy. It is also demonstrated that AL patients who do not achieve early haematological responses can still achieve excellent haematological responses overall. IgM-associated AL is a rare entity, with poor haematological responses and heterogeneous treatment approaches. We report that rituximab-bendamustine results in good, durable haematological responses in IgM-associated AL. It is also demonstrated in this work that carfilzomib is a treatment option in AL patients with severe neuropathic involvement, whose treatment options are limited due to the neurotoxicity of standard treatment options in AL.

^{18}F-florbetapir is a novel imaging tracer that is licensed in imaging brain amyloid deposits. We demonstrate in a pilot study of cardiac AL patients that all had cardiac uptake with ^{18}F-florbetapir PET imaging. There were no false negatives. While further work is needed to establish whether there is any difference in cardiac uptake in AL and ATTR patients, we show that this is the first tracer to consistently show cardiac uptake in cardiac AL. This is an important finding, as this tracer may serve a crucial role in the non-invasive diagnostic algorithm in cardiac amyloidosis.

Ethical approval

All patients included in the research studies in this thesis provided explicit informed consent. Patients who attended the UK National Amyloidosis Centre signed a written consent form that was approved by the Royal Free Hospital Ethics Committee (REC Ref 06/Q0501/42, 09/H0715/58). Patients that were seen at the Amyloidosis Centre at University Hospital Heidelberg (included in the collaborative study detailed in Chapter 6) provided informed consent in accordance with the Declaration of Helsinki. The administration of radioactive isotopes was approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health.

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Abbreviations

AApoA1: Hereditary Apolipoprotein A1 amyloidosis

Aβ2M: β2-microglobulin associated amyloidosis

AFib: hereditary fibrinogen amyloidosis

AGel: hereditary gelsolin amyloidosis

AL: immunoglobulin light chain associated amyloidosis

ALECT2: leukocyte chemotactic factor 2-associated

ALP: alkaline phosphatase

ASCT: autologous stem cell transplantation

ATTR: transthyretin-associated amyloidosis

BMDex: bortezomib-melphalan-dexamethasone

BNP: brain natriuretic peptide

BR: rituximab-bendamustine

CR: complete response

CTD: cyclophosphamide-thalidomide-dexamethasone

CyBorD: cyclophosphamide-bortezomib-dexamethasone

dFLC: difference in involved and uninvolved light chains

DPD: ^{99m}Tc-labelled 3,3-diphosphono-1,2-propanodicarboxylic acid

ECOG: Eastern Cooperative Oncology Group

ECV: extracellular volume

GCSF: granulocyte colony stimulating factor

GFR: glomerular filtration rate

HMDP: ^{99m}Tc-labelled hydroxymethylene disphosphonate

ICC: international consensus criteria

IgM-AL: IgM-associated AL amyloidosis

ITT: intention to treat

LV: left ventricle

MDex: melphalan-dexamethasone

MGUS: monoclonal gammopathy of undetermined significance

MRD: minimal residual disease

MRI: magnetic resonance imaging

NAC: UK National Amyloidosis Centre

NHL: non-Hodgkin lymphoma

NT-proBNP: N-terminal pro-brain natriuretic peptide

NYHA: New York Heart Association

OHT: orthotopic heart transplantation

ORR: overall haematological response rate

OS: overall survival

PET: positron-emission tomography

PFS: progression-free survival

PYP: ^{99m}Tc-labelled pyrophosphate

RI: retention index

SAA: serum amyloid A protein

SAP: serum amyloid protein

SUV: standardised uptake variable

TNT: time to next treatment

TRM: transplant-related mortality

TTR: transthyretin

VGPR: very good partial response

WM: Waldenström's macroglobulinaemia

Chapter One: Introduction

The amyloidoses are a rare group of disorders characterised by the extracellular deposition of insoluble amyloid fibrils. These fibrils arise when a protein fails to adopt its functional, physiologic conformational state. Such proteins form toxic, insoluble highly ordered fibrillary aggregates with an abnormal β-pleated sheet (predominantly antiparallel) structure.(1) Amyloid deposits are identifiable through their characteristic apple-green birefringence under a polarised light microscope after staining with Congo Red. Deposits are made up of rigid, non-branching fibrils with a diameter of 7-10nm.

Amyloid fibril formation and deposition can cause progressive and potentially fatal multi-organ dysfunction. Over recent years, there have been advances in mass spectrometric diagnosis of precursor proteins in amyloidosis. Thirty-six amyloidogenic proteins have been identified to date (2) and ~15 can cause systemic amyloidosis. (3) The clinical phenotype of amyloidosis can vary, depending on the precursor protein type.

Pathogenesis

Several factors can increase the propensity of amyloid fibrils to form in-vivo: 1) a sustained pathological increase in the concentration of the protein (as in the case of serum amyloid A protein (SAA) in chronic inflammatory disorders and β2-microglobulin in end-stage renal disease); 2) intrinsically unstable

amyloidogenic proteins (e.g. acquired proteins in monoclonal immunoglobulin light chain-associated amyloidosis (AL) or inherited proteins in hereditary amyloidoses); 3) ageing (e.g. wild-type transthyretin-associated (ATTR) amyloidosis and atrial natriuretic peptide amyloidosis). (3, 4)

Whilst amyloid precursor proteins have heterogeneous structures, the amyloid fibrils that they potentially form are morphologically indistinguishable.(5) As well as their fundamental fibrillar structure, all amyloid deposits contain non-fibrillar components. Serum amyloid protein (SAP) is a circulating glycoprotein from the pentatraxin family that is bound by all types of amyloid fibril (independent of the precursor protein type) via a calcium-dependent interaction that confers partial resistance to proteolytic degradation of amyloid fibrils.(6) A number of proteoglycans are also universally found in amyloid deposits, including heparin sulphate which appears to accelerate the native state of SAA into its amyloidogenic state(7), and also accelerates the formation of amyloid fibrils in AL amyloidosis(8, 9) and ATTR amyloidosis(10). Other common elements in amyloid deposits include extracellular matrix components such as laminin, entactin, type IV collagen, and chaperone proteins, such as apolipoprotein E and clusterin.(11) These proteins form a scaffold, enabling the initial phases of fibril nucleation. Amyloid fibril formation occurs through a process of nucleated growth. Monomers of amyloid-forming proteins can remain in solution for long periods in a lag period. Once a critical nucleus is formed, fibril formation begins, followed by rapid ongoing fibrillary assembly. Partly

unfolded amylogenic precursor proteins are rapidly incorporated into the growing fibrils.

Specific amyloidogenic precursor proteins deposit predominantly in certain organs, although the reasons for this are unestablished (Table 1.1).

Table 1.1: Common types of amyloidosis.

Type of amyloidosis	Proportion of patients seen at UK NAC (n=5100, from 1987-2012)(4)	Acquired or hereditary	Aetiology	Precursor protein	Organ involvement
Systemic immunoglobulin light chain amyloidosis (AL)	68%	Acquired	Plasma cell dyscrasia	Immunoglobulin light chains	Cardiac Renal Liver Peripheral nerve Autonomic nerve Gastrointestinal Lung
Systemic AA amyloidosis	12%	Acquired	Inflammatory disorder (e.g. rheumatoid arthritis, juvenile idiopathic arthritis)	Serum amyloid A protein	Renal Liver (rarely cardiac)
Hereditary transthyretin	6.6%	Hereditary	TTR gene mutation	Abnormal TTR	Cardiac

(ATTR) amyloidosis					Peripheral neuropathy Autonomic neuropathy
Wild-type transthyretin-associated amyloidosis	3.2%	Acquired		Wild-type TTR	Cardiac Carpal tunnel syndrome
β2-microglobulin associated amyloidosis (Aβ2M)	1.8%	Acquired	Dialysis-related	β2-microglobulin	Carpal tunnel syndrome Arthropathy
Hereditary fibrinogen amyloidosis (AFib)	1.7%	Hereditary	Fibrinogen-α gene mutation	Abnormal fibrinogen	Renal Liver Cardiac (very rare)
Hereditary Apolipoprotein A1 amyloidosis (AApoA1)	0.8%	Hereditary	ApoA1 gene mutation	Abnormal ApoA1	Renal Liver Cardiac Peripheral neuropathy
Hereditary lysozyme amyloidosis (ALys)	0.3%	Hereditary	Lysozyme mutation	Abnormal lysozyme	Renal Liver

Hereditary leucocyte cell-derived chemotaxin amyloidosis (ALECT2)	0.3%	Acquired	Aetiology unknown	LECT2	Renal Liver
Hereditary gelsolin (AGel) amyloidosis	0.1%	Hereditary	Gelsolin mutation	Abnormal gelsolin	Cranial nerve Corneal lattice dystrophy Renal

For example, amyloid deposition within the kidneys leading to clinical dysfunction is a hallmark of systemic AA amyloidosis, leukocyte chemotactic factor 2-associated (ALECT2) amyloidosis and fibrinogen Aα chain amyloidosis. (3) Peripheral nerve involvement is found in ATTR and AL amyloidosis. Amyloid deposits can involve virtually any organ in AL amyloidosis. Factors that are likely to contribute to this curious organ tropism include high local precursor protein concentrations, tissue–specific glycosaminoglycans, pH, the presence of local proteolytic enzymes or cellular receptors and collagen binding(12).

The exact mechanism of tissue damage in amyloidosis has not been fully elucidated. However, interstitial amyloid deposition disrupts tissue architecture, adversely affecting organ function – but prefibrillar aggregates can also contribute. The relative contribution of amyloid deposits and

prefibrillar aggregates to organ dysfunction may vary depending upon the type of amyloidosis and organs affected. There is evidence to suggest that prefibrillar oligomers may themselves be directly toxic in Alzheimer's disease (13) and ATTR amyloidosis (14). Amyloidogenic immunoglobulin light chains may be directly toxic to cardiac myocytes in AL amyloidosis through induction of oxidative stress, cellular dysfunction and apoptosis.(15) In AL amyloidosis, reduction of the circulating monoclonal immunoglobulin light chains with treatment can result in improvements in the levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) (a prognostic marker of cardiac dysfunction in AL amyloidosis), despite no early improvement in myocardial amyloid load as assessed by echocardiography.(16)

Only a small proportion of immunoglobulin light chains are amyloidogenic. Indeed, only 10% of patients with myeloma develop coexisting AL amyloidosis. The λ isotype of light chains may predispose to amyloidogenicity. The $V_{\lambda VI}$ variability subgroup in particular was found to be predominant among monoclonal lambda light chains from patients with AL amyloidosis, and was found exclusively on amyloidosis-associated proteins.(17) Two germline genes belonging to the lambdaIII and lambda VI families (3r and 6a) contributed equally in encoding 42% of amyloidogenic λ light chains, and these two gene segments have a strong association with amyloidosis if their prevalence is compared with those in polyclonal conditions.(18) The variable domains of light chains mutate during the immune response, and some of these physiologic mutations may give rise to an aggregation-prone state.(19, 20).

Protein instability also has a role in amyloidogenicity. For example, TTR monomers are less stable than TTR tetramers, and have an increased propensity to auto-aggregate and assemble as amyloid fibrils.(21) Single point mutations can destabilise lysozyme's structure, with partially folded monomers being produced which are able to auto-aggregate and form fibrils.(22)

Amyloid regression is poorly understood, although macrophage activity is thought to play a role. Amyloid-β immunotherapy studies implicated macrophages in amyloid clearance, but this was opposed by ongoing aggregation of newly secreted Aβ.(23)

Epidemiology

There are few epidemiological studies in amyloidosis. An early population-based study in the USA in 1992 reported the incidence of amyloidosis as three to five cases per million.(24) Death certificate data in England estimated the incidence of amyloidosis as one per 100,000.(25) Table 1.1 outlines the proportion of patients seen with different types of amyloidosis at the UK National Amyloidosis Centre (NAC).(4)

Systemic AL amyloidosis is the most prevalent type of amyloidosis in developed countries. There has been a progressive fall in the proportion of patients seen at the UK National Amyloidosis Centre (NAC) with newly

diagnosed AA amyloidosis, likely due to the increasing use of effective biologic therapies in the inflammatory arthropathies. There has been a concomitant increase in the number of patients seen at the UK NAC with wild-type transthyretin amyloidosis (ATTR), partly due to improving awareness of this disorder and the increasing use of cardiac MRI (magnetic resonance imaging) in investigation of patients with unexplained heart failure and/or ventricular hypertrophy. The true incidence of wild-type ATTR amyloidosis is unknown, but a population based autopsy study identified TTR amyloid deposits in 25% of cardiac tissue samples from those over 80 years old.[26]

Systemic AA amyloidosis

Amyloid deposits in systemic AA amyloidosis are derived from serum amyloid AA protein (SAA), an acute phase reactant. SAA is an apolipoprotein synthesised by hepatocytes in response to transcriptional stimuli from proinflammatory cytokines (e.g. interleukin-1, interleukin-6 and tumour necrosis factor alpha).[26] SAA is usually found in the serum at low levels. Sustained overproduction of SAA is essential for development of AA amyloidosis, although curiously, only a small proportion of patients with chronic inflammatory diseases develop systemic AA amyloidosis. [27, 28]

In a large study of patients at the UK National Amyloidosis Centre diagnosed with systemic AA amyloidosis from 1990-2005, the most common underlying inflammatory disorder was chronic inflammatory arthritis (60% of patients

with AA amyloidosis, most commonly rheumatoid arthritis). Other underlying causes were chronic infection (e.g. bronchiectasis, intravenous drug use, tuberculosis), periodic fever syndromes (e.g. Familial Mediterranean fever), inflammatory bowel disease and malignancy.(29) A proportion of patients have idiopathic AA amyloidosis. Obesity is now recognised as a risk factor in development of AA amyloidosis(30), and mouse models have demonstrated that obesity is associated with chronic inflammation and AA amyloidogenesis. (31, 32)

The predominant feature of AA amyloidosis is renal dysfunction, with 97% of patients presenting with proteinuria and/or an elevated creatinine in Lachmann et al's study.(29) While 23% of patients in the latter study had liver uptake on ^{123}I-serum amyloid P (SAP) scintigraphy (indicative of hepatic amyloid deposits), only 6% had an elevated alkaline phosphatase. Other involved organs in systemic AA amyloidosis can include the spleen, adrenal glands and gastrointestinal involvement – although symptomatic involvement of these organs is uncommon. Cardiac involvement in AA amyloidosis is exceptionally rare.

The treatment of systemic AA amyloidosis is by means of robustly controlling the underlying inflammatory disorder in order to suppress circulating SAA levels. In turn, this maximises the likelihood of amyloid stabilisation or regression, the latter allowing for improvement in amyloid-related organ dysfunction. Survival is prolonged in those patients with a persistently low

circulating SAA concentration. (30) Surgical excision of highly inflammatory tumours such as localised Castlemann's disease has led to substantial reduction of acute phase reactant levels and scintigraphic regression of amyloid deposits. (33) Malignancies successfully treated with chemotherapy have also been associated with amyloid regression. (34) High dose colchicine is effective in autoinflammatory conditions such as Familial Mediterranean fever and can result in amyloid regression.(35) Immunomodulatory therapies in inflammatory joint disease and inflammatory bowel disease have also successfully induced remission of associated AA amyloidosis. (36, 37)

ATTR amyloidosis

ATTR amyloidosis is classified based on the sequence of the TTR gene, into wild-type ATTR amyloidosis (absence of a TTR genetic mutation) and hereditary ATTR amyloidosis (presence of a TTR genetic mutation). TTR is a tetrameric protein that is present in all human serum and is physiologically responsible for transportation of thyroxine and retinol-binding protein. The majority of transthyretin is synthesised in the liver, and a small proportion (<5%) is produced in the choroid plexus of the brain and retinal pigment epithelium. The TTR gene is present on chromosome 18. Single point mutations can destabilise the TTR tetramer. TTR tetramers with at least one mutant subunit are kinetically or theromodynamically unstable and release monomers in physiologic conditions. (38) These monomers have an ability to misfold and aggregate into polypeptides, resulting in TTR oligomers (that

cause direct tissue toxicity) and amyloid fibrils. Wild-type ATTR amyloidosis is associated with a normal TTR genetic sequence, and it is unclear why the wild-type protein kinetically destabilises and aggregates.

Clinical features and disease course

The clinical phenotype is more variable in hereditary ATTR amyloidosis than wild-type ATTR amyloidosis and depends upon the site of TTR deposition. (39) The phenotype can comprise variable manifestations of cardiomyopathy (with arrhythmias and heart failure), peripheral neuropathy and autonomic neuropathy. Patients with hereditary ATTR amyloidosis often have a mixed phenotype. Peripheral and autonomic neuropathy are less common and less pronounced in wild-type ATTR amyloidosis, where heart failure is the predominant feature.

More than 120 amyloidogenic TTR variants have been identified to date and their phenotypic picture, too, is variable. The commonest TTR mutation worldwide is Val122Ile, present in 3% of Afro-Caribbeans and which tends to present in older age with a predominantly cardiac phenotype (progressive heart failure symptoms and ventricular wall hypertrophy). (40) The Val30Met mutation is the second commonest TTR mutation globally (with major clusters in Portugal, Japan and Sweden) and has a predominantly neurological phenotype. The Thr60Ala mutation originates in the Republic of Ireland and occurs predominantly in males and neuropathy is a primary feature. Hereditary ATTR amyloidosis is inexorably progressive, with a

median survival of 2-5 years in those with familial amyloid cardiomyopathy. (41, 42) In patients with familial amyloid polyneuropathy, median survival is approximately 8-10 years. (43)

Wild-type ATTR amyloidosis is largely a disease occurring in older adults and predominantly in males. The median survival from diagnosis is 3.5 years. Cardiac biomarkers have been used in staging of the condition. The Mayo clinic have outlined a staging system using thresholds of troponin T>0.05ng/ml and NT-proBNP>3000pg/ml, whereby Stage I was defined as both biomarkers below the threshold, stage II as one biomarker above the threshold and Stage III as both biomarkers above the thresholds. The median survival in these stages was 66, 42 and 20 months respectively.(44) The UK National Amyloidosis Centre have described a staging system in wild-type ATTR amyloidosis, whereby the thresholds were NT-proBNP<3000pg/ml and eGFR>45ml/min.(45) The median prognosis in Stage 1 (NT-proBNP<3000pg/ml and eGFR>45ml/min), Stage 2 (NT-proBNP>3000pg/ml or eGFR<45ml/min) and Stage 3 (NT-proBNP>3000ng/L and eGFR<45ml/min) was 69.2 months, 46.7 months and 24.1 months, respectively.

Treatment options

1) Medical management

The mainstay of management in ATTR amyloidosis is heart failure therapy, with salt restriction and diuretics. Given the high incidence of

atrial arrhythmias in this cohort, anticoagulation should also be considered.

2) Organ transplantation

Orthotopic liver transplantation has been used in hereditary ATTR amyloidosis, as the majority of transthyretin is produced in the liver. A study of 1940 patients with mutant transthyretin production who underwent transplantation with a liver producing only wild-type transthyretin showed that 80% of the patients survived for at least ten years, and 20 year survival was 55%.(46) Neuropathic progression was halted, but there was no recovery of existing amyloid-related organ dysfunction and there was substantial mortality in patients with advanced disease. The use of liver transplantation in this condition has much reduced in view of emerging therapies, and orthotopic liver transplantation is not indicated in wild-type ATTR amyloidosis. Orthotopic heart transplantation is an option in a very selected group of patients.

3) Stabilising transthyretin tetramer

   i) <u>Diflunisal</u>

   Diflunisal is a non-steroidal anti-inflammatory medication that binds within the two thyroxine binding sites of transthyretin, stabilising the tetramer and preventing amyloid fibril formation. A Phase III randomised controlled study of diflunisal in patients with hereditary ATTR polyneuropathy showed an improvement in neuropathic symptoms, compared to placebo. (47) Single

centre studies have also shown a survival benefit in patients with ATTR cardiomyopathy and is well tolerated. (48, 49)

ii) Tafamidis

Tafamidis binds to the thyroxine-binding sites on transthyretin, stabilising the tetramer and slowing down its dissociation into monomers, therefore preventing amyloid formation. A randomised controlled study of tafamidis compared to placebo in patients with hereditary Val30Met ATTR amyloid polyneuropathy showed slowing of progression of neuropathy with the former therapy. (50) The ATTR-act study compared the efficacy of tafamidis compared to placebo in patients with wild-type and hereditary ATTR amyloidosis. In the latter study, tafamidis resulted in lower all-cause mortality and cardiovascular hospitalisations(51).

4) TTR-specific oligonucleotides

i) Patisiran

Patisiran is a small interfering RNA which targets transthyretin and reduces TTR messenger RNA levels. This reduces subsequent synthesis of transthyretin, thereby reducing the amount of misfolded TTR monomers that are able to aggregate and form amyloid deposits.(38) A recent Phase III study showed a significant improvement in the modified Neuropathy Impairment Score in patients with hereditary ATTR polyneuropathy who were treated with patisiran, compared to placebo.(52) Subgroup analysis of patients with ATTR

cardiomyopathy within the study revealed a reduction in NT-proBNP, fewer cardiovascular hospital admissions and lower all-cause mortality with patisiran compared to placebo.(53)

    ii)   Inotersen

Inotersen is an anti-sense RNA construct. A phase III study of inotersen compared to placebo in patients with familial amyloid polyneuropathy revealed an improvement in the modified Neuropathy Impairment Score with inotersen.(54) However, there was thrombocytopenia reported with inotersen, with mean platelet counts being significantly lower with inotersen compared to placebo, and one patient who received inotersen suffered a fatal intracranial haemorrhage.(54) The mechanism of thrombocytopenia with inotersen is unclear.

β2-microglobulin associated amyloidosis

β2-microglobulin is a constituent of the human HLA class I major histocompatibility complex and circulates as a monomer. (55) β2-microglobulin is metabolised in the kidneys, and serum β2 levels are elevated in patients with renal failure, most commonly those on dialysis. The increased serum levels of β2-microglobulin act as a precursor protein for amyloid deposition. Traditional dialysis membranes did not allow for removal of β2-microglobulin. Although newer membranes do, β2-microglobulin production still exceeds clearance with dialysis. Manifestations of amyloid

deposition in this condition include carpal tunnel syndrome, arthralgia, juxta-articular bone cysts and resulting pathological fractures. Visceral involvement (most commonly cardiac), where present, is usually subclinical.

Hereditary fibrinogen amyloidosis (AFib)

Hereditary fibrinogen amyloidosis is an autosomal dominant condition and is the commonest type of hereditary renal amyloid in the UK. (56) Patients with hereditary fibrinogen amyloidosis present with renal disease (proteinuria +/- abnormal renal excretory function), and typically progress to end-stage renal disease. Extra-renal involvement is rare. In a UK observational study of 71 patients with the disease, family history of renal disease was often absent – suggesting a variable penetrance. Among 44 patients who reached end-stage renal disease in this study, the median survival was 9.3 years and the median graft survival of twelve renal transplants in the study was 6 years.

Hereditary Apolipoprotein A1 amyloidosis

Apolipoprotein A1 is a high-density lipoprotein that is important in cholesterol transport.(57) It is produced by the liver and intestines and degraded renally. Hereditary ApoA-1 amyloidosis is a rare, autosomal dominant form of amyloidosis with variable penetrance. The ApoA-1 gene is found on Chromosome 11. Twenty amyloidogenic mutations have thus far been described. The phenotype varies depends upon the variant, with Gly26Arg, Trp50Arg, Leu60Arg, Del70-72, Leu75Pro and Leu64Pro all characterised by renal involvement and hepatosplenomegaly. (58) The disease course is

usually slowly progressive. Amyloid deposition can arise in the kidneys, liver, spleen and heart, giving rise to chronic kidney disease, hepatomegaly and cardiomyopathy. Progression of renal disease is slow. Some variants (Leu90Pro, Arg173Pro, Leu174Ser and Leu178His) are associated with skin deposits and a progressive cardiomyopathy. Renal transplantation is a potential treatment option in patients with renal disease, as graft survival in such patients is excellent even in patients with amyloid recurrence within the graft.(58) Liver transplantation can favourably alter the natural history of the condition, with regression of visceral amyloid.

Hereditary lysozyme amyloidosis

Lysozyme is a bacteriolytic enzyme produced in macrophages in the gastrointestinal tract and hepatocytes.(59) Hereditary lysozyme amyloidosis is a rare autosomal disease that was first described in 1993 by Pepys et al. (60) Affected individuals were found to be heterozygous for point mutations in the lysozyme gene, and six different mutations have been reported thus far.(59) The disease exhibits high clinical penetrance and a family history is usually present, unlike other forms of hereditary systemic amyloidosis. Most patients have hepatic amyloid, although liver function tests are usually relatively preserved. However, hepatic rupture and haemorrhage has been reported in this condition – possibly due to the extent of hepatomegaly. Other manifestations include soft tissue amyloidosis (presenting with sicca syndrome, bruising, lymph node enlargement), renal dysfunction progressing to end-stage renal disease (although speed of progression is variable) and

gastrointestinal haemorrhage. Orthotopic liver transplantation has been used in cases of liver rupture, and renal transplantation for end-stage renal disease in this condition has been associated with good graft survival.

Leucocyte cell-derived chemotaxin amyloidosis (ALECT2)

ALECT2 amyloidosis was first reported after being discovered in a nephrectomy specimen from a patient with co-existing renal cell carcinoma. (61) LECT2 is found in the liver and is a chemotactic factor to neutrophils, with high levels of induction with infection in zebrafish studies. (62) The human LECT2 gene is found on chromosome 5, and encodes 151 amino acids. There is no mutation in the LECT2 gene associated with ALECT2 amyloidosis, but nearly all patients in a recent series were found to be homozygous for a polymorphism that results in a substitution of isoleucine with valine at position 40 in the mature protein.(63) The disease has been mainly reported in individuals from the Indian subcontinent, Egypt and Hispanics. Low level proteinuria and abnormal renal excretory function are often the presenting features of the condition. While hepatic amyloid is common, liver synthetic function is usually preserved. Cardiac involvement is absent. The natural history of the condition is slow, and median estimated overall survival in one series was over 15 years. (63)

Hereditary gelsolin (AGel) amyloidosis

Hereditary gelsolin amyloidosis has autosomal dominant inheritance and is one of the most common heritable disorders among the Finnish

population(64). Gelsolin is a ubiquitous calcium-activated, actin modulating protein that is implicated in many biological processes. Clinical manifestations of this disease include corneal lattice dystrophy, cranial nerve abnormalities and cutis laxa. Patients commonly present at age 25-35 years with corneal lattice dystrophy. Renal and cardiac involvement are rare. The severity of the clinical phenotype is variable, although homozygotes experience rapid progression. Treatment of the disease is largely supportive, but corneal transplantation and reconstructive surgery are potential treatment options.

Localised AL amyloidosis

Localised amyloidosis is characterised by amyloid deposition that is confined to a single site. Localised deposits of amyloid can occur almost anywhere in the body and are usually presumed to be of light-chain type, due to the presence of a monoclonal B-cell dyscrasia within the affected tissue. (65) Commonly reported sites include the urinary tract, respiratory tract, larynx, skin and eyelids. It is an indolent disease that rarely evolves to systemic amyloidosis and has no effect on life expectancy. Treatment is dependent upon symptoms. Tracheobronchial deposits can be particularly symptomatic and challenging to manage, although radiotherapy can result in some symptomatic benefit.

Systemic AL amyloidosis

Systemic AL amyloidosis is the commonest form of systemic amyloidosis. It is characterised by deposition of a misfolded monoclonal light chain that is secreted from a malignant plasma cell clone.(66) The disease is associated with potentially catastrophic visceral involvement, and cardiac involvement predicts outcome.

Epidemiology

Population studies estimate an incidence of AL amyloidosis of 8.9-12 affected individuals per million per year.(24) The prevalence of AL was previously estimated to be 8.8-15.5 affected individuals per million person-years, although this has increased to 40-58 affected individuals per million person-years (likely due to improved overall survival of patients).(25, 67) The mean age at presentation of AL is around 63 years, and just over half of patients with the disease are male. (67) Patients with a monoclonal gammopathy of undetermined significance (MGUS) are more likely to develop systemic AL amyloidosis compared to individuals without MGUS, with a relative risk of 8.8 in the former. (68) In a cohort of patients who were newly diagnosed with multiple myeloma, bone marrow biopsy, fat pad aspirate and organ biopsy data revealed that 38% had amyloid deposition at one or more site.(69) Around 10% of patients with myeloma have coexisting overt AL amyloidosis at presentation, and 1% of patients with pre-existing myeloma will go on to develop AL amyloidosis.(66, 70)

A large genome-wide association study has also characterised a germline susceptibility to AL amyloidosis. (71) Single nucleotide polymorphisms at 10 loci showed evidence of an association with AL, most significant of which was rs9344 at the splice site of cyclin D1, promoting translocation (11;14). The latter was only marginally significant in multiple myeloma, suggesting that cyclin D1 is a more prominent driver in AL amyloidosis.

Mechanism of fibrillogenesis in AL amyloidosis

AL amyloidosis is caused by a low level B cell clone that produces, in the majority (80%), an excess of lambda immunoglobulin chains. In the remainder, there is an over-production of kappa immunoglobulin light chains. There is a high frequency of chromosomal translocation (11;14) within the amyloidogenic B cell clone. Hyperdiploidy is less common in AL amyloidosis than it is in myeloma(72), as are other poor-risk myeloma genetic abnormalities such as t(4;14) and 17p deletions(73). Somatic mutations in IGLV (which encodes the light chain variable region) result in the production of amyloidogenic variable light chain domains through alteration of fold stability and protein dynamics. (74) Aggregation of amyloidogenic light chains can occur due to disruption of extracellular chaperones. Protein aggregation is also promoted by interaction of amyloidogenic light chains with the tissue microenvironment (extracellular matrix components, shear forces, proteases and metals). (66) Light chain oligomers form highly organised fibrils. Organ damage can result through amyloid deposition within parenchymal tissue and through soluble prefibrillar forms that cause direct

tissue damage through proteotoxicity and increased cellular oxidative stress with resulting mitochondrial damage and reduced cell viability. (15, 66, 75, 76)

When a specific concentration of misfolded proteins is reached, a critical nucleus forms that acts as a catalyst for further protein aggregation and fibrillogenesis. The concentration of misfolded light chains that is necessary for fibril elongation is lower than the concentration required for forming the critical fibrillary nucleus. (66) Amyloid deposits are largely resistant to degradation, although there is some slow clearance by macrophages.(77)

<u>Clinical features</u>

A survey by the Amyloid Research Consortium showed that over a third of patients are diagnosed with AL amyloidosis over a year after initial symptom onset.(78) The clinical features of systemic AL amyloidosis are protean, but the commonest at diagnosis are nephrotic syndrome with or without renal insufficiency, congestive cardiomyopathy, sensorimotor and/or autonomic neuropathy and hepatomegaly. (73) Fatigue and weight loss are common but non-specific symptoms, and patients are mostly diagnosed after organ-specific symptoms arise.

<u>Cardiac involvement</u>

Around 70% of patients have cardiac involvement (79) and the presence and extent of cardiac involvement is predictive of outcome.(80) The cardiomyopathy is restrictive and gives rise to symptoms of congestive cardiac failure.(73) Arrhythmias, particularly atrial fibrillation, are common and are associated with a sudden deterioration in cardiac performance and clinical condition. Atrial thrombi can also occur in patients with atrial fibrillation, or even those patients in sinus rhythm.

<u>Renal involvement</u>

Over half of patients with AL amyloidosis have renal involvement(79), which is usually glomerular and gives rise to nephrotic syndrome.(73) Marked albuminuria (as opposed to isolated Bence Jones proteinuria) in patients with myeloma raises suspicion of AL amyloidosis. Loss of excretory function is also common, but very rarely without proteinuria. Symptoms of renal involvement include peripheral oedema, fatigue, and breathlessness due to pleural and pericardial effusions.

<u>Peripheral and autonomic neuropathy</u>

Approximately a fifth of patients present with neuropathic involvement.(79) This is most commonly a peripheral symmetrical sensory neuropathy, with numbness, paraesthesiae and pain.(73) Motor neuropathy is rare. Autonomic

neuropathy can occur, giving rise to postural hypotension, impotence, weight loss and gastrointestinal dysmotility.

Gastrointestinal and hepatic involvement

Gastrointestinal tract involvement can be focal or diffuse, and symptoms relating to its involvement can vary.(73) Macroglossia occurs in 10% of patients and is pathognomonic of AL amyloidosis. It can lead to airway obstruction, sleep apnoea, speech and eating difficulties. Symptoms of gastrointestinal involvement include diarrhoea, nausea, weight loss and rectal bleeding. A quarter of patients have hepatomegaly at presentation, although not all have abnormal liver function tests. Hepatomegaly can result from direct amyloidotic infiltration or due to congestive hepatopathy.

Bleeding symptoms and coagulation abnormalities

The coagulation screen is abnormal in half of AL amyloidosis patients and bleeding symptoms are reported in almost a third.(81) The commonest abnormalities are prolongation of the thrombin time. Prolongation of the thrombin time is associated with hepatic amyloid infiltration and nephrotic syndrome. Widespread small vessel fragility is also a common feature, with cutaneous ecchymoses and periorbital purpura being one of the distinctive presenting features. Specific coagulation factor deficiencies are also noted, most commonly Factor X deficiency. This is postulated to occur due to adsorption of Factor X by amyloid deposits in the spleen. However, this is likely to be an oversimplification as the amyloid load within the spleen has

not been shown to correlate with Factor X levels.(81) Other acquired coagulation factor deficiencies reported in systemic AL amyloidosis include Factor IX, II, VII and V. Vitamin K factor malabsorption may result from gastrointestinal amyloidotic infiltration.

Other organ systems

Other clinical features include skin and soft-tissue thickening, seronegative arthropathy, hoarse voice (due to vocal cord infiltration), hypoadrenalism (due to adrenal gland infiltration), hypothyroidism (due to thyroid gland infiltration), lymphadenopathy and pulmonary infiltration.(73)

Diagnosis

A diagnosis of AL amyloidosis should be considered in any patient with heart failure with preserved ejection fraction, proteinuria, peripheral neuropathy, autonomic neuropathy or hepatomegaly – especially in a patient with a monoclonal gammopathy or myeloma. (66) Amyloidosis is a histological diagnosis, and work-up includes assessment of amyloid type and identification of extent of organ involvement.

Histology

Histological confirmation of amyloid deposition from a biopsy of an affected organ is the gold standard diagnostic test in amyloidosis. Characteristically, amyloid deposits give rise to pathognomonic apple-green birefringence when

viewed under cross-polarised light. Alternatively, abdominal fat pad fine needle aspiration is a low risk and widely used 'screening' diagnostic test that can obviate the need for biopsy of an affected organ. The specificity of the latter test is high, but its diagnostic sensitivity is variable (reported as 50-90%).(82, 83) A large study of patients with cardiac amyloidosis found poor diagnostic sensitivity of fat aspiration in wild-type ATTR amyloidosis and V122Ile-associated cardiac amyloidosis, but 84% sensitivity in AL amyloidosis.(84) Rectal and labial biopsies are other potential sites for screening biopsy. However, a negative screening biopsy does not exclude the diagnosis of amyloidosis, and should be followed by biopsy of an organ that is suspected to be involved if clinical suspicion is sufficient. Patients with amyloid deposits detected on bone marrow biopsy and an absence of organ involvement have a 2.7% chance of developing systemic amyloidosis.(85) If amyloid deposits are identified, it is important to establish whether the involvement is localised or systemic. Common sites of localised involvement include the skin, larynx, urinary tract and bowel.

Immunohistochemistry

Immunohistochemical typing of amyloid remains challenging. Fibril protein specific antibodies are available to enable assessment of amyloid type, but sensitivity and specificity remain poor in AL amyloidosis. Particular difficulties arise with immunohistochemical staining of AL deposits due to the presence of background normal immunoglobulin, and because light chain epitopes that are recognised by antisera may be lost during fibril formation and tissue

fixation.(73) Commercial antisera that detect kappa and lambda immunoglobulin light chains are usually directed against epitopes on the constant region of the immunoglobulin light chain, but in AL amyloidosis usually only a fragment of intact light chain is deposited and this tends to be the variable portion.(79) Additionally, a variety of antisera are required to exclude other forms of systemic amyloidosis, and few centres possess these. Immunofluorescence is a more reliable technique, but it requires frozen amyloidotic tissue.(86) Immunoelectron microscopy combines immunohistochemistry and electron microscopy and is more reliable in assessment of amyloid type, but not widely available.

Proteomic analysis

Laser dissection and mass spectrometry accurately confirms and types amyloid deposits, and can be performed using small quantities of formalin fixed amyloidotic tissue.(86) The technique was initially validated by the Mayo group in 2009 in predominantly endomyocardial biopsy specimens, and found to be highly sensitive and specific for identification and typing of amyloid deposits.(87) Amyloid deposits are removed from a slide, and peptides are sequenced by mass spectrometry. The sequences are then identified in reference libraries of proteins for identification. A recent large UK study showed high concordance between Congo Red staining and mass spectrometry for the identification of amyloid samples.(86) Mass spectrometry was found to be superior to immunohistochemistry in establishing amyloid type.

DNA analysis

DNA analysis is useful in distinguishing AL amyloidosis from hereditary forms of amyloidosis. A family history of amyloidosis may not always be present due to incomplete penetrance. In a study of 350 patients with a diagnosis of AL amyloidosis, genetic analysis was undertaken and revealed that 9.7% had been misdiagnosed with AL amyloidosis; the commonest amyloidogenic mutations were of the fibrinogen A alpha-chain and TTR.(88) Therefore, genetic analysis should be undertaken where there is clinical suspicion of hereditary systemic amyloidosis, or in patients where confirmation of AL amyloidosis cannot be obtained. Particular phenotypes that should rouse suspicion of a hereditary form of systemic amyloidosis include the presence of amyloid polyneuropathy and cardiomyopathy (ATTR amyloidosis) and an exclusively renal presentation (fibrinogen amyloidosis). Patients with AL amyloidosis may have a co-existing and incidental hereditary amyloidosis mutation – therefore results of DNA analysis should be interpreted along with clinico-pathological findings.

Assessment of plasma cell dyscrasia

AL amyloidosis is caused by expansion of an indolent B-cell clone, that produces an immunoglobulin light chain. This is usually lambda type in 75-80% of cases, and kappa in the remaining cases. The implicated light chains consist of all or part of the variable domain of the light chain, although intact light chains can occasionally be present. Monoclonal intact immunoglobulins are present in around 50% of patients.

1) Measurement of serum free light chains

The free light chain assay is a nephelometric measurement of kappa and lambda light chains circulating as light chain monomers or dimers, and that are not bound to immunoglobulin heavy chains.(89) A monoclonal elevation of serum free light chains along with an abnormal kappa:lambda ratio can be identified in 98% of patients with AL amyloidosis, even in those who do not have an intact circulating monoclonal immunoglobulin.(90) The high sensitivity of the nephelometric free light chain assay means that it is able to identify even small plasma cell clones, which are often found in AL amyloidosis. Elevated free light chains are not specific for systemic AL amyloidosis, however. A monoclonal elevation in free light chains can be found in other B-cell disorders, such as myeloma, light chain deposition disease, monoclonal gammopathy of uncertain significance (MGUS) and plasmacytoma. Serum free light chains can also accumulate in patients with impaired renal function, making interpretation of this test difficult in this setting.(91) The kappa:lambda ratio is used in such cases, although the range for a normal kappa:lambda ratio is altered in progressive renal failure.(92) The kappa:lambda ratio is also skewed by significant drops in the uninvolved free light chains that are induced by chemotherapy, and to that end, the difference in involved and uninvolved light chains (dFLC) is now widely used in monitoring treatment response.(89) This measurement is also applicable in patients with renal impairment.(93) A high dFLC measurement is associated with more substantial bone

marrow plasmacytosis, poor performance status and more severe cardiac involvement. A 50% decrease in dFLC with treatment is associated with better survival, and is more predictive of overall survival than reduction in the paraprotein – therefore the former parameter is key to measurement of haematologic response in AL amyloidosis.(94) While the nephelometric light chain assay is highly sensitive (it can detect free light chains down to 1mg/L), the assay is prone to imprecision.(95) Given this, a baseline dFLC of ≥50mg/L is required for assessment of dFLC response.(96)

2) Serum and urinary electrophoresis and immunofixation

Approximately 50% of patients with AL amyloidosis have a detectable monoclonal paraprotein in the serum or urine by electrophoresis, although this is usually low level.(97) Routine serum electrophoresis is often negative in AL patients due to the modest size of the clone, and therefore it is essential to perform serum and urine immunofixation.

3) Bone marrow examination

Bone marrow aspirate and trephine in AL amyloidosis are commonly morphologically normal or show only a modest plasmacytosis. Patients with greater than 10% bone marrow plasma cell infiltration have a poorer prognosis.(98) Multiparameter flow cytometry immunophenotyping can detect phenotypically aberrant plasma cells in 97% of patients with AL amyloidosis, even in the case of smaller clones. (99) Phenotypically aberrant plasma cells in AL amyloidosis

often have simultaneous infra-expression of CD19 and CD45, with or without overexpression of CD56; the immunophenotypic profile is similar to that seen in myeloma. Immunophenotyping is also prognostic: patients with greater than 1% bone marrow plasma cells have shorter overall survival. Next-generation sequencing has revealed that the mutational landscape in AL amyloidosis is less complex than that seen in myeloma, with the former associated with a lower frequency of mutations (BRAF, TP53, NRAS, KRAS and CCND1).(100) Interphase fluorescence in situ hybridisation (iFISH) has revealed that hyperdiploidy in AL amyloidosis is less common (11%) than in myeloma and MGUS.(72) Hyperdiploidy in AL amyloidosis is associated with an intact immunoglobulin, kappa light chain restriction, older age, and bone marrow plasmacytosis. Cytogenetic abnormalities occur in 59% of AL amyloidosis without myeloma, of which the commonest are IGH rearrangement, t(11;14) (40-60% of patients) and monosomy 13/del(13q).(101, 102) First-line treatment with bortezomib has been shown to be less beneficial in patients with t(11;14), with poorer overall survival and haematological responses in those with the mutation compared to those without.(102, 103) Similar findings have been reported in patients treated with immunomodulatory-based regimens.(102) Trisomy 13 is also associated with poorer survival.(102) iFISH is therefore prognostic and may be used to guide treatment.

Evaluation of organ involvement

Uniformly accepted criteria for the definition of organ involvement were developed in 2005(104) and updated in 2010(105) to include N-terminal pro hormone beta natriuretic peptide (NT-proBNP). Organ involvement is defined as:

- Cardiac

    o Mean left ventricular wall thickness on echocardiography>12mm, no other cause found

    o NT-proBNP>332ng/l in absence of renal failure or atrial fibrillation

- Renal

    o 24 hour urinary protein>0.5g/day, predominantly albumin

- Liver

    o Total liver span > 15cm in the absence of heart failure or alkaline phosphatase (ALP) >1.5 times institutional upper limit of normal

- Nerve

    o Peripheral: symmetric lower extremity sensorimotor peripheral neuropathy

    o Autonomic: gastric emptying disorder, pseudo-obstruction, voiding dysfunction, unrelated to direct organ infiltration

- Gastrointestinal tract:

    o Direct biopsy verification with symptoms

- Lung:

    o Direct biopsy verification with symptoms

    o Interstitial radiographic pattern

- Soft tissue

  o Tongue enlargement, clinical

  o Arthropathy

  o Claudication, presumed vascular amyloid

  o Skin

  o Myopathy by biopsy or pseudohypertrophy

  o Lymph node (may be localised)

  o Carpal tunnel syndrome

Prognostic factors

The extent of cardiac involvement is the main determinant of outcome in AL amyloidosis. Cardiac troponins are sensitive, specific markers of cardiac muscle injury. N-terminal pro brain natriuretic peptide (NT-proBNP) is released by myocardial cells in response to cardiac wall stress. Both biomarkers are incorporated in the widely used Mayo staging system.(80) The thresholds in this staging system are NT-proBNP>332ng/l and cardiac troponin-T>0.035ng/ml. Stage 1 pertains to both biomarkers below these thresholds, Stage 2 to one biomarker above threshold and Stage 3 to both biomarkers above the thresholds. Median overall survival in Stage 1, Stage 2 and Stage 3 disease is 26.4, 10.5 and 3.5 months, respectively. Measurement of cardiac troponin T using a high sensitivity assay is also a prognostic marker.(106) An NT-proBNP>8500ng/l and systolic blood pressure<100mmHg have been found to define an especially poor risk group, with a median overall survival of three months. (107) The amyloidogenic light chain burden has been incorporated into a revised Mayo

2012 prognostic score, whereby patients were assigned a score of 1 for each of dFLC≥180mg/l, cardiac troponin T≥0.025ng/ml and NTproBNP≥1800pg/ml, with scores of 0, 1, 2 and 3 corresponding to Stages I, II, III and IV, respectively.(108) The median overall survival in these groups was 94.1, 40.3, 14 and 5.8 months, respectively.

The clearance of natriuretic peptides is impacted by glomerular filtration, and NT-proBNP is almost exclusively cleared through glomerular filtration.(109) B-type natriuretic peptide (BNP) is also actively removed from the bloodstream, through binding with natriuretic peptide receptor C and protease hydrolysis. NT-proBNP is therefore more influenced than BNP by renal dysfunction, and BNP has been found to be a better prognostic marker than NT-proBNP in patients with advanced renal dysfunction (eGFR<15ml/min).(109)

Cardiac imaging also yields prognostic insights. Namely, the mean basal strain, a measure of longitudinal left ventricular function, is predictive of clinical outcome.(110) The extent of late gadolinium enhancement on cardiac MRI is also prognostic.(111)

The size of the plasma cell clone is also prognostic, and patients with more than 10% plasma cells in the bone marrow have a poorer prognosis than those with less than 10% (median overall survival 16.2 months and 46 months respectively).

Cytogenetic abnormalities are prognostic in AL amyloidosis. The commonest cytogenetic abnormality is t(11;14), reported in approximately half of patients with the disease.(102, 103) A large study by the Mayo group concluded that bortezomib-based and immunomodulatory therapy-based regimens were associated with poorer haematological responses in patients with t(11;14), compared to patients without this translocation.(102) The rate of VGPR/better in the bortezomib group was 52% and 77% in patients with and without the translocation. While there was no known difference in survival in patients with t(11;14) and those without, the study reported that patients with t(11;14) who received bortezomib-based therapy had shorter OS compared with patients who lacked this translocation (median OS 15 and 27 months, respectively). Similar survival findings were apparent in patients treated with immunomodulatory based therapy (median OS 12 and 32 months, respectively). The presence of t(11;14) did not impact upon overall survival in patients treated with autologous stem cell transplantation (ASCT), however. In the same study, trisomies were present in a quarter of AL patients, and found to confer a poorer prognosis (median 29 months OS, compared to 69 months in patients without trisomies). Patients with trisomies who were treated with melphalan were found to fare poorly, compared to those without trisomies (median overall survival 15 months and 32 months, respectively). Therefore, cytogenetic findings may be prognostic and are a key consideration when selecting treatment approach.

<u>Assessment of treatment response</u>

Assessment of response to treatment is twofold: haematological response and improvement in amyloidotic-organ dysfunction.

1) Haematological response

    The reduction in the monoclonal amyloidogenic free light chain component with treatment is directly associated with survival in this condition. A large collaborative study demonstrated that absolute concentration of free light chains after chemotherapy (rather than percentage reduction) best predicted survival, irrespective of baseline free light chain levels.(96) In contrast to myeloma, it is the free light chain response in AL amyloidosis that correlates better with survival than reduction of the monoclonal intact immunoglobulin. (94)

    Consensus criteria define a complete haematological response (CR) to treatment as normal serum free light chains and the absence of a detectable monoclonal protein in the serum or urine by immunofixation.(96) A very good partial response (VGPR) is defined as a reduction of dFLC to 40mg/l, and a partial response as a dFLC decrease of greater than 50%. Patients who achieve a CR or VGPR have the best survival outcomes, and the objective of treatment is therefore to achieve a VGPR or better. In patients with a dFLC<50mg/l at baseline who have a monoclonal paraprotein>5g/l by electrophoresis, standard myeloma response criteria are used in assessment of paraprotein response.(112)

The current criteria stipulate that patients with a presenting dFLC<50mg/L cannot be assessed for light chain response, and such patients are therefore often excluded from clinical trials.(96) This subgroup (which comprises of approximately 20% of patients) has been characterised and haematologic parameters defined and validated in recent studies.(113, 114) Patients with a dFLC<50mg/l had a smaller bone marrow plasmacytosis, but no significant differences in cytogenetic anomalies.(114) Cardiac involvement was less frequent and less severe, whereas renal involvement was more common. A low-dFLC response to treatment was defined for such patients with presenting dFLC 20-50mg/l, who had a post-treatment dFLC<10mg/l. A low-dFLC response was predictive of survival (median OS not reached in those in a low-dFLC response, compared to 92 months in those who weren't). Similarly, a low-dFLC response predicted renal survival, with significantly fewer patients requiring dialysis in this patient group.

2) Organ response

Consensus criteria for the definition of organ response and progression were defined in 2012 and are as follows:(115)

Table 1.2: International consensus criteria for assessment of organ response.

	Response	Progression
Cardiac	NT-proBNP response (>30% and >300ng/l decrease in patients with baseline NT-proBNP≥650ng/l) or NYHA class response (≥2 class decrease in subjects with baseline NYHA class 3 or 4)	NT-proBNP progression (>30% and 300ng/l increase) or cardiac troponin progression (≥33% increase) or ejection fraction progression (≥10% decrease)
Renal	50% decrease (at least 0.5g/day) of 24 hour proteinuria (baseline proteinuria>0.5g/day). Creatinine and creatinine clearance must not worsen by 25% over baseline.	50% increase (at least 1g/day) of 24 hour proteinuria to >1g/day or 25% worsening of serum creatinine or creatinine clearance
Liver	- 50% decrease in abnormal alkaline phosphatase value	50% increase of alkaline phosphatase above the lowest documented value

	- Decrease in liver size radiographically by at least 2cm	
Peripheral nervous system	Improvement in electromyogram nerve conduction velocity (rare)	Progressive neuropathy by electromyography or nerve conduction velocity

3) Measurement of minimal residual disease (MRD) in AL amyloidosis

The aim of treatment in AL amyloidosis is rapid reduction of amyloidogenic light chains. A complete haematological response is associated with prolonged overall survival, but such a response may still not allow for recovery of amyloid-related organ dysfunction and may result in eventual relapse. Identification of residual clonal disease is therefore an area of much interest in AL amyloidosis. In myeloma, studies consistently show that patients in a complete response who have persistent minimal residual disease (MRD) have inferior progression-free survival (PFS) compared to those patients in a complete response who are MRD-negative.(116-119) The identification of MRD by next generation sequencing and next generation flow cytometry have been introduced into the International

Myeloma Working Group's consensus criteria for response in myeloma.(112)

In AL amyloidosis, evaluation of MRD in treated patients is of great interest although studies thus far have been relatively small. Multiparameter flow cytometry is able to detect clonal bone marrow plasma cells in 97% of patients with AL amyloidosis.(99, 120) The Mayo clinic demonstrated that, at the end of treatment, AL patients with ≥0.1% monotypic plasma cells had a shorter PFS and OS than those with percentage of monotypic plasma cells below this threshold (2 year PFS 31% vs 87%, 2 year OS 87% vs 98%).(121) In patients in a VGPR or better, the monotypic plasma cell 0.1% threshold predicted patients that were likely to undergo progression, but the threshold was not predictive of OS.

A EuroFlow-based next generation flow cytometry (NGF) has been recently designed, to allow for better sensitivity compared to conventional multiparameter flow cytometry MRD method.(112, 122) This also allows for standardised, validated detection of MRD in myeloma. The method relies on two eight-colour combinations that combine surface antigens for the identification of phenotypically aberrant clonal plasma cells, and cytoplasmic light chain restriction to confirm clonality. The technique includes an initial bulk lysis step and software algorithms that been developed to allow rapid and automated identification of clonal plasma cells. The technique is able to

accurately quantify tumour plasma cells at a level down to five per million cells. It has also been explored in AL amyloidosis. A study by Kastritis et al evaluated the presence of MRD by this technique in 20 patients with AL amyloidosis who were in a CR based on negative serum and urine immunofixation, a normal FLC ratio and normal FLCs and a negative bone marrow biopsy.(123) The NGF MRD technique allowed for high sensitivity levels, approaching 10^{-6}. 60% of patients in the study were MRD positive, despite being in a CR. No baseline factors were found to be associated with a higher probability of MRD negativity in this small study. Among the cardiac responders, ¾ were MRD negative. Similarly, Palladini and colleagues evaluated MRD by NGF and found that 6/11 of AL patients in a haematologic CR were MRD negative, and MRD negative patients were more likely to achieve cardiac and renal responses.(124)

Next-generation sequencing has been incorporated into the consensus criteria for assessment of MRD in myeloma and there is much interest in its role in AL amyloidosis. (112) The approach identifies the specific clonal rearrangements affecting the original progenitor B cell that develops into a malignant plasma cell. A baseline sample must be available. The technique involves amplification of genomic DNA using locus-specific primers targeting the IGH complete locus, IGH incomplete locus, immunoglobulin K locus and immunoglobulin L locus. The amplified product is then sequenced and a clone is identified based on frequency. A recent

study in AL identified an initial clone in 29 of 37 newly diagnosed AL patients, and data on these clones after treatment is awaited.(125)

<u>Imaging</u>

1) Serum amyloid P component scintigraphy

Serum amyloid P (SAP) is a non-fibrillar glycoprotein that is ubiquitous in amyloid deposits. Plasma SAP is in constant equilibrium with SAP in amyloid deposits, leaving the circulation and depositing within amyloid fibrils, possibly because of specific calcium-dependent binding affinity for them.(126) It is this concept that led Hawkins et al to develop radiolabelled SAP as an imaging tracer for amyloid deposits.(127) Scintigraphy with iodine-123 labelled SAP results in high quality images because a high proportion of tracer is deposited in amyloid and retained for prolonged periods, in contrast to circulating SAP which undergoes rapid catabolism and excretion. Radiolabelled SAP localises rapidly and specifically to amyloid deposits in proportion to quantity of amyloid present. It identifies visceral amyloid deposits in the liver, kidneys, adrenal glands, spleen and bones. There is complete concordance between hepatic uptake on SAP scintigraphy and the presence of amyloid deposits on liver histology, but the imaging technique is able to identify liver involvement in over 30% of cases with normal biochemical liver function tests.(128) Cardiac, nerve and gastrointestinal amyloid, however, is poorly visualised.(127) Its inability to visualise cardiac amyloid may be due to

ventricular blood-pool content, the lack of a fenestrated endothelium in the myocardium preventing transfer of the large SAP molecule to the amyloidotic interstitium within the short half-life of ^{123}I isotope.(129) SAP scintigraphy is available at the UK National Amyloidosis Centre and is performed routinely in patients that are referred with proven or suspected amyloidosis. It is useful in its assessment of extent and distribution of organ involvement by amyloid, and for evaluating effects of treatment. The tracer is rapidly catabolised and excreted, and the dose of radiation is equivalent to a plain X-ray of the lumbar spine.(127, 130)

2) Echocardiography

Echocardiographic features that are suggestive of cardiac amyloidosis include concentric ventricular wall thickening with normal or small ventricular cavities, thickened valves and dilated atria.(73) The ejection fraction is usually preserved. Impaired systolic function is usually a late feature of the disease and associated with poor prognosis.(131) The presence of left ventricular wall thickening with low voltage complexes on ECG is suggestive of an infiltrative cardiomyopathy, and amyloidosis is one of the most likely differential diagnoses. Diastolic dysfunction is common, as are abnormal longitudinal strain and strain rate analysis.

3) Cardiac magnetic resonance imaging (MRI)

Cardiac MRI is becoming increasingly popular in the diagnosis of cardiac amyloidosis. Gadolinium is used as a contrast agent, and is distributed in the expanded extracellular space of the myocardium due to amyloid deposition.(131) Global and subendocardial late gadolinium enhancement occurs after gadolinium contrast injection in cardiac amyloidosis and correlates well with histological evidence of amyloid deposition. The pattern of late gadolinium enhancement in the myocardium is heterogeneous, and can range from global transmural, subendocardial or patchy and focal – which may represent the deposition pattern of interstitial amyloid.(132) Incidental findings of atrial enhancement or atrial thrombi can also be detected. Measurement of native T1 and extracellular volume (ECV) fraction have been shown to be higher in the myocardia of patients with cardiac amyloidosis. The presence of late gadolinium enhancement is associated with increased risk of all-cause mortality in patients with AL amyloidosis(133) and the degree of myocardial oedema on cardiac MRI is also a prognostic indicator.(134)

Cardiac MRI is especially useful in differentiating cardiac amyloidosis from other causes of concentric hypertrophy. For example, hypertrophic cardiomyopathy may share similar echocardiographic features such as concentric hypertrophy, bi-atrial dilation, reduced longitudinal function and decompensated biventricular restrictive disease, but has different tissue characterisation findings on cardiac MRI compared to cardiac amyloidosis.(111)

There are some limitations to this imaging modality. Firstly, severe

renal dysfunction is a contraindication to gadolinium use – although

there is some research to suggest that native myocardial T1 may

enable diagnosis of cardiac amyloidosis without the need for

gadolinium contrast.(135) Moreover, there are service delivery issues

with this imaging modality (the need for complex protocols, specialist

interpretation and costly infrastructure) such that it is not widely

available currently.

4) Bisphosphonate bone tracers

Radionuclide bone scintigraphy with technetium-labelled

bisphosphonates have been shown to localise to cardiac amyloid

deposits, although early results were heterogeneous and the basis for

localisation was unknown. Recent studies of bone scintigraphy have

shown that ^{99m}Tc-labelled 3,3-diphosphono-1,2-propanodicarboxylic

acid (DPD), ^{99m}Tc-labelled pyrophosphate (PYP) and ^{99m}Tc-labelled

hydroxymethylene disphosphonate (HMDP) are sensitive, specific

tracers for imaging cardiac ATTR amyloid deposits.(136-138) Bone

scintigraphy may detect cardiac ATTR amyloid deposits early on,

before abnormalities are evident on echocardiography or cardiac

MRI.(139, 140) Low grade cardiac uptake with bone tracers can occur

in some patients with AL amyloidosis (138). In a large multicentre

study, Gillmore et al confirmed >99% sensitivity of bone scintigraphy

for cardiac ATTR amyloid.(141) Specificity for cardiac ATTR amyloid

was, however, only 86% and this was due to uptake in patients with cardiac AL amyloidosis. However, the combination of high grade cardiac uptake and the absence of a monoclonal protein in the serum or urine had a specificity and positive predictive value for cardiac ATTR amyloidosis of 100%. Bone scintigraphy has therefore provided an important opportunity for non-biopsy diagnosis in patients with high grade cardiac uptake on bone scintigraphy and the absence of a monoclonal gammopathy.

Treatment and prognosis

Significant advances have been made in AL amyloidosis over the last 20 years. The median survival has nearly doubled (4 year OS 50% from 2008-2012, 4 year OS 28% before 2005).(4) However, nearly a quarter of all patients die within a few months of diagnosis. Less than a quarter of patients achieve a complete, durable haematological remission with survival of >10 years.(142) The function of amyloidotic organs improves in only a quarter of patients on an intention-to-treat basis.

Treatment for AL amyloidosis is based on anti-myeloma therapy that suppresses the underlying plasma cell disorder, along with supportive care measures to manage the organ-related complications of amyloid deposition.(143) The aim of treatment is to rapidly suppress the production of the amyloid precursor and enable reabsorption of amyloid deposits, allowing for swift improvement in amyloidotic organ dysfunction and an improvement

in quality of life and survival. It is profoundly challenging to provide maximal tolerated effective therapy in a frail patient with severe multi-organ dysfunction, in whom a rapid and deep response is essential but whose functional reserves render them particularly vulnerable to treatment toxicity.

All patients with symptomatic systemic AL amyloidosis with organ involvement, significant soft tissue involvement, coagulopathy or neuropathic involvement should be considered for treatment. Assessment of treatment response should take place after every cycle of chemotherapy. The UK NAC recommends switching treatment after three cycles of therapy if initial first-line treatment is not associated with at least a partial response, although there is a lack of evidence to guide this approach.(143)

Plasma cell directed therapies

1) Autologous stem cell transplantation (ASCT)

   High-dose intravenous melphalan conditioning followed by ASCT has been used as treatment for AL amyloidosis in selected patients since the 1990s.(144) An early randomised trial of high dose melphalan and ASCT compared to standard dose melphalan and dexamethasone demonstrated inferior outcomes with ASCT. (145) Transplant-related mortality (TRM) was also alarmingly high in this study (24%). The disappointing outcomes in the ASCT arm have been attributed to inclusion of patients with severe cardiac amyloidosis, patients with

involvement of three or more organs and attenuated doses of melphalan in the ASCT arm.

Refined selection criteria for ASCT and improvements in supportive care have resulted in a reduction in TRM. The Mayo group retrospectively analysed their ASCT data from 1996-2009 and 2009-2011.(146) TRM in the former group was 10.5%, compared to 1.1% in the latter. NT-proBNP and troponin levels were predictive of TRM, and cut-offs of NT-proBNP>5000ng/L and troponin T>0.06ng/L were proposed as cardiac exclusion criteria for ASCT.

In contrast to the early randomised study of ASCT compared to melphalan-dexamethasone (145), subsequent large studies have shown good haematological responses and OS with ASCT. D'Souza et al analysed ASCT data from the Center for International Blood and Marrow Transplant Research Database and reported a CR rate of 37%. (147) The 5 year OS in this study was 77% and centres that performed more than four AL transplantations per year had better survival outcomes. The TRM in this study had also reduced with time, having been 20% from 1995-2000 to 5% from 2007-2012. Lower doses of melphalan conditioning were found to be an independent predictor of relapse on multivariate analysis.

Between 1994-2014, 629 patients with AL amyloidosis have undergone ASCT at Boston University. (148) Inclusion criteria for

ASCT were: left ventricular ejection fraction≥40%, absence of symptomatic pleural effusions, absence of uncompensated heart failure or arrhythmias resistant to medical management, oxygen saturation≥95% on room air, lung diffusion capacity≥50% predicted, supine systolic blood pressure ≥ 90mmHg and performance status ≤2. Overall TRM was 7.5%, with TRM of 3.4% after 2005. The CR rate was 34.8% and median OS was 7.63 years. The median OS was 10.47 years in patients receiving 200mg/m^2 melphalan, compared to 5.15 years in those who received 100-140mg/m^2. A series of 421 patients from the same centre reported that 78% of evaluable patients achieved an organ response at 1 year and median event-free survival was 2.6 years. In patients in a CR, the median event-free survival was 8.3 years.(149)

The Mayo clinic have also reported their 20 year experience of ASCT in AL amyloidosis.(150) A total of 672 patients underwent ASCT between 1996-2016 and the median OS was 10 years. From the period of 2010-2016, the CR rate was 39%, median OS was not reached and TRM had fallen to 2.4% (having been 14.5% at the beginning of the study).

Whilst there is a large body of evidence on the efficacy of ASCT in AL amyloidosis, stringent selection criteria that have evolved to minimise TRM render only a fifth of patients transplant-eligible. The role of attenuated conditioning doses has been explored. In a retrospective

analysis of patients who underwent full-intensity melphalan conditioning compared to reduced-dose conditioning, CR rates were 53% and 37% respectively. Organ response rates were better in the full-intensity group (74% vs 59%), as were PFS (4 year PFS 55% vs 31%) and OS (86% vs 54%). The OS and PFS were also better in the full-intensity group irrespective of Mayo cardiac staging.

There is some evidence that induction before ASCT may improve outcomes. In a randomised trial of induction with bortezomib followed by ASCT, compared to ASCT without induction therapy, 2 year OS was 95% in the induction group compared to 69.4% in the non-induction group.(151) Overall haematologic response rates (ORR) and organ responses were also better in the induction group (ORR 92% vs 69%; organ response 75% vs 54%). Patients with a bone marrow plasmacytosis of greater than 10% at presentation should be considered for induction therapy before ASCT.(98)

2) Proteasome inhibitor-based therapy

Bortezomib-based therapy has become a mainstay of treatment in AL amyloidosis, particularly in patients who are ineligible for ASCT. Bortezomib is a potent, selective inhibitor of the 26S proteasome, a multi-subunit protein complex that is ubiquitous in all eukaryotic cells.(152) In addition to damaged or abnormal proteins, proteasomes degrade proteins that are involved in the regulation of cell-cycle

progression, oncogenesis and apoptosis. Proteasomal degradation is coupled with extraction of aberrant proteins from the endoplasmic reticulum (ER), and proteasome inhibition causes accumulation of misfolded secretory proteins in the ER and hence ER stress. This stress then causes apoptosis. This is particularly desirable in AL, as although the misfolded amyloidogenic light chains negotiate transport across the stringent ER quality control checkpoints, their accumulation initiates a signalling cascade known as the unfolded protein response – whereby transcription of genes enchancing protein folding and degradation are increased, entry of proteins into the ER and stability of mRNA encoding secretory proteins are selectively inhibited. If this process is overwhelmed and insufficient for eliminating misfolded proteins from the ER, apoptosis is activated.

A prospective Phase 1/2 study of single-agent bortezomib in relapsed AL demonstrated good haematological responses (68.8% and 66.7% in patients treated with weekly bortezomib and twice weekly bortezomib, respectively.(153) CR rate was 37.5% and 24.2% respectively. One year PFS was 72.2% and 74.6%, and one year OS was 93.8% and 84.0%, respectively. The rate of Grade≥3 neuropathy was higher in the group treated twice a week.

A large European collaborative retrospective study of 230 patients treated upfront with cyclophosphamide-bortezomib-dexamethasone (CyBorD) demonstrated an ORR of 60%, with 43% of

VGPR/better.(154) Cardiac response was achieved in 17% of patients, and renal response in 25% of patients. In a matched case-control study of patients treated upfront with bortezomib-melphalan-dexamethasone (BMDex) compared to patients treated with melphalan-dexamethasone (MDex), the CR rate was 23% and 19%, respectively.(155) There was no difference in survival between the two groups. In a matched comparison of patients treated with CyBorD versus cyclophosphamide-thalidomide-dexamethasone (CTD), ORR was 71% vs 79.7% (p=0.32).(156) The CR rate was higher in the CyBorD group (40.5% vs 24.6%, p=0.046). There was no difference in OS, although PFS was better in the CyBorD group (28 months and 14 months, p=0.039). Bortezomib is therefore a suitable option in patients that are ASCT-ineligible but are still candidates for systemic chemotherapy, provided that there are no contraindications (e.g. severe peripheral neuropathy, fibrotic lung disease).(66)

Cytogenetic findings should also be considered in treatment decisions, as treatment with bortezomib plus melphalan-dexamethasone can overcome effects of a 1q21 gain (which confers a poorer outcome with oral melphalan), or t(11;14) (which confers a poorer outcome with bortezomib). (102, 103, 142, 157, 158) Similarly, a large long-term follow-up study of patients with AL treated with high dose melphalan and ASCT had a higher rate of CR if they possessed t(11;14) than those without (CR rate 41.2% vs 20%, p=0.02)(158).

Event-free survival was also better in the former group, although there was no significant difference in OS.

Carfilzomib is a second-generation highly selective, irreversible proteasome inhibitor with reported efficacy in newly diagnosed and relapsed myeloma. Carfilzomib has fewer off-target effects and lesser neurotoxicity than bortezomib, due to its marked selectivity for the chemotrypsin-like active site of the proteasome.(159-161) A Phase 1/2 study of carfilzomib in relapsed/refractory AL resulted in an ORR of 63% and a CR/VGPR rate of 45.8%.(162) However, twice-weekly dosing was associated with substantial cardiac, pulmonary and renal toxicity.

Ixazomib is the first orally administered, reversible, second-generation proteasome inhibitor. In a Phase 1/2 study of relapsed/refractory AL patients, ixazomib was administered on Days 1, 8, 15 of a 28 cycle for up to 12 cycles.(163) Dexamethasone was added in patients with less than a PR after 3 cycles. The maximum tolerated dose was 4mg. Common adverse events included nausea, skin rash, diarrhoea, fatigue, dyspnoea and fatigue. The ORR was 52%, and organ responses were seen in 56% of patients. The 1 year PFS and OS were 60% and 85%, respectively.

3) Melphalan-dexamethasone (MDex)

The combination of oral melphalan and dexamethasone was previously considered standard therapy for AL patients who were ineligible for ASCT. A large Italian study of 259 patients treated with oral melphalan and dexamethasone reported an ORR of 76% in patients treated with 40mg dexamethasone, and 51% in patients with advanced cardiac disease who were treated with an attenuated (20mg) dose of dexamethasone.(164) The CR rate was 31% and 12%, respectively. The median OS was 7.4 years and 20 months, respectively. Toxicity was largely equivalent in both groups, and the most common severe adverse event was fluid retention. This treatment protocol remains an option in ASCT-ineligible patients who have severe peripheral neuropathy, in whom bortezomib is contraindicated.

4) Immunomodulator therapy

Thalidomide, combined with cyclophosphamide and dexamethasone (CTD), is another treatment option in patients who are ineligible for ASCT. Previous reports of this treatment regimen have yielded an ORR of 68%-79% and a CR rate of 21-24.6%. (156, 165) However, there is considerable toxicity with this treatment regimen in AL patients and common adverse events include marked oedema, constipation and fatigue.(166) (167) Symptomatic bradycardia, skin rash and deep vein thromboses were also reported.

The combination of lenalidomide with melphalan and dexamethasone in upfront treatment yielded an ORR of 38-68% and a CR rate of 7-42%.(168-170). There was significant myelosuppression with the regimen (168) and a dose escalation study revealed a maximal tolerated dose of lenalidomide 15mg once daily.(169) The combination of lenalidomide with cyclophosphamide and dexamethasone in upfront AL has been evaluated in Phase II studies with ORR of 46-60% and 18-43% VGPR/better.(171-173) Haematologic toxicity was also common with this regimen, as well as fatigue, rash and oedema. A study of lenalidomide-dexamethasone in relapsed AL revealed an ORR of 61%, with 20% complete response, and outcomes were independent of previous thalidomide/bortezomib therapy.(174) Those patients on prolonged treatment were more likely to achieve an organ response.

Pomalidomide is a next generation immunomodulatory agent licensed in relapsed myeloma. Three Phase II studies explored the efficacy of pomalidomide in combination with dexamethasone in patients with relapsed AL amyloidosis.(175-177) The maximum tolerated dose was 4mg.(176) The ORR was 48-68%. The median OS and PFS were 26-28 months and 14-16 months, respectively.(175, 177) Common Grade ≥3 adverse events included fluid retention, infection, myelosuppression and fatigue. A retrospective study of 29 patients with relapsed AL treated with pomalidomide-dexamethasone revealed an ORR of 46% with none of the patients achieving a complete

response.(178) The median OS was 27 months and median PFS was 15 months.

5) Bendamustine

Bendamustine, a purine and alkylator combination, is used in myeloma, chronic lymphocytic leukaemia and non-Hodgkin lymphoma. It also has a role in relapsed AL. A small Phase II study of bendamustine-dexamethasone in AL relapsed/refractory AL resulted in an ORR of 50% (22% CR/VGPR).(179) 38% of patients had a renal response, and 7% had a cardiac response. The median PFS was 9.4 months and OS was 18.1 months. Common adverse events included gastrointestinal symptoms, fatigue, neutropenia and anaemia. The Italian and Pavia amyloidosis groups have reported a retrospective analysis of 130 AL patients treated with bendamustine and prednisolone.(180) The majority (90%) had relapsed/refractory disease. The ORR was 35% with a CR/VGPR rate of 10%. Cardiac responses were achieved in 12% of patients and renal responses in 31%. The median OS was 21 months, and median PFS was 9 months.

6) Daratumumab

Daratumumab is a human IgG1k monoclonal antibody with high-affinity binding to CD38.(181) CD38 is uniformly and highly expressed on myeloma cells. Daratumumab induces death of myeloma plasma cells through complement-dependent cytotoxicity, antibody-dependent

cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis and apoptosis.(182) Daratumumab has proven efficacy in myeloma as monotherapy and in combination with standard myeloma treatment regimens.(183-188) The use of daratumumab with these combinations have resulted in excellent MRD-negativity rates and the drug has not been associated with renal or cardiac toxicity.

Investigators at Boston University have reported a prospective Phase II study of daratumumab monotherapy (16mg/kg intravenous) in 22 patients with relapsed AL amyloidosis. The CR/VGPR rate was 86%.(189) The renal and cardiac response rates were 67% and 50% respectively. There were no Grade 3-4 infusion-related reactions, and Grade 3-4 adverse events included respiratory infections (18%) and atrial fibrillation (18%). Three of the patients in this study were solid organ transplant recipients, and there was no adverse effect on graft function with daratumumab.

A French prospective Phase II study of daratumumab in 40 relapsed AL patients showed a CR/VGPR rate of 47.5%.(190) The median time to haematological response was one week. Cardiac responses occurred in 29% and renal responses in 30%. The two year OS was 74%.

A large real-world German series of 168 relapsed-refractory AL patients treated with daratumumab-dexamethasone (n=106) or

daratumumab-bortezomib-dexamethasone (n=62) demonstrated an ORR of 64% and 66%, respectively.(191) The three month VGPR rate was 48% and 55%, respectively. The median PFS was 11.8 months in the daratumumab-dexamethasone group, and 19.1 months in the daratumumab-bortezomib-dexamethasone group. Median OS was 19.1 months and not reached, respectively. Cardiac responses were 22% and 26%, respectively. Grade 3/4 infective complications occurred in 16% and 18% of patients, and lymphopenia in 20% and 17%. Hyperdiploidy and gain 1q21 conferred poorer OS and PFS in the daratumumab-dexamethasone group, and t(11;14) was associated with a better PFS. A multivariate analysis revealed dFLC>180mg/L and nephrotic-range albuminuria were adverse factors for PFS.

The latter three studies reveal differing outcomes. (189-192) The duration of therapy in the French study was 6 months, whereas it was 24 months in the Boston University study. The median duration of therapy in the German study was 5 months. The two Phase II studies excluded patients with bone marrow plasmacytosis>30% and NT-proBNP>8500ng/L, but the German series included such patients. A third of patients in the German series had an NT-proBNP >8500ng/L. The median dFLC levels were also strikingly different: 81mg/L, 164mg/L and 136mg/L in the Boston University, French and German studies – and this may reflect why haematological and organ responses were substantially better in the Boston University study.

Importantly, however, no patients discontinued treatment because of toxicity in any of the three studies.

Results from the ANDROMEDA study (NCT03201965), a Phase III randomised open-label study comparing CyBorD with or without subcutaneous daratumumab in newly diagnosed AL have been recently presented in oral abstract form.(193) A total of 388 patients were randomised to receive daratumumab-CyBorD (n=195) or CyBorD alone (n=193). The study exclusion criteria were stage IIIB cardiac involvement, eGFR<20ml/min and presence of symptomatic myeloma. 37% of patients had Stage IIIA cardiac involvement. The median follow-up was 11.4 months, and the median duration of treatment was 9.6 months in the daratumumab-CyBorD group and 5.3 months in the CyBorD group. The number of patients requiring further treatment in the two groups was 19 and 79, respectively. The CR rate was 53% in the daratumumab-CyBorD group and 18% in the CyBorD group, and the overall response rate was 92% and 77% respectively. Among responders, the median time to ≥VGPR/CR was 17/60 days in the daratumumab-CyBorD group and 25/85 days in the CyBorD group. The cardiac response rate was 42% in the daratumumab-CyBorD arm, compared to 22% in the CyBorD group (p=0.0029). The renal response rate was 54% and 27% respectively (p<0.0001).The commonest (>5%) grade 3/4 adverse events were lymphopenia (daratumumab-CyBorD 13%/CyBorD 10%), pneumonia (8%/4%), cardiac failure (6%/5%), neutropenia (5%/3%) and peripheral oedema

(3%/6%). Systemic administration-related reactions occurred in 7% of daratumumab-CyBorD patients – all of which were Grade 1/2 and almost all occurred during first dose of daratumumab. Publication of this study and data on survival outcomes is awaited, but these findings are clearly promising.

7) Venetoclax

Venetoclax is a highly selective B-cell lymphoma-2 (Bcl-2) inhibitor that is licensed in treatment of relapsed/refractory chronic lymphocytic leukaemia. Venetoclax is also effective in myeloma, particularly in patients with t(11;14).(194) Case reports also describe its efficacy in AL amyloidosis although its role has yet to be explored in a trial setting. (195-197)

Relapse

Although haematologic progression and organ progression are defined in the consensus criteria, currently there is no consensus of when to re-treat patients.(115, 198) Approximately a third of patients undergo relapse after ASCT and a large study by the Italian group found that 35% of patients who received non-transplant upfront therapy progressed to next treatment after a median follow-up of 3.5 years. (199, 200) Patients with a rising NT-proBNP (cardiac progression) had shorter survival. (200) The study identified 'high-risk dFLC progression' as a 50% increase in dFLC from the value reached after upfront therapy to an absolute value of >20mg/L that corresponded to at least 20% of the baseline value observed at diagnosis. High-risk dFLC

progression preceded cardiac progression by a median of 6 months in 85% of cases.(142, 200) Patients who were re-treated with the same therapy that they received upfront were likely to need re-treating sooner than those treated with an alternative regimen, but there was no difference in OS.(201)

The Mayo Clinic assessed timing of second-line therapy in 235 patients initially treated with ASCT.(202) The median time to next treatment was 24.3 months, and patients with organ progression at the time of second-line therapy had inferior survival. The study reported that patients in a VGPR or better had a longer time to develop organ progression after haematologic relapse/progression (24 vs 3.2 months, p=0.007). However, almost a quarter of patients with organ progression had a baseline dFLC<50mg/L and the majority of these patients had a dFLC<50mg/L at the time of organ progression.

Supportive care

Given the multisystem nature of AL amyloidosis, patients benefit from multidisciplinary care – particularly with cardiology and renal input.

1) Supportive care in cardiac disease

Patients with cardiac involvement often do not tolerate B-blockers, and their sinus tachycardia is usually physiological in an attempt to maintain cardiac output.(142) Diuresis is often necessary, and loop diuretics are most commonly used. Bumetanide is generally preferred to furosemide due to better oral bioavailability. In patients

with atrial arrhythmias, amiodarone is the best tolerated agent although digoxin is also an option at small doses. Atrial ablation can also be considered. Ventricular arrhythmias are similarly common, although the role of implantable cardiac defibrillators in long-term survival in AL is unclear (203) and pulseless electrical activity is commonly a pre-terminal event.

Doxycycline has been shown to reduce amyloid fibril formation in a transgenic mouse model in AL.(204) A retrospective case-matched study of outcomes in 30 patients with cardiac AL treated with doxycycline and standard therapy compared to 73 matched controls showed a survival benefit with doxycycline.(205)

The role of orthotopic heart transplantation (OHT) in AL has been limited due to the risk of disease progression in other organs, as well as the possibility of amyloid recurrence in the transplanted heart. A long-term follow up study of AL patients treated with OHT at the Mayo Clinic supported the use of OHT with strict selection criteria (predominant cardiac involvement, no evidence of myeloma, good clonal response to chemotherapy).(206) However, most AL patients do not fall in this group.

2) Supportive care in renal disease

Medical management of nephrotic syndrome generally comprises of diuretic therapy and strict fluid and salt restriction.(207)

Prophylactic anticoagulation should be considered. In patients with marked hypoalbuminaemia in the context of nephrotic syndrome, diuretics alone may not result in sufficient diuresis.(142) Some amyloid physicians utilise albumin infusions in such patients, although the evidence for this is limited.

Approximately one-third of patients with nephrotic syndrome will proceed to renal replacement therapy.(207) Haemodialysis can be a challenge in cardiac AL patients, who are often hypotensive. Peritoneal dialysis can be considered but may not result in sufficient offloading. Renal transplantation is an option in those patients whose underlying plasma cell disorder is well controlled, and whose performance status and absence of other significant comorbidities permit this approach. There is a risk of amyloid recurrence in the graft. In a UK study of 22 AL patients who underwent renal transplantation, the 5 year OS was 67% and none of the patients suffered renal graft failure due to amyloid recurrence during a median follow up of 4.8 years.(208) In a Mayo Clinic series of 19 AL patients who underwent renal transplantation, two patients experienced amyloid recurrence within the graft with a median follow-up of 3.5 years.(209)

3) Orthostatic hypotension

Postural hypotension in AL can be multifactorial. Impaired autonomic function, cardiac dysfunction, diuretics,

antihypertensives and primary adrenal failure due to amyloid deposition within the adrenal glands can all be contributors.(207) For symptomatic orthostatic hypotension, lower limb compression stockings can be used to augment venous return. Midodrine is an orally active alpha-adrenergic agonist that can be helpful, with careful upwards titration of the dose (side effects include tachycardia and supine hypertension). Fludrocortisone is less effective and often poorly tolerated due to co-existing fluid retention.

4) Peripheral neuropathy

Clinical improvement in peripheral neuropathy is rare despite a good haematological response to chemotherapy.(142) Management is largely symptomatic – gabapentin, pregabalin and duloxetine can be useful options at alleviating neuropathic pain.

Amyloid-directed therapy

Plasma cell directed therapy reduces amyloidogenic burden, but does not degrade amyloid deposits.(142) Existing amyloid deposits are slowly resorbed when production of the amyloid precursor is suppressed. Therefore there is much interest in the development of drugs that are able to target existing amyloid deposits.

1) NEOD001

NEOD001 is a humanised form of murine monoclonal antibody 2A4 that binds to an epitope that is unique to the misfolded light chain protein.(210) This light chain epitope is possibly exposed during misfolding and aggregation but is not available in the native conformation of a light chain or fully formed immunoglobulin.(210, 211) In a mouse model, 2A4 promoted AL amyloid clearance by phagocytosis.(211) Interim data was reported from a Phase I/II dose-escalation/ expansion study of NEOD001 in 27 patients with pre-treated AL (partial response or better) and persistent organ dysfunction.(210) No serious adverse events were reported. The most frequent adverse events were fatigue, upper respiratory tract infection, cough and dyspnoea. The recommended dose was 24mg/kg and pharmacokinetics supported intravenous dosing every 28 days. 8/14 (57%) had cardiac responses and 9/15 (60%) had renal responses. The data was felt to be encouraging in that organ responses were higher than is often the case with standard chemotherapies.

PRONTO (NCT02632786) was a subsequent Phase IIb multi-centre, randomised, double blind, placebo-controlled clinical study of NEOD001 in AL patients who responded to previous systemic therapy but had persistent cardiac dysfunction. The PRONTO study failed to meet its primary (cardiac response during 12 months of treatment) or secondary (change in Short-form 26 Physical Component Summary Score, 6 minute walk test, NT-

proBNP rate of change, renal best response and change in peripheral neuropathy NIS-LL score) endpoints.

VITAL (NCT02312206) was a Phase III multicentre, randomised, double-blind, placebo-controlled clinical trial of NEOD001 vs placebo in treatment-naïve AL patients with cardiac dysfunction. Both arms received standard of care. The primary endpoint was a composite of all-cause mortality and cardiac hospitalisations as events. A futility analysis was carried out, based on 103/156 events having occurred, and there was no significant difference between the two groups.(212) The VITAL study was therefore discontinued and it was recommended that all studies of NEOD001 be discontinued.(213)

i)   CAEL-101

CAEL-101, formerly 11-1F4, is a murine monoclonal antibody that recognises an amyloid-associated conformational epitope in human light chain-related fibrils. 11-1F4 was administered to mice with AL amyloidomas (induced by subcutaneous injection of human AL extracts).(214) The amyloidomas reduced in size compared to controls. A Phase I study explored the use of the antibody labelled with I[124], followed by imaging with PET/CT.(215) Uptake was noted in organs that had biopsy-proven amyloid. These findings have led to a Phase I study (NCT02245867) of 27 patients who were treated with 11-1F4.(216) (217) There were no

Grade 4/5 adverse events or dose-limiting toxicities. Two patients developed a Grade 2 rash after the infusion. Of evaluable patients, 61% demonstrated an organ response, with a median time to response of 2 weeks after the start of treatment. A randomised Phase 2/3 trial for newly diagnosed patients is currently awaited.

ii)   CPHPC (miridesap) and anti-SAP (dezamizumab)

All amyloid deposits contain SAP, a non-fibrillar plasma glycoprotein.(6) SAP binds reversibly to all amyloid fibrils. CPHPC is a small molecule drug that is able to deplete circulating SAP and removes some SAP from systemic amyloid deposits. (218, 219). Administration of anti-human-SAP antibodies to mice with amyloid deposits containing human SAP triggered a potent, complement-dependent macrophage-derived giant cell reaction, resulting in destruction of the amyloid deposits.(220) CPHPC was followed by administration of a fully humanised monoclonal IgG1 anti-SAP antibody to activate macrophage destruction of SAP-containing amyloid deposits in tissues in an open-label, dose-escalation Phase I clinical study.(221) Fifteen patients with systemic amyloidosis were treated with CPHPC to deplete circulating SAP, followed by the anti-SAP antibody. There were no serious events. Infusion reactions occurred in initial recipients, although these were reduced when slowing the infusion rate in subsequent patients. Improvements in liver function were noted six weeks after treatment, along with a reduction in hepatic amyloid load on SAP

scintigraphy and MRI. A reduction in renal amyloid load and shrinkage of an amyloid-laden lymph node were also seen. Further attempts to evaluate the safety, pharmacokinetics and dose-response effects of up to three cycles of miridesap followed by dexamizumab in 23 patients with systemic amyloidosis confirmed that the treatment was well tolerated, except self-limiting early onset rashes.(222) Dose-related clearance of hepatic amyloid was demonstrated, alongside improved liver function tests. Regression of amyloid within the spleen and kidneys was also visualised by ^{123}I-SAP scintigraphy. No adverse cardiac events were noted in six subjects with cardiac amyloidosis. Unfortunately, a Phase II study in cardiac amyloidosis (NCT03044353) was initiated in 2018 but later terminated due to an unfavourable risk-benefit profile.

Aims and Objectives

Outcomes in AL amyloidosis have improved, and this thesis will focus on contemporary outcomes in the disease, particularly in subgroups that present treatment challenges. It will also explore a novel imaging tracer that may play a future role in the diagnostic pathway.

Bortezomib-based therapy is commonly used in upfront and relapsed/refractory myeloma and has become the mainstay of treatment in newly diagnosed AL amyloidosis. Previous studies have reported outcomes with bortezomib-based therapy in AL patients, but none have examined how sustained these responses are by measuring the time to next treatment. (223-226) Chapter 3 will examine outcomes in the largest cohort of AL patients treated with upfront bortezomib-based therapy. It will explore the impact of a 'stringent dFLC response' (which we define as a difference in involved and uninvolved light chains less than 10mg/L after treatment) in all patients in view of recent studies that demonstrate the use of this response criterion in patients with low level presenting light chains.(113, 114)

The British Society Haematology guidelines in AL amyloidosis advise review of treatment response after three cycles of first-line chemotherapy, with a view to switching therapy in patients who have not achieved a haematological response.(143) However, clinicians face a dilemma of how to manage such patients as there is a paucity of data to guide best approach.

Chapter 4 will examine outcomes in patients who do not achieve a haematological response after three cycles of bortezomib-based therapy.

Patients with AL amyloidosis and advanced cardiac involvement have dismal survival outcomes. Practically, the management of such patients is exceedingly difficult. Such patients have poor physiological reserves and are particularly vulnerable to treatment toxicity. Patients with advanced cardiac involvement are also excluded from the overwhelming majority of clinical trials. Chapter 5 will assess treatment outcomes in AL patients with advanced cardiac involvement (defined by NT-proBNP greater than 8500ng/L) with the aim of informing treatment decisions in this group.

ASCT has latterly been associated with prolonged OS, and good haematological and organ responses.(148, 150) The application of stringent selection criteria has also markedly reduced transplant-related mortality. However, only a minority of patients with AL amyloidosis meet these selection criteria for ASCT at presentation. Chapter 6 will examine the safety and efficacy of deferred ASCT in initially transplant-ineligible patients who go on to have reversal of exclusion criteria with good responses to bortezomib-based therapy.

IgM-associated AL amyloidosis is exceptionally rare, and the treatment approach in such patients is heterogeneous.(227) Rituximab-bendamustine is a chemotherapy regimen favoured in patients with non-Hodgkin

lymphoma. Chapter 7 will assess efficacy and toxicity with this treatment in patients with IgM-associated AL amyloidosis.

Up to a fifth of patients with AL amyloidosis have amyloid-related peripheral or autonomic neuropathy. Treatment of such patients is challenging, as bortezomib is contraindicated in patients with substantial neuropathy given its neurotoxic profile. Chapter 8 will explore outcomes in a group of such patients treated with carfilzomib, a second-generation highly selective proteasome inhibitor with lesser neurotoxicity.

^{18}F-florbetapir is a novel imaging tracer with high affinity for beta amyloid deposits in the brain. A previous pilot study has demonstrated cardiac uptake with this tracer in patients with AL and ATTR cardiac amyloidosis.(228) Chapter 9 will detail a larger pilot study using this tracer in patients with cardiac AL, examining in particular whether there is any difference in uptake with interval imaging and depending on treatment response.

Chapter Two: Materials and Methods

Declaration

I have designed the studies, and collected and analysed data. I performed this work during my role as a Clinical Research Fellow at the UK National Amyloidosis Centre (NAC), University College London (Royal Free Hospital Campus). Chapter Nine was a collaborative study between the NAC and the Amyloidosis Centre at University Hospital Heidelberg, Germany. In the latter study, data from the patients at the Amyloidosis Centre at University Hospital Heidelberg was collected by Dr Ute Hegenbart and Dr Stefan Schonland.

Multiple diagnostic methods were performed by other individuals at the National Amyloidosis Centre and Royal Free Hospital and they were as follows:

- Haematological and biochemistry investigations were performed by Pathology laboratory services at Royal Free Hospital.
- Histological and immunohistochemical analyses were performed by Janet Gilbertson.
- Gene sequencing was performed by Dr Dorota Rowczenio and Hadija Trojer.
- Echocardiography was performed by Sevda Ozer and Babita Pawarova.
- ^{123}I-SAP was performed by David Hutt, Raymond Vito and Florentina Simona-Grigore.

- ^{18}F-Florbetapir imaging was performed by the Nuclear Medicine Department, Royal Free Hospital and images were reported by Dr Thomas Wagner, Nuclear Medicine physician.

Patients

All patients included in this thesis were seen at the NAC, apart from the patients managed at the Amyloidosis Centre, University Hospital Heidelberg, in Chapter 6. A Microsoft Access database is maintained at the NAC with details of all patients referred to the centre. All patients provide explicit informed written consent for this purpose.

All patients at the NAC undergo a standardised review at their first consultation. Initial review includes history taking, clinical examination, functional assessment, blood tests, imaging (echocardiography and ^{123}I-SAP scintigraphy, fat aspiration (where indicated) and review of histological material. Patients are subsequently reviewed six monthly thereafter, with particular attention paid to assessment of haematological response status, organ response and measurement of amyloid load by ^{123}I-SAP scintigraphy.

Functional assessment

The Eastern Cooperative Oncology Group (ECOG) performance status scale was published in 1982 and has proven universally important in standardised assessment of functional status.(229) All patients have assessment of ECOG score performed at every visit to the NAC. The score is found in Table 2.1.

Table 2.1: ECOG performance status(229)

Grade	
0	Fully active, able to carry on pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry out any self-care; totally confined to bed or chair
5	Dead

The extent of heart failure symptoms is assessed using the New York Heart Association's functional classification (NYHA). The score is found in Table 2.2.

Table 2.2: New York Heart Association's functional classification score

Class	Patient symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations or dyspnoea.
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitations or dyspnoea.
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitations or dyspnoea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

All patients at the NAC undergo a six minute walk test at their first visit and at subsequent visits, if screening questions determine them to be well enough to perform this. The aim of the six minute walk test is to ascertain functional exercise capacity, and it is carried out as per standardised guidelines.(230) Improvement in the six minute walk test performance after chemotherapy has been associated with haematological and organ responses in patients with AL. (231)

Definition of organ involvement and organ response

Amyloidotic organ involvement is defined by international amyloidosis consensus criteria, found in Table 2.3.(104) Histological proof from a biopsy performed of an affected organ is required or biopsy from another other site (e.g. fine needle abdominal fat aspiration, minor salivary gland biopsy, rectal biopsy).

Table 2.3: Definition of organ involvement, as defined by international amyloidosis consensus criteria.(104)

Organ	**Definition of amyloidotic organ involvement**
Heart	Echocardiogram: mean wall thickness>12mm, no other cardiac cause

Kidney	24 hour urine protein>0.5g/day, predominantly albumin
Liver	Total liver span>15cm in the absence of heart failure or alkaline phosphatase>1.5 times institutional upper limit of normal
Nerve	Peripheral: clinical; symmetric lower extremity sensorimotor peripheral neuropathy. Autonomic: gastric-emptying disorder, pseudoobstruction, voiding dysfunction, not related to direct organ infiltration
Gastrointestinal tract	Direct biopsy verification with symptoms
Lung	Direct biopsy verification with symptoms Interstitial radiographic pattern
Soft tissue	Tongue enlargement, clinical Arthropathy Claudication, presumed vascular amyloid Skin Myopathy by biopsy or pseudohypertrophy

	Lymphadenopathy (may be localised)
	Carpal tunnel syndrome

Organ response and progression are also defined by international amyloidosis consensus criteria, found in Table 1.2.(115)

^{123}I-SAP scintigraphy

All new patients at the NAC undergo ^{123}I-SAP scintigraphy on first visit. Patients undergo serial SAP scintigraphy on an approximately annual basis, where clinically indicated. Female patients of child-bearing age are asked to confirm that they are not pregnant through signature of a pregnancy declaration form. Thyroid blockade with potassium iodide 60mg is administered before patients receive 200ug of SAP intravenously with 190MBq of radiolabelled iodine (^{123}I). The dose of tracer is equivalent to 3.8mSv of radiation. Whole body imaging is obtained six or 24 hours after tracer administration with a General Electric Starcam gamma camera (GE Medical systems, Slough, UK).

The images are subsequently reviewed by senior clinicians at the NAC and graded as follows:

- Normal – no abnormal tracer localisation
- Small – abnormal uptake in one or more organs, normal intensity in the blood pool

- Moderate - abnormal uptake in one or more organ with diminished blood pool

- Large – abnormal uptake in one or more organ with no evidence of tracer in the blood pool, despite adjustment of the grey scale to encompass the involved organs.

Follow-up scans are performed (if clinically indicated) and graded as showing:

- Unchanged appearances

- Progression – increase in tracer uptake within an affected organ

- Regression – reduction in tracer uptake within an affected organ

Cardiac assessment

All patients routinely undergo cardiac assessment, and this includes blood pressure monitoring, measurement of cardiac biomarkers, electrocardiogram (ECG), echocardiography. DPD and cardiac MRI were performed when clinically indicated indicated.

Cardiac biomarkers

The Mayo staging system is the current prognostic classification in AL and relies upon the use of cardiac biomarkers.(80) All AL patients at the NAC undergo testing of cardiac biomarkers at their first visit, and their NT-proBNP is monitored at subsequent visits. The Mayo classfication is as follows: Stage 1 is defined as NT-proBNP<332ng/L and cardiac troponin T<50ng/L; stage 2

is defined as NT-proBNP>332ng/L or cardiac troponin T>50ng/L; stage 3 is defined as both NT-proBNP>332ng/L and cardiac troponin T>50ng/L.(80)

Blood pressure measurement

All patients undergo assessment of their blood pressure at every visit to the NAC. This is performed while the patient is supine for five minutes, followed by a blood pressure measurement performed when the patient is standing.

ECG

All patients have an ECG performed at every visit to the NAC, with a calibration of 10mm/mV and a speed of 25mm/s. Low voltage complexes on an ECG are regarded as suspicious of cardiac amyloidosis.

Echocardiography

All patients underwent echocardiography with two-dimensional and M-mode settings (GE Vivid 7 system). Criteria from the British Society of Echocardiography were used in the evaluation of left ventricular systolic and diastolic function, ventricular wall thickness and atrial diameter.

Assessment of a clonal disorder

Serum immunoglobulins were measured on a BN^{TM}II system nephelometer (Siemens, Germany). Serum protein electrophoresis and immunofixation (Sebia, France) were carried out in all patients at baseline, and on subsequent follow-up. Blood tests for serum free light chains were performed

in all patients at presentation, serially during treatment (usually repeated after every cycle), and 1-3 monthly thereafter. A latex-enhanced immunoassay was used in measurement of serum free light chains (The Binding Site, Birmingham, United Kingdom) on a Behring BNII auto-analyser (Dade Behring, Marburg, Germany).(232) The assay comprises of antibodies that are directed against serum free light chain epitopes within whole immunoglobulin molecules. The sensitivity of the assay is <5mg/L and reference ranges are arrived at by testing sera from 100 healthy blood donors. Serum free light chains, along with serum and urine electrophoresis and immunofixation are included in assessment of haematologic response, found in Table 2.4.

Table 2.4: Haematologic response criteria(115)

Haematologic response	Criteria
Complete response	Normalisation of the free light chain levels and ratio, negative serum and urine immunofixation
Very good partial response	Reduction in the dFLC to <40mg/L
Partial response	Greater than 50% reduction in the dFLC
Non-response	Less than a PR
Progression	From CR, any detectably monoclonal protein or abnormal free light chain ratio (light chain must double)

	From PR, 50% increase in serum M protein to >0.5g/dl or 50% increase in urine M protein to 20mg/day (A visible peak must be present) Free light chain increase of 50% to >100mg/L

A baseline dFLC>50mg/L was regarded as being at a level that was evaluable for response. In patients with AL and normal FLC or dFLC<50mg/L at presentation, standard criteria of response used in myeloma are applicable (with M-protein>5g/L regarded as assessable for response).

Histology and immunohistochemistry

Diagnostic tissue was processed using Puchtler's alkaline alcoholic Congo Red method.(233) Formalin-fixed, de-paraffinised tissue sections measuring 6-8µg were counterstained with Mayer's haematotoxylin under tap water. The slides were then immersed in ethyl alcohol and stained in fresh Congo Red solution. Slides were rinsed, dehydrated and cleared before being mounted in DPX. Cross-polarised microscopy using a 10x objective was used to interpret slides. Known positive controls (validated by mass spectrometry) were processed alongside diagnostic material.

Formalin-fixed, de-paraffinised 2µm sections of amyloidotic tissue then underwent immunohistochemical testing. A panel of anti-human monospecific antibodies reactive to SAA (Eurodiagnostica, Huntington, UK), AL kappa, lambda, transthyretin and lysozyme (Dako Ltd, Denmark House, Ely, UK), fibrinogen Aα(Calbiochem) and Apolipoprotein AI (Genzyme

Diagnostics) were used as appropriate. Interpretation of the slides was carried out blindly by two independent and experienced workers. Laser microdissection and mass spectrometry was performed in parallel.(87)

Gene sequencing

Gene sequencing was performed in patients with suspected hereditary amyloidosis. Blood was taken in an EDTA tube and then frozen. Genomic DNA was isolated by a rapid method Polymerase chain reaction (PCR) using 'Ready-To-Go' tubes (GE Healthcare), amplifying the coding regions for Apolipoprotein AI (exons 3 and 4), Fibrinogen Aα (the 5' end of exon 5) and transthyretin (exons 2, 3 and 4), HotStar Taq DNA Polymerase kit (Qiagen) was used in amplifying the coding region for the lysozyme gene (exon 2). The primers used are found in Table 2.6.

Table 2.6: The primers used in genotyping hereditary amyloidosis.

Gene (exon)	Forward primer sequence	Reverse primer sequence
Apolipoprotein (3)	5'-GGCAGAGGCAGCAGGTTTCTCAC-3'	5'-CCAGACTGGCCGAGTCCTCACCTA-3'
Apolipoprotein (4)	5'-CACTGCACCTCCGCGGACA-3'	5'-CTTCCCGGTGCTCAGAATAAACGTT-3'
Fibrinogen (5)	5'-AGCTCTGTATCTGGTAGTACT-3'	5'-ATCGGCTTCACTTCCGGC-3'
Lysozyme (2)	5'-GTTATATTGTTCGTTGGTGT-3'	5'-CATTTGTATTGAGTCTCAATTC-3'

Transthyretin (2)	5'-TTTCGCTCCAGATTTCTAATAC-3'	5'-CAGATGATGTGAGCCTCTCTC-3'
Transthyretin (3)	5'-GGTGGGGGTGTATTACTTTGC-3'	5'-TAGGACATTTCTGTGGTACAC-3'
Transthyretin (4)	5'-GGTGGTCAGTCATGTGTGTC-3'	5'-TGGAAGGGACAATAAGGGAAT-3'

[18]F-Florbetapir PET

[18]F-florbetapir PET imaging was used in the study detailed in Chapter 9, in order to assess cardiac uptake with this tracer in patients with cardiac AL. The patients in the study in Chapter 9 underwent dynamic PET imaging for 60 minutes using a Siemens Biograph PET/CT machine, after which they received a mean intravenous administration of 331 MBq (range 294-370MBq) of ^{18}F-florbetapir. The heart was placed in the centre of the field of view. CT imaging was performed over this area using automatic exposure control (CARE Dose 4D, CARE kV – Siemens Healthcare) with exposure parameters of 65mAs and 120kV. After dynamic imaging, patients were asked to void. A half body acquisition PET-CT was subsequently performed. Images underwent iterative reconstruction (2 iterations, 21 subsets) using time of flight information and point spread function modelling, with 2mm Gaussian post filtering. The 60 minute list mode data was reconstructed into 37 frames (12 frames of 5 s each, 6 frames of 10 s each, 4 frames of 30 s each, 6 frames of 60 s each, 8 frames of 300 s each, and 1 frame of 600 s).

Images were assessed by a nuclear medicine physician using HERMES workstation (HERMES Medical Solutions). The dynamic images (0-60 minutes) were assessed visually for cardiac uptake. The delayed half body images were visually analysed for uptake in the heart and other organs. All acquired data for all time frames was analysed and interpreted. For the dynamic images of the heart, the nuclear medicine physician assessed the presence of uptake in the myocardium and its localisation. For delayed half body images, cardiac uptake was assessed in the same manner. For extra-cardiac uptake, comparison was made with normal tracer distribution as described in the literature(228) and as inferred from this study's patient population.

<u>Statistical analysis</u>

Statistical analysis was performed using SPSS Version 24 (SPSS, Chicago, IL) and Graph Pad Prism (Version 5.03). Specific statistical methods are discussed separately in the corresponding results chapters.

Chapters Three-Nine: Results

Chapter Three: Outcomes in 915 patients with newly diagnosed AL amyloidosis treated with bortezomib-based therapy

This chapter is written in the context of my publication:

A prospective observational study of 915 patients with systemic AL amyloidosis treated with upfront bortezomib.

Manwani R, Cohen O, Sharpley F, Mahmood S, Sachchithanantham S, Foard D, Lachmann HJ, Quarta C, Fontana M, Gillmore JD, Whelan C, Hawkins PN, Wechalekar AD. Blood 2019; 134(25):2271-2280

Introduction

Treatment in AL amyloidosis is based upon rapid suppression of amyloidogenic serum free light chains, and the magnitude of reduction predicts organ response and survival. The main upfront treatment choice is between a high dose melphalan autologous stem cell transplant (ASCT) or combination chemotherapy. Only approximately 20% of patients are eligible for upfront ASCT, as significant cardiac involvement or poor performance status preclude the majority from this treatment at presentation.

Whilst such ASCT-ineligible patients tended to receive a combination of oral melphalan and dexamethasone, bortezomib-based regimens have become standard therapy in AL. Reece et al reported the first prospective Phase 2 study of single-agent bortezomib in relapsed AL.(153) In the latter study, patients were either treated with 1.6mg/m^2 once weekly bortezomib (35 day cycle) or 1.3mg/m^2 twice weekly. The ORR was 68.8% and 66.7%, respectively, with CR rates of 37.5% and 24.2%. The median time to best response was 3.2 and 1.2 months, respectively. Cardiac responses were achieved in 13% and renal responses in 29% of patients. Grade 3 toxicity was more frequent in the twice weekly regimen.

Kastritis et al reported a retrospective series of 94 patients with relapsed and newly diagnosed AL, who were treated with bortezomib +/- dexamethasone, resulting in an ORR of 71% and 25% CR rate.(224) Two further retrospective

studies have described excellent ORR (81.4-94%) and CR rates (41.9-71%) with the use of bortezomib-cyclophosphamide-dexamethasone (CyBorD) in upfront and relapsed patients.(225, 226) A large retrospective European collaborative study of CyBorD in upfront AL reported an ORR of 60%, with a CR rate of 23%. (154) Similarly, a large multicentre retrospective European study showed that upfront CyBorD was unable to overcome the poor outcomes in Mayo Stage III cardiac involvement, with a median survival of 4.6 months and 17% CR rate.(223) Previous studies have not reported in detail about time-to-next treatment (TNT) and long-term organ responses in AL with bortezomib.

Haematologic responses in AL are defined by international amyloidosis consensus criteria (ICC) published in 2012.(96) These utilise the dFLC measure in response assessment, as this parameter is more predictive of outcomes in AL than M-protein response.(94) The ICC stipulate that a very good partial response (VGPR, dFLC<40mg/L) is the goal of therapy, whilst >50% reduction in dFLC (partial response (PR)) is inadequate. The ICC also stipulate that the presenting dFLC value must be ≥50mg/L in order to be 'measurable' for response assessment, but this excludes 20% of patients from response assessment. Recently, 'low-dFLC' response criteria have been reported(113, 114), whereby a reduction in dFLC after treatment to <10mg/L predicted favourable overall and renal survival in patients with presenting dFLC of 20-50mg/L.

There is also much interest in AL regarding the prognostic role of minimal residual disease (MRD) testing. The Mayo clinic demonstrated that, at the end of treatment, AL patients with ≥0.1% monotypic plasma cells had a shorter PFS and OS than those with percentage of monotypic plasma cells below this threshold (2 year PFS 31% vs 87%, 2 year OS 87% vs 98%).(121) Given data on low-dFLC response and the value of MRD, we posited that a target of dFLC<10mg/L (termed a 'stringent dFLC response') may be important in all AL patients, irrespective of baseline light chain levels. This chapter reports upon outcomes in the largest cohort of AL patients treated with upfront bortezomib and explore the impact of dFLC<10mg/L on outcomes.

Methods

All patients from a prospective observational study of newly diagnosed AL (ALchemy) treated with upfront bortezomib-based regimens from February 2010-August 2017 were included. Patients were treated at their local centres as per nationally agreed protocols. All patients underwent a review at the UK National Amyloidosis Centre three months after starting bortezomib-based therapy and then at least six monthly thereafter for comprehensive assessment. Investigations for the study were performed at the UK National Amyloidosis Centre, where data was collected and analysed. Dose modifications, number of treatment cycles and steroid doses were at the discretion of locally treating physicians. Patients were treated with intravenous bortezomib until 2013, and subcutaneous bortezomib thereafter. Decisions for a change in treatment took into account the individual dFLC

response, extent of organ damage, improvement in organ function and performance status.

Diagnosis of AL was confirmed with biopsy immunohistochemistry and/or proteomic analysis. All patients underwent serial biochemical tests for organ function, serum free light chains, serum and urine protein electrophoresis and immunofixation, cardiac biomarkers, echocardiography and/or cardiac MRI (unless contraindicated). Organ involvement and responses were defined by ICC.(104, 115) Organ responses were assessed at 12 and 24 months from the time of treatment initiation. The European modification of Mayo 2004 staging was used, with Stage III stratified into IIIa (NT-proBNP <8500ng/L) and IIIb (NT-proBNP ≥8500ng/L).(107)

Haematologic responses at 6 months were assessed by the international consensus criteria in patients with presenting dFLC>50mg/L(96). CR was defined as negative serum and urine immunofixation, and normal serum free light chain ratio (0.26-1.65). VGPR was defined as dFLC<40mg/L, and PR as >50% dFLC reduction.

Patients with presenting dFLC 20-50mg/L were regarded as achieving a low-dFLC PR if dFLC<10mg/L was reached after treatment. Patients with dFLC<20mg/L at presentation were included in survival analysis but excluded from response assessment (20mg/L is the lowest dFLC analysed in recent low-dFLC studies(113, 114)). Absolute dFLC at 6 months was assessed in all patients. Given recent reports of low-dFLC response(113, 114), we assessed absolute dFLC at 6 months in all patients and termed 6 month dFLC<10mg/L as a 'stringent dFLC response'. Outcomes were

assessed in those with stringent dFLC responses and those without.

Outcomes were also assessed in patients who achieved dFLC>10 mg/L but

<40mg/L (i.e. VGPR patients without a stringent dFLC response). Survival

outcomes were analysed using the Kaplan-Meier method with comparisons

done using the log-rank test.

TNT was defined as time from first-line therapy to beginning of second-line

therapy. Patients that died without having progressed to second-line

treatment were excluded from analysis of TNT. Haematological progression

was an indication for second-line treatment. While some criteria require a

substantial increase in FLC to define progression, patients often cannot wait

until this threshold is reached. The novel criteria of high-risk progression

defined by the Italian amyloidosis group are critically important(200) but not

universally adopted – we are incorporating these in our decision-making

algorithms but they were not routinely used for the duration of this study.

Conversely, in some patients, second-line treatment was deferred after

multidisciplinary discussions taking into account all factors in the patients'

disease status including organ function, performance status, light chain

burden, frailty and co-morbidities. Since chemotherapy does not directly

impact end-organ damage or its improvement, it is challenging to capture the

true benefit of treatment when a patient dies of amyloid-related organ

dysfunction. Hence, we have used TNT in a real world attempt to capture

the true impact of benefit or loss of benefit from front-line treatment.

All p-values were two-sided with a significance level of <0.05; median values were used to dichotomise continuous variables. Factors that were significant on univariate analysis were further assessed in multivariate modelling by Cox's regression analysis. Statistical analysis was performed using SPSS version 24. Approval for analysis and publication was obtained from NHS institutional review board; written consent was obtained from all patients in accordance with the Declaration of Helsinki.

Results

Patients

915 patients underwent bortezomib-based therapy from February 2010-August 2017 (Figure 3.1) and Table 3.1 demonstrates annual patient recruitment to the study. Baseline characteristics are described in Table 3.2.

Figure 3.1. Flow diagram of patient recruitment and those included in response assessment.
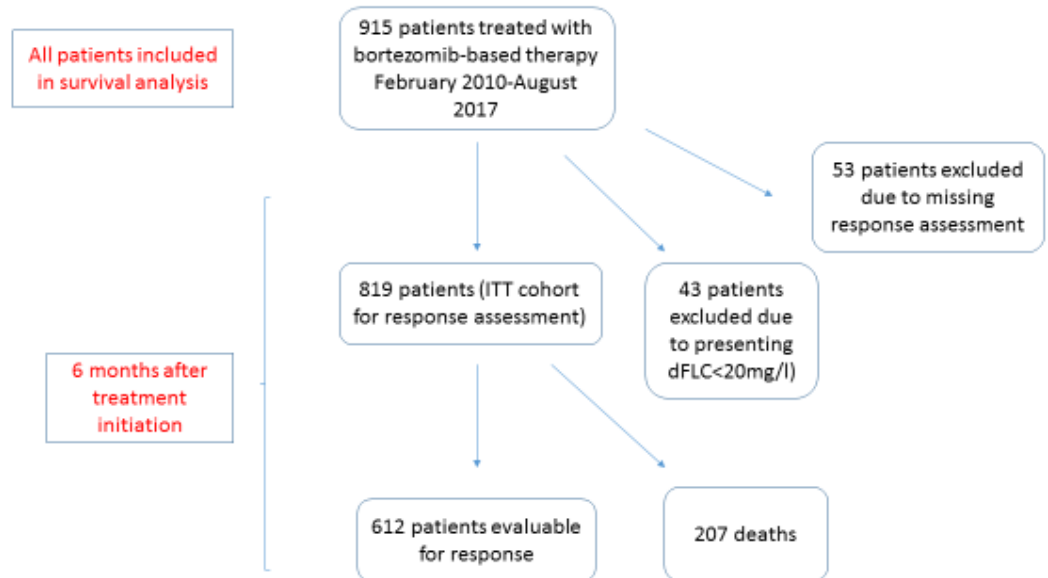
Table 3.1. Patients recruited to the study per year.

Year	Total patients treated	Number of patients with absent response data
2010	7	0
2011	46	0
2012	83	3
2013	113	5
2014	170	11
2015	188	10
2016	191	16
2017	117	8

Table 3.2: Baseline characteristics.

n=915	Median (range) / n(%)
Median age (years)	66 (29-89)
Male:Female	540 (59%): 375 (41%)
NYHA class	
1	223 (24.4%)
2	446 (48.7%)
3	108 (11.8%)
4	4 (0.1%)
Unrecorded	134 (15%)
ECOG	
0	205 (22.4%)
1	363 (40.0%)
2	259 (28.3%)
3	49 (5.3%)
4	0
Unrecorded	39 (4.0%)
Cardiac involvement	653 (71.4%)
Median NT-proBNP (ng/l)	2228 (29-93776)
Median high-sensitivity cardiac troponin T (ng/l)	54 (1-458)
Mayo Stage	
I	144 (15.7%)
II	302 (33%)

III, NT-proBNP ≤ 8500ng/l	344 (37.6%)
III, NT-proBNP>8500ng/l	125 (13.7%)
Median systolic blood pressure (mmHg)	118 (63-198)
Median LV wall thickness (mm)	13 (6-23)
Median LV ejection fraction (%)	58 (11-80)
Renal involvement	623 (68.1%)
Median serum creatinine (umol/l)	97.5 (26-1124)
Median GFR (ml/min)	64 (3-100)
Median proteinuria (g/24h)	3.14 (0.08-36.05)
Liver involvement	124 (13.5%)
Median serum bilirubin (umol/l)	6 (1-449)
Median ALP (units/l)	90 (26-2142)
Soft tissue involvement	124 (13.5%)
Peripheral nerve involvement	57 (6.2%)
Autonomic nerve involvement	53 (5.8%)

GI involvement	28 (3.0%)
Median number of involved organs	2 (1-5)
Involved light chains: Kappa Lambda No monoclonal light chain excess	186 (20.3%) 680 (74.3%) 49 (5.4%)
Median dFLC (mg/l)	180 (0-15898)
IgG/IgA/ IgM/IgD/light chain/no detectable serum paraprotein	239 (26.1%) / 93 (10.2%) / 26 (2.8%) / 4(0.04%) / 61 (6.7%) / 492 (53.8%)
Median serum monoclonal protein (g/l)	4
Median duration between diagnosis and treatment initiation (days)	27 (0-98)

The proportion of patients with cardiac, renal, liver, peripheral nerve, autonomic and gastrointestinal involvement was: 71.4%, 68.1%, 13.5%, 6.2%, 5.8% and 3.0%. Mayo (2004) Stage I, II and III disease was found in 15.7%, 33% and 51.3% patients, respectively. The median NT-proBNP and dFLC were 2228ng/L (range 29-93776) and 180mg/L (0-15898), respectively.

All were treated with bortezomib: CyBorD 94.9%, bortezomib-dexamethasone 2.9%, bortezomib-thalidomide-dexamethasone 1.3%, bortezomib-melphalan-prednisolone 0.4%, bortezomib-melphalan-dexamethasone (BMDex) 0.2%, bortezomib-lenalidomide-dexamethasone 0.2% and bortezomib-melphalan-thalidomide-dexamethasone 0.1%. The median number of chemotherapy cycles was 5 (1-9). Patients found not to be in a haematological response at three months continued bortezomib with addition of either cyclophosphamide or thalidomide.

Haematologic responses

Of 915 total patients, response analysis excluded 43 patients due to presenting dFLC<20 mg/L (the lowest dFLC analysed in recently reported 'low-dFLC' studies(113, 114)) and 53 patients due to missing response data. The latter group did not attend for follow-up at our centre at 12 months, but survival data for these patients is available and has been incorporated into survival analysis. 819 patients were therefore included in haematologic response intent-to-treat (ITT) analysis. Evaluable response analysis included 612 patients (this excluded the patients who died before response assessment). Haematologic responses are shown in Figure 3.2.

Figure 3.2: Haematologic responses in the cohort.

Haematological responses

	CR	VGPR	PR	Low-dFLC PR	Non-response
Stage III (evaluable)	38	32	19	1	10
Stage III (ITT)	23	19	11	4	46
All cohort (evaluable)	35	27	20	5	13
All cohort (ITT)	25	20	16	4	35
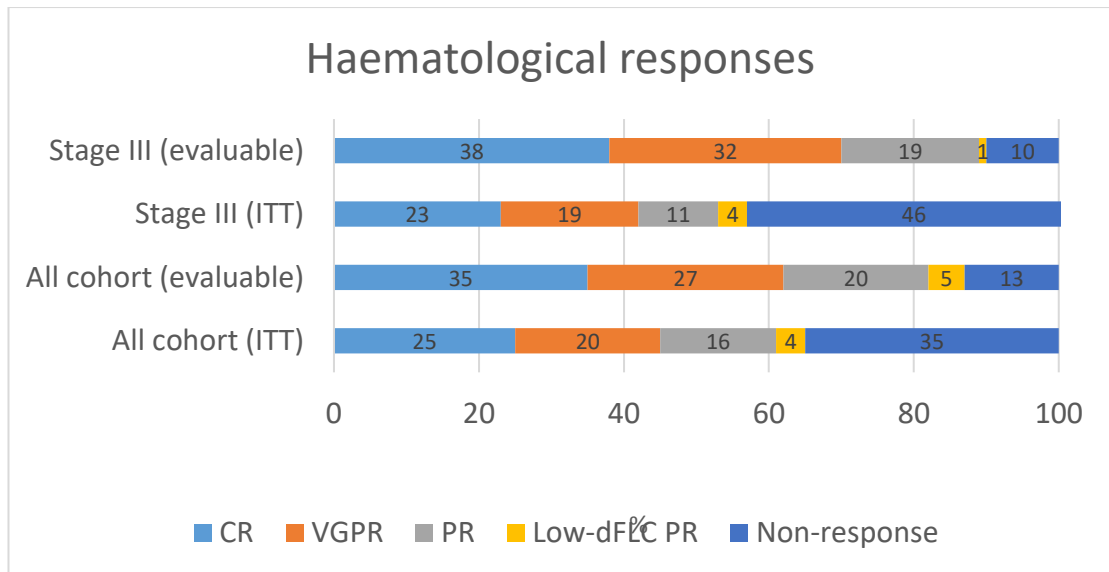
Haematologic responses (ITT) were: CR 25%, VGPR 20%, PR 16%, low-dFLC PR 4%, non-response 35% (including 207 deaths (25%)). Evaluable haematologic responses (n=612) were: CR 35%, VGPR 27%, PR 20%, low-dfLC PR 5% and non-response 13%. Of 421 patients with Mayo Stage III disease, haematologic responses (ITT) were: CR 23%, VGPR 19%, PR 11%, low-dFLC PR 1%, non-response 46% (including deaths 39%)). Evaluable haematologic responses (i.e. deaths excluded, n=256) in Stage III patients were: CR 38%, VGPR 32%, PR 19%, low-dFLC PR 1% and non-response 10%. ITT haematological responses in Stage IIIB disease were: CR 13%, VGPR 17%, PR 8%, low dFLC-PR 1% and non-response 61% (including deaths 59%). Evaluable haematological responses (deaths excluded) in Stage IIIB patients included: CR 33%, VGPR 41%, PR 18%, low-dFLC PR 2% and non-response 6%.

The proportion of patients in a CR with presenting dFLC 50-200mg/L, 201-600mg/L and >600mg/L was 58%, 28% and 14%, respectively.

Overall survival and time-to-next-treatment

Median follow-up for all patients and living patients was 23 and 32 months, respectively. Median OS (ITT, n=915) was 72 months (Figure 3.3).

Figure 3.3: Median overall survival (OS) and time-to-next-treatment (TNT).
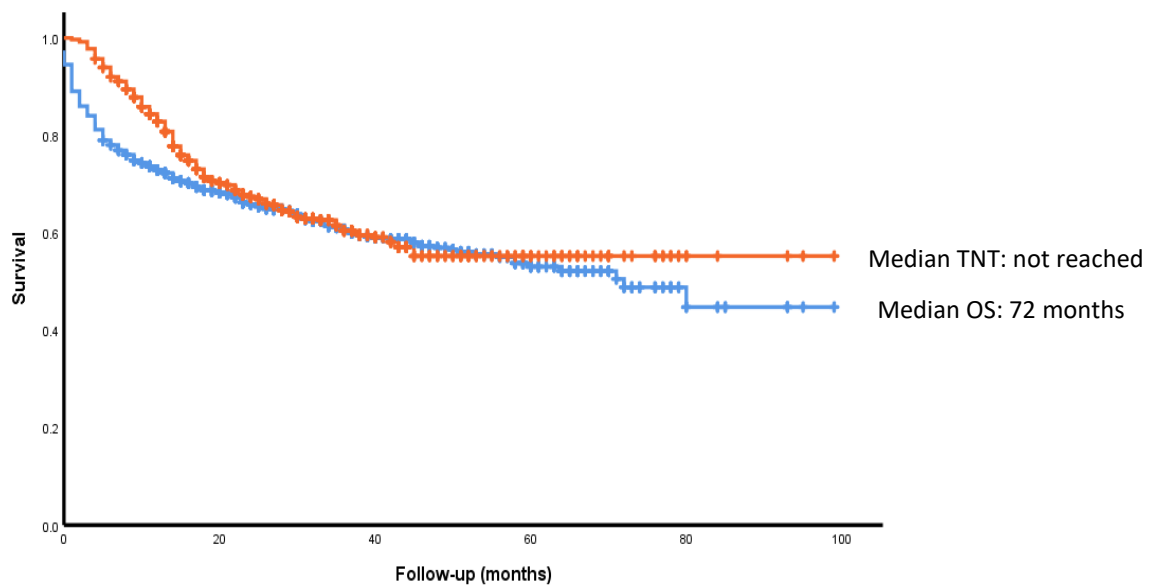
289 patients (31.6%) died without progressing to second-line treatment. Of the remaining 626 patients, the median TNT was not reached and 55% had not progressed to further treatment at 7 years. Median OS in Mayo Stage I, II, IIIa and IIIb was: not reached, 80, 36 and 4 months (p<0.0001) (Figure 3.4).

Figure 3.4: Median overall survival (OS) by Mayo cardiac staging.
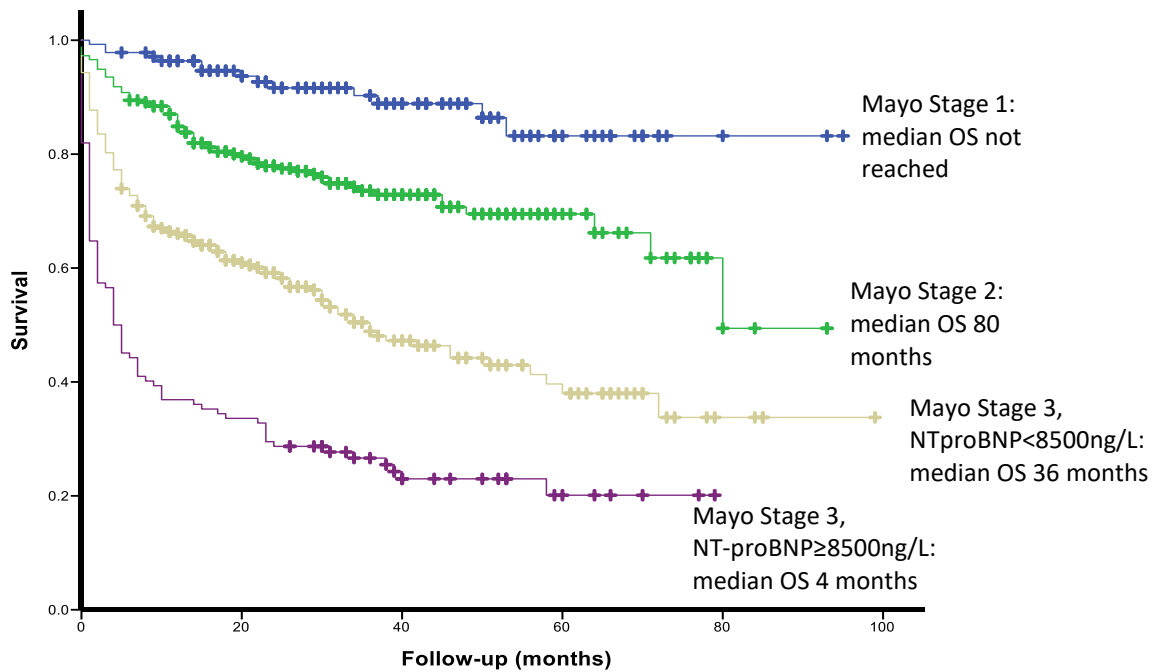
Median TNT was not reached in Mayo Stage I, II, IIIa and was 38 months in Stage IIIb. Median OS was not reached in patients in CR or low-dFLC PR; median OS was 71 and 39 months in patients in PR and non-response, respectively. Median TNT in patients in CR, VGPR and low-dFLC response was not reached; it was 17 and 13 months in those in a PR and non-response, respectively (Figure 3.5).

Figure 3.5: Median time-to-next-treatment (TNT) in patients by
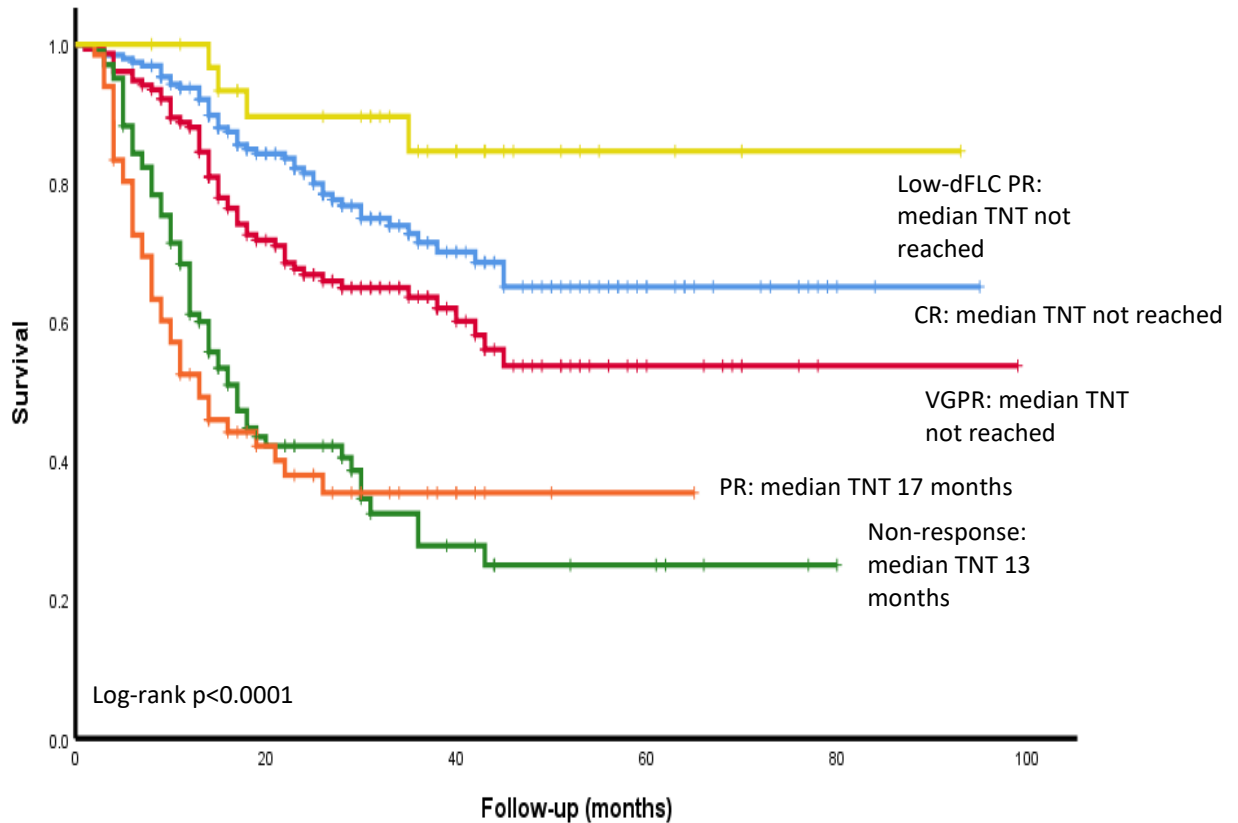
At one, five and seven years, 98%, 77% and 60% of CR patients did not require further treatment, respectively.

Of patients considered transplant-eligible upfront (i.e. age<70 years, NT-proBNP<5000ng/L, cardiac troponin T<60ng/L, serum creatinine<150umol/L, and organ involvement<3) but were treated with upfront bortezomib instead, the median OS and TNT were not reached. At five years, 78% were still alive and 71% had not progressed to next treatment.

Assessing impact of achieving a stringent dFLC response (dFLC<10mg/L) at 6 months

Absolute 6 month dFLC responses (ITT, n=819) were: dFLC<10 mg/L 30%; dFLC 10-20mg/L 11%; dFLC 20-30mg/L 6%; dFLC 30-40mg/L 5%, dFLC 40-50mg/L 3%, dFLC>50mg/L 20% and deaths 25% (Table 3.3).

Table 3.3: Absolute 6 month dFLC response (ITT=819).

6 month dFLC	% response in each category	Cumulative total number of patients in a response
<10mg/L	30%	30%
10-20mg/L	11%	41%
20-30mg/L	6%	47%
30-40mg/L	5%	52%
40-50mg/L	3%	55%
>50mg/L	20%	75%

Presenting dFLC levels did not impact upon achievement of stringent dFLC responses. Presenting dFLC for all 246 patients reaching a stringent dFLC response were: <50mg/L 22%, 50-200mg/L 45% and >200mg/L 33%. Of

patients in a stringent dFLC response, 13% had an abnormal serum free light chain ratio.

Median OS and TNT were significantly better for patients achieving a stringent dFLC response compared to lesser responses (even if they achieved a VGPR by ICC). Median OS was not reached in patients achieving a stringent dFLC response or dFLC 10-40mg/L, compared to 53 months in patients with dFLC≥40mg/L (log-rank $p < 0.0001$) (Figure 3.6).

Figure 3.6: Median overall survival (OS) by absolute 6 month difference in involved and uninvolved light chains (dFLC) value.
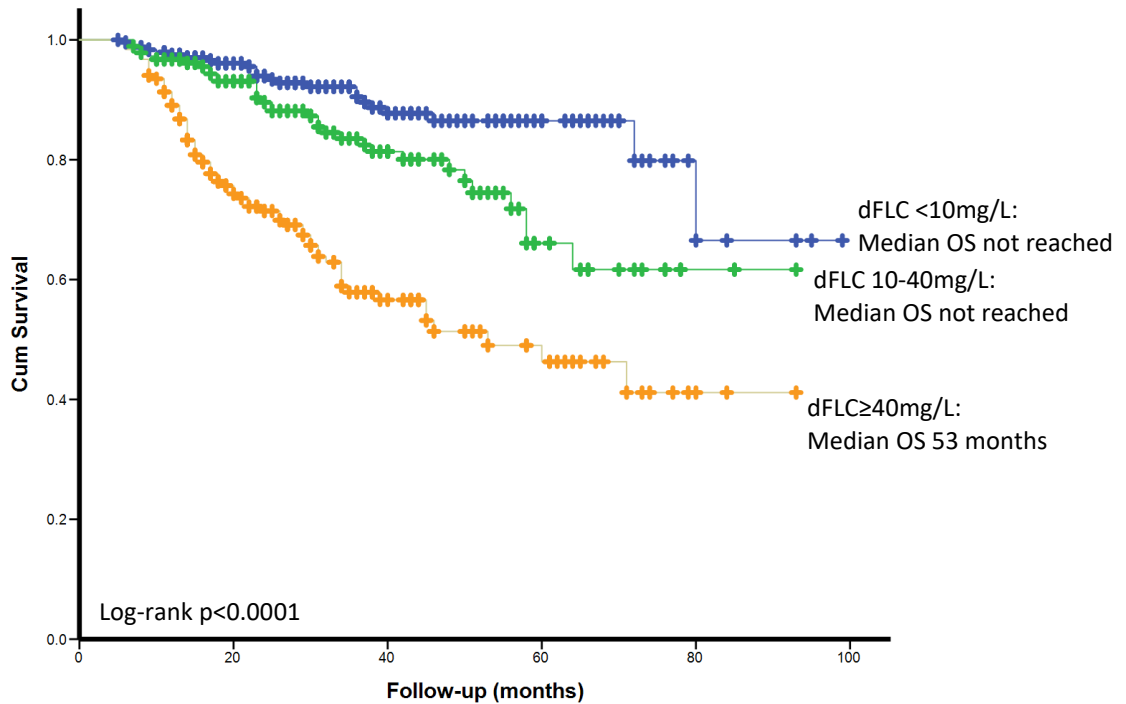
At one, three and five years, 98%, 92% and 86% of patients in a stringent dFLC response were alive. In the dFLC 10-40mg/L group, 97%, 84% and 66% of patients were alive at these time periods. Median TNT in patients with 6 month dFLC<10mg/L, 10-40mg/L and dFLC>40mg/L was not reached, 38 and 13 months (log-rank p<0.0001) (Figure 3.7).

Figure 3.7: Median time-to-next-treatment (TNT) by absolute 6 month dFLC value.
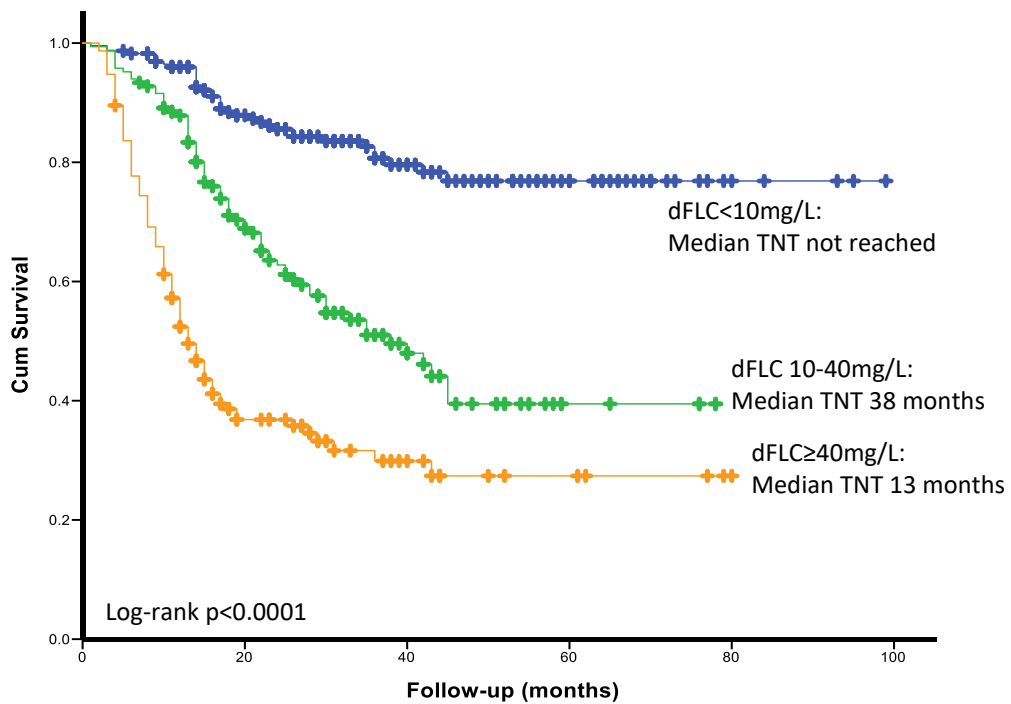
There was a significant difference in OS between CR patients with stringent dFLC responses (n=145, median not reached) compared to CR patients without stringent dFLC responses (n=67, median not reached) (p=0.033) (Figure 3.8).

Figure 3.8: Median OS in patients with a CR with and without a stringent dFLC response.
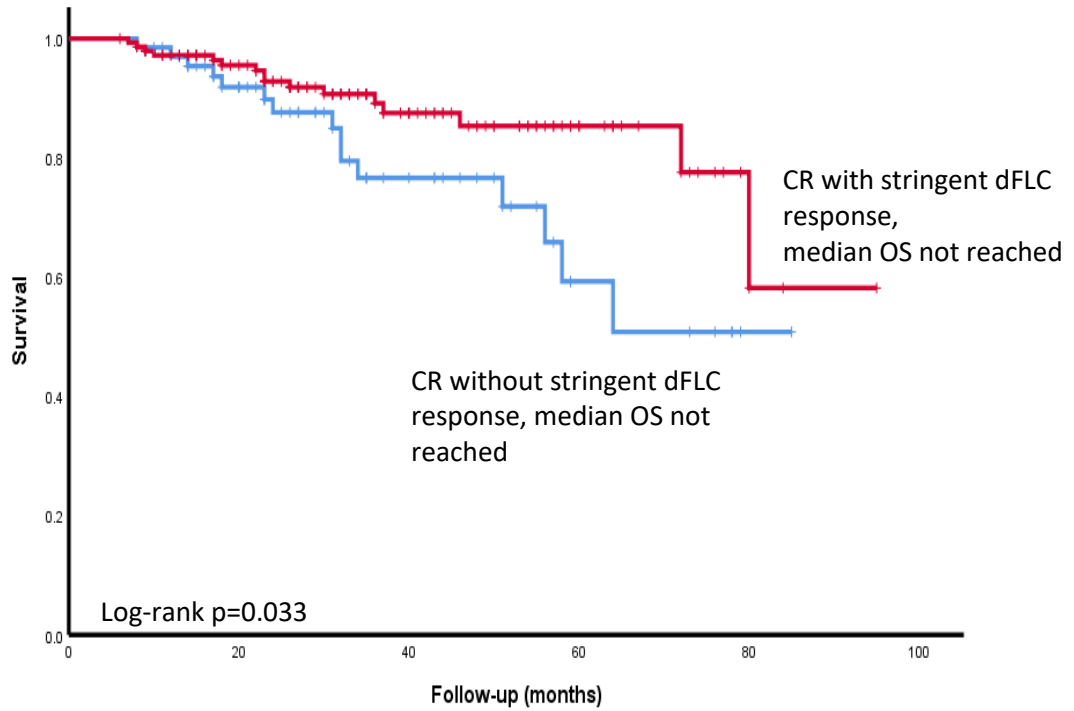
The median TNT in CR patients with stringent dFLC response was not reached, compared to 38 months in CR patients without a stringent dFLC response (p<0.001) (Figure 3.9).

Figure 3.9: Time-to-next-treatment (TNT) in CR patients with and without a stringent dFLC response.
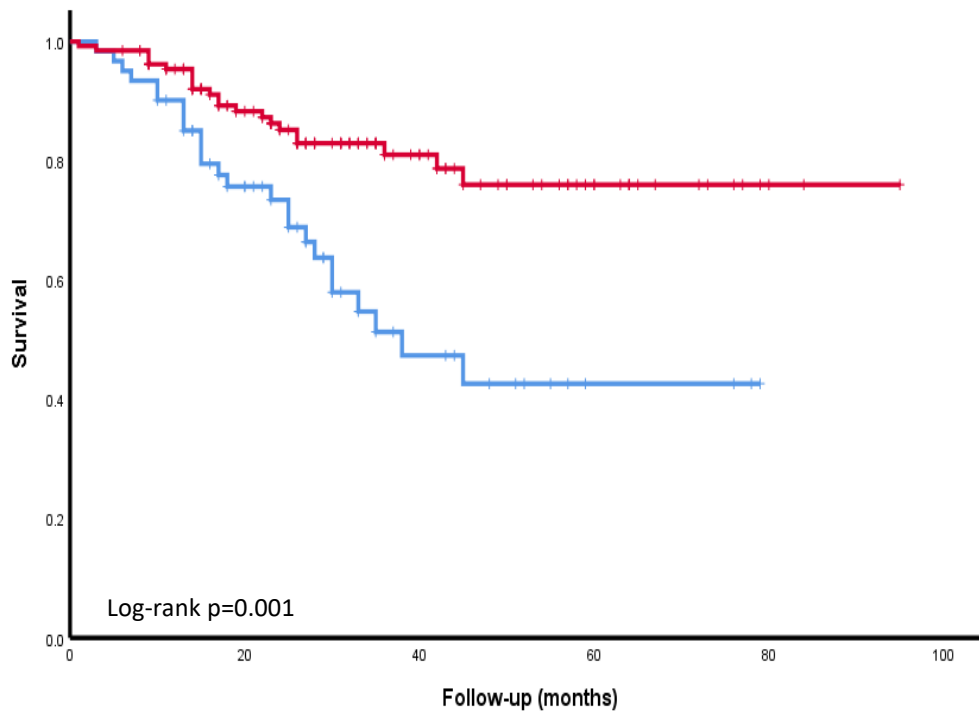
Figure 3.10: Median OS in VGPR patients with and without a stringent dFLC response.
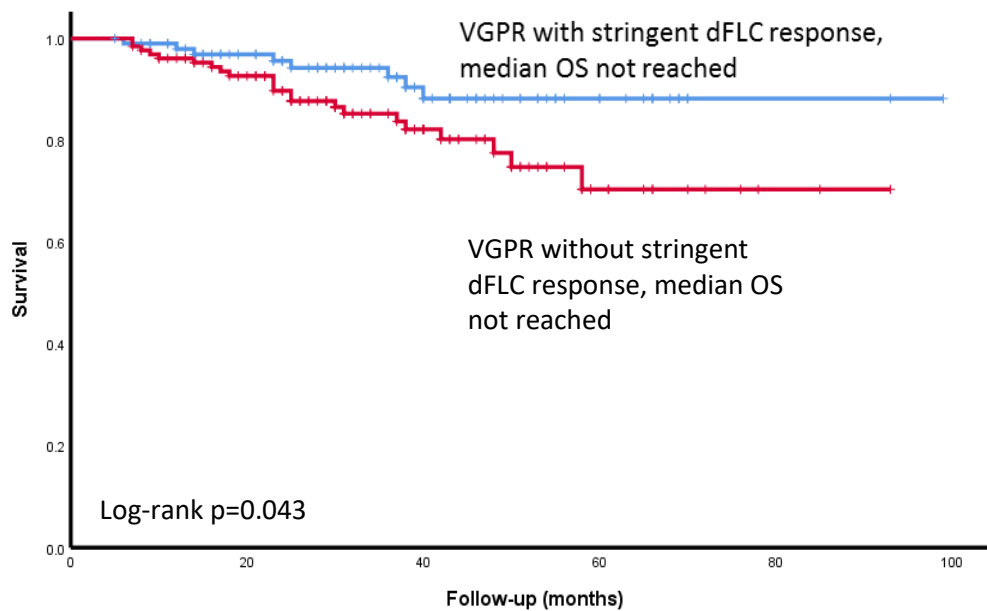
The median TNT in VGPR patients with and without a stringent dFLC response was not reached and 40 months, respectively (p<0.001) (Figure 3.11).

Figure 3.11: Median time-to-next treatment (TNT) in VGPR patients with and without a stringent dFLC response.
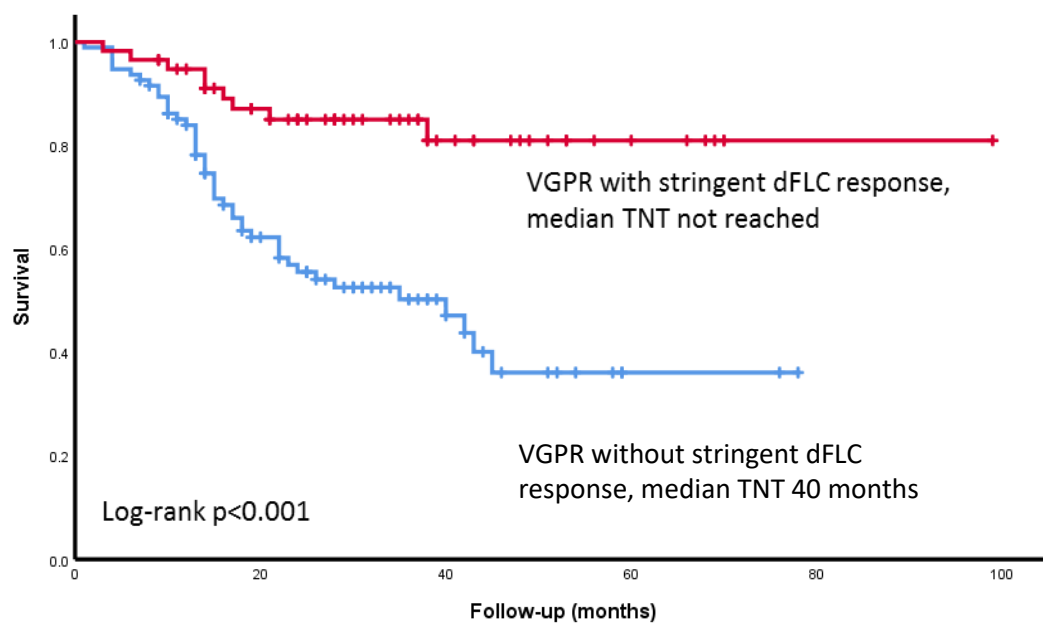
The median OS was not reached in patients with a CR and those with a stringent dFLC response without CR, although OS appeared to be better in the latter (p=0.089).

In a multivariate analysis, 6 month dFLC was predictive of TNT, independent of Mayo disease stage and presenting serum free light chains (Table 3.4).

Table 3.4: Multivariate model incorporating Mayo disease stage, presenting difference in amyloidogenic involved and uninvolved light chains (dFLC) and 6 month absolute dFLC values.

	Hazard ratio	p-value	95% confidence interval
OS			
Mayo disease stage			
1	2.05	0.06	0.96-4.34
2	3.7	0.01	1.74-7.86
3	6.49	<0.001	2.96-14.21
Presenting dFLC<180mg/L	1.3	0.04	1.01-1.68
ECOG			
1	1.25	0.29	0.83-1.9
2	2.12	<0.001	1.39-3.22
3	2.3	0.004	1.3-4.08
TNT			
Mayo disease stage			
1	1.01	0.96	0.70-1.47
2	0.84	0.40	0.56-1.26

3	0.91	0.73	0.52-1.58
Presenting dFLC<180mg/L	0.94	0.70	0.71-1.26
6 month dFLC			
<10mg/L			
10-20mg/L	2.76	<0.001	1.72-4.43
20-30mg/L	2.78	<0.001	1.74-4.46
30-40mg/L	3.27	<0.001	1.82-5.87
40-50mg/L	3.81	<0.001	2.32-6.28
>50mg/L			

22.9% of patients in a dFLC response had amyloidogenic light chains of kappa isotype. These patients had a better OS than those with lambda light chain isotype (p=0.033). The median was not reached in both groups; 100% alive in 5 years with kappa light chains and 83% alive at 5 years with lambda light chains. Of patients with a stringent dFLC response, patients with lambda light chain preponderance were more likely to have cardiac involvement (72.5%) than kappa (54.5%), (p=0.026). In the overall cohort, 61% of patients with kappa light chain preponderance had cardiac involvement, compared to 68% with lambda light chain preponderance (p=0.073). There was no significant difference in median OS or TNT between these two groups in the overall cohort. There was no difference in TNT

between the two light chain isotypes. The proportion of patients in a stringent dFLC response with reduced creatinine clearance (<30ml/min) was 44/246 (17.9%). Of these 44 patients, 7 (15.9%) had kappa amyloidogenic light chains. There was no difference in median OS and TNT in patients with severe renal dysfunction (eGFR < 30 ml/min) who had a kappa or lambda light chain preponderance.

Organ responses

Organ responses were assessed at 12 and 24 months. Table 3.5 outlines organ responses at 12 months according to 6 month haematological response.

Table 3.5: Organ responses 12 months after treatment according to 6 month haematological response.

6 month haematological response	12 month cardiac response	12 month renal response	12 month liver response
Complete response	67/113 (59.3%)	32/148 (21.6%)	9/20 (45%)
CR, dFLC<10mg/L	43/72 (59.7%)	28/100 (28%)	6/16 (37.5%)
CR, dFLC≥10mg/L	24/41 (58.5%)	4/48 (8.3%)	3/4 (75%)
Very good partial response	55/119 (46.2%)	23/122 (18.9%)	9/21 (42.9%)
Partial response	21/59 (35.6%)	12/78 (15.4%)	6/17 (35.3%)
Non-response	5/25 (20%)	6/56 (10.7%)	2/7 (28.6%)

On an ITT basis, 517 patients with cardiac involvement were assessable. At 12 months, 32.5% achieved cardiac responses, 21.7% did not, and 45.8% had died. By 24 months, 88 patients had progressed to next treatment (including 41 who had previously been in a cardiac response). Of the remaining patients, cardiac responses at 24 months were as follows: 28.6% in a cardiac response, 11.6% were not and 59.8% had died. dFLC responses were assessed in patients who were alive at 6 months. 12 month cardiac responses were achieved in 76/125 (61%) of those with stringent dFLC

responses at 6 months, compared to 90/199 (45%) of patients with lesser

responses (p=0.005) (Figure 3.12).

Figure 3.12: Cardiac responses according to absolute dFLC values at 6 months.
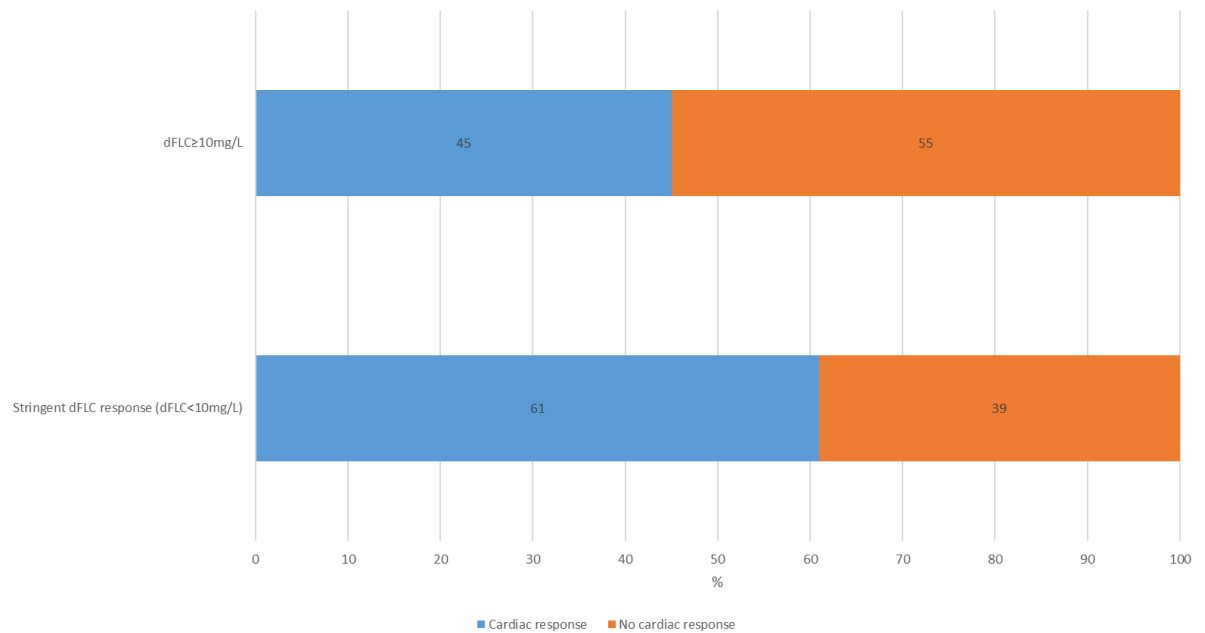
On an ITT basis, 611 patients with renal involvement were assessable for

renal response: 15.4% achieved renal responses, 59.9% did not and 24.7%

had died. At 24 months, 148 patients had progressed to next treatment

(including five that had achieved a renal response at 12 months). Of the

remaining patients, renal responses were as follows: renal response 26%,

non-response 32% and deaths 42%. 12 month renal responses were

achieved in 55/204 (27%) in patients with stringent 6 month dFLC responses,

compared to 38/267 (14%) with lesser responses (p=0.001).

On an ITT basis, 95 patients with liver involvement were assessable for liver response, 30% achieved liver responses, 45.3% did not and 25.3% had died. A stringent dFLC response did not affect liver responses (39.2 % vs. 29.2% respectively (p=0.31)).

Toxicity

Table 3.6 details reported toxicity, the commonest of which was Grade 1-2 lethargy, constipation, fluid overload and sensory neuropathy.

Table 3.6: Grade 1-2 and Grade 3-4 toxicity experienced by patients included in this cohort.

	Grade 1-2	Grade 3-4
Lethargy	55.7%	6.5%
Fluid overload	24.1%	4.8%
Non-neutropenic infection	6.2%	4%
Neutropenia	2.5%	0.2%
Febrile neutropenia	0.04%	0.001%
Hypotension	12.3%	2%
Sensory neuropathy	21.1%	1.3%
Motor neuropathy	0.8%	0.07%
Diarrhoea	13.1%	1.3%
Constipation	25.5%	0.03%
Rash	7.4%	0.03%

Discussion

In this study, we report outcomes in the largest unselected cohort of AL patients treated with upfront bortezomib (mainly CyBorD) therapy. This cohort captures nearly all AL patients seen at our centre, two-thirds of whom presented with cardiac involvement and half with Mayo Stage III disease. Haematologic response rates were high (ORR 65%) with half achieving VGPR/better in an ITT analysis. TNT was excellent, with median TNT not reached for responders at 7 years – data that rivals outcomes reported with upfront ASCT. However, we remain unable to abrogate the high incidence of early mortality in AL, with 40% of Stage III patients dying within six months of diagnosis – data that is not captured in prospective clinical trials due to selection bias. Lastly, achieving a stringent dFLC response resulted in significantly better TNT, and translated into two-thirds achieving cardiac responses. Achievement of a stringent dFLC response may therefore be a new potential therapy goal in AL.

Treatment in AL has evolved, and the Mayo group reported that the glass ceiling of poor outcomes has been overcome.(234) The pro-apoptotic effect of proteosomal inhibition is a desirable mechanism of treatment since AL plasma cells are substantially more sensitive to proteasome inhibition due to added burden of proteotoxicity.(235, 236) While ASCT is associated with excellent haematologic responses and OS(148), only 20% of patients are ASCT-eligible and there are concerns about treatment-related toxicity outside of experienced centres. Bortezomib-based therapy makes up the mainstay of treatment for most newly diagnosed AL patients at our centre.

Table 3.7 summarises outcomes with upfront bortezomib in previous AL studies.

Table 3.7: Summary of studies with upfront bortezomib-based therapy in systemic AL amyloidosis.

Bortezomib-containing regime	Number of newly diagnosed patients treated with bortezomib	Stage III patients	Median follow-up (months)	ITT ORR	ITT CR	Organ response	Median OS	Median PFS or TNT
Current cohort (mainly CyBorD)	915	51.3%	23 months in all patients (32 months in living patients)	65%	15%	ITT: Cardiac 32.5% Renal 15.4% Liver 30%	72 months	Median TNT not reached (55% had not progressed to next treatment at 7 years)
CyBorD (223)	60	100%	11.8	68%	17%	Cardiac 32% Renal 23% Liver 25%	1 year OS 57%	Not documented
CyBorD (156)	69	58%	12.7	71%	40.6%	Evaluable responses: Cardiac 35% Renal 43% Liver 53%	1 year OS 65.2%	Median PFS 28 months
Bortezomib, dexamethasone (237)	49 treated with bortezomib-dexamethasone (26 with twice weekly bortezomib, 23 with once weekly bortezomib)	28% (twice weekly bortezomib), 48% (once weekly bortezomib)	57	77%	39%	Cardiac 45% Renal 53%	1 year OS 67%; 4 year OS 43%	1 year PFS 58%; 4 year PFS 26%
CyBorD(154)	230	49%	25 (living patients)	62%	21%	Cardiac 17% Renal 25% Liver 32%	5 year OS 55%	Median PFS 13 months

Bortezomib, melphalan, dexamethasone(238)	53	15%	25 (living patients)	81%	CR/VGPR 64%	Cardiac 38% Renal 48%	1 year OS 80%	Not documented

We reported a matched comparison of CyBorD compared to cyclophosphamide-thalidomide-dexamethasone with ORR of 71% and CR of 40.6% in the former group.(156) A multicentre study of 60 treatment-naïve Mayo Stage III AL patients treated with CyBorD revealed an ORR of 68%, with a poorer CR rate (17%).(223) A European multicentre retrospective study of 230 AL patients treated upfront with CyBorD demonstrated an ORR of 60% and CR of 21%, with inferior outcomes in Stage III patients.(154) An international randomised phase III study of melphalan-dexamethasone compared to BMDex revealed significantly better ORR in the latter group (81%) than the former (56%).(238) The heterogeneous outcomes in these studies likely reflect their differing sample sizes and proportions of patients with severe cardiac involvement. Haematologic and organ responses in our study were similar to the European collaboration study of CyBorD(154) and the multicentre study of CyBorD in Stage III patients(223), reflecting the large proportions of Stage III patients in these studies.

There is a paucity of data on duration of response with upfront bortezomib in AL. Our European multicentre study of CyBorD reported that 55% of patients were alive at five years, and median PFS was 13 months.(154) Four-year OS in AL reported by the Mayo group was 31% in 2000-2004, 54% in 2005-2009 and 60% in 2010-2014.(234) The current cohort shows that the contemporary OS of unselected AL patients was 72 months, confirming a progressive improvement in OS in AL over the last decade at our centre(4) and others.

PFS and TNT after chemotherapy (particularly bortezomib-based regimes) remain less robustly studied compared to those reported after ASCT. The median PFS in the Mayo study was 6, 11 and 16 months in 2000-2004, 2005-2009 and 2010-2014, respectively(234). The Mayo study reported a median PFS of 53 and 8 months in the ASCT and non-ASCT group treated from 2010-2014, respectively. However, most patients were not treated with upfront bortezomib. Outcomes with ASCT in AL have dramatically improved in recent years, with median OS of 7-10 years;(148, 239) the median PFS has been reported as 2.6-4 years in two large retrospective analyses of patients treated with ASCT.(149, 239) In this study, patients that would be regarded as eligible for upfront ASCT but were treated with upfront bortezomib instead had excellent outcomes – with 78% still alive and 71% having not progressed to further treatment at five years.

The median TNT was not reached and 55% did not progress to next treatment at 7 years. Of note, 98% of patients in a CR did not require further treatment at one year, and 77% and 60% at five and seven years, respectively. Two key messages are apparent. Firstly, bortezomib cannot overcome the high early mortality rates that plague AL. Secondly, patients achieving haematologic responses, especially deep responses, have a prolonged TNT almost rivalling those achieved with upfront ASCT. Most patients in this study were treated with CyBorD. Perhaps, given remarkable early data with BMDex in the recently concluded phase III study(238), the latter may induce more durable responses.

Light chains drive AL and their reduction has been key to improving outcomes. Current ICC derive from the pivotal international series reported in 2012(96). Most patients in the latter series were not treated with an upfront bortezomib-based regime and deep responses were less frequent. Hence, a dFLC of <40mg/L (VGPR) was considered as 'adequate' and the goal of therapy. Low-dFLC criteria (for patients not assessable by standard criteria due to baseline dFLC<50mg/L) have subsequently been identified, whereby a low-dFLC response is defined as dFLC<10mg/L in patients with presenting dFLC 20-50mg/L, predictive of overall and renal survival. The importance of light chain burden has also been demonstrated in a recent study by the Mayo group, whereby an involved free light chain level (iFLC) of <20mg/L translated in better organ responses, PFS and OS. In the latter study, normalisation of the serum free light chain ratio was not associated with better outcomes.(240)

We demonstrate here that low-dFLC response criteria can be extended to all AL patients irrespective of presenting dFLC. Patients achieving a stringent dFLC response have excellent outcomes. Due to already excellent survival in patients with a deep response, at this time, there is no significant apparent difference in OS using dFLC response criteria; although this may become apparent on longer follow up. However, the TNT in the group achieving absolute dFLC<10mg/L is markedly better than those with lesser responses (even if dFLC was below the threshold of a VGPR (<40mg/L)). Even in patients who had achieved a conventional CR by ICC, 84% with stringent dFLC responses did not progress to further treatment at three years, compared to 72% of patients in a CR without stringent dFLC responses.

More importantly, stringent dFLC responses translated into strikingly high cardiac responses, with 61% of patients with stringent dFLC responses achieving cardiac responses, compared to 32% in the entire cohort.

This study's strength is its ability to capture a large 'real-world' AL cohort treated in multiple centres. However, it only includes patients seen at the UK National Amyloidosis Centre. While most UK patients are seen here for assessment, there is a referral bias in that the sickest patients are unable to travel to our centre. Due to its observational nature, exact treatment regimens and doses were at the discretion of the locally treating physician. Whilst there was an attempt to capture toxicity, this was reported at the discretion of the local physicians. Due to lack of source data verification, it is likely that toxicity data has been missed and likely to be under-reported. This remains a significant limitation. The proportion of patients in a CR with and without stringent dFLC responses was small, limiting comparison of outcomes between a stringent dFLC response and CR. Importantly, we have evaluated the importance of a stringent dFLC response in patients treated with only bortezomib-based therapy. The impact of stringent dFLC response remains to be ascertained in patients treated with other therapies.

In conclusion, this study confirms the substantial impact of bortezomib in improving long term outcomes in AL. We show that patients responding to therapy have a long TNT perhaps approaching that seen with ASCT. High early mortality, unfortunately, remains a feature of AL and this glass ceiling has not yet been breached. Finally, this is the first study to apply the new 'low-dFLC' response criteria to an unselected AL population. Achieving

absolute dFLC<10mg (a stringent dFLC response) predicts prolonged overall survival, TNT and led to impressive organ responses. If these findings are validated in an independent international collaborative cohort, a stringent dFLC response may potentially become the new goal of therapy in AL.

Chapter Four: Outcomes in 99 AL amyloidosis patients who do not achieve early haematological responses with upfront CyBorD

Background

High dose melphalan with autologous stem cell transplantation (ASCT) is associated with good haematologic and organ responses in AL but the majority of newly diagnosed patients are ineligible for this treatment due to the extent of their cardiac involvement. In transplant-ineligible AL amyloidosis patients, bortezomib-based combinations have become standard therapy. Early studies of bortezomib-based therapy reported overall response rates of 60-94%, with 23-71% achieving a CR.(154, 224-226) An international multicentre series of 60 newly diagnosed AL amyloidosis patients with advanced cardiac involvement who were treated with CyBorD reported an ORR of 68% and CR rate of 17%.(223) The median time to haematologic response in one study of patients treated with CyBorD was two months.(225) Our group reported a median time to maximal response of 4.1 months with this treatment combination.(226)

A dilemma often faced by clinicians lies in the management of patients who do not have early haematological responses to upfront therapy. At our centre, it is standard practice to review treatment response after three cycles of CyBorD and consider treatment modifications in the absence of a haematological response after three cycles of CyBorD. However, there is a paucity of evidence to guide the best approach in such cases. We report here the outcomes of a cohort of patients at our centre who were treated with CyBorD and had not achieved a haematological response after three cycles.

Methods

In a prospective observational study of newly diagnosed AL (ALchemy) from June 2011-October 2017, 107 patients treated upfront with CyBorD were identified as not having achieved a haematological response after three cycles of therapy. Diagnosis of AL was confirmed on biopsy by immunohistochemistry +/- proteomic analysis. All patients underwent serial assessment including biochemical tests for organ function, serum free light chains, serum and urine protein electrophoresis and immunofixation, cardiac biomarkers and echocardiography. Organ involvement and responses were defined by international consensus criteria.(104, 115) Patients underwent review of haematological response after three cycles of treatment, with consideration of treatment modification in the absence of haematological response. Haematological and organ response assessment were subsequently conducted at the end of treatment. Kaplan-Meier analysis was undertaken to establish overall survival (OS) and time-to-next-treatment (TNT) with comparisons done using the log-rank test. TNT was defined as the time to next treatment from the time of three cycle review (not including any treatment modifications at the three cycle review).

Results

Patients

A total of 107 patients were treated with CyBorD for newly diagnosed AL amyloidosis from June 2011-October 2017 and found not to be in a

haematological response after three cycles. Eight patients opted to have no further treatment and are therefore excluded from this analysis.

The median age was 65 years (range 38-83 years), with male:female ratio of 68%:32%. The number of patients with cardiac, renal, liver, peripheral nerve, autonomic, soft tissue and gastrointestinal involvement was: 69 (70%), 65 (66%), 15 (15%), 4 (4%), 8 (8%), 7 (7%) and 4 (4%). At presentation, the median NT-proBNP and troponin T were 1939ng/L (range 25-30483ng/L) and 39ng/L (3-339ng/L), respectively. The proportion of patients with NYHA class 1, 2, 3 and 4 symptoms was: 34%, 54%, 12% and 0%, respectively. The proportion of patients with ECOG status 0, 1, 2 and 3 was 28%, 45%, 21% and 6%, respectively. The median left ventricular wall thickness was 13mm (range 6-19mm) and the median left ventricular ejection fraction was 59% (range 21-76%). Patients with Mayo Stage I, II and III disease comprised 18%, 37% and 45% of the cohort, respectively. Ten per cent of patients had a presenting NT-proBNP>8500ng/L. The median serum creatinine and 24 hour proteinuria were 91umol/L (range 36-613) and 3.1g/24 hours (range 0.1-18.4g/24 hours), respectively. The median serum bilirubin and ALP were 5 (2-27) and 80 units/L (38-615), respectively. The involved light chain type was kappa in 23% and lambda in the remainder. The median difference in involved and uninvolved light chains at presentation was 182mg/L (range 0-12650mg/L).

The CyBorD treatment protocol and steroid doses were at the discretion of the treating physician. After three cycles of treatment, all patients had not achieved a haematological response (haematological response defined as >50% reduction in presenting dFLC) and underwent interim treatment review +/- modification.

Of the 99 patients that opted to have further treatment, 34 patients continued CyBorD (34.3%), 25 (25.3%) patients changed treatment to cyclophosphamide-bortezomib-thalidomide-dexamethasone (CVTD), 14 (14.1%) patients were treated with bortezomib-thalidomide-dexamethasone (VTD), 13 (13.1%) patients underwent treatment with lenalidomide-dexamethasone, four patients (4%) were treated with cyclophosphamide-thalidomide-dexamethasone. One patient each went on to have treatment with oral melphalan-dexamethasone, high dose melphalan and autologous stem cell transplantation, bendamustine-thalidomide-dexamethasone, melphalan-prednisolone-thalidomide, rituximab-bendamustine, bortezomib-lenalidomide-cyclophosphamide-dexamethasone and daratumumab. The median number of cycles post-interim review was 3 (1-26).

Haematological responses

Haematological responses are found in Table 4.1.

Table 4.1: Haematological and organ responses by treatment.

	CyBorD	VTD	CVTD	Lenalidomide-dexamethasone
Haematological response				
CR	1/34 (2.9%)	0	2/25 (8%)	0/13
VGPR	11/34 (32.4%)	2/14 (14.3%)	6/25 (24%)	3/13 (23.1%)
PR	8/34 (23.5%)	7/14 (50%)	9/25 (36%)	5/13 (38.5%)
NR	14/34 (41.2%, including 2 deaths)	8 (32%, including one death)	8/25 (32%, including one death)	5/13 (38.5%, including one death)
Organ response				
Cardiac	3/20 (15%)	3/13 (23%)	1/15 (7%)	1/10 (10%)
Renal	2/25 (8%)	1/9 (11%)	2/17 (12%)	1/7 (14%)
Liver	1/5 (20%)	1/2 (50%)	2/3 (66%)	1/2 (50%)

Haematological responses (on an ITT basis) after completion of treatment were as follows: CR 5 (5%), VGPR 27 (27.2%), PR 32 (32.3%), non-response 35 (35.4%, including seven deaths (7.1%)). Patients that continued

CyBorD had the following haematological responses: CR 1 (2.9%), VGPR 11 (32.4%), PR 8 (23.5%), non-response 14 (41.2%) (including two deaths, 5.9%). Patients that switched to CVTD had the following responses: CR 2 (8%), VGPR 6 (24%), PR 9 (36%), non-response 8 (32%) (including one death, 4%). Patients that switched to VTD had the following responses: CR 0, VGPR 2 (14.3%), PR 7 (50%), non-response 8 (32%, including one death (4%). Patients treated with lenalidomide-dexamethasone had the following responses: CR 0, VGPR 3 (23.1%), PR 5 (38.5%), non-response 5 (38.5%, including one death (7.7%)). Of the four patients treated with CTD, haematological responses were: CR 1 (25%), VGPR 0, PR 1 (25%), non-response 2 (50%, including 1 death (25%)).

There was no significant difference in the proportion of haematological responses in the group that continued CyBorD compared to those that were treated with VTD/CVTD.

Organ responses

Of 69 patients with cardiac involvement, nine (13%) achieved a cardiac response, 41 (59%) did not, 17 (25%) died and two (3%) had no follow-up data. Of 65 patients with renal involvement, eight (12%) achieved a renal response; 46 (71%) did not, eight (12%) died and three (5%) had no follow-up data. Five of fifteen patients (33%) with liver involvement achieved a hepatic response; seven (47%) did not, two (13%) died and one (7%) had no follow-up data.

Organ responses by treatment are found in Table 4.1. There was no

significant difference in the proportion of organ responses in the group

treated with ongoing CyBorD and those treated with VTD/CVTD.

Overall survival (OS)

The median OS from the time of starting treatment was 34 months (Figure

4.1).

Figure 4.1: The median OS from the time of starting treatment was 34 months.
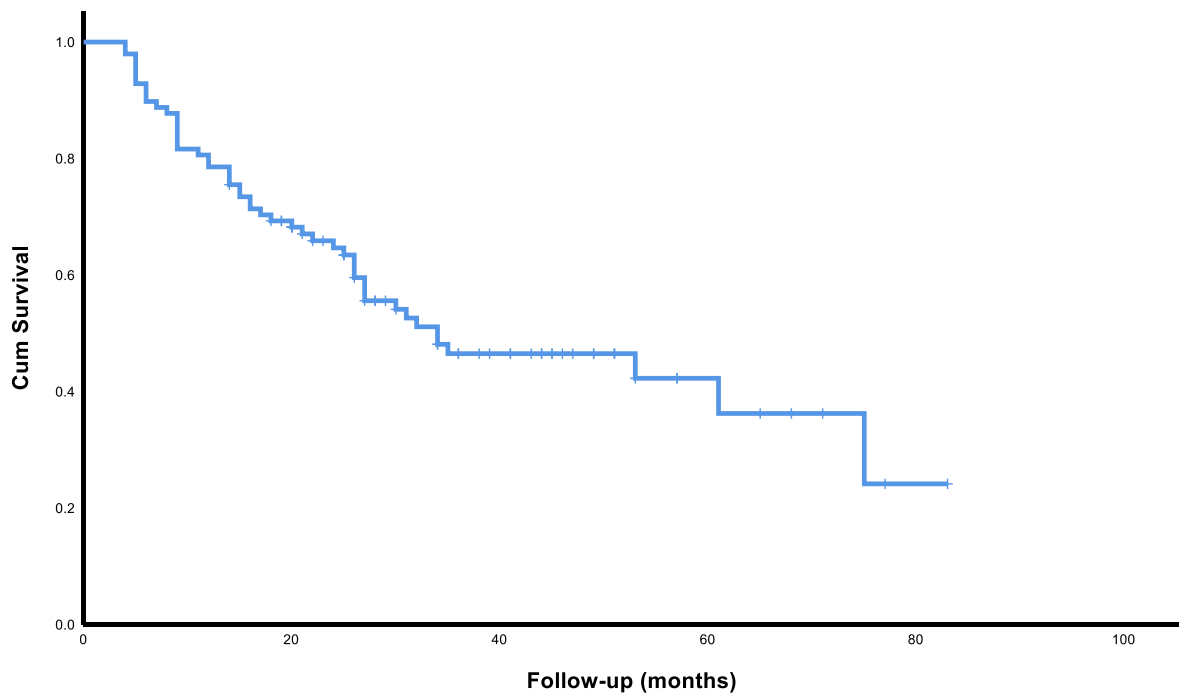
The estimated 3 year OS for patients in a CR, VGPR, PR and NR was: 100%, 78%, 54% and 36%, respectively (p=0.0001). The median OS from start of treatment for patients in a CR/VGPR was 61 months, compared to 26 months in those patients who were not (p=0.03). The median OS in patients treated with CyBord, CVTD, VTD and lenalidomide was: 61 months, not reached, 31 months and 16 months (p=0.54).

The median OS from time of three cycle review was 30 months. The 3 year OS from this timepoint for patients in a CR, VGPR, PR and non-response was 100%, 80%, 53% and 35%, respectively (p=0.002). The median OS for patients in a CR/VGPR was 57 months from the time of three cycle review, compared to 23 months in those who were not (p=0.027). The median OS from the time of three cycle review for patients treated with CyBorD, CVTD, VTD and lenalidomide was 57 months, not reached, 28 months and 13 months, respectively (p=0.538).

Time-to-next-treatment (TNT)

Fifty three patients (53.5%) progressed to another line of treatment (modifications made after three cycle review were not regarded as another line of treatment in this study). Patients that died without progressing to a second line of treatment were excluded from analysis of TNT. The median TNT after three cycle review was 11 months (Figure 4.2).

Figure 4.2: The median TNT from the time of three cycle review was 11 months.
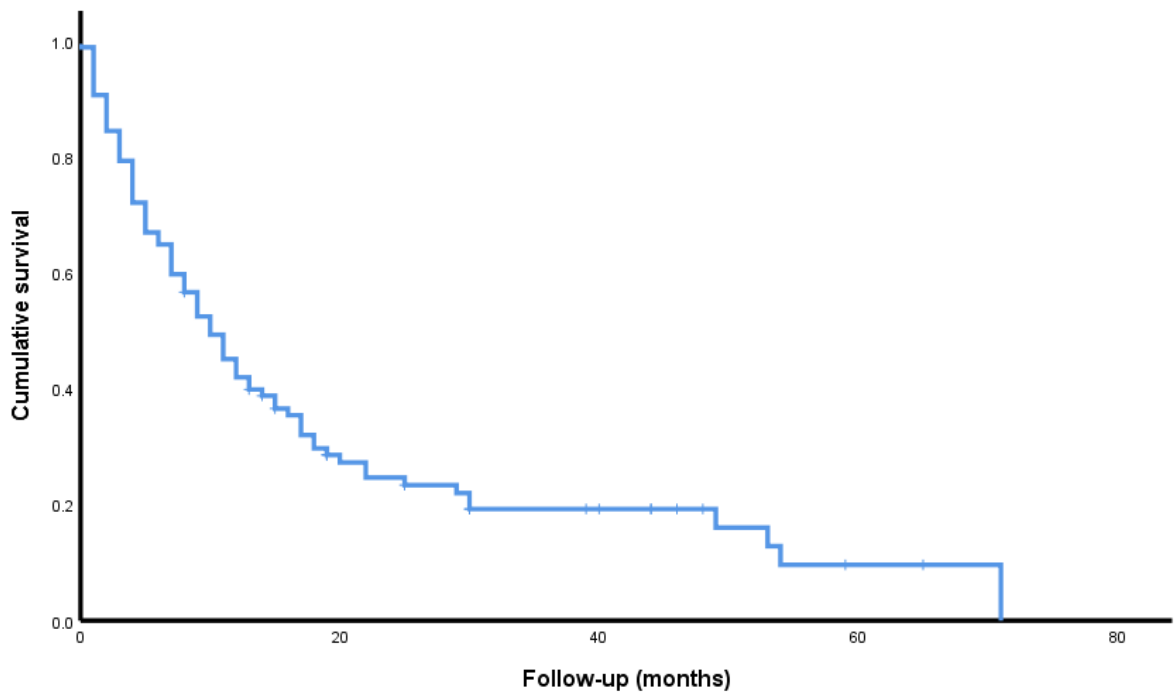
The median TNT for patients in a CR, VGPR, PR and non-response was: 49 months, 25 months, 9 months and 5 months, respectively. The median TNT for patients in a CR/VGPR was 49 months, compared to 7 months who were not (p<0.001). The median TNT in patients treated with CyBorD, CVTD, VTD and lenalidomide was 11, 11, 8 and 15 months (p=0.048).

Discussion

In this study, we report outcomes in AL amyloidosis patients without early haematological responses to upfront CyBorD. Outcomes in AL amyloidosis

have improved over the past decade. High dose melphalan and ASCT yield excellent haematologic responses, organ responses and survival. Two large studies of ASCT in AL amyloidosis reported a median OS of 7-10 years, and CR rates of ~40%. (148, 150) However, only a fifth of all AL amyloidosis patients are eligible for ASCT, given the burden of organ (particularly cardiac) involvement and poor performance status in many patients at presentation. In the majority of patients, therefore, upfront bortezomib-based regimes have evolved to become the mainstay of therapy.

Chapter 3 revealed that, in 915 patients treated with upfront bortezomib-based regimes, the median OS was six years. The median OS was not reached in patients who achieved a CR or VGPR, and was 5.9 years in PR patients and 3.25 years in non-responders. The latter study also reported that 55% of patients did not require further treatment at seven years, and the median TNT was not reached in CR/VGPR patients.

In patients who have excellent haematological responses with bortezomib, survival outcomes are excellent. Chapter 5 shows that, even in patients with very advanced cardiac involvement, those that have rapid early haematological responses have better survival than those who do not. A dilemma arises when patients do not have rapid haematological responses. At our centre, we advise interim review of response assessment after three cycles of chemotherapy – but there is no current evidence base to guide whether any treatment modifications should be made, and if so, what these

should be. This is the first study to examine outcomes in patients that do not have early haematological responses to upfront CyBorD, whose treatment was either subsequently continued or modified.

We show that a third of patients who are haematological non-responders after three cycles of CyBorD can still go on to achieve a CR or VGPR. Such patients have good outcomes overall, with a median OS of over five years and a median TNT of around four years. This should instil some confidence that patients who do not achieve early haematological responses can still go on to achieve good haematological responses overall, and benefit from survival outcomes that are better than one would anticipate. However, survival outcomes are poorer than those seen in the study of 6 month haematological responses in all patients treated with CyBorD in Chapter 3, where patients who achieved a CR/VGPR had a median OS and TNT that were not reached. Given that this current cohort captures patients who do not rapidly benefit from light chain reduction, the persistent presence of amyloidogenic circulating light chains leaves them vulnerable to ongoing organ damage (particularly cardiac) that is likely to affect their long term outcomes. It is interesting to note that the baseline median dFLC was not substantially elevated (182mg/L) in this cohort. Unfortunately, as these patients had diagnostic bone marrow analyses performed at multiple centres, their cytogenetic profile is unknown.

Regrettably, only a third of patients in this cohort were able to achieve good haematological responses overall. A third of patients achieved a PR and a third were non-responders. This suggests that ongoing treatment options offered to such patients were not able to drastically upgrade haematological responses in most patients. Two-thirds of the cohort continued bortezomib-based therapy. A third of patients continued CyBorD, a third underwent addition of thalidomide to CyBorD or substitution of cyclophosphamide with thalidomide, 13.1% were switched to lenalidomide-based therapy, and the remaining patients were treated with other regimens. Only a third of patients achieved a CR/VGPR in the CyBorD group and CVTD group. The proportion of patients achieving a CR/VGPR was even smaller in the VTD group, although numbers in this group were small. It is curious, however, that patients in the CyBorD or CVTD group had a higher proportion of patients in a CR/VGPR than those in the VTD group. The median TNT was also shorter in the latter group. It is possible that ongoing cyclophosphamide may play an important role, although clearly overall numbers in this study were small and results should therefore be interpreted with caution.

There was no difference in median OS by treatment choice. As well as the TNT findings above, patients treated with lenalidomide appeared to have a longer TNT. The duration of lenalidomide treatment was longer than all other treatment options in this study, and longer TNT (from time of treatment review/modification to next line of treatment) in this group reflects the drug's maintenance role.

Since CyBorD became our baseline therapy for non-transplant patients in 2013, our practice has been to add in thalidomide, or use this as a substitute for cyclophosphamide. This study, however, shows no significant difference in haematological responses, OS and PFS between those who receive ongoing treatment with CyBorD, compared to those who receive ongoing treatment with VCTD or VTD. This is important as thalidomide is associated with significant toxicity in AL patients, including fluid overload, exacerbation of congestive cardiac failure, fatigue and cardiac arrhythmias.(166, 167) It is therefore less favoured by many clinicians.

Organ responses in this study were disappointing, with only 13% achieving cardiac responses and 12% achieving renal responses. These findings reflect the importance of rapid suppression of amyloidogenic monoclonal light chains with chemotherapy, in order to maximise the likelihood of achieving an organ response.

While it is encouraging that some patients who do not achieve early haematological responses can still achieve good haematological responses and survival outcomes overall, our findings suggest that continuation of bortezomib-based therapy in these forms has only a modest effect on haematological responses, organ responses and survival. A switch to another agent should therefore be considered. The addition of thalidomide did not result in better haematological responses, OS or PFS compared to

continuing CyBorD. Other treatment modifications were heterogeneous and overall patient numbers too small to arrive at any meaningful conclusions as to what optimal next-line therapy should be in such patients. This is a significant limitation of this study.

Recent data on the use of daratumumab in relapsed-refractory AL amyloidosis is encouraging. A Phase II study by Boston University investigators has reported a CR/VGPR rate of 86%, with 67% achieving of patients achieving renal responses and 50% achieving a cardiac response.(189) A Phase II study by French investigators of daratumumab monotherapy in relapsed-refractory AL resulted in more modest haematological and organ responses, with 47.5% achieving a VGPR/better.(190) The proportion of patients achieving organ responses in the latter study was also lower: 31% achieved a renal response and 30% achieved a cardiac response. However, the duration of daratumumab treatment was only 6 months in the latter study, compared to 24 months in the former study. A large real-world German series of daratumumab in 168 relapsed-refractory AL patients has reported good haematological responses with daratumumab-dexamethasone or daratumumab in combination with CyBorD.(191) The 3 month VGPR/better rate in these groups was 48% and 55%, respectively. Reassuringly, there was no renal or cardiac toxicity in these three studies – and haematological responses were rapid. Daratumumab as monotherapy, or in combination with CyBorD, is therefore a potentially effective treatment option in such patients.

In conclusion, AL patients who do not achieve early haematological responses to upfront CyBorD can still go on to achieve excellent overall haematological responses with good survival outcomes. Patients should therefore be considered for ongoing therapy despite initially disappointing haematological responses, if their clinical condition and wishes are amenable to ongoing therapy. However, based on the findings of this study alone, it is not possible to make specific treatment recommendations for such patients. International Phase III studies in refractory AL are crucial to robustly guide the approach to management in this complex patient group.

Chapter Five: Outcomes in patients with very advanced (Stage IIIb) cardiac AL amyloidosis

This chapter is written in the context of my publication:

Rapid Haematologic Responses Improve Outcomes in Patients With Very Advanced (Stage IIIb) Cardiac Immunoglobulin Light Chain Amyloidosis. Richa Manwani, Darren Foard, Shameem Mahmood, Sajitha Sachchithanantham, Thirusha Lane, Cristina Quarta, Taryn Youngstein, Tamer Rezk, Helen J Lachmann, Julian D Gillmore, Marianna Fontana, Carol Whelan, Philip N Hawkins, Ashutosh Wechalekar.

Haematologica, 103 (4), e165-e168 April 2018.

Background

Outcomes in AL amyloidosis are heterogeneous, but cardiac involvement is a key survival predictor. Cardiac troponin-T and NT-proBNP are sensitive, specific markers of myocyte damage and critically determine prognosis in AL. They form the basis of the widely used Mayo Clinic 2004 cardiac AL staging system.(80)

The initial Mayo study reported median OS in Stage I (NT-proBNP<332ng/L and Troponin-T<0.035µg/L), II (NT-proBNP>332ng/L or Troponin-T>0.035 µg/L) and III (NT-proBNP>332ng/L and Troponin-T>0.035µg/L) AL as 26.4, 10.5 and 3.5 months, respectively.(80) This staging system has been refined, incorporating difference in involved and uninvolved serum free light chains (dFLC).(108) A European collaboration reported median OS of 7.1 months in Stage III and defined an ultra-high risk subgroup with Stage IIIb involvement (NT-proBNP>8500 ng/L and Troponin-T>0.035µg/L), associated with the poorest survival (4 months).(107) The initial Mayo study was a retrospective analysis of 242 patients with newly diagnosed AL between 1979-2000; the European study captured patients from 2004-2013. Modest improvement in outcomes in the latter study may be due to novel agent availability, disease awareness and improved supportive care. There is a paucity of outcome data in the ultra-high risk Stage IIIb subgroup - such patients are generally excluded from clinical trials. We therefore report outcomes of 179 patients with Stage IIIb cardiac AL, showing that treatment responses can impact survival even in this poor risk cohort.

Methods

All patients from ALchemy (a prospective observational study of all newly diagnosed AL patients at the UK National Amyloidosis Centre) with Mayo Stage IIIb (Troponin-T>0.035µg/L and NT-proBNP>8500ng/L) cardiac AL from 2009-2015 were included (n=179). Patients were treated according to nationally agreed protocols in the British Society of Haematology guidelines(242) (current protocols available at http://www.ucl.ac.uk/amyloidosis/nac/chemotherapy_protocols). Organ involvement, hematologic and amyloidotic organ responses were assessed according to amyloidosis consensus criteria.(115) Primary outcome measures were OS and impact of hematologic response on survival.

Results

179 patients were included. Table 5.1 shows baseline characteristics.

Table 5.1: Baseline characteristics.

n=179	Median (range)	Frequency (%)
General		
Median age (years)	66.3 (41.4 –89.4)	
Male		102 (57%)
Female		77 (43%)
ECOG performance status		
0		29 (16%)
1		118 (66%)
2		32 (18%)
3		0

4		
Median 6-minute walk test (n=68, metres)	184 (46-651)	
Involved light chain type Kappa Lambda		38 (21%) 141 (79%)
Median dFLC (mg/L)	396 (0.7 - 12788)	
dFLC>400mg/L		87 (49%)
Median serum monoclonal paraprotein (g/L)	5 (0 – 54)	
Serum paraprotein > 5g/L		60 (34%)
Organ involvement		
Cardiac involvement		179 (100%)
Renal involvement		132 (73%)
Liver involvement		29 (16%)
Peripheral nerve involvement		12 (7%)
Autonomic involvement		15 (8%)
Soft tissue involvement		31 (17%)
Gastrointestinal tract involvement		10 (6%)
Median serum creatinine (umol/L)	126 (49-684)	
Median 24 hour urinary protein (g/24 hours)	1.96 (0.1 - 56.8)	
Median serum albumin (g/L)	35 (14-49)	
Median bilirubin (umol/L)	10 (2-70)	
Median ALP (ULN 129 units/L)	104 (35-1602)	
Cardiac Parameters		
NYHA class 1-2 3-4 Not recorded		87 (49%) 67 (38%) 25 (13%)
Median systolic BP (mmHg)	107 (79-171)	
Systolic BP ≤110 mm Hg		97 (54%)
Median NT-proBNP (ng/L)	14762 (8500-147940)	
NT-proBNP > 8500ng/L		179 (100%)
Median cardiac troponin T (ng/L)	156 (39 – 874)	
Median left ventricular ejection fraction (%)	49 (23-75)	
Median left ventricular wall thickness (mm)	15 (10-21)	

Left ventricular ejection fraction <55%		128 (72%)

Median age was 66.3 years (41.4-89.4 years). 44% had NYHA class 3-4 symptoms and 18% had ECOG score≥3. Median NT-proBNP was 14762ng/L (8500-147940ng/L). Median LV wall thickness was 15mm (10-21mm); median LV ejection fraction (LVEF) was 49% (23-75%). 132 (73%) had renal involvement and 29 (16%) had liver involvement. Thirty (17%) patients died prior to treatment. These patients were very unwell and opted for supportive care only. First-line treatment included: cyclophosphamide, thalidomide and dexamethasone (CTD) 27%; cyclophosphamide, bortezomib and dexamethasone (CyBorD) 39%; bortezomib and dexamethasone 7%; melphalan and dexamethasone 2%; lenalidomide and dexamethasone 1%; other 7%.

On an intention-to-treat (ITT) basis (including all patients), 6 month hematologic responses were: complete response (CR) 35 (20%), very good partial response (VGPR) 25 (14%), partial response (PR) 32 (18%) and non-response 87 (48%) (including deaths prior to/after treatment initiation). Thirty seven patients (21%) achieved CR/VGPR at Day 30. On an ITT basis, median OS was 6 months (Figure 5.1).

Figure 5.1: Median OS of this cohort.
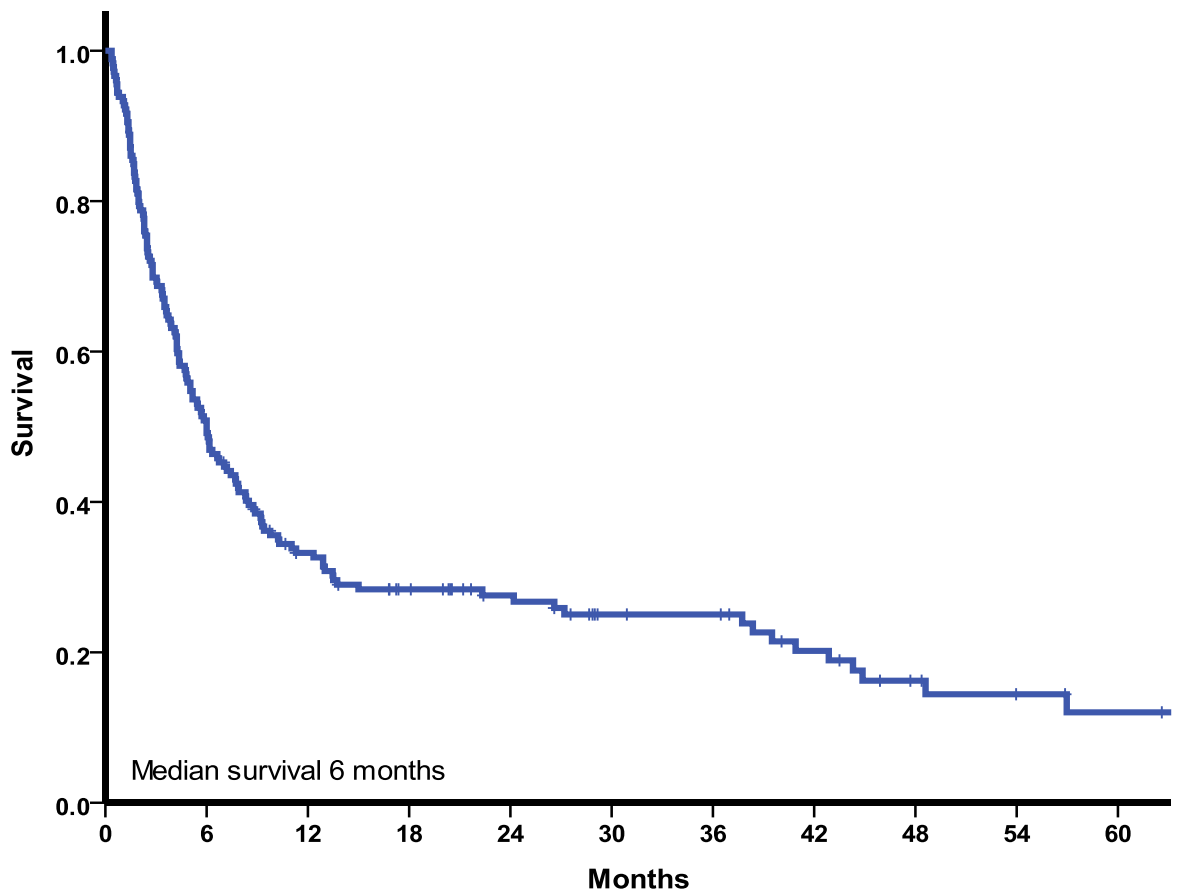
Patients in a CR/VGPR by Day 30 of treatment had median OS of 26 months, compared to 5 months in non-CR/VGPR (Figure 5.2).

Figure 5.2: Median OS in patients depending on haematological response at Day 30.
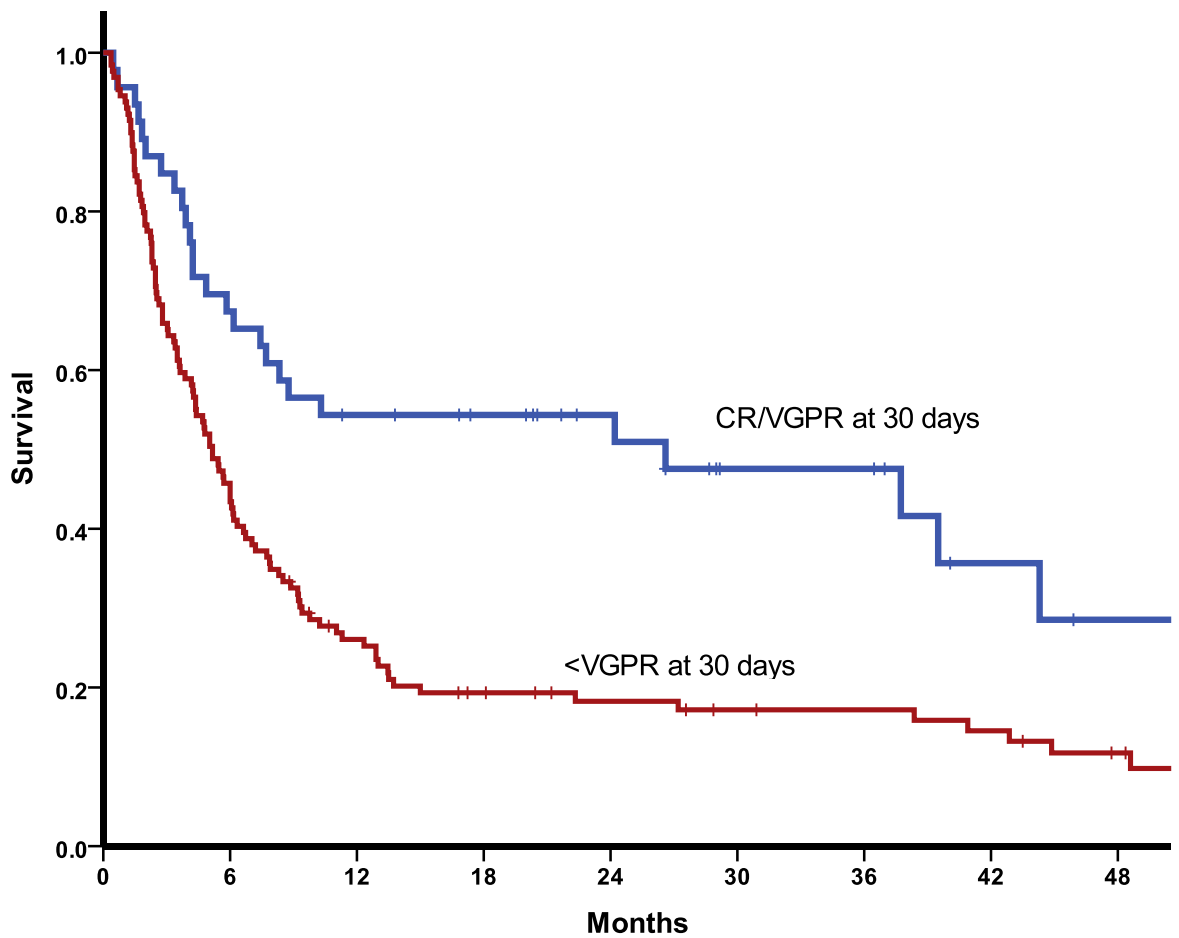
Median OS in patients achieving overall CR/VGPR, PR and non-response at 6 months was 38 months, 7 months and 2.6 months respectively (log rank p<0.0001) (Figure 5.3).

Figure 5.3: Median OS by 6 month haematological response.
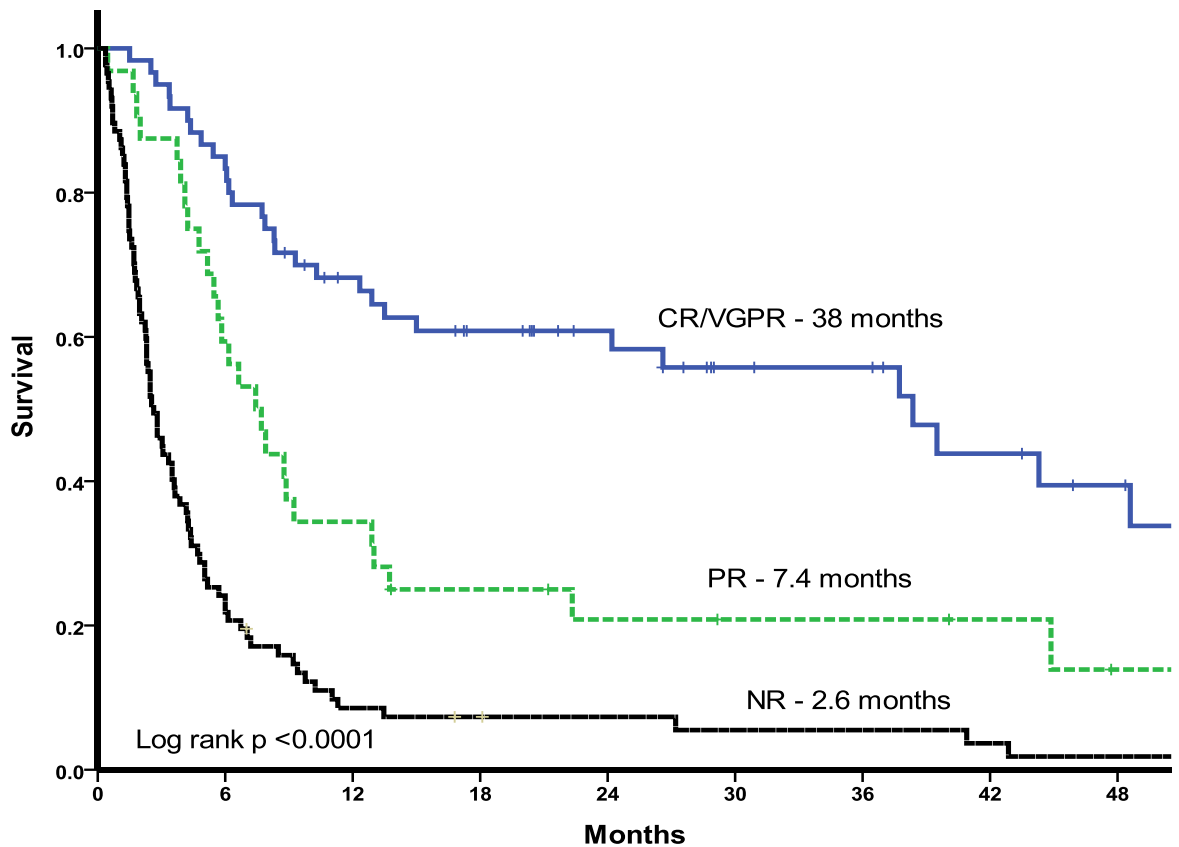
A landmark analysis showed that of 76 patients still alive at 6 months who had achieved a CR, VGPR, PR and non-response at 1 month (after 1 cycle of chemotherapy), the proportion alive at 12 months was 86%, 74%, 74% and 33%, respectively. Table 5.2 shows hematologic responses by treatment.

Table 5.2: Hematologic responses by treatment. (CTD: cyclophosphamide, thalidomide and dexamethasone; CyBorD: cyclophosphamide, bortezomib and dexamethasone).

	N	Hematologic response at 30 days (ITT)					Hematologic response at 6 months (ITT)				
		CR	VGPR	PR	Non-response, including deaths	Deaths	CR	VGPR	PR	Non-response, including deaths	Deaths
CTD	48	4 (8%)	3 (6%)	21 (44%)	12 (25%)	6 (13%)	11 (23%)	5 (10%)	13 (27%)	19 (40%)	18 (38%)
CyBorD	70	12 (17%)	13 (19%)	23 (33%)	15 (21%)	9 (13%)	20 (29%)	16 (23%)	12 (17%)	22 (31%)	20 (29%)
Bortezomib, dexamethasone	13	1 (8%)	1 (8%)	5 (38%)	5 (38%)	4 (31%)	3 (23%)	2 (15%)	3 (23%)	5 (39%)	4 (31%)
Melphalan, dexamethasone	4	1 (25%)	0	0	2 (50%)	0	1 (25%)	1 (25%)	1 (25%)	1 (25%)	1 (25%)
Lenalidomide, dexamethasone	3	0	2 (67%)	0	1 (33%)	0	0	1 (33%)	0	2 (67%)	2 (67%)
Other	11	0	0	3 (27%)	8 (73%)	3 (27%)	0	0	3 (27%)	8 (73%)	7 (64%)

Of patients treated with CTD or CyBorD, 14% and 36% achieved a

CR/VGPR at 1 month (p<0.01), respectively. The proportion of patients

treated with CTD or CyBorD that achieved a 6 month CR/VGPR was 33%

and 52% (p=0.04), respectively. There was a suggestion of better OS with

CyBorD compared to CTD but the difference was not statistically significant

(possibly due to small patient numbers and proportion of CTD patients also

achieving a VGPR/better within one cycle) (Figure 5.4).

Figure 5.4: Median OS by treatment type.
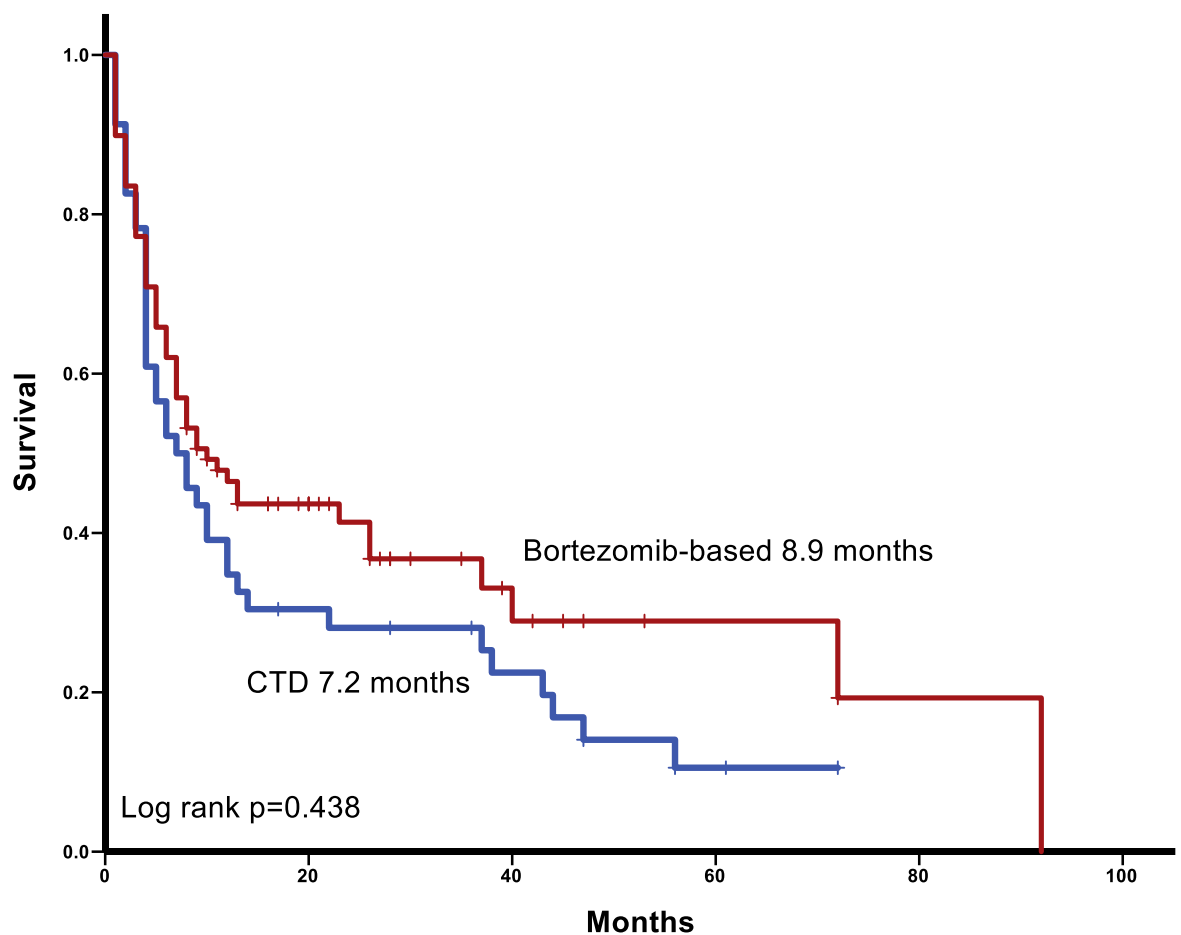
Univariate and ROC analysis revealed that LVEF<55%, dFLC>400 mg/L and

systolic blood pressure (SBP)<110 mmHg were predictors of poor survival.

Median OS for patients with values above/below the threshold was: dFLC <

vs. > 400 mg/L – 7 months vs. 3 months; LVEF > vs. < 55% - 10 months vs. 5 months; SBP > vs. < 110 mg Hg – 10 months vs. 5 months. In a multivariate model, not achieving a CR/VGPR at 6 months (HR 5.3, p<0.001; 95%CI 3.8-8.2), LVEF<55% (HR1.5 p=0.044; 95%CI 1.05-2.1), dFLC>400 mg/L (HR 1.3 p=0.076; 95%CI 0.9-1.8) and SBP<110mmHg (HR 1.55 p=0.023; 95% CI 1.05-2.1) were independent predictors of mortality.

Discussion

This study, focusing exclusively on Stage IIIb AL, highlights the complex heterogeneity of this disease. There is likely referral bias in the cohort: very unwell patients may be unable to travel to our centre. That withstanding, median OS is 6 months, slightly better than survival previously described in advanced cardiac involvement. Stage IIIb AL presents a challenging dichotomy. Half of the patients lived long enough to complete treatment and be assessed for response. Strikingly, those achieving a rapid response at Day 30 or overall CR/VGPR at 6 months had markedly better survival than ever reported in this patient cohort. However, the other half of patients died, unable to benefit from treatment - perhaps their disease was too advanced to enable hematologic response to improve survival. The European collaboration identified Stage IIIb as a separate cohort(107) but patients are heterogeneous. Hypotension, poor systolic function and high presenting light chains (previously reported as poor prognostic factors in AL) were further determinants of survival. The European study reported lower hematologic responses (32% achieved ≥PR (ITT))(107), probably because it included patients from 2002-2010 with a smaller proportion treated with novel agent

based combination therapy compared to the current cohort (7% vs. 48% treated with a bortezomib-based regime, respectively). Hematologic responses to CyBorD in this cohort are similar to those in a previous multicentre study of Stage III patients treated with CyBorD.(223)

These data generate important hypotheses requiring study in prospective trials. The encouraging survival of patients who respond to therapy should engender confidence in designing trials for this patient cohort, thus far excluded from all prospective AL trials.(115, 243) The marked improvement in outcomes for early responders suggests that perhaps in some patients, light chain toxicity is a critical factor that is potentially reversible with chemotherapy-induced hematologic response. Others may have a combination of light chain toxicity and true amyloid deposition that may not be rapidly amenable to chemotherapy by the same extent. Specialist cardiac magnetic resonance imaging with T2 sequences showing oedema may help delineate these findings and is part of an ongoing study at our centre.

Although rapid haematological response in one month improves survival, the challenge is to guide these fragile patients through chemotherapy, where toxicity has a high chance of leading to cardiac mortality. Patients not achieving a reduction in dFLC by the end of cycle 1 require review of their chemotherapy regime, with addition of other agents if feasible. In both ITT and landmark analyses, we show that early response appears to translate into survival benefit at 12 months. However, haematological response does

not immediately impact upon amyloidotic visceral dysfunction: patients can succumb to effects of end-organ damage despite excellent haematological responses. This may partly explain the lack of difference in outcomes with CyBorD vs CTD, despite better haematological responses in the CyBorD group. The small numbers limit utility of subset analysis but larger studies across international centres are planned to validate these results. Given the better early responses, bortezomib-based regimes would still be the recommended first-line for these patients.

Treatment regimens offering rapid haematological responses with minimal toxicity are the holy grail of this disease. Use of genetic markers to identify markers of clonal sensitivity and targeted therapies (such as venetoclax in patients with t(11;14)) require further study. Anti-plasma cell monoclonal antibodies such as daratumumab have demonstrated rapid responses with good tolerance, suggesting a role for this early in the disease course but further studies are needed to evaluate its use in patients with advanced cardiac involvement.(244) Small molecules such as doxycycline or p38 MAP kinase inhibitors may help reduce light chain cardiotoxicity. Immunotherapy agents such as NEOD001, a monoclonal antibody binding to an epitope unique to misfolded light chains, may enable acceleration of cardiac amyloid fibril clearance and phase I data suggests the possibility of rapid cardiac responses.(245) Such agents may have a crucial role in early treatment with a dual mechanism.

In conclusion, treatment of advanced cardiac AL remains a major unmet medical need. Whilst confirming the fragility and mortality of this population, these data shine a ray of hope that rapid responses with novel agent based treatment can change outcomes in even this very advanced patient group. Larger international collaborative studies and novel imaging may help to tease out factors impacting survival. Crucially, this study shows that this patient population must be included in future prospective clinical trials of anti-amyloid and novel anti-plasma cell therapy.

Chapter Six: Deferred autologous stem cell transplantation in systemic AL amyloidosis

This chapter is written in the context of my publication:

Introduction

Cardiac involvement in AL amyloidosis is a critical determinant of survival. High-dose melphalan with autologous stem cell transplantation (ASCT) in AL can result in high haematological response rates, with over a third of patients achieving complete haematological response (148, 246), translating into promising organ responses.(247) Response to ASCT appears to be durable with a median overall survival (OS) of 7.6 years reported by the Boston group.(148) Bone marrow plasma cell infiltration greater than 10% prior to ASCT is a poor prognostic factor.(98) Transplant-related mortality (TRM) has historically been high (early reports of up to 40%) in unselected patients.(248) The recognition that advanced cardiac involvement is associated with higher TRM has led to refined patient selection strategies with a reduction in TRM to ~5%(246) and most recently 2.4% reported by the Mayo group.(150) However, stringent selection criteria, which vary in each country, render the majority of patients with cardiac amyloidosis transplant-ineligible.

Modern chemotherapy agents can induce haematological and organ responses in AL, including those with cardiac involvement, but durability of response remains uncertain.(223, 224, 226) No study has demonstrated the prolonged progression-free survival (PFS) patients treated with non-transplant regimes akin to that achieved with ASCT. It is now apparent that a proportion of patients with significant cardiac involvement will substantially improve after achieving a good response to chemotherapy. While studies

have examined the role of bortezomib-based induction chemotherapy immediately prior to ASCT, no studies to date have specifically focused on the role of deferred ASCT in transplant-ineligible patients. (151, 225, 249, 250)

We therefore report a retrospective cohort of AL patients (from two large European amyloidosis centres) with advanced cardiac involvement who were considered transplant-ineligible at presentation, but achieved haematologicalal and organ responses with chemotherapy, allowing them to undergo ASCT later.

Methods

All patients with systemic AL amyloidosis ineligible for ASCT at presentation who had initial induction chemotherapy and then underwent deferred ASCT from September 2011 to July 2017 were included in this study. Reasons for transplant-ineligibility are listed in Table 6.1 and differed slightly between centres.

Table 6.1: Reasons for transplant-ineligibility at presentation.

Heidelberg exclusion criteria for ASCT	At presentation (n=9)	At ASCT (n=9)
Severe cardiac failure	6	0
ECOG PS≥2	3	0
Systolic BP≤90mmHg	2	0
Gastrointestinal bleeding	1	0
UK NAC exclusion criteria for ASCT	**At presentation (n=13)**	**At ASCT (n=13)**
NT-proBNP>1000ng/L	13	1 (patient had an NT-proBNP response after induction chemotherapy)
Systolic BP≤90mmHg	1	0
Serum albumin<20g/L	1	0
eGFR<40ml/min	1	0
Large load on SAP scintigraphy	2	0
ECOG PS>2	1	0

Amyloidosis of AL type was confirmed on immunohistochemistry or by proteomic analysis of biopsy specimens. Exclusion of mutations in genes for hereditary amyloidosis was carried out as appropriate. All patients underwent serial protocolized assessment including biochemical tests for organ function, serum free light chain measurement, serum and urine protein electrophoresis and immunofixation, cardiac biomarker assessment, echocardiography and/or cardiac MRI (unless contraindicated). Organ involvement and haematologicalal response were defined as per international amyloidosis consensus criteria.(96, 104, 115) Progression was defined as haematological progression, time to next treatment or death.

All patients underwent ASCT as per local protocols. Stem cells were mobilized with G-CSF only in patients treated at the UK National Amyloidosis Centre, and with cyclophosphamide priming in the patients at Heidelberg. Transplant conditioning was undertaken with intravenous melphalan, followed by infusion of autologous hematopoietic stem cells. Dose reduction of melphalan was used in patients with severe renal impairment. Standard institutional protocols were followed for post-transplantation supportive care and antimicrobial prophylaxis.

Primary outcome variables were TRM (defined as death within 100 days post-ASCT), haematological and organ response, PFS and OS.(115) Statistical analysis was performed using SPSS version 24. Approval for analysis and publication was obtained from the institutional review boards, and written consent was obtained from all patients in accordance with the Declaration of Helsinki. Survival outcomes were analysed using the Kaplan-Meier method. All p-values were two sided with a significance level of <0.05.

Results

Twenty two patients were included in this study. Nine were from Heidelberg University Amyloidosis Centre and thirteen from the UK National Amyloidosis Centre. Baseline characteristics are presented in Table 6.2.

Table 6.2: Baseline characteristics

	At presentation	At ASCT	p value
Median age (years)	54 (range 39-69)	56 (range 40-70)	**<0.001**
Male:Female	15 (68%):7(32%)		
NYHA class 1 2 3 4 Not recorded	1 (4.5%) 17 (77.3%) 3 (13.7%) 0 1 (4.5%)	13 (59.1%) 9 (40.9%) 0 0 0	**0.0013**
ECOG 0 1 2 3 4	1 (4.5%) 12 (54.6%) 8 (36.4%) 1 (4.5%) 0	16 (72.7%) 6 (27.3%) 0 0 0	**<0.0001**
Cardiac involvement	22 (100%)		
Median NT-proBNP (ng/L)	2924 (range 624-28737)	415 (range 118-2853)	**<0.0001**
Median cardiac troponin T (ng/L)	62.5 (range 9-1885)	13 (range 5-76)	**0.0142**
Mayo stage I II III (Stage III patients with NT-proBNP>8500ng/L)	0 3 (13.6%) 19 (86.4%) (2 (9.1%))		
Median systolic blood pressure (mmHg)	114 (range 80-137)	114 (range 100-142)	0.2107
Median mean left ventricular wall thickness (mm)	15.0 (range 8-19)	14.0 (range 7.5-17.5)	0.0861
Median LV ejection fraction (%)	55 (range 38-67)	57 (range 39-65)	0.285
Renal involvement	8 (36.3%)		
Median creatinine (umol/L)	77 (range 46- 537)	80.5 (range 53-162)	0.697
Median GFR (ml/min)	97 (range 10 - 219)	88 (range 41-124)	0.313
Median proteinuria (g/24h)	0.35 (range 0.1 – 4.7)	0.1 (range 0.1-4.1)	**0.0356**
Liver involvement	4 (18.2%)		

Median ALP (units/L)	75.5 (range 42-340)	70.5 (range 40-177)	0.2987
Soft tissue involvement	9 (40.9%)		
Peripheral nerve involvement	2 (9.1%)		
Autonomic nerve involvement	2 (9.1%)		
GI involvement	5 (22.7%)		
Median number of involved organs	2 (1-5)		
Kappa:Lambda	9 (36%): 13 (64%)		
Median involved FLC (mg/L)	691.5 (range 135-9594)	36.5 (range 0.2-950)	**<0.0001**
Median dFLC (mg/L)	562 (range 118-6830)	25.1 (range 1.6-911)	**0.0002**
Median serum monoclonal protein (g/L)	1 (range 0-16)	0 (range 0-6)	**0.0015**
Detectable serum paraprotein	8 (36%)	2 (9%)	**0.0011**
Serum paraprotein: light chain only /IgG/IgA/IgM/IgD	3 (17%)/ 5 (23%)/1(5%)/0/0		

The median age at presentation was 54 years (range 39-69 years). The number of patients with NYHA class 1, 2, 3 and 4 symptoms at presentation was as follows: 1 (4.5%), 17 (77.3%), 3 (13.7%) and 0, respectively. The number of patients with ECOG score 0, 1, 2, 3 and 4 was: 1 (4.5%), 12 (54.6%), 8 (36.4%), 1 (4.5%) and 0, respectively. The number of patients with Mayo cardiac stage I, II and III patients were as follows: 0, 3 (13.6%) and 19 (86.4%), respectively. Of the Stage III patients, 2 patients had an NT-proBNP>8500ng/L (stage IIIb as per the European variation of the original Mayo staging system (107)). At diagnosis, median NT-proBNP and median Troponin T were 2924ng/L (range 624-28737ng/L) and 62.5ng/L

(range 9-1885ng/L), respectively. Baseline median left ventricular wall thickness was 15mm (range 8-19mm) and median LV ejection fraction was 55% (range 38-67%). The number of patients with renal, liver, soft tissue, peripheral nerve, autonomic nerve and gastrointestinal involvement was as follows: 8 (36.3%), 4 (18.2%), 9 (40.9%), 2 (9.1%), 2 (9.1%) and 5 (22.7%), respectively. The median baseline involved free light chains were 691.5mg/L (range 135-9594 mg/L), with a median difference in involved and uninvolved light chains of 562 mg/L (range 118-6830 mg/L).

All patients were regarded as transplant-ineligible at presentation, most commonly because of advanced, clinically significant cardiac involvement (reasons for transplant ineligibility are found in Table 6.1). All patients were treated with chemotherapy upfront. All patients had been treated with bortezomib-based therapy prior to ASCT. Treatment included bortezomib-dexamethasone in 5 (23%), cyclophosphamide–bortezomib–dexamethasone 13 (59%), bortezomib-lenalidomide-dexamethasone 2 (9%) and cyclophosphamide–thalidomide–dexamethasone 2 (9%), respectively. Seven (32%) patients switched chemotherapy after a median of three initial cycles of treatment (range 1-4) due to suboptimal initial haematological responses. Of these seven patients, three went on to be treated with a bortezomib-alkylator-dexamethasone regime, three went on to be treated with a bortezomib-immunomodulatory-dexamethasone combination and one went on to have immunomodulator therapy. The median number of total frontline cycles was 6 (range 4-9). Haematological responses after chemotherapy on an ITT basis were: complete haematological response

(CR) 11 (50%), very good partial response (VGPR) 8 (36%) and partial response 3 (14%). At six months, 14 patients (64%) achieved a cardiac response, 5/8 (63%) with renal involvement achieved a renal response and 3/4 (75%) with liver involvement achieved a liver response.(104)

The median time from presentation to ASCT was 14.5 months (range 6-45 months). The indication for ASCT was haematological progression in 10 (45%) patients and as a consolidation procedure in the remainder. All patients had resolution of ASCT exclusion criteria, enabling them to undergo ASCT (Table 6.1). There were no serious adverse events or deaths during stem cell mobilisation. At the time of ASCT, median dFLC was 25.1mg/L (range 1.6-911mg/L). At the time of ASCT, all patients had NYHA Class 1-2 symptoms compared to 81.8% at presentation (p=0.0013) and ECOG 0-1 prior to ASCT, compared to 59% of patients with ECOG 0-1 at presentation (p<0.0001). The median NT-proBNP prior to ASCT was 415 ng/L (range 118-2.853 ng/L), having been 2924 ng/L (624-28737 ng/L) at presentation (p<0.0001) and 1497 ng/L (237-5167 ng/L) 6 months after diagnosis (Figure 6.1).

Figure 6.1: Serial median NT-proBNP measurements at baseline, 6 months post diagnosis, prior to ASCT and 6 months post-ASCT (n=22). Median NT-proBNP at baseline, 6 months post diagnosis, prior to ASCT and 6 months post-ASCT were: 2924ng/L (624-28737ng/L), 1497ng/L (range

415ng/L (range 118-2853ng/L), 415ng/L (range 118-2853ng/L) and 554ng/L (range 186-2589ng/L), respectively.
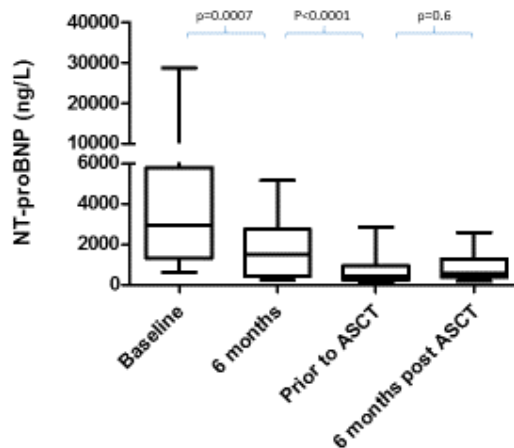
The mean left ventricular wall thickness was 15mm (range 8-19mm), compared to 14mm (range 7.5-17.5mm) at the time of ASCT (p=0.086). The median left ventricular ejection fraction at presentation was 55% (range 38-67%), compared to 57% (range 39-65%) at time of ASCT (p=0.285). Median cardiac troponin T was 13ng/L (range 5-76 ng/L), having been 62.5 ng/L (range 9-1885 ng/L) at baseline (p=0.0142). Median systolic blood pressure was 114 mmHg (range 80-137) at baseline and 114mmHg (range 100-142) at the time of ASCT (p=0.21). Median proteinuria had improved from 0.35g/24h (range 0.1-4.7) to 0.1g/24h (range 0.1-4.1) at ASCT (p=0.036).

Median follow up was 20.5 months (range 6 - 74 months). At three months post-ASCT, on an ITT basis, the number of patients in a CR, VGPR, PR and no-response were: 12 (54.5%), 7 (31.8%), 2(9.1%) and 1 (4.5%). The patient who had not achieved a haematologic response to ASCT had undergone ASCT for hematological progression. The number of patients in a CR, VGPR, PR and non-response at six months post ASCT on an ITT basis was: 11 (50%), 6 (27.3%), 4 (18.2%) and 1 (4.5%, died at six months) respectively. Cardiac responses were assessed in 21 patients (the remaining patient died at six months). Of these 21 patients, 18 (85.7%) had achieved a cardiac organ response compared to presentation. Eleven patients had an NT-proBNP less than 650ng/L at the time of ASCT. Of the remaining ten patients, four patients had a cardiac response compared to NT-proBNP at the time of ASCT, four had no cardiac response compared to NT-proBNP at the time of ASCT and two had evidence of cardiac progression compared to NT-proBNP at the time of ASCT. Median NT-proBNP at six months post-ASCT was 554ng/L (range 186-2589ng/L) (Figure 6.1). The median dFLC at the time of ASCT in the group that underwent ASCT for consolidation was 13 mg/L (range 1.6-36 mg/L). In the group that underwent ASCT for relapse, the median dFLC was 61 mg/L (range 24.3-911mg/L), (p=0.0002).

There was no TRM in the cohort. The median OS of the cohort from time of ASCT has not yet been reached (Figure 6.2).

Figure 6.2: Overall survival (OS) and progression-free survival (PFS) from time of ASCT. Median overall survival from ASCT was not reached in the cohort. Median progression free survival of the cohort from time of ASCT was 54 months.
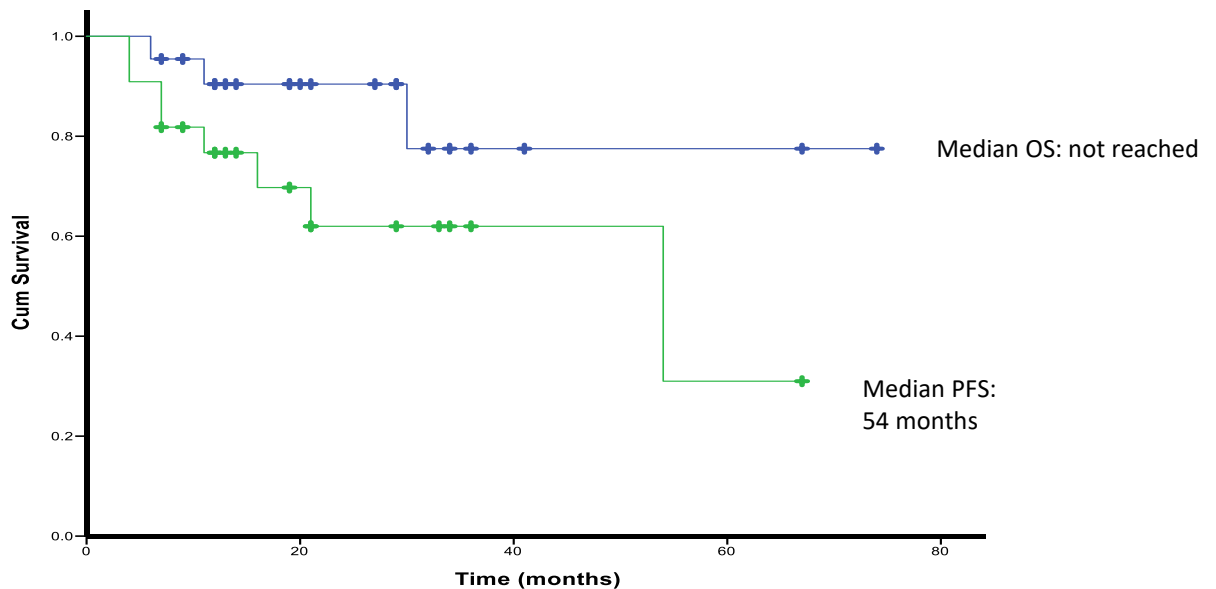
Three patients (13.6%) died (at 6, 11 and 30 months after ASCT). The cause of death in these patients was: congestive cardiac failure in 2 patients and chemotherapy-related sepsis (in a patient who developed haematological progression 4 months after ASCT). Eight patients (36%) progressed after ASCT (defined as hematological progression, progression to next treatment or death). The median PFS in the cohort was 54 months

(Figure 6.2). The median OS and median PFS were not reached in the group that underwent ASCT for consolidation (Figure 6.3).

Figure 6.3: **Overall survival (OS) and progression-free survival (PFS) in patients undergoing deferred ASCT for relapse or consolidation.** A) Median OS in the patients who underwent deferred ASCT for consolidation was not reached; patients who underwent deferred ASCT for relapse had a median OS of 30 months. B) Median PFS in patients who underwent for ASCT for consolidation was not reached; patients who underwent deferred ASCT for relapse had a median PFS of 11 months.
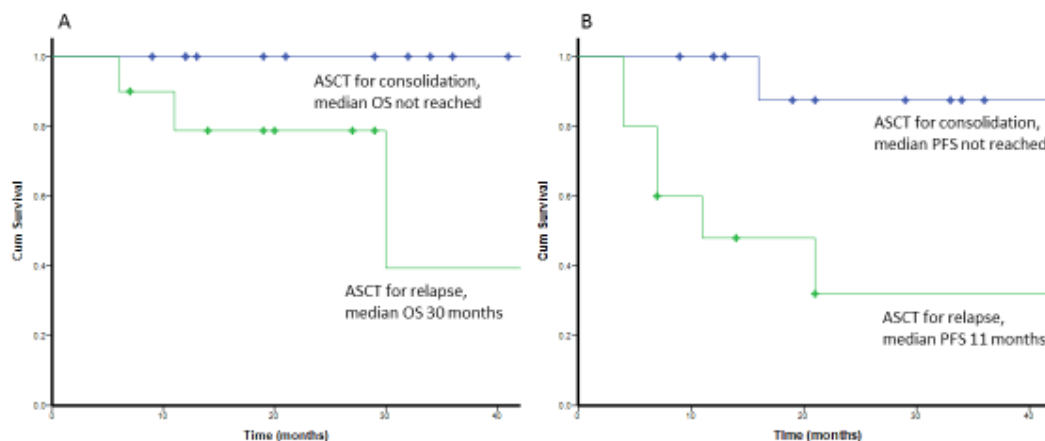
The median OS and median PFS were 30 months and 11 months, respectively, in the group that underwent ASCT for haematological progression.

Discussion

ASCT can induce good haematologic and organ responses in systemic AL amyloidosis, as well as prolonged overall survival(148, 150). Historically, ASCT in AL has been fraught with a substantial risk of transplant-related mortality (248) often due to cardiac involvement, which is a key factor in patient selection.(246) However, given that most patients with AL have some degree of cardiac involvement, upfront ASCT is not a realistic treatment option for the majority of patients. We show in this cohort that patients with advanced cardiac involvement at presentation who were considered transplant-ineligible can be treated with chemotherapy, achieving haematological responses that enable improvements in organ function, allowing them to be safely transplanted at a later date.

ASCT remains an important treatment modality in AL since none of the standard chemotherapy regimens using novel agents have shown the prolonged PFS reported with ASCT. In patients with multiple myeloma, it is clear that ASCT still has a role in patients treated with novel agent-based combination regimes and increases the rate of MRD negativity, associated with longer PFS.(251) Since rigorous patient selection is key to achieving reduced transplant-related morbidity and mortality in AL, such selection strategies have become routine in assessment for ASCT. There remains no international consensus on specific exclusion criteria, but all centres concur that "significant" cardiac involvement is a contraindication to transplantation. In our centres, only 10-15% of all patients assessed are considered suitable for transplantation. Importantly in this disease, mortality is concentrated in

the early months after diagnosis when there is ongoing organ damage due to amyloidogenic light chain toxicity, along with toxicity from treatment regimens and ASCT. Mortality in this disease falls rapidly after the first 6-12 months, hence a deferred transplantation approach is attractive.

Previous studies have examined the role of ASCT later in the disease course. In the seminal study of seventeen patients treated with bortezomib, cyclophosphamide and dexamethasone, Mikhael et al demonstrated that this treatment regimen rendered three transplant-ineligible patients to become eligible for ASCT. (225) Cornell et al went on to explore the role of bortezomib-based induction in 28 transplant-ineligible patients with AL. (249) A third of patients in the latter study achieved haematological and organ responses with bortezomib-based therapy, subsequently enabling them to undergo ASCT. However, only four patients had advanced cardiac involvement that improved enough to enable transplant eligibility. A single centre retrospective cohort study by Hong et al demonstrated favourable outcomes in 20 patients treated with bortezomib-based therapy prior to ASCT, but only 10% had advanced cardiac involvement. (250) Similarly, Scott et al studied outcomes in 31 patients with AL who underwent ASCT, of which 12 received bortezomib-based induction therapy. (151) The latter authors demonstrated superior overall haematological and organ response rates in the bortezomib pre-treated group. Six patients were initially deemed transplant-ineligible but became eligible for ASCT after bortezomib-based therapy but only one patient had advanced cardiac disease at presentation. In a recent report of the Mayo group's 20 year experience of ASCT in AL,

38% of patients underwent bortezomib-based treatment before ASCT in 2010-2016, compared to only 3% from 2003 -2009. However, only a minority underwent ASCT more than 12 months after diagnosis and survival outcomes in Mayo stage (2012) III/IV cardiac involvement were poorer. It is unclear what proportion of patients that had deferred transplants had advanced stage disease or achieved organ responses with induction chemotherapy prior to deferred ASCT. (150)

Unlike previous retrospective cohorts, patients in this cohort had Mayo Stage II/Stage III cardiac involvement at presentation (the majority with Stage III involvement). After induction chemotherapy, 86% achieved a CR/VGPR and 64% achieved a cardiac response by international amyloidosis consensus criteria. (115) Median NT-proBNP fell from 2924 (624-28737ng/L) at presentation to 415ng/L (range 118-2853ng/L) prior to ASCT. ECOG score and NYHA class improved in the group, with all patients having ECOG score 0-2 and NYHA class 1-2 symptoms at ASCT.

Haematological responses six months post-ASCT were excellent, with 77.3% of patients achieving CR/VGPR on an evaluable basis. These translated into impressive cardiac organ responses – with 85.7% in a cardiac response six months post-ASCT. Despite the majority of patients having advanced cardiac involvement at presentation, there was no TRM in the group.

PFS and OS were significantly poorer in the group that underwent ASCT for haematological progression compared to those who underwent ASCT as consolidation (Figure 6.3). These findings are in contrast to an earlier retrospective study by one of our groups, which suggested that OS and PFS were not affected by timing of transplantation (i.e. ASCT at relapse or as consolidation).(252) The latter study included patients from 2003-2012 and none of the patients received a bortezomib-based combination first-line (having largely been treated with cyclophosphamide-thalidomide-dexamethasone or vincristine-doxorubicin-dexamethasone, compared to 91% patients in this study who received a bortezomib-based combination first-line). It is possible that those patients who relapse having been previously treated with bortezomib-based therapy have poor risk disease, accounting for worse outcomes after ASCT compared to those who are treated for consolidation. Median dFLC at the time of ASCT were significantly higher in the relapse group compared to the consolidation group, and this may also account for inferior outcomes in the relapse group. Larger studies are essential to address the question of optimal timing of ASCT.

There is another important question of the role of ASCT in patients that are refractory to first-line bortezomib-based therapy. Given the relatively conservative approach to ASCT at our centres, we routinely do not undertake autologous stem cell transplantation in patients who are refractory to therapy. Such patients proceed to second-line non-ASCT chemotherapy options to aim for deeper clonal responses. Seven patients in this cohort did not respond to initial chemotherapy and switched to second-line treatment

(predominantly with bortezomib-based therapy) and achieved haematological responses prior to autologous stem cell transplantation.

This retrospective small study needs to be interpreted within its limitations. It features a carefully selected patient cohort and does not capture 'all-comers' to our centres. Other experienced transplant centres may have deemed a proportion of patients included in this study to be suitable for upfront transplants. However, centres with lesser experience still need to be extremely cautious in cardiac AL patients due to the risk of TRM.

In conclusion, our data highlight that deferred ASCT can be undertaken safely in a consolidation or haematological progression setting in selected patients with advanced cardiac AL who are initially transplant-ineligible but go on to achieve organ responses. The approach of novel agent-based chemotherapy followed by deferred ASCT may allow a greater proportion of patients with systemic AL amyloidosis to undergo ASCT. Larger studies with longer follow-up are required to confirm our findings, assess durability of response and clarify optimal timing of ASCT.

Chapter Seven: Treatment of IgM-associated immunoglobulin light chain amyloidosis with Rituximab-Bendamustine

This chapter is written in the context of my publication:

Introduction

AL amyloidosis with an IgM monoclonal protein (IgM-AL) accounts for 5-7% of patients with AL and has a distinct phenotype with a higher prevalence of lymph node, neuropathic and lung involvement, less frequent cardiac involvement and lower amyloidogenic light chains.(227) Cardiac, neuropathic and liver involvement are predictive of mortality. A reduction in both intact monoclonal protein and free light chains are important for response assessment and prognosis in IgM-AL.   A very good partial response (VGPR) is associated with better overall survival (OS) and organ responses but is challenging to achieve in IgM-AL due to an unclear underlying diagnosis (usually lymphoplasmacytic lymphoma), and lack of treatment uniformity. In a large retrospective collaborative study, we reported the use of twenty-two different front-line regimens.(227) Rituximab-based regimens are often used, in combination with bortezomib, alkylating agents or purine analogs; outcomes remain poor with few complete responses.(227, 253, 254) The Mayo group demonstrated an overall haematological response rate (ORR) of 89% in 12 patients with IgM-AL treated with autologous stem cell transplantation – but given the older age of patients with IgM-AL, this is often not a suitable option.(255) Treatment of relapsed/refractory patients is particularly challenging and recent experience with ibrutinib was disappointing. (256)

Bendamustine, which has features of both an alkylating agent and purine analog, is used alongside rituximab in treatment of newly diagnosed and

relapsed/refractory non-Hodgkin lymphoma(257) and recently in relapsed/refractory Waldenström's Macroglobulinaemia (WM) with ORR of 83.3% in 30 patients.(258) Bendamustine has been used in relapsed/refractory AL (with a plasma cell dyscrasia) setting with disappointing responses.(259) There have been no studies focusing on the use of rituximab-bendamustine (BR) in patients with IgM-AL. We report efficacy of BR in untreated and relapsed/refractory IgM-AL.

Methods

All patients treated with BR between 2011-2017 were identified from the UK National Amyloidosis Centre database. Twenty-seven patients were included in this study: 22 received BR as first-line therapy and 5 as second-line. Twenty-five had a serum IgM M-protein. Two had lymphoplasmacytic lymphoma on bone marrow examination but had a serum IgG M-protein (instead of IgM).

AL was confirmed on biopsy immunohistochemistry or by proteomic analysis. All patients underwent serial assessment including biochemical tests for organ function, serum free light chains, serum and urine protein electrophoresis and immunofixation, cardiac biomarkers, echocardiography and/or cardiac MRI (unless contraindicated). Organ involvement and haematological responses were defined as per international amyloidosis consensus criteria.(96, 104, 115) Primary outcome variables were haematological and organ response, OS and time-to-next-treatment (TNT). Median progression-free survival (PFS) appeared similar to median OS:

deaths are likely to have been confounding PFS as few patients had

haematologic progression or progression to next treatment. PFS has

therefore been omitted from this paper in place of TNT, which we felt to be

more meaningful here.

<u>Results</u>

Table 7.1 shows baseline characteristics.

Table 7.1: Baseline characteristics.

n=27	Median (range) / n(%)
Median age (years)	70 (range 56-86)
Male:Female	16 (59%):11 (41%)
NYHA class 1 2 3 4	 12 (44%) 14 (52%) 1 (4%) 0
ECOG 0 1 2 3 4	 7 (26%) 15 (56%) 5 (18%) 0 0
Cardiac involvement	17 (63%)
Median NT-proBNP (ng/L)	978 (range 42-5708)
Median cardiac troponin T (ng/L)	31.5 (range 3-122)
Mayo Stage I II IIIA (NT-proBNP ≤8500ng/L) IIIB (NT-proBNP>8500ng/L)	 8 (30%) 13 (48%) 6 (22%) 0
Median systolic blood pressure (mmHg)	119 (range 100-164)
Median left ventricular wall thickness (mm)	11 (range 8.5-18)
Median LV ejection fraction (%)	60 (range 36-72)
Renal involvement	17 (63%)
Median serum creatinine (umol/L)	98 (range 46-493)
Median GFR (ml/min)	68 (range 10-100)
Median proteinuria (g/24h)	0.8 (range 0.1-34.6)
Liver involvement	6 (22%)
Median serum bilirubin (umol/L)	6 (range 2-50)
Median ALP (units/L)	105 (range 25-1879)
Soft tissue involvement	2 (7%)
Peripheral nerve involvement	6 (22%)

Autonomic nerve involvement	4 (15%)
Lymph node involvement	13 (48%)
GI involvement	0
Median number of involved organs	1 (range 1-4)
Involved light chains: Kappa Lambda No monoclonal light chain excess	7 (26%) 12 (44%) 8 (30%)
Median dFLC (mg/L)	59.8 (range 2.2 – 856.4)
Median serum monoclonal protein (g/L)	11.5 (range 1-30)

The median age was 70 years (range 56-86) and all had ECOG performance status of ≤ 2. Cardiac, renal, liver, peripheral nerve, autonomic nerve, soft tissue and lymph node were seen in 17 (63%), 17 (63%), 6 (22%), 6 (22%), 4 (15%), 2 (7%) and 13 (48%) respectively. The Mayo 2004 cardiac stage was: Stage I 30%, Stage II 48% and stage III 22%. Median NT-proBNP was 978ng/L (range 42-5708ng/L) and median serum creatinine was 98umol/L (46-493umol/L). The median M-protein was 11.5g/L (1-30g/L); 19 patients (70.4%) had a serum free light chain excess. Twenty one patients had available bone marrow data: three normal, one showed plasma cell infiltration, fourteen had lymphoplasmacytic lymphoma and three had NHL not specifically classified. Five patients were treated with BR for refractory disease, previous therapies included bortezomib-cyclophosphamide-dexamethasone in two patients and in one case each: rituximab-bortezomib-dexamethasone, rituximab-cyclophosphamide-vincristine-prednisolone and rituximab-cyclophosphamide-dexamethasone.

The median number of BR cycles was 5 (range 1-8). The median number of previous cycles in the second-line group was 6 (range 4-8). Three patients received two-monthly rituximab maintenance after first-line therapy with BR. All three remain on rituximab maintenance, with treatment duration thus far of 8, 10 and 11 months.

Haematological response rate on an intention-to-treat (ITT) and evaluable basis was 59% and 76%, respectively. On an ITT basis, complete response (CR) was seen in 11%, very good partial response (VGPR) 37%, partial response (PR) 11% and no-response (NR) 41% (including 22% deaths). In the first-line group, haematological responses were: CR 14%, VGPR 32%, PR 14% and NR 40% (including 18% deaths). The evaluable response rate was: CR 17%, VGPR 39%, PR 17% and NR 27%. Of three patients treated with two-monthly rituximab maintenance, one remains in VGPR, one is in CR (having been in VGPR at time of completing BR) and one is in PR (having not achieved a haematological response at time of completing BR). In the five patients treated with BR for refractory AL, three (60%) achieved a VGPR and two were non-responders (including one death). 3/17 (18%) patients with cardiac involvement achieved NT-proBNP responses and 3/17 (18%) patients achieved renal responses by consensus criteria.

Median follow-up was 18 months (range 3-55 months). Median OS was not reached (Figure 7.1).

Figure 7.1: Overall survival and time-to-next-treatment. This shows the overall survival (OS) on ITT basis and evaluable time-to-next-treatment (TNT). The median OS and median TNT were not reached. OS at 1 year and 3 years was 65% and 56%. At 1 and 3 years, only 88% and 79% of evaluable patients did not require further treatment.
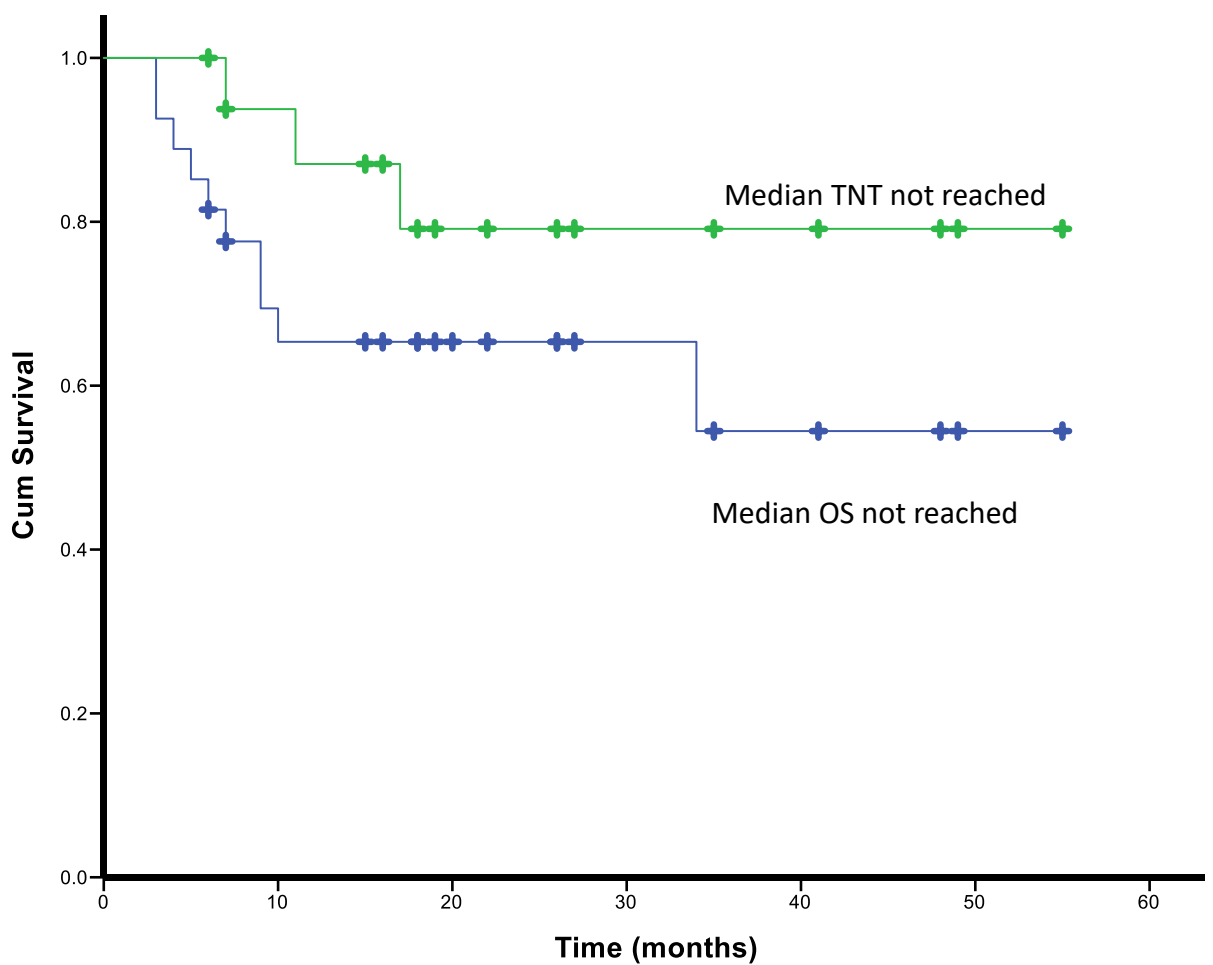
reached with 88% and 79% of evaluable patients in a haematological response at 1 and 3 years, respectively. Median OS was not reached in patients who achieved VGPR/better, compared to 9 months in patients who did not (Figure 7.2).

Figure 7.2: OS according to haematological response. Median OS was not reached in those patients who achieved a very good partial response (VGPR) or better with 92% alive at 2 years; median OS was 9 months in patients who did not achieve a VGPR.
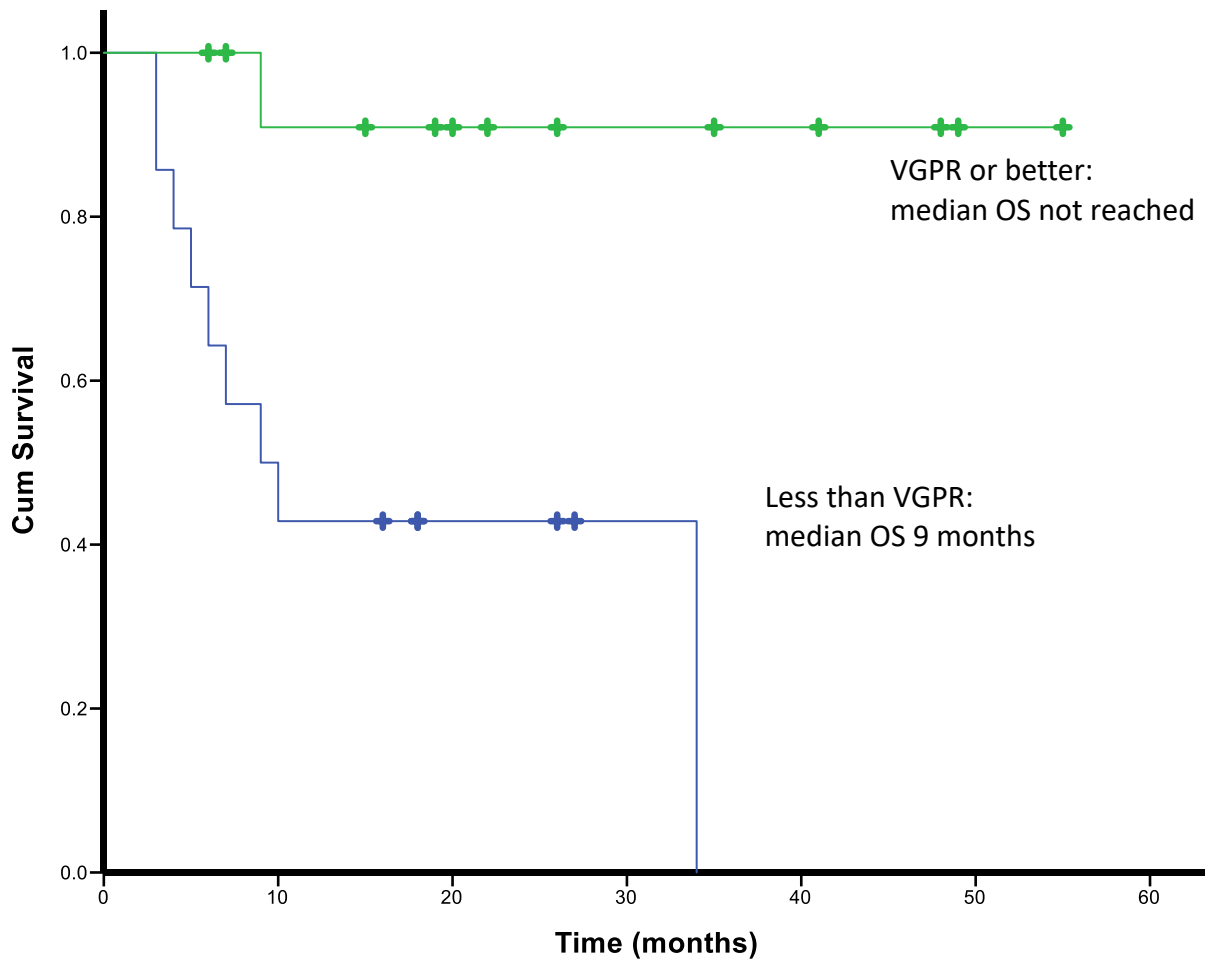
<u>Discussion</u>

Data from this small retrospective study demonstrate an excellent ORR (ITT) of 59% and evaluable ORR of 76% in patients treated with BR first-line. Impressively, 48% achieved VGPR/better (ITT). Sixty percent of patients treated with BR second-line achieved a VGPR. Furthermore, rituximab maintenance after BR upgraded responses in the few patients that received this therapy.

Treatment in IgM-AL has been historically heterogeneous, with poor responses and no complete responses with alkylator treatment (responses ~27-38%(253, 260, 261)) or purine-analogue/anthracycline-containing therapy (227, 253). Outcomes with bortezomib-based therapy are mixed: VGPR/better 27% and 42% in two studies.(227, 262). Combining bortezomib with rituximab improved responses with ORR of 78%.(254) With a high prevalence of disease-related neuropathy in IgM-AL, there is reticence in using bortezomib. Furthermore, bortezomib is not funded in the UK for lymphoproliferative disorders. The few patients eligible for ASCT appear to have good outcomes (ORR 89% and 67% organ responses) but treatment-related mortality was 8%. (255) In a large European collaborative study, only 1.8% of patients were treated by ASCT.(227) The efficacy of ibrutinib in WM without AL (ORR of 91%) (263) was not replicated in AL. A retrospective study of ibrutinib in eight patients with relapsed/refractory IgM-AL yielded only two haematological responses and poor tolerability.(256)

In conclusion, this study suggests that first-line BR leads to high response rates in IgM-AL and these are better than most previously reported. Bendamustine is not neurotoxic, dosing is not affected by renal impairment and there is no known cardiac toxicity, rendering BR a widely applicable regime in IgM-AL. Response appears durable and maintenance rituximab may upgrade depth of response. The limitation of this study is one shared with almost all literature in IgM-AL given its rarity: it is small and retrospective. Larger collaborative studies are needed to confirm these results and combination with novel proteasome inhibitors should be explored to further improve outcomes.

Chapter Eight: Outcomes with carfilzomib in AL amyloidosis patients with peripheral and autonomic neuropathy

This chapter is written in the context of my publication:

Carfilzomib is an effective upfront treatment in AL amyloidosis patients with peripheral and autonomic neuropathy. Manwani R, Mahmood S, Sachchithanantham S, Lachmann HJ, Gillmore JD, Yong K, Rabin N, Popat R, Kyriakou C, Worthington S, Sharpley F, Smith M, Shah R, Cheesman S, Hawkins PN, Wechalekar AD. British Journal of Haematology 2019 Dec; 187(5):638-641

Background

Treatment in systemic AL amyloidosis is based upon eradication of monoclonal amyloidogenic immunoglobulin light chain production with chemotherapy or ASCT. The latter, although associated with excellent haematological and organ responses, is unsuitable in most patients due to extent of amyloid-related organ dysfunction. Bortezomib, a proteasome inhibitor, has become the standard backbone of first-line therapy in ASCT-ineligible AL patients. Although neuropathy risk has been partially mitigated by subcutaneous, once-weekly administration, a third of patients treated with bortezomib develop peripheral neuropathy often necessitating its discontinuation.(226) Treatment of AL patients presenting with predominant amyloid-related peripheral or autonomic neuropathy (9-20% of AL patients) remains challenging as bortezomib is contraindicated and alkylator-based regimes do not achieve the same depth of response.

Carfilzomib is a second-generation highly selective, irreversible proteasome inhibitor with well documented efficacy in newly diagnosed and relapsed myeloma, including in patients with bortezomib-resistant disease. Due to marked selectivity for the chemotrypsin-like active site of the proteasome(159), it has fewer off-target effects and in-vitro and animal studies have demonstrated its lesser neurotoxicity.(160, 161) Studies in myeloma have demonstrated a low incidence of treatment-emergent

neuropathy with carfilzomib, with almost no ≥grade 3 treatment-emergent peripheral neuropathy(264-269) A Phase III study of carfilzomib-dexamethasone compared to bortezomib-dexamethasone demonstrated that the proportion of patients with grade ≥2 peripheral neuropathy was significantly higher in the bortezomib group (32%), compared to the carfilzomib group (6%).(270)

Carfilzomib is therefore potentially attractive in AL patients presenting with amyloid-related peripheral and/or autonomic neuropathy, but data on its use in AL is scant. In a phase I/II study of carfilzomib in 28 patients with relapsed/refractory AL, the maximal tolerated dose was 36mg/m^2 biweekly. (162) A quarter of patients had neuropathic disease. The overall response rate was 63%, with 45.8% achieving a complete response (CR)/very good partial response (VGPR), but twice-weekly dosing was associated with substantial cardiac, pulmonary and renal toxicity. Worsening peripheral or autonomic neuropathy were not reported. A study of carfilzomib with thalidomide and dexamethasone in relapsed/refractory AL is ongoing at our centre, but excludes patients with significant neuropathy (ClinicalTrials.gov identifier NCT02545907). We report here a small cohort of AL patients with significant cardiac, peripheral nerve and autonomic involvement treated with carfilzomib at presentation.

Methods

Five patients with newly diagnosed AL who had cardiac, peripheral nerve and autonomic involvement were treated with carfilzomib at the UK National Amyloidosis Centre and University College London Hospitals. Bortezomib was contraindicated in all patients due to peripheral and autonomic nerve involvement. AL was confirmed on biopsy immunohistochemistry or proteomic analysis. All underwent serial biochemical tests for organ function, cardiac biomarkers, serum free light chains, serum and urine protein electrophoresis and immunofixation, echocardiography and cardiac MRI. Organ involvement was defined by international amyloidosis consensus criteria.(104) Haematological responses were assessed according to AL response criteria.(115)

Results

The median age was 62 years (40-69 years). All had cardiac involvement: Mayo (2004) Stage II and III (80)disease in 4/5 and 1/5 patients, respectively. Two patients had ECOG score 1 and three had ECOG score 2. One patient and four patients was in NYHA class 1 and 2, respectively. The median NT-proBNP was 975ng/L (536-4681ng/L) and median serum troponin T was 43ng/L (37-90ng/L). Two patients had renal involvement. The median serum creatinine was 63umol/l (33-82umol/L), median eGFR 100ml/min (86-100ml/min) and median 24 hour proteinuria was 0.2g/24 hours (0.1-8.9g/24 hours). All had amyloid-related peripheral neuropathy and autonomic neuropathy. The median supine systolic blood pressure was 122mmHg (91-

177mmHg), with a median postural blood pressure fall of 27mmHg (20-44mmHg). No patients had liver involvement; the median serum bilirubin was 4µmol/l (2-12µmol/l) and median ALP was 74 units/L (62-110 units/L). The involved amyloidogenic light chain in all patients was lambda, with median presenting serum lambda light chains of 122mg/L (64-208mg/L) and median difference in involved and uninvolved light chains (dFLC) of 97.8mg/L (58.4-199.2mg/L).

All received weekly intravenous carfilzomib on days 1, 8 and 15 (28 day cycle), combined with corticosteroids (intravenous methylprednisolone in three and dexamethasone in two patients). One patient each received additional lenalidomide or pomalidomide, respectively, for cycle 1. The median number of cycles was 3 (range 1-6). The carfilzomib dose was 20 mg/m^2 on Day 1 and 27 mg/m^2 from Day 8 onwards. Two patients had dose escalation to 36 mg/m^2 after cycle 1 in the absence of CR and one patient had a further dose increase to 56mg/m^2 from cycle 4 (achieving a CR after the first dose at this dose level).

Haematological responses were rapid with 80.6% (22-99.4%) reduction in the median dFLC after one cycle. Figure 8.1 demonstrates dFLC values with each cycle.

Figure 8.1: Serial dFLC (difference in involved and uninvolved light chains) values of patients in this cohort on carfilzomib treatment.
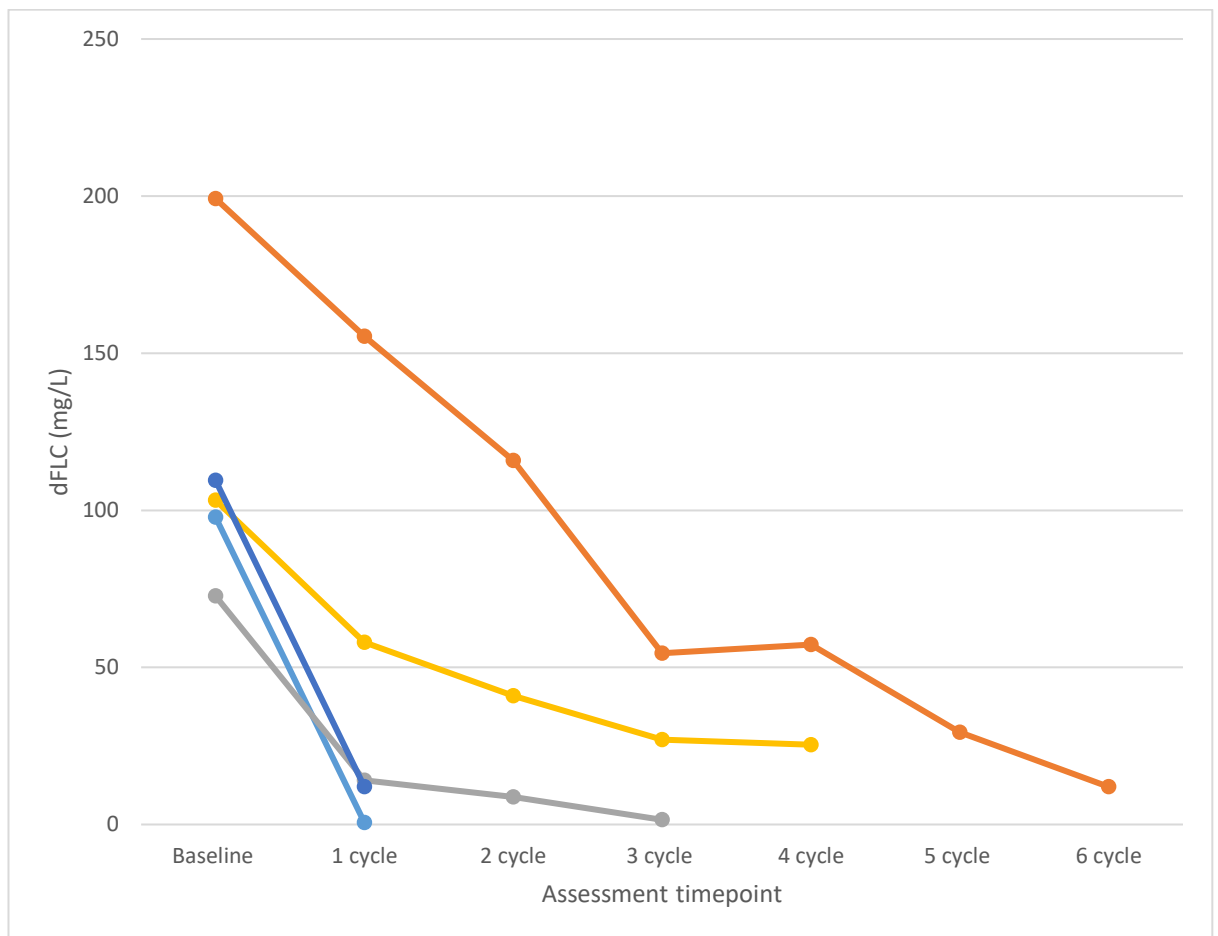
Three patients achieved a CR and two achieved a VGPR (one of the latter patients remains on carfilzomib-methylprednisolone). Both patients treated with an immunomodulatory agent achieved a CR at the end of cycle 1. One patient achieved a cardiac response at 12 months, two did not, and two have not reached this assessment time-point. Of two patients with renal involvement, one did not achieve a renal response and the other has not reached the 12 month assessment time-point.

In view of the small patient cohort in this cohort, Kaplan-Meier survival analysis was not performed. One patient (in a CR) suffered a neck of femur fracture and died of post-operative complications. Although both patients treated with immunomodulatory combinations achieved a rapid CR, they discontinued treatment after one cycle due to drug-related problems: grade 3 fatigue and fluid retention with pomalidomide (this patient was severely nephrotic with serum albumin <20g/L) and a grade 3 rash with lenalidomide, respectively. The former patient progressed to requiring further treatment with an alternative regimen two months later. There have been no other progression events in the group.

Other grade 3 toxicity included transient acute kidney injury and hypotension in the patient treated with pomalidomide. Grade 1-2 toxicity included fatigue (4 patients), nausea (2 patients), diarrhoea (2 patients) and acute kidney injury (1 patient). All patients had an echocardiogram after cycle 1 and 2 with no deterioration in cardiac function. There was no worsening of peripheral or autonomic neuropathy.

Discussion

This is the first report of carfilzomib in AL with significant neuropathy. Crucially, all patients had peripheral nerve, autonomic nerve and cardiac involvement at presentation and bortezomib was contraindicated due to extent of neuropathy. None experienced worsening peripheral or autonomic neuropathy with carfilzomib. None of the patients had any significant

cardiovascular toxicity as assessed by echocardiography. Haematological responses were rapid and excellent, with all patients achieving a VGPR/better, and 3/5 patients in a CR. The data is too limited to assess organ responses especially as follow-up is short and overall patient numbers small. This is a small, retrospective study with selected patients – none of whom presented with severely advanced cardiac involvement (all patients had an NT-proBNP<8500ng/L) or severe renal impairment. However, this study suggests that single agent carfilzomib has significant activity in AL. Whilst previous experience with carfilzomib in AL has demonstrated significant cardiac toxicity with a biweekly dosing regime(162), a once weekly protocol could be potentially administered without substantial cardiac toxicity and exacerbation of peripheral/autonomic neuropathy. Transient renal toxicity was noted in two patients, and close renal monitoring is critical in all AL patients treated with carfilzomib.

In conclusion, this data suggests that carfilzomib may be a potentially effective early treatment option in selected AL patients with peripheral and/or autonomic neuropathy (without severe cardiac or renal involvement) in whom bortezomib would be contraindicated. A prospective study in such patients is needed to confirm these findings.

Chapter Nine: A pilot study demonstrating cardiac uptake with 18F-florbetapir PET in AL amyloidosis patients with cardiac involvement

This chapter is written in the context of my publication:

A pilot study demonstrating cardiac uptake with 18F-florbetapir PET in AL amyloidosis patients with cardiac involvement. Manwani R, Page J, Lane T, Burniston M, Skillen A, Lachmann HJ, Gillmore JD, Fontana M, Whelan C, Hawkins PN, Wagner T, Wechalekar AD. Amyloid. 2018 Dec;25(4):247-252

Background

Cardiac involvement is a feature of both systemic AL amyloidosis and ATTR amyloidosis, whereby the extracellular space of the heart is expanded by an amorphous fibrillary material, causing a restrictive cardiomyopathy. (271) Cardiac involvement is the critical determinant of outcomes in AL amyloidosis. Early diagnosis is key, as treatment delay in AL can lead to stark outcomes. Histological diagnosis is the gold standard but the risks of endomyocardial biopsy are not insignificant – especially in elderly, frail patients with other comorbidities.

Imaging has been a cornerstone of diagnosing cardiac amyloidosis. Echocardiography is the most widely used method but continues to lack specificity and sensitivity. (272) Cardiac MRI overcomes some of these limitations but is contraindicated in a substantial proportion of patients, and is only available in specialist centres with interpretation often dependent on the use of specific protocols. (273) ^{123}I-labeled serum amyloid P component (^{123}I-SAP) scintigraphy binds specifically to amyloid deposits in viscera and is helpful to quantify and localise amyloid deposition, but is only available in two centres in the world and does not demonstrate cardiac amyloid deposits. (127) Bone seeking tracers such as ^{99m}Tc-DPD or ^{99m}Tc-PyP have a very high sensitivity for imaging cardiac ATTR amyloidosis but are less sensitive in detecting other types of cardiac amyloidosis. (136)

PET modalities have emerged in cardiac amyloidosis. Antoni et al reported increased cardiac uptake with ^{11}Carbon-Pittsburgh compound B (^{11}C-PIB) in patients with ATTR and AL cardiac amyloidosis. (274) Lee et al reported a significant increase in ^{11}C-PIB myocardial uptake in chemotherapy-naïve AL patients compared to already treated patients, but this tracer is only available in centres with a cyclotron. (275)

Dorbala's group performed a pilot study of PET imaging with ^{18}F-florbetapir, a tracer with a high affinity for beta amyloid in the brain, in patients with ATTR and AL cardiac amyloidosis. (228) They found that left ventricular retention index (RI), target-to-background ratio, left ventricular myocardial standardised uptake values (SUV) and left ventricular myocardial to liver SUV were higher in patients with cardiac amyloidosis compared to controls. They also reported higher cardiac retention indices in patients with cardiac AL amyloidosis compared to patients with ATTR cardiac amyloidosis, although sample sizes were small and this finding was not statistically significant. Autoradiography studies have demonstrated ^{18}F-florbetapir uptake in autopsy cases of cardiac AL and ATTR amyloidosis, but no uptake in control samples. (276) Osborne et al reported delayed myocardial washout with ^{18}F-florbetapir imaging in 8 subjects with cardiac amyloidosis compared to 3 control subjects. (277) ^{18}F-florbetaben PET has shown similar promise in another study, differentiating between cardiac amyloidosis and hypertensive heart disease. (278) Uptake with 18F-florbetapir PET at extra-cardiac sites of amyloid deposition (spleen, tongue, parotids, fat, lungs and kidneys) has also been recently described. (279)

Currently, clinical cardiac response to chemotherapy in patients with systemic AL amyloidosis is defined by an improvement in NT-proBNP after chemotherapy. (115) This biomarker response has been found to predict long term survival, but other factors such as renal insufficiency, causes of heart failure independent of AL amyloidosis, immunomodulatory agents and acute heart failure can also lead to elevation of NT-proBNP and may not necessarily reflect true organ response. Echocardiographic and cardiac MRI imaging, whilst useful after treatment, are not used in routine evaluation of organ response.

In this pilot study, we evaluated cardiac uptake with ^{18}F-florbetapir PET in patients with systemic AL amyloidosis with cardiac involvement before and after treatment, as well as its serial utility in monitoring.

Materials and methods

All patients were recruited to a prospective study of ^{18}F-florbetapir in cardiac amyloidosis from September 2016 to June 2017. The inclusion criteria were: confirmed diagnosis of AL amyloidosis defined by amyloid deposition confirmed on a tissue biopsy and documented AL amyloid fibril typing, and evidence of cardiac involvement as defined by international amyloidosis consensus criteria. (104) The exclusion criteria were: inability to lie flat, NYHA Class IV heart failure, pregnancy or unwilling to undergo pregnancy test prior to study (in women of child bearing potential), the presence of any

contraindication to undergoing PET imaging, and a known allergy to Amyvid. All patients were aged 40 years or over.

The primary outcome was ^{18}F-florbetapir uptake in the heart. The variables of the study were left ventricular retention index (RI), mean and maximum left ventricular myocardial standardised uptake variable (SUV_{mean} and SUV_{max}). ^{18}F-florbetapir RI was calculated by the method described by Dorbala et al, as the mean LV myocardial tissue radiotracer concentration between 10 and 30 minutes after injection of ^{18}F-florbetapir, divided by the integral of the blood pool ^{18}F-florbetapir time activity curve from 0 to 20 minutes. (228) A volume of interest in the left ventricular cavity was used by Dorbala's group. However, our group felt that counts in the left ventricular wall were spilling over into the blood pool volume of interest, potentially giving falsely low results in some patients. An approach was therefore taken using a volume of interest in the aorta for the blood pool instead, as this was less likely to include counts from other areas of uptake.

All patients underwent serial protocolized assessment at the UK National Amyloidosis Centre including full biochemical tests for organ function, serum free light chain measurement as well as serum and urine protein electrophoresis and immunofixation, cardiac biomarker measurement, echocardiography, cardiac MRI (unless contraindicated), and ^{123}I-SAP scintigraphy. Organ involvement was defined as per international amyloidosis consensus criteria. (104)

Imaging Protocol:

Patients underwent list mode dynamic PET imaging for 60 minutes using a Siemens Biograph PET/CT machine following a mean intravenous administration of 331 MBq (range 294-370MBq) of ^{18}F-florbetapir. The heart was placed in the centre of the field of view. CT imaging was performed over this area using automatic exposure control (CARE Dose 4D, CARE kV – Siemens Healthcare) with exposure parameters of 65mAs and 120kV. After dynamic imaging, the patient was asked to void. A half body acquisition PET-CT was then performed. Images underwent iterative reconstruction (2 iterations, 21 subsets) using time of flight information and point spread function modelling, with 2mm Gaussian post filtering. The 60 minute list mode data was reconstructed into 37 frames (12 frames of 5 s each, 6 frames of 10 s each, 4 frames of 30 s each, 6 frames of 60 s each, 8 frames of 300 s each, and 1 frame of 600 s).

Images were assessed by a nuclear medicine physician using HERMES workstation (HERMES Medical Solutions). The dynamic images (0-60 minutes) were assessed visually for cardiac uptake. The delayed half body images were visually analysed for uptake in the heart and other organs. All acquired data for all time frames was analysed and interpreted. For the dynamic images of the heart, the nuclear medicine physician assessed the presence of uptake in the myocardium and its localisation. For delayed half body images, cardiac uptake was assessed in the same manner. For extra-

cardiac uptake, comparison was made with normal tracer distribution as described in the literature and as inferred from this study's patient population.

The study had approval from the institutional review board, and written consent was obtained from all patients in accordance with the Declaration of Helsinki. Statistical analysis was conducted using SPSS Version 24 software.

Results

Baseline characteristics are found in Table 9.1.

Table 9.1: Baseline characteristics

Overall cohort (n=15)	Median (range) / n(%)
Age	58 (46-73)
Male	11 (73%)
Female	4 (24%)
ECOG: 0-2 3-4	 15 (100%) 0
NYHA: I-II III-IV	 15 (87%) 2 (13%)
Median NT-proBNP (ng/L)	2287 (114-6739)
Median troponin T (ng/L)	59 (35-259)
Median LV wall thickness (mm)	16 (13-18)
Median LV ejection fraction (%)	54 (29-63)
Median 6 minute walk test (metres)	472 (138-662)
Median serum creatinine (umol/L)	114 (66-203)
Median 24 hour urinary protein (g/24h)	0.3 (0.1-7.2)
Median serum albumin (g/L)	42 (22-49)
Median bilirubin (umol/L)	8 (5-38)
Median ALP (ULN 129 units/L)	106 (45-1702)
Organ involvement: Cardiac Renal Liver Autonomic nerve Peripheral nerve Soft tissue Gastrointestinal	 15 (100%) 8 (53%) 4 (24%) 2 (12%) 1 (6%) 4 (24%) 0
Cardiac Mayo stage at presentation : I II III	 0 0 15 (100%)
Haematological response at time of imaging: CR VGPR PR No haematological response	 9 (60%) 2 (13%) 1 (7%) 3 (20%)
NT-proBNP response at time of imaging	6 (40%)
No NT-proBNP response at time of imaging	9 (60%)

Fifteen patients with systemic AL amyloidosis were included. Two patients underwent repeat imaging – before starting chemotherapy and after completion of treatment. All had cardiac involvement. Median age was 58 years (range 46-73 years). All patients had an ECOG score of 0-2 and the majority had NYHA class I-II symptoms. Median NT-proBNP was 2287 ng/L (range 114 – 6739 ng/L). All patients had Mayo Stage III cardiac involvement at presentation. (80) Median ventricular wall thickness and left ventricular ejection fraction on echocardiography were 16mm (range 13 - 18mm) and 54% (range 29-63%), respectively.

Two patients were treatment-naïve at the time of the study, and two were early in their first cycle of chemotherapy (although one of the latter had achieved a haematological partial response at the time of imaging). At the time of imaging, the haematological response status was: patients in a complete haematological response (CR) - 9/12 (75%), very good partial response (VGPR) - 2/12 (16.7%), partial response (PR) - 1/12 (8.3%). Of the three remaining patients, two were treatment-naïve and one was too early into chemotherapy for response assessment. Six patients had achieved a cardiac response as per international amyloidosis consensus criteria at the time of imaging. (104)

All patients had cardiac uptake with ^{18}F-florbetapir (Figure 9.1).

Figure 9.1: Myocardial uptake with ^{18}F-florbetapir was demonstrated in all patients in the study. a) Diffuse, intense myocardial uptake is seen in a patient with cardiac AL amyloidosis undergoing first cycle of chemotherapy. b) Markedly intense myocardial uptake with ^{18}F-florbetapir in a patient with cardiac AL amyloidosis, prior to chemotherapy initiation. c) Diffuse, low grade myocardial uptake in a patient with cardiac AL amyloidosis in a complete clonal response three months after completion of chemotherapy.
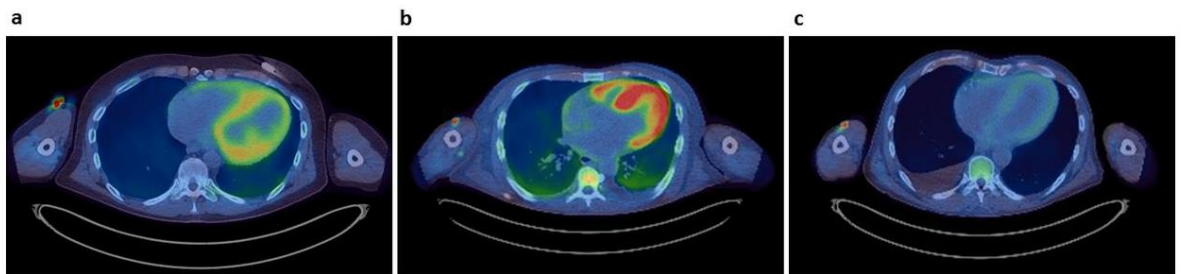
There were no false negative scans. Primary outcome variables are presented in Table 9.2.

Table 9.2: Primary outcome variables.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Diagnosis	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL	AL
LV retention index (min^{-1})	0.05	0.22	0.29	0.05	0.11	0.06	0.23	No data	0.11	0.19	0.17	0.25	0.22	0.14	0.13
Myocardial SUV max	1.6	6.5	12.4	0.8	2.1	5.8	15.7	5.4	7.2	7.2	9.9	9	9.4	7.2	7.9
Myocardial SUV mean	1.1	3.6	7.1	0.8	1.2	3.6	6.8	2.2	3.2	2.7	5.3	4.8	4.5	2.7	4.6

The median LV retention index was 0.16 min^{-1} (0.05 – 0.29 min^{-1}). Median myocardial SUV_{max} and SUV_{mean} was 7.2 (range 0.8 – 15.7) and 3.6 (range 0.8 - 7.1), respectively. The pattern of uptake was diffuse. Uptake in the liver was seen in all cases as this tracer is excreted by the liver into the biliary system.

Previous reports suggested a lack of uptake in patients not "actively depositing" amyloid protein i.e. those in a haematological response. In this cohort, all patients in a clonal response still showed cardiac uptake. There was a suggestion that there was greater cardiac uptake in the treatment-naïve patients, although the number of treatment-naïve patients was too small for meaningful statistical analysis of difference in cardiac uptake between the two groups (Table 9.3).

Table 9.3: Relationship between primary outcome variables and clinical parameters.

	LV retention index (min^{-1})	LV myocardial SUV$_{max}$	LV myocardial SUV$_{mean}$
AL patients that were treatment-naïve (n=2), mean	0.21 (range 0.17-0.25)	9.45 (range 9 – 9.9)	5.05 (range 4.8-5.3)
AL patients established on treatment (n=13), mean	0.14 (0.05-0.29)	7.2 (range 0.8-15.7)	3.6 (range 0.8-7.1)
NT-proBNP response (n=6), mean	0.19 (range 0.11-0.29) p=0.68	7.3 (range 2.1-12.4) p=0.52	3.9 (range 1.2-5.2) p=0.73
No NT-proBNP response (n=9), mean	0.16 (range 0.05-0.25)	8.7 (range 1.6-15.7)	4.3 (range 1.1-6.8)
Haematological partial response (PR) or better (n=12), mean	0.14 (range 0.05-0.29) p=0.11	6.38 (range 0.8-12.4) p=0.07	3.38 (range 0.8-7.1) p=0.07
No haematological PR or better (n=3), mean	0.21 (range 0.17-0.25)	11.5 (range 9-15.7)	5.63 (range 4.8-6.8)

Patients that had not achieved at least a partial haematological response appeared to have higher LV retention indices, myocardial SUV_{mean} and myocardial SUV_{max}, although these findings were not statistically significant (Table 9.3). There was no statistically significant correlation with primary outcome variables and achievement of a cardiac response by NT-proBNP criteria. (115)

There was no statistically significant correlation between the retention indices of ^{18}F-florbetapir with global longitudinal strain rate, left ventricular ejection fraction, mean ventricular thickness, myocardial extracellular volume fraction (ECV) on cardiac MRI, serum troponin-T, serum NT-proBNP, difference in involved and uninvolved light chains (dFLC), and kappa:lambda ratio.

Comparison between initial and repeated ^{18}F-florbetapir imaging

Three patients with AL had repeated ^{18}F-florbetapir imaging. At the time of initial imaging: one patient was treatment-naïve; one was early into first cycle of chemotherapy (but had achieved a haematological PR); one was on chemotherapy and in a haematological CR. All three patients were in a haematological CR at the time of repeat imaging and two had achieved an NT-proBNP response. There was no significant difference in primary outcome variables between initial and repeated imaging (Table 9.4).

Table 9.4: Repeated ^{18}F-florbetapir imaging in three cardiac AL patients.

	Initial ^{18}F-florbetapir imaging						Repeat ^{18}F-florbetapir imaging						
Patient	Treatment	Haematologic response	NT-proBNP response	LVRI (min^{-1})	SUV max	SUV Mean	Treatment	Interval (months)	Haematologic response	NT-proBNP response	LVRI (min^{-1})	SUV max	SUV mean
1	Day 15, cycle 1 CyBorD	Partial response	No	0.05	1.6	1.1	7 months post chemotherapy	2	Complete response	Yes	0.06	1.4	1.0
3	Carfilzomib, pomalidomide, dexamethasone cycle 11	Complete response	Yes	0.29	12.4	7.1	Carfilzomib, pomalidomide, dexamethasone Cycle 14	8	Complete response	Yes	0.19	11.8	4.7
12	Pre-chemotherapy	Not applicable	No	0.25	9	4.8	Completed 2 cycles of chemotherapy	8	Complete response	No	0.21	10.7	5.2
Difference in primary outcome variables between initial and repeat imaging											p=0.3	p=0.71	p=0.5

<u>Discussion</u>

This is the largest study to date reporting on the use of ^{18}F-florbetapir PET imaging in AL patients with cardiac involvement. It is the first study to include patients that underwent ^{18}F-florbetapir imaging prior to starting chemotherapy, and the first to include repeat ^{18}F-florbetapir PET imaging at later timepoints. Crucially, this study also captures patients in differing haematological responses. It has previously been suggested that patients with normal light chains may have "inactive" amyloid deposits, which may not be associated with cardiac uptake with ^{18}F-florbetapir. There was a suggestion of greater cardiac uptake in patients with higher light chains than those in a good light chain response. However, all patients in this cohort had demonstrable cardiac uptake, irrespective of serum free light chain burden or NT-proBNP response. This is an important novel observation that offers new insight into the argument of 'active vs. 'inactive' amyloid hearts.

Two patients in this study underwent ^{18}F-florbetapir imaging before starting chemotherapy. These patients appeared to have increased cardiac uptake compared to the rest of the cohort. Similarly, the three patients in this cohort who had not achieved at least a partial haematological response (two treatment-naïve patients and one in the first week of chemotherapy) appeared to have increased cardiac uptake, compared to those who did not. It must be emphasised that the latter two findings were not statistically significant. There remains a distinct possibility, however, that patients with particularly active clonal disease may have a higher cardiac disease burden,

manifesting in increased cardiac uptake – but this relationship needs to be explored further in a study with larger patient numbers. Differing cardiac uptake in 'active' and 'inactive' amyloid deposits would have a potential role in monitoring treatment response as currently cardiac organ response after chemotherapy is defined as a 30% fall in NT-proBNP (115), but there are drawbacks of solely using a biomarker in assessment of cardiac response, when this biomarker is confounded by many other factors (e.g. renal insufficiency, immunomodulatory agents, acute fluid status and other causes of heart failure).

Three patients underwent follow-up imaging. At the time of initial imaging, one of these patients was treatment-naïve and subsequently went on to have chemotherapy, one patient had recently started chemotherapy and one had achieved a complete haematological response with chemotherapy. All patients were in a complete haematological response at the time of repeat imaging. There was no significant difference in cardiac uptake on initial and repeated imaging. However, the interval between imaging was short (range 2-8 months) and future studies need to include greater numbers of patients and assess further the optimal timing of repeat ^{18}F-florbetapir imaging post-treatment.

No correlation was identified between primary outcome variables and cardiac biomarkers, LV ejection fraction, ventricular strain, ventricular wall thickness and ECV on cardiac MRI. The reasons for this are uncertain, but possibilities

include a "saturation" effect due to a very high tracer affinity for amyloid, the heterogeneity of patients included in this cohort, or the relatively small sample size. Whilst the uptake in all cases suggests a role for this tracer in diagnosis of cardiac amyloidosis, the lack of correlation with other markers may limit the utility of this tracer as a marker of prognosis in patients with more advanced cardiac amyloidosis.

The pattern of cardiac uptake in this study was diffuse and not restricted to the ventricular base as seen in ^{99m}Tc-DPD or ^{99m}Tc-PyP imaging. The exact reason for this discrepancy is unclear but is likely to be due to the different mechanisms of tracer binding to amyloid deposits. ^{99m}Tc-DPD or ^{99m}Tc-PyP probably bind to calcium in amyloid deposits, whereas ^{18}F-florbetapir is fibril-specific. On endomyocardial biopsies, amyloid deposition can be seen throughout the myocardium and hence, we believe that diffuse cardiac uptake with ^{18}F-florbetapir reflects total amyloid deposition at the time of imaging.

The findings of this study need to be interpreted within its limitations. The overall number of patients in this study is small. As there were no control subjects included in the cohort, it was not possible to evaluate specificity of this tracer for cardiac amyloidosis. Interval imaging was only performed in three AL patients and was carried out at differing timepoints in patients - further studies are needed to assess cardiac uptake at standardised timepoints before and after chemotherapy. Additionally, the utility of this

tracer in early cardiac amyloidosis has yet to be defined as all patients in this study had clearly identifiable cardiac involvement.

[18]F-florbetapir is licensed for detecting brain beta amyloid deposits in Alzheimer's disease. During the numerous studies of patients with Alzheimer's disease, imaging was limited to the head only and no systemic imaging details are available. Dorbala et al reported the first use of ^{18}F-florbetapir in 9 patients with cardiac amyloidosis (5 AL, 4 ATTR) and 5 control subjects. (228) Increased myocardial tracer uptake was seen in all amyloid subjects and none of the controls.

The small number of total subjects in studies thus far limits definitive conclusions on sensitivity and specificity, but both appear promising and make ^{18}F-florbetapir potentially attractive in distinguishing cases of true cardiac amyloid from non-amyloid pathologies which can share some echocardiographic and cardiac MRI appearances with amyloidotic infiltration. This is the first tracer to consistently demonstrate myocardial uptake in cardiac AL amyloidosis. This, if confirmed in larger studies, will be a step change in our approach to the diagnosis of cardiac AL amyloidosis.

The striking appeal of ^{18}F-florbetapir is in its seemingly high affinity for cardiac amyloid deposits, as demonstrated by our study and previous literature. (228, 276, 280) The exact place for this tracer in the amyloid

diagnostic paradigm remains unclear. Larger studies are needed to clarify if this tracer will complement or, indeed, supplant the current methods of imaging cardiac amyloidosis.

Chapter Ten: Conclusion

This thesis assesses the outcomes of patients with systemic AL amyloidosis who are treated with standard upfront therapy. It also explores complex subgroups in whom treatment options are often challenging, such as those with advanced cardiac involvement, severe neuropathic involvement, IgM-associated AL and early non-responders. A novel imaging modality, ^{18}F-florbetapir PET, is also studied.

Chapter 3 reports analysis of the outcomes of 915 patients treated with bortezomib-based therapy. Bortezomib-based therapy has become the standard upfront treatment option in AL, as most patients have substantial organ involvement and/or poor performance status at presentation that precludes them from undergoing ASCT. While other studies have reported on outcomes with bortezomib-based therapy, none have explored outcomes in such a large cohort or assessed the duration of response in patients treated with this regime.

Chapter 3 demonstrates that haematological responses with bortezomib-based therapy are good, with two-thirds of patients attaining a haematological response. Half of patients treated achieved excellent haematological responses (that is, CR/VGPR/low dFLC PR). Unfortunately, a third of the group did not respond to therapy (of which the majority comprised of patients who died before assessment of treatment response at six

months). This highlights that early mortality rates in AL have not been overridden by modern treatment regimes.

The median OS in the latter cohort was six years, which supports the improvement in survival reported by the Mayo group.(234) Approximately 30% of patients died before progressing to another treatment, but of the remainder, the median TNT was not reached. The OS and TNT were particularly excellent in those patients who achieved a CR/VGPR/low dFLC PR (median OS and TNT not reached in these groups).

A deep light chain response is key to good outcomes in AL. Chapter 3 also examined the role of a 'stringent dFLC response', whereby the post-treatment dFLC was less than 10mg/L. Patients who achieved a stringent dFLC response had better OS and TNT, compared to those who did not. Importantly, patients who achieved a CR and a stringent dFLC response had better survival outcomes than those in a CR without a stringent dFLC response. Cardiac and renal responses were also significantly better in patients with stringent dFLC responses, compared to patients with lesser responses.

Chapter 4 reports outcomes in a group of AL patients who did not achieve early haematological responses. It is encouraging to note that despite being initial non-responders, a third of this cohort went on to achieve a CR/VGPR with further treatment and were able to benefit from good OS (61 months)

and TNT (49 months). However, organ responses in the cohort were poor and two-thirds of patients did not drastically improve their haematological responses with ongoing treatment. The latter group had disappointing outcomes, with a median OS of 49 months and a median TNT of 7 months. This chapter highlights that patients who do not achieve early haematological responses should still be considered for further treatment. However, due to limitations of the study, it is not possible to make specific recommendations on what the optimal subsequent treatment regimen would be in such patients.

Chapter 5 assesses outcomes in patients with very advanced cardiac AL. Stage IIIb cardiac involvement is defined as an NT-proBNP>8500ng/L, and a European collaboration reported particularly poor outcomes in this subgroup of patients. (107) We show in Chapter 5 that in a cohort of 179 patients with Stage IIIb cardiac AL who were seen at the NAC, there was a dichotomy of outcomes. A third of patients achieved excellent haematological responses (CR/VGPR), These patients fared much better than previously reported in patients with advanced cardiac AL, with a median OS of 38 months. Conversely, patients who did not respond to therapy or achieved a PR had dismal survival. Patients treated with CyBorD were more likely to achieve an excellent haematological response than those treated with CTD. Importantly, we found that patients with Stage IIIb cardiac AL who achieve good haematological responses have much better outcomes than anticipated in this poor subgroup. This highlights that patients with Stage IIIb cardiac AL should still be included in clinical trials, where possible (currently this is

generally an exclusion criterion in most clinical trials). The focus of treatment in this subgroup should be to achieve rapid and deep haematological responses, with CyBorD as a favoured upfront regime. Unfortunately, a substantial proportion of patients in this subgroup simply do not have the functional reserves to tolerate treatment – or do achieve a haematological response but still go on to succumb to their overwhelmingly severe disease. Treatments with a minimal toxicity profile that are able to rapidly reduce amyloidotic organ burden are drastically needed.

Chapter 6 focuses on outcomes in a selected group of patients who were ASCT-ineligible at presentation (predominantly due to the extent of their cardiac involvement). These patients subsequently went on to have ASCT after achieving organ responses and improvements in performance status with bortezomib-based chemotherapy. All patients were treated with bortezomib-based therapy and the majority of patients had Mayo Stage III disease at presentation. Their NYHA class, ECOG score, median NT-proBNP and proteinuria improved with induction therapy, enabling them to subsequently undergo ASCT. Haematological responses were excellent, with two-thirds of patients achieving CR/VGPR. There was no TRM. The median OS was not reached and median PFS was 54 months. Interestingly, patients who underwent deferred ASCT at the time of disease progression fared worse than those patients who underwent the treatment as initial consolidation therapy. The findings of Chapter 6 highlight that patients with severe cardiac involvement can still avail ASCT at a subsequent point, with organ responses after induction therapy. This is an important finding as,

while ASCT is associated with excellent long term outcomes in AL, the majority of patients are unable to undergo upfront ASCT.

Chapter 7 reports outcomes in a cohort of patients with IgM-associated AL amyloidosis who were treated with rituximab-bendamustine. IgM-associated AL is a rare and distinct entity, where treatments have historically been heterogeneous and haematological responses generally poor. ASCT has been associated with good haematological responses but patients with IgM-associated AL often tend to be older (the median age of the cohort in this chapter was 70 years old) and ASCT is rarely an accessible option. In this group, haematological response rates with rituximab-bendamustine were better than previously reported in this condition, with an ITT ORR of 59% (48% CR/VGPR). Responses were also durable, with 79% of evaluable patients not requiring further treatment at three years. These results are promising, although given the rarity of this disease, this was a small cohort of patients.

Chapter 8 reports on outcomes in a small cohort of AL patients with significant peripheral and autonomic neuropathy who were treated with carfilzomib. Due to the extent of their neuropathic involvement, bortezomib was contra-indicated. All patients had cardiac involvement (although none had Stage IIIb involvement). Haematological responses were rapid, with a median 80% reduction in dFLC after the first cycle. All patients achieved a CR/VGPR. There was no worsening in neuropathy, and no worsening of

cardiac involvement. This was a very small patient cohort, but AL patients with substantial neuropathic involvement are extremely challenging to treat given the limited treatment options available, and these results show that carfilzomib is a potential option in such patients.

Chapter 9 outlines results from a pilot study of a novel imaging technique, ^{18}F-florbetapir PET, in cardiac AL amyloidosis. A smaller previous pilot study demonstrated cardiac uptake in patients with cardiac AL and ATTR amyloidosis, and absence of uptake in five control patients.(228) In our study in Chapter 9, fifteen AL patients with cardiac involvement underwent ^{18}F-florbetapir PET and cardiac uptake was observed in all. Cardiac uptake appeared higher in those patients who were treatment-naïve or early on in treatment. There were no false negatives. There was no apparent difference in cardiac uptake indices in patients who underwent repeated imaging after chemotherapy. The numbers in this study were very small and the timing of imaging was heterogeneous (patients underwent imaging at differing timepoints of their treatment journey). However, given that were no false negatives and control subjects had absence of cardiac uptake in a previous study(228), this tracer appears to be a sensitive imaging technique in cardiac amyloidosis. The ideal cardiac imaging technique in amyloidosis would i) detect amyloid deposits with high sensitivity and specificity, ii) differentiate between different forms of cardiac amyloidosis, reducing the need for endomyocardial biopsies. The role of ^{18}F-florbetapir PET in the amyloidosis diagnostic paradigm is unclear. It would be helpful to confirm the sensitivity of the technique for cardiac amyloid deposits, as well as establishing

specificity and exploring any difference in cardiac uptake in ATTR vs AL

amyloidosis.

Future studies

Chapter 3 reported outcomes in the largest cohort of AL amyloidosis patients treated with bortezomib-based therapy. Overall survival and time-to-next-treatment were better than historically reported and may rival those of ASCT, particularly in those patients who had very deep haematological responses. There are very few prospective randomised controlled trials in AL. The only study of ASCT versus standard chemotherapy concluded that there was no survival benefit with ASCT compared to standard chemotherapy.(145) The latter study had significant limitations, however, as it included patients with advanced cardiac involvement. A contemporary prospective study of ASCT versus bortezomib-based chemotherapy would be of paramount importance in AL. A study of outcomes with ASCT performed in the UK is currently under way, as well as a matched comparison of outcomes with ASCT versus bortezomib-based therapy.

Chapter 4 demonstrated that patients who are initially refractory to CyBorD can still go on to achieve excellent haematological responses and good survival outcomes. However, the majority of patients who do not achieve haematological responses after three cycles of CyBorD have poorer outcomes and the treatment options outlined in Chapter 4 were not effective in improving haematological responses in the majority of patients. Clinical trials of novel chemotherapy agents, such as daratumumab, are essential in the refractory setting.

Chapter 5 examined outcomes in very advanced (Stage IIIb) cardiac involvement. Survival outcomes in patients with excellent haematological responses were much better than previously reported in this high-risk patient cohort. These outcomes should engender confidence in the inclusion of patients with Stage IIIb AL within future clinical trials.

Chapter 6 reported outcomes after deferred ASCT in patients with AL who were ASCT-ineligible at presentation, but achieved haematological and organ responses with bortezomib to enable them to avail ASCT later. Patients who underwent ASCT for haematological progression had inferior survival outcomes compared to patients who underwent ASCT as consolidation. In the clinical arena, there is often a dilemma of how to manage patients who achieve a CR with upfront standard chemotherapy – and whether these patients should undergo consolidative ASCT or to reserve this as a treatment option in the event of relapse. The findings of this study were interesting in this regard, but this was a selected, small group and this question needs to be addressed in larger, ideally prospective studies.

Chapter 7 captures a patient cohort with IgM-associated AL amyloidosis who were treated with rituximab-bendamustine. IgM-associated AL is a rare and distinct entity, characterised by more frequent neuropathic, lung and lymph node involvement, and lesser cardiac involvement. Haematological responses have historically been poor in this subgroup, due to an often unclear underlying diagnosis and heterogeneous treatment approaches.

Novel therapies such as BTK inhibitors and BCL2 inhibitors that have expanded the armamentarium in Waldenström's macroglobulinaemia are largely unexplored in IgM-associated AL. There are no randomised controlled studies to date that have enrolled solely IgM-associated AL patients due to the rarity of the disease. An international registry of patients with IgM AL is much needed and would better inform treatment strategies in this challenging disorder.

Chapter 9 reported a pilot study of ^{18}F-florbetapir, a novel imaging tracer in amyloidosis that resulted in cardiac uptake in a group of AL patients with cardiac involvement. A previous study has described cardiac uptake in amyloidosis patients with cardiac involvement, and absence of uptake in control subjects.(228) Great advances have been made in the imaging landscape of amyloidosis. Findings of ventricular wall hypertrophy and abnormal longitudinal strain on echocardiography are not specific for amyloidosis. Cardiac MRI with gadolinium is becoming increasingly popular in the diagnosis and monitoring of cardiac amyloidosis. However, it also has its limitations and is contraindicated in patients with pacemakers or severe renal impairment. DPD imaging is sensitive for ATTR cardiac amyloid deposits, but less sensitive in other types of amyloidosis. An imaging modality that is highly sensitive and specific for cardiac amyloid deposits, as well as able to confidently differentiate between AL and ATTR amyloid deposits would be highly useful in the diagnostic paradigm. Larger studies are needed to confirm the sensitivity and specificity of ^{18}F-florbetapir for

amyloid deposits, and to establish whether there is any difference in uptake

between cardiac AL and cardiac ATTR deposits.

Publications arising from thesis work

A prospective observational study of 915 patients with systemic AL amyloidosis treated with upfront bortezomib.

Manwani R, Cohen O, Sharpley F, Mahmood S, Sachchithanantham S, Foard D, Lachmann HJ, Quarta C, Fontana M, Gillmore JD, Whelan C, Hawkins PN, Wechalekar AD. Blood 2019; 134(25):2271-2280

Rapid Haematologic Responses Improve Outcomes in Patients With Very Advanced (Stage IIIb) Cardiac Immunoglobulin Light Chain Amyloidosis. Richa Manwani, Darren Foard, Shameem Mahmood, Sajitha Sachchithanantham, Thirusha Lane, Cristina Quarta, Taryn Youngstein, Tamer Rezk, Helen J Lachmann, Julian D Gillmore, Marianna Fontana, Carol Whelan, Philip N Hawkins, Ashutosh Wechalekar. Haematologica, 103 (4), e165-e168 April 2018.

Deferred autologous stem cell transplantation in systemic AL amyloidosis. Richa Manwani, Ute Hegenbart, Shameem Mahmood, Sajitha Sachchithanantham, Charalampia Kyriakou, Kwee Yong, Rakesh Popat, Neil Rabin, Carol Whelan, Tobias Dittrich, Christoph Kimmich, Philip Hawkins, Stefan Schönland, Ashutosh Wechalekar. Blood Cancer J. 2018 Nov 5; 8(11): 101

Treatment of IgM-associated immunoglobulin light chain amyloidosis with rituximab-bendamustine. Manwani R, Sachchithanantham S, Mahmood S, Foard D, Sharpley F, Rezk T, Lane T, Quarta C, Fontana M, Lachmann HJ, Gillmore JD, Whelan C, Hawkins PN, Wechalekar AD. Blood 2018 Aug 16; 132(7): 761-764

A pilot study demonstrating cardiac uptake with 18F-florbetapir PET in AL amyloidosis patients with cardiac involvement. Manwani R, Page J, Lane T, Burniston M, Skillen A, Lachmann HJ, Gillmore JD, Fontana M, Whelan C, Hawkins PN, Wagner T, Wechalekar AD. Amyloid. 2018 Dec;25(4):247-252

Carfilzomib is an effective upfront treatment in AL amyloidosis patients with peripheral and autonomic neuropathy. Manwani R, Mahmood S, Sachchithanantham S, Lachmann HJ, Gillmore JD, Yong K, Rabin N, Popat R, Kyriakou C, Worthington S, Sharpley F, Smith M, Shah R, Cheesman S, Hawkins PN, Wechalekar AD. British Journal of Haematology 2019 Dec; 187(5):638-641

Successful treatment of systemic AA amyloidosis associated with underlying Hodgkin lymphoma. Manwani R, Wrench D, Wechalekar A, Lachmann H. British Journal of Haematology 2018 Sep;182(5):619.

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