Novel treatments for systemic amyloidosis

Systemic amyloidoses are potentially lethal diseases. Current treatment is directed against an ongoing supply of precursor proteins. Treatment modalities include liver transplantation for transthyretin-related (ATTR) amyloidosis, elimination of inflammation in amyloid A (AA) amyloidosis and intensive chemotherapy in immunoglobulin light chain (AL) amyloidosis. These therapies often elicit substantial morbidity and mortality or insufficient effectiveness. Therefore, the search for new, more effective therapies without serious side-effects remains mandatory. Since cardiac involvement is vital when deciding on therapy, the introduction of N-terminal probrain natriuretic peptide (NT-proBNP) and troponin are of great importance. Determination of serum free light chains has become indispensable in the diagnosis and follow-up of AL amyloidosis. Manipulation of amyloid deposition and degradation might become possible in the future by the use of eprodisate in AA amyloidosis, 4′-iodo-4′-deoxydoxorubicin in AL amyloidosis, diflunisal in ATTR amyloidosis and CPHPC in all types of amyloidosis. New potent precursor-lowering drugs, such as thalidomide, lenalidomide and bortezomib, need to find their place in treatment protocols in AL amyloidosis.

KEYWORDS: bortezomib ■ diflunisal ■ eprodisate ■ FLC ■ free light chain ■ lenalidomide ■ NT-proBNP ■ systemic amyloidosis ■ thalidomide

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Amyloidosis is characterized by the deposition of protein fibrils with a β-sheeted structure. This structure is responsible for the insolubility of the protein fibrils, their resistance to proteolysis and binding affinity of Congo red dye, and the green birefringence observed with polarized light. In systemic amyloidosis, deposition of amyloid fibrils in (vital) organ systems leads to organ dysfunction and, if untreated, eventually leads to death. A prerequisite for adequate treatment is a proper diagnostic and prognostic work-up [1,2].

Major types of systemic amyloidosis

Different types of systemic amyloidosis are recognized depending on the type of precursor protein of the amyloid fibril [3]. The three main types are amyloid A amyloidosis (AA), immunoglobulin light chain amyloidosis (AL) and transthyretin-related amyloidosis (ATTR). In AA amyloidosis, the precursor protein is serum amyloid A protein (SAA), an acute-phase reactant. This type of amyloidosis is seen in patients with chronic inflammation, for example in inflammatory rheumatic or gastro–intestinal diseases, and in patients with chronic infections or hereditary fever syndromes. In AL amyloidosis, the precursor protein is a κ or λ immunoglobulin light chain. The underlying monoclonal plasma cell dyscrasia is often of low-grade origin and usually lacks the malignant sheets of immature plasma cells in the bone marrow as seen in multiple myeloma (MM). ATTR amyloidosis is an autosomal–dominant hereditary disease caused by various point mutations of transthyretin (TTR), or is a disease of the very old (senile type). Transthyretin is almost entirely produced in the liver and is a transport protein of thyroid hormone and retinol-binding protein.

A fourth main type of systemic amyloidosis is $A\beta_2M$ amyloidosis, which is only seen in patients with longstanding dialysis. The precursor β_2-microglobulin is excreted by the kidney, and therefore high serum concentrations are found in patients on dialysis, and kidney transplantation prevents further deposition of amyloid. Apart from these four main types, some other types of hereditary systemic amyloidosis can be encountered, such as AApoAI, AApoAII, ACys, AFib, AGel (with a focus in Finland) and ALys amyloidosis, with apolipoprotein A-I and A-II, cystatin, fibrinogen α-chain, gelsolin and lysozyme being the respective precursors [3]. All these types are very rare, and therefore we refer to the literature for an overview of the clinical features [4–6]. In patients with fibrinogen-derived amyloidosis (AFib), kidney transplantation or combined kidney and liver transplantation can be considered [7]. However, owing to their rarity, there is no consensus within the amyloid community regarding optimal treatment for these uncommon types of systemic amyloidosis. In this review, we will

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focus on the three main types: AA, AL and ATTR amyloidosis, and, in particular, AL amyloidosis, since a number of drug trials in this disease that have been published during the last 5 years.

Organ involvement in the different types of systemic amyloidosis

Disease manifestations differ among the three major forms of systemic amyloidosis. In AA amyloidosis, renal involvement is observed very frequently (in approximately 90% of cases). Less frequent manifestations are enteropathy, autonomic neuropathy, splenomegaly, hepatomegaly and cardiomyopathy. Peripheral neuropathy is extremely rare in AA amyloidosis. In AL amyloidosis, clinical manifestations can be very diverse, for example: cardiomyopathy, peripheral and autonomic neuropathy, hepatomegaly, splenomegaly, nephrotic syndrome and renal failure, diarrhea, arthropathy, carpal tunnel syndrome, glossomegaly and purpura, which usually affects the neck and periorbital area. In ATTR amyloidosis, the most prominent manifestations are peripheral and autonomic neuropathy, but cardiomyopathy, enteropathy, renal failure and eye involvement are also often observed [2].

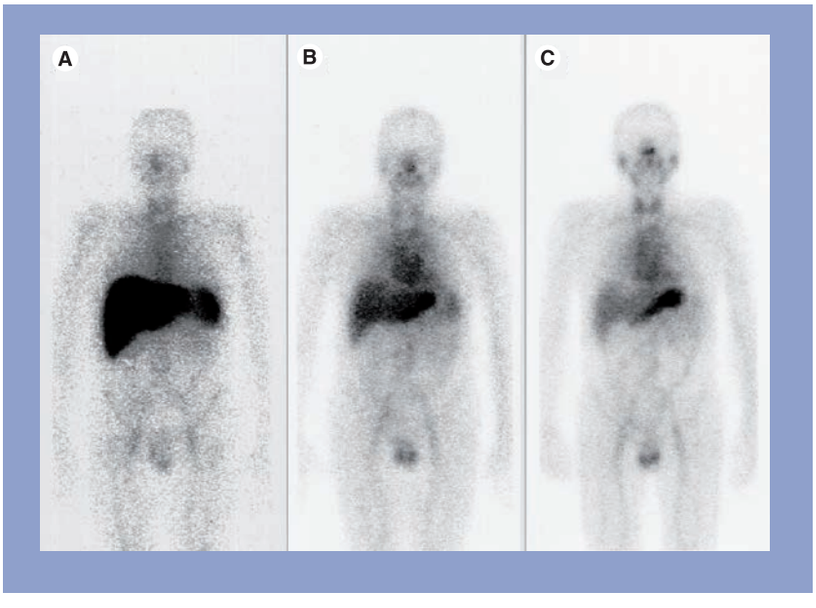

Figure 1. ^{123}I-serum amyloid P component scintigraphy of a patient with immunoglobulin light chain amyloidosis who achieved a complete hematological response after high-dose melphalan and peripheral blood stem-cell transplantation with a 2-year follow-up. Anterior total body views 24 h after administration of serum amyloid P. **(A)** At presentation: clearly increased uptake in liver and spleen, with decreased blood-pool activity in heart and large blood vessels. **(B)** After 1 year: decreased uptake in liver and spleen and some blood-pool activity visible. **(C)** After 2 years: normalization of uptake in liver and spleen, and normal blood-pool activity in heart and blood vessels. Some radioactive degradation products are visible in the stomach.

Diagnosis & typing of systemic amyloidosis

When systemic amyloidosis is suspected, diagnosis can be made by Congo red analysis of a tissue specimen showing the classic apple-green birefringence in polarized light. Rectum and gingiva are tissue sites of choice for a biopsy to look for systemic amyloidosis. Abdominal fat tissue aspiration is, in our opinion, the primary procedure, being an elegant and easy-to-perform technique, with high diagnostic sensitivity and specificity [8]. The next step is determination of the type of amyloidosis. In many cases, the medical history and clinical picture already point to one of the three types of systemic amyloidosis. In AA amyloidosis, immunohistochemistry is diagnostic, providing that sensitive and specific antibodies are applied. In ATTR and AL amyloidosis, immunohistochemistry is less reliable owing to the heterogeneity of amyloid deposits, loss of epitopes in the fibril structure and nonspecific adherence of immunoglobulins to amyloid deposits or the background [9]. The gold standard for typing amyloid is direct peptide sequencing of the amyloid [10]. In suspected AL amyloidosis, the serum free light chain (FLC) assay, which has a much greater sensitivity for finding monoclonal plasma cell dyscrasia than conventional immuno-electropheresis of serum and urine, is of great diagnostic value [11–13]. DNA analysis of the *TTR* gene to detect a TTR mutation is helpful for detecting or excluding one of the known hereditary types of ATTR amyloidosis in cases with cardiomyopathy and/or neuropathy. In cases with a clinical picture of nephropathy, DNA analysis of non-TTR mutations, such as fibrinogen, is useful to avoid misdiagnosis of AL amyloidosis [14]. Scintigraphy using ^{123}I-labeled serum amyloid P component (SAP, see below) is diagnostic in approximately 90% of patients with AA and AL amyloidosis [15–17].

Assessment of organ involvement

Before deciding on therapy, assessment of organ involvement is crucial for establishing therapeutic eligibility and for evaluation of the effect of therapy. In 2004, an internationally accepted classification of organ involvement and organ response was established [18]. Since all amyloid deposits contain SAP, scintigraphy using ^{123}I-labeled SAP (Figure 1) can show specific uptake in organs such as the liver, spleen, kidneys, adrenals, bone marrow and joints, and therefore may be of additional value [15–17]. Limitations of SAP scintigraphy are that this technique is

not available in most countries, it is not helpful for showing cardiac involvement and it is less diagnostic in ATTR amyloidosis, relating to the different distributions and burdens of amyloid in this disorder [17].

Estimating prognosis

The final step of clinical work-up is estimating prognosis. Prognosis of untreated amyloidosis is poor, with median survival being less than 1 year for AL amyloidosis and AA amyloidosis has a median survival of 2–4 years [4,19]. In AL amyloidosis, prognosis depends upon the number of organs involved and the type and severity of vital organ involvement, and especially the presence of cardiac involvement and response to therapy [20,21]. In AA amyloidosis, prognosis improves considerably after successful treatment of the underlying inflammation.

Therapeutic options

Therapy in systemic amyloidosis is aimed at reducing or eliminating the precursor-protein production, thereby prohibiting further deposition of amyloid fibrils in organs. Given the hypothesis that amyloidosis is a dynamic process of deposition and removal, resolution of amyloid deposits may be expected [15]. This has been called the 'precursor–product' concept. Since the precursor protein is different in the three major types of systemic amyloidosis, therapeutic options are different. In AA amyloidosis, therapy is aimed at effectively suppressing inflammation and thereby reducing SAA levels [22]. This can be achieved using antibiotic and surgical treatment in infectious diseases, DMARDs or biologicals in inflammatory rheumatic diseases, biologicals in inflammatory bowel diseases and hereditary autoinflammatory diseases, and colchicine in preventing AA amyloidosis in familial Mediterranean fever [23]. In AL amyloidosis, therapy is aimed at eliminating the FLC by applying chemotherapeutic regimens, as are used in MM. Owing to major organ involvement, especially cardiac involvement, these chemotherapies are often less well tolerated compared with in MM, and treatment-related mortality (TRM) is often much higher than in uncomplicated MM. Therefore, assessment of eligibility for specific chemotherapeutic regimens by assessing the number of major organs involved and the severity of organ involvement is essential.

Since transthyretin in ATTR amyloidosis is almost entirely produced by the liver, liver transplantation will result in a substantial decrease of the variant TTR concentration in the blood. This was initially reported to result in overall improvement. However, after conducting more than 1000 liver transplantations, it has become clear that in a substantial percentage of patients, disease progression is not prevented in all organ systems. In particular, amyloid cardiomyopathy may progress after liver transplantation, presumably reflecting the propensity for normal wild-type TTR to be deposited as amyloid in the heart [24–27]. Progression of amyloid deposition in the heart has been observed, particularly in patients with some non-Met30 TTR mutations [24,25].

A completely different approach is to look for new drugs that may be able to interfere with amyloid deposition or to stimulate removal of amyloid deposits.

In this review, we will briefly highlight new diagnostic modalities in systemic amyloidosis that are of importance in deciding on the type of therapy and in the follow-up of therapy. We will extensively discuss new therapeutic modalities for the different types of systemic amyloidosis that have been developed over the last 5 years. Since new modalities are primarily in the field of chemotherapy, our focus will be on the treatment of AL amyloidosis. In all types of systemic amyloidosis, adequate supportive care treatments are essential, for example, the use of salt restriction, diuretics and angiotensin-converting enzyme in nephrotic syndrome, care in using conduction-influencing drugs, implantation of a pacemaker in case of symptomatic bradycardia, adequate oral or intravenous feeding and adequate treatment of diarrhea [2].

New diagnostic modalities

N-terminal brain-natriuretic peptide & cardiac troponins

Heart involvement in AL amyloidosis is, by far, the major prognostic determinant and conditions the therapeutic strategy [20,21]. Criteria of heart involvement and response to therapy in systemic amyloidosis are defined by observing the presence of low voltage on electrocardiogram, the mean left ventricular wall thickness and using the New York Heart Association classification criteria of heart failure [18]. These criteria have unsatisfactory sensitivity and/or specificity.

New cardiac markers have been developed. Probrain natriuretic peptide (proBNP) is a propeptide produced by myocytes in response to increased ventricular wall stress. After release, it is cleaved into two fragments: the active BNP and a leader sequence, the N-terminal

proBNP (NT-proBNP). Levels of NT-proBNP are higher in women and increase with age; therefore, reference limits are different for men and women and for individuals under 50 or over 50 years of age. Dialysis causes altered metabolism of NT-proBNP.

Cardiac troponins are highly specific markers of myocardial injury. Both NT-proBNP and cardiac troponins have proved to be very sensitive and specific in determining cardiac injury and increased ventricular wall stress in AL amyloidosis, and are independent prognostic factors for survival [28,29].

A staging system using these objective and easily reproducible biochemical criteria has been developed [30]. This staging system is a predictor of survival and can be used as a prognostic determinant. In stage 1, both of the cardiac markers (troponin and NT-proBNP) are below the threshold, in stage 2, only one marker is below the threshold and in stage 3, both markers are above the threshold. Palladini *et al.* demonstrated that NT-proBNP is a rapidly reacting marker in successfully treated AL amyloidosis without overt decrease of amyloid heart deposits, suggesting that cardiac toxicity of amyloidogenic FLC is independent of amyloid load [31]. In a study of 200 patients with AL amyloidosis, a fall in NT-proBNP was significantly related to better outcome, irrespective of hematological response [32].

Therefore, cardiac troponins and NT-proBNP are an important part of the diagnostic and prognostic arsenal in assessing cardiac involvement and effect of therapy in AL amyloidosis. Cardiac troponins appear not to be a sensitive marker in ATTR amyloidosis [33]. The TTR nature of ATTR amyloidosis seems to be less harmful to cardiac myocytes than that of the light chains of AL amyloid. However, BNP is also a sensitive marker for cardiomyopathy in ATTR amyloidosis [33].

Recently, prognostic utility of serum uric acid in AL amyloidosis has been demonstrated in patients with cardiac involvement. This appeared to be independent of loss of renal function or use of diuretics [34]. The exact relationship between serum uric acid and cardiac function is still elusive.

Utility of free light chain assay in AL amyloidosis: the importance of reaching complete hematological response

Since the availability of the very specific and sensitive FLC assay, the importance of reaching a complete normalization of FLC has been demonstrated by several authors. In 2003, it was demonstrated in a group of 164 patients with AL amyloidosis, that a more than 50% reduction of FLC was associated with a substantial survival benefit compared with those patients whose FLC did not fall by half (5-year survival: 88 and 39%, respectively; $p < 0.0001$). This effect was observed regardless of the type of chemotherapy used [13].

In 2005, in a study with 66 patients with AL amyloidosis, it was demonstrated that FLC improvement correlated with complete hematological response as measured by immunofixation and bone marrow studies, but a clear advantage was that FLC improvement was more readily detected early following treatment [35].

Dispenzieri *et al.* demonstrated in 2006, in 93 AL amyloidosis patients who underwent intensive treatment with melphalan and peripheral blood stem-cell transplantation (PBSCT), that attainment of a low absolute level of FLC, rather than a percentage reduction, is the best immunoglobulin FLC predictor for hematologic response (and not FLC ratio), organ response and overall survival after PBSCT [36]. A landmark study (to eliminate bias from early death) of 213 patients who survived 6 months after stem-cell transplantation, demonstrated that achieving a complete hematological response translates to longer survival ($p < 0.001$) [37].

All these studies established the importance of FLC measurement before and following therapy, and underscore the importance of reaching a complete normalization of FLC in terms of survival benefit. Treatment strategies should be directed to complete normalization of FLC and can be guided by their early effect on serum FLC concentration. These data suggest that those patients who do not normalize their absolute FLC concentration after therapy could be considered for further therapy. Therapy should always be balanced against toxicity, and one should keep in mind that organ improvement has been noticed after reaching a partial response of the amyloidogenic light chain [38], although organ response rates are higher after reaching a complete response (CR) [13]. One should be aware that in case of renal insufficiency, absolute outcomes of FLC are unreliable and the ratio of $\kappa:\lambda$ can be used instead (ratio should be normal). Recently, high-flux hemofilters have been used in acute renal failure, aiming to increase the removal of FLCs from the circulation [39]. Whether this approach may have some additional benefit in AL patients with acute renal failure needs to be studied.

New treatment modalities

Prohibiting formation or stimulating degradation of amyloid fibrils in AA & ATTR amyloidosis

Effective suppression of inflammation in AA amyloidosis is the most effective treatment and remains the cornerstone of therapy [22,40]. Infections such as tuberculosis and leprosy should be cured completely with antibiotics. Sometimes, surgical therapy may be necessary in infections such as osteomyelitis and bronchiectasis. Chronic inflammatory diseases such as Crohn's disease, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and juvenile idiopathic arthritis can often be treated effectively with DMARDs such as methotrexate. In nonresponders to DMARDs, a new generation of drugs – so-called biologicals directed against TNFα, IL1β, CD20 (rituximab) and abatacept (CTLA4Ig, a human fusion protein that consists of a cytotoxic lymphocytic-associated type 4 antigen bound to the Fc part of the IgG1) – should be considered and may have a dramatic effect on inflammation in these chronic inflammatory diseases [41]. Familial Mediterranean fever, a hereditary autoinflammatory syndrome, has been treated effectively with colchicine for many years. Other hereditary autoinflammatory diseases, such as TNF-receptor-associated periodic syndrome, can often be treated successfully with anti-TNF drugs, and cryopyrin-associated periodic syndromes usually respond dramatically to IL1-inhibition [42]. Long-acting IL1 antagonists are currently being investigated in cryopyrin-associated periodic syndromes [43]. A recently developed and promising biological agent is tocilizumab, an IL6-receptor antagonist that directly suppresses the production of SAA [44]. Castleman's disease can be treated with tocilizumab and also with surgery [45].

If the serum concentration of the precursor SAA, an acute-phase protein, can be normalized to levels below 4 mg/l, amyloid will often regress and survival will increase. The risk of death was 17.7 times as high among patients with SAA concentrations in the highest octile (≥155 mg/l) than among those with concentrations in the lowest octile (<4 mg/l), and the risk of death was four times higher in the next-to-lowest octile (4–9 mg/l) [40].

In 2007, Dember *et al.* published a multicenter, randomized, double blind, placebo-controlled trial to evaluate the efficacy and safety of eprodisate in patients with AA amyloidosis and kidney involvement [46]. Eprodisate is a negatively charged, sulfonated molecule that has structural similarities to heparin sulfate. It is suggested that glycosaminoglycans, such as heparansulfate, are critical in the pathogenensis of amyloidosis. Interactions between amyloidogenic proteins and glycosaminoglycans promote fibril assembly and stabilize amyloid deposits in tissues. Eprodisate is designed to interfere with interactions by competively binding to precursor protein SAA, thereby prohibiting polymerization of amyloid fibrils and deposition of the fibrils in tissues. In this study, 183 patients with AA amyloidosis were randomly assigned to receive either eprodisate or placebo. Eprodisate slowed the decline of renal function in AA amyloidosis (hazard ratio: 0.58; 95% CI: 0.37–0.93; p = 0.02). There was no significant effect on progression to end-stage renal disease or on risk of death in this 2-year study.

Another approach is to stabilize the native structure of the precursor protein and thus, prevent its transition to a misfolded protein. This is under investigation using diflunisal in patients with ATTR amyloidosis. Diflunisal binds to the thyroxine-binding sites of the TTR tetramer, thereby stabilizing the protein and counteracting its tendency to transform into a β-pleated amyloid configuration [47,48]. Another drug with a similar activity of TTR tetramer stabilization that is also under study is Fx-1006A [49]. Stabilization of TTR, and thus inhibition of aggregation, can be induced *in vitro* using sulfite and epigallocatechin-3-gallate (EGCG), an extract of green teas [50,51]; however, no animal or clinical studies have been performed as of yet. EGCG has also been described to have an effect on plasma cells *in vitro* and in one case with AL amyloidosis [52,53]. A small clinical study with EGCG has been scheduled to start soon in patients with AL amyloidosis.

Drugs that stimulate degradation of amyloid fibrils are continually being found. Doxycycline appears to disrupt ATTR amyloid in a mouse model [54], and a dose-finding study has recently begun in patients with ATTR amyloidosis. Clearance of amyloid may be enhanced by active or passive immunization and also by removing SAP from the blood and amyloid deposits using R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid (CPHPC) [55]. The idea behind CPHPC is that SAP protects amyloid from degradation, and after removal of SAP, the body can begin degrading amyloid. Whether this approach will be useful in patients has not yet been studied. 4'-iodo-4'deoxydoxorubicin (IDOX) is

a drug that initially seemed to be promising as an amyloid degrading agent, but the first clinical study did not demonstrate a distinct effect of the used dose on amyloid resolution [56].

Precusor–product concept-based new treatment modalities in AL amyloidosis

Before the 1990s there was no therapy for AL amyloidosis. The first effective therapy, described in 1996 and 1997, was oral melphalan and prednisone (MP) which prolonged survival from 6–9 to 16–18 months [57,58]. This therapy is only effective in 25% of patients and another disadvantage is the slow rate of response that can take up to 6 months. Therefore, the search began for rapidly effective therapies. Since 1998, multiple studies using high-dose melphalan (HDM) or intermediate-dose melphalan (IDM) and PBSCT have been described [21,38,59–61]. The hematological response rates (53–83%) and organ reponse rates (42–83%) following HDM and PBSCT were high. The single-center study that includes the highest number of patients (n = 277, of whome 155 were treated with HDM) is a study by Skinner *et al.,* published in 2004. Median survival of the HDM-treated patients in this study was 7.8 years, with a 5-year survival rate of 61% [21]. In a case–control study, the group of patients treated with HDM and PBSCT demonstrated superior survival compared with the case–control group. The 1-, 2- and 4-year overall survival rates differed significantly (89 and 71%, 81 and 55%, and 71 and 41%, respectively; $p < 0.001$) [62]. In addition, quality of life is measurable and PBSCT resulted in sustained improvement of this important parameter [63].

The main problem of these first studies using intensive treatment turned out to be the high TRM, ranging between 12 and 43%. Therefore, criteria of eligibility have been developed for high-dose therapy. Overt cardiac involvement, involvement of more than two vital organ systems and age are the main exclusion criteria [21,64], reducing the group of patients eligible for this kind of therapy substantially. In a retrospective study by Dispenzieri *et al.,* only 18% of patients were eligible [62]. IDM followed by PSCT demonstrated lower TRM (maximum of 15%) at the cost of lower response rates (25–30%) [21,61], a lower median survival of 2.9 years and 5-year survival of 41% [21]. Improvement in TRM over the years (in experienced amyloid centers ranging from 0–5% since 2004) may be due to improved patient selection, improvement in supportive care and cumulative experience [65,66].

Whether induction therapy before HDM/IDM plus PBSCT has advantages has not yet been investigated. Two small studies with induction therapy before HDM showed good feasibility and survival outcome using 2–3 cycles of vincristine, doxorubicin and dexamethasone (VAD) [66,67], a regimen that demonstrated a rapid hematological response and at least stabilization of organ function. A randomized, controlled trial using two cycles of MP, a regimen known to take many months to be effective, as induction therapy prior to HDM plus PBSCT, demonstrated no benefit, and showed even worse survival in cardiac involvement [68].

In addition, the application of mobilization chemotherapy has not been established. Since in most cases of AL amyloidosis the plasma cell load prior to PBSCT is very low, mobilizing chemotherapy is not inevitable, although the number of collected stem cells are significantly higher than with granulocyte colony-stimulating factor (G-CSF) alone [69].

Given the high response rates with long-standing duration, the improvement in quality of life and good survival outcome, we consider intensive treatment with HDM or IDM plus PBSCT preferential therapy. Owing to the high percentage of patients not eligible for this intensive treatment, it is still imperative to search for new therapies with a rapid, long-standing response without serious adverse effects or high TRM. Below, we describe new treatment modalities over the last 5 years in AL amyloidosis. Dosages of the different treatment regimens are shown in Table 1.

High-dose dexamethasone

Dexamethasone has been demonstrated to be effective in the treatment of MM [70]. The presumed mechanism is a cytotoxic effect on plasma cells in the bone marrow. In 1999, in a Phase II trial of 19 patients (of whom 14 were pretreated), it was shown that high-dose dexamethasone was of occasional benefit in AL amyloidosis [71]. If patients responded, the regimen was changed to 40 mg less frequently – taken on days 1–4 at 4-weeks intervals. Palladini *et al.* had preliminary experience of the above-mentioned schedule of high-dose dexamethasone being rather toxic in AL patients. In a small study of 23 patients in 2001, they observed a 35% response in a median time of 4 months, without significant toxicity, using a modified high-dose dexamethasone regimen of up to eight cycles if response was recorded [72]. Both studies considered dexamethasone to be a

Table 1. Therapeutic regimens in immunoglobulin light chain amyloidosis.

Abbreviation	Drugs and dosage schedule
HDM	Melphalan intravenously: 200 mg/m^2 in 2 days intravenously
IDM	Melphalan intravenously: 100–140 mg/m^2 in 2 days intravenously
HDex	Dexamethasone orally: 40 mg on days 1–4, 9–12 and 17–20 at 4-week intervals
Modified HDex	Dexamethasone orally: 40 mg on days 1–4 at 3-week intervals or 20 mg instead of 40 mg
MDex	Melphalan: 0.22 mg/kg or 10 mg/m^2 + dexamethasone orally: 40 mg on days 1–4 every 4 weeks
TDex	Thalidomide orally: 100–400 mg continuously + dexamethasone orally: 20–40 mg on days 1–4 every 3 weeks
LDex	Lenalidomide orally: 15 mg for 21 days followed by 7 days rest + dexamethasone: 20–40 mg on days 1–4 (and 15–18) every 4 weeks
BDex	Bortezomib intravenously: 1.3 mg/m^2 on days 1,4,8 and 11 + dexamethasone orally: 40 mg on days 1–4 every 3 weeks
MTD	Melphalan orally: 0.22 mg/kg on days 1–4, dexamethasone orally: 20 mg on days 1–4 every 4 weeks + continuous thalidomide orally: 100 mg/day
CTD	Cyclophosphamide: 500 mg once a week + thalidomide orally: 100–200 mg/day + dexamethasone orally: 40 mg on days 1–4 and 9–12 every 4 weeks
CTDa	Cyclophosphamide: 500 mg on day 1,8 and 15 + thalidomide orally: 50–200 mg/day + dexamethasone orally: 20 mg on days 1–4 and 9–12

BDex: Bortezomib plus dexamethasone; CTD: Cyclophosphamide, thalidomide and dexamethasone; CTDa: Attenuated cyclophosphamide, thalidomide and dexamethasone; HDex: High-dose dexamethasone; HDM: High-dose melphalan; IDM: Intermediate-dose melphalan; LDex: Lenalidomide and dexamethasone; MDex: Melphalan plus oral dexamethasone; MTD: Melphalan, thalidomide & dexamethasone; TDex: Thalidomide plus dexamethasone.

bridging therapy to more aggressive and effective therapies and suggested that the addition of other potentially effective therapies might increase the response rate.

Melphalan & high-dose dexamethasone compared with high-dose melphalan plus peripheral blood stem-cell transplantation

In a study of 46 patients with AL amyloidosis who were ineligible for HDM and PBSCT, the addition of melphalan to oral dexamethasone (MDex) resulted in a response rate of 67%, with 33% complete remissions, without significant toxicity [73]. Extended follow-up of this study demonstrated durable response for at least 3 years in 70% of patients. In relapsing patients, the amyloid clone remained sensitive to MDex, and CR was restored by repeating treatment [74].

Given these relatively good results, debate began as to whether HDM and PBSCT should be regarded as the best therapy, in the light of MDex having both a better toxicity profile and TRM compared with HDM plus PBSCT. Randomized, controlled trials comparing different treatment regimens in AL amyloidosis are scarce. In 2007, Jaccard *et al.* published a randomized trial comparing HDM (or IDM in cases of >65 years of age, ejection fraction less than 35%, creatinine clearance less than 30 ml/min or severe liver disease) followed by PBSCT with MDex for up to 18 cycles in patients with newly diagnosed AL amyloidosis. The results were analyzed on an intention-to-treat basis with overall survival as the primary end point. A total of 50 patients were enrolled in each group and median follow-up was 3 years. Pretreatment drop-out was 13 out of 50 in the intensive melphalan group compared with two out ot 50 in the MDex group. The outcome of treatment with HDM/IDM and PBSCT was not superior to MDex. Median overall survival was even significantly longer (56.9 months) in the MDex-assigned group compared with the HDM/IDM plus PBSCT group (22.4 months; p = 0.04) [75]. A major criticism of this study was that the high TRM (24%) in the PBSCT group in this multicenter study (29 centers) was twice, or even four times, the rate of experienced single centers [76]. The 4-year survival of patients treated with HDM/IDM and PBSCT was only 40%. In the Netherlands, we conducted a prospective multicenter trial between 1996 and 2006 by the Dutch–Belgian Hemato–Oncology Cooperative Group (HOVON) in 69 previously untreated patients with AL amyloidosis. Patients were treated with induction therapy with VAD followed by intensive melphalan plus PBSCT. TRM was 11%, approximately 50% lower than that reported in the study by Jaccard *et al.* The 4-year overall survival among all our patients was 62%, and the 4-year survival rate after transplantation was 78% [67,77], substantially higher than in the study by Jaccard *et al.* As mentioned previously, TRM substantially improved after 2004, being less than 10%. To minimize criticism regarding the high TRM in the HDM/IDM group, Jaccard *et al.* performed a landmark analysis of patients surviving 6 months

after randomization who actually received their assigned treatment. This analysis demonstrated that ten out of 28 patients died in the HDM/IDM group, whereas eight out of 37 patients died in the MDex group (p = 0.38). Subdividing patients into low-risk and high-risk groups demonstrated no significant difference in outcome.

In addition, questions were raised regarding the enrolment of severely affected patients in the HDM group of the study by Jaccard *et al.* (more than three organs were affected in 36% of patients), thereby introducing bias in favor of MDex. Moreover, a substantial percentage (27%) of patients in the HDM-assigned group received a reduced dose of 140 mg/m^2 [76].

Therefore, the results of this multicenter study need to be confirmed in studies by amyloidosis centers with much experience in high-dose therapy. Furthermore, a recent study in 73 patients suggested that patients with plasmocytosis above 20% had worse overall survival and progression-free survival compared with patients with plasmocytosis of less than 20% when treated with MDex, irrespective of hematological response. This effect of plasmocytosis on overall survival and progression-free survival was not noticed in a retrospective group of 342 patients treated with HDM and PBSCT [78].

In addition, one should realize that melphalan therapy is toxic for the bone marrow and will affect stem-cell harvest. Therefore, treatment with MDex will exclude patients for intensive therapy in the future. Therefore, MDex could be used as a first-line therapy for those patients who are ineligible for HDM and PBSCT.

Thalidomide plus dexamethasone

The putative mechanism of thalidomide is believed to be by antiangiogenesis and by promoting plasma-cell death [79]. Thalidomide is effective in refractory and relapsed MM [80], and *in vitro* it overcomes drug resistance of myeloma plasma cells in synergy with dexamethasone [81]. Thalidomide as a single therapy in AL amyloidosis has been shown to be associated with a high incidence of thalidomide adverse reactions, and this low tolerability limits its dosing and applicability [82,83]. In a study of 31 patients with AL amyloidosis whose disease had been refractory to, or relapsed after, first-line therapy, thalidomide (100 mg/day with 100 mg increments every 2 weeks up to 400 mg) in combination with modified HDex (20 mg dexamethasone) was evaluated. First-line therapy had previously been MP (42%), PBSCT (32%) or HDex (26%). Even in patients who received less than 400 mg/day of thalidomide, a substantial response rate (35% hematological response) was observed. The response rate was higher among patients receiving 400 mg/day thalidomide (73% hematological response). Probably, the relatively low dose of dexamethasone reduced the response rate. Median time to hematological response was 3.6 months (range: 2.5–8.0 months). Only 35% of patients tolerated the full dose of 400 mg thalidomide. Overall, 65% of patients experienced severe (grade 3 or higher) thalidomide-related toxicity, consisting mainly of symptomatic bradycardia, fatigue and constipation. This occurred even though patients with symptomatic neuropathy had been excluded from the study. The observation in this study that 50% of patients treated initially with HDex responded to thalidomide plus dexamethasone (TDex), indicates that combination therapy with thalidomide can overcome treatment failure with dexamethasone [84].

In conclusion, this study demonstrates that TDex is effective in AL amyloidosis treatment and has the potential to induce a rapid response but, even in combination with dexamethasone, the low tolerability of thalidomide limits its applicability. TDex might be useful as an induction therapy before HDM and PBSCT, although the use of induction therapy in AL amyloidosis without the manifestation of underlying MM is debatable. In previously untreated MM, thalidomide induction treatment increased hematological response rate before and after intensive therapy with HDM and PBSCT [85].

Melphalan, thalidomide & dexamethasone

Patients with AL amyloidosis and heart failure have a very poor prognosis and need a rapidly effective treatment but cannot tolerate aggressive therapy. In MM, the addition of thalidomide to MP improves the response rate and reduces the response time to a median of 1.4 months [86]. Palladini *et al.* prospectively studied MTD in a small study of 22 patients with AL amyloidosis with advanced cardiac involvement. Eight patients (36%) obtained a hematological response and one patient demonstrated a CR. Response was reached after three cycles in 90% of patients. In four of these responders, NT-proBNP reduction of over 30% from baseline was observed at the time of hematological response. Hematological and organ response rates in all patients completing three cycles were 50 and 25%, respectively. Approximately 20% of the patients discontinued thalidomide

due to adverse reactions. Median survival was 5.3 months. Nearly all patients died of cardiac amyloidosis. Only those patients with a decrease of NT-proBNP survived longer, with a median follow-up of 28.3 months (range: 24.2–36.1) [87].

Cyclophosphamide, thalidomide & dexamethasone

In MM, the combination of orally administered cyclophosphamide, thalidomide and dexamethasone (CTD) has been evaluated, resulting in hematological response rates of 61–71% [88,89]. In a study of 75 patients with AL amyloidosis, CTD was given until a stable hematological response was reached. In patients older than 70 years of age, and in those with New York Heart Association class II, an attenuated regimen was administered (CTDa). Thalidomide maintenance therapy was considered for responders, depending on tolerance. In 31 patients, therapy was first-line and in 44 cases, therapy was for refractory or relapsed disease. Ten patients (13%) had undergone prior intensive therapy with HDM and PBSCT. A hematological response occurred in 48 of 65 evaluable patients (74%). There was no significant difference in hematological response rate between newly diagnosed or relapsed patients, although an extension of this study (in 202 patients) demonstrated a slight difference in response rate between newly diagnosed and relapsed patients (67 vs 54%, respectively), but without difference in CR rate. Median estimated survival was 41 months, and 3-year estimated survival was 100% in complete responders, 82% in partial responders and 0% in nonresponders (in the extended study of 202 patients, the respective figures were 92, 68 and 46%). Cessation of therapy due to toxicity was necessary in 8% of all patients, and toxicity was observed in 52% of grade 2 or higher patients, more often in those treated with CTD (60%) compared with CTDa (50%). In the extended study grade 3 toxicity was 21%. Therefore, the CTD combination is less toxic than TDex but strongly depends on the dosage of thalidomide used. The most common severe toxicity observed was fluid retention. TRM was 4%. Time to response was not specifically mentioned in the manuscript [90,91].

Lenalidomide with & without dexamethasone

Lenalidomide is an analogue of thalidomide, but *in vitro* it has been demonstrated to be 50–200-times more potent than thalidomide [92]. The combination of lenalidomide and dexamethasone (LDex) has proved to be a potent induction therapy in patients with newly diagnosed MM [93]. Treatment responses have been observed in patients who previously failed thalidomide treatment and fewer non-hematological side effects were observed than with thalidomide [93,94].

In 2007, two studies with lenalidomide in AL amyloidosis were published. Both studies showed the maximum tolerated dose of lenalidomide to be 15 mg, and lenalidomide alone was less effective than the combination with dexamethasone. Dispenzieri *et al.* described 22 patients with previously treated and untreated AL amyloidosis, with the median number of affected organs being two and with cardiac involvement in 64% of patients. After three cycles of lenalidomide alone, only one patient had achieved a hematological response and ten of 22 patients (45%) terminated active treatment. Dexamethasone was added to lenalidomide in the 11 remaining patients and nine out of 11 of these patients (82%) demonstrated a hematological response. Eventually, ten out of 22 patients (45%) reached a hematological response, with a median time of 6.2 months from the start of the study with lenalidomide only. Toxicity was observed in a large number of patients and only four patients could tolerate the 25 mg dose. Grade 3 or higher toxicity was mainly due to cytopenia, and to a lesser extent was due to skin rash, edema, dyspepsia, trombosis and fatigue. In 23% of patients there was a grade 1 neuropathy [95].

Sanchorawala *et al.* described 34 patients with AL amyloidosis of whom 38% had cardiac involvement. A total of 31 patients (91%) had received previous chemotherapy – 56% had received HDM and PBSCT and 21% thalidomide. Study protocol was similar to the study by Dispenzieri *et al.* [95], with the exception of an intermediate dose of dexamethasone instead of high-dose dexamethasone being added after three cycles of single-agent lenalidomide without hematological response.

In addition, aspirin was given as standard treatment to prevent thrombo–embolic complications. Evaluation after three cycles of therapy was available in 24 patients. Hematological response was seen in seven out of 24 patients (29%) treated with lenalidomide alone, and another nine patients (38%) responded to the combination with dexamethasone. The overall hematological response rate was 16 out of 34 (47%). Median time to respond was 6 months from the start of lenalidomide-only treatment. Toxicity

was similar to the study by Dispenzieri, but respiratory infections unrelated to neutropenia were observed in 50% of patients. The authors suggested adding antibiotic prophylaxis in treatment protocols [96]. Nearly identical results were obtained in a recent study of 40 patients with refractory or relapsed AL amyloidosis [97].

Overall, LDex is effective in AL amyloidosis, and because of the different toxicity profile compared with thalidomide and bortezomib, lacking neuropathy and less frequent fluid retention, this is a good regimen for patients with neuropathy and probably for patients with cardiac involvement.

Bortezomib plus dexamethasone

Bortezomib is a proteasome inhibitor that downregulates the NF-κB pathway and enhances sensitivity of cancer cells to traditional chemotherapy *in vitro* [98]. In addition, proteasome inhibitors are bound to cause accumulation of misfolded secretory proteins in the endoplasmic reticulum (ER) and hence cause ER stress. The additional stress imposed on the ER machinery by amyloidogenic light chains may increase the sensitivity of amyloidogenic plasma cells to bortezomib [99].

In MM, the addition of dexamethasone to bortezomib (BDex) results in superior outcome [100]. Kastritis *et al.* treated 18 AL amyloidosis patients with with BDex for up to six cycles. Dose reduction depended on toxicity. A total of 11 patients (61%) had been pretreated. The majority of patients had cardiac involvement (81%). It is of note that the median bone marrow plasmocytosis was 20%, suggesting a high proportion of patients suffered from MM. Hematological response was observed in 15 out of 16 evaluatable patients (94%) and seven (44%) showed complete hematological response. Median time to response was 0.93 months (range: 0.7–1.5 months). Median follow-up was 9.5 months (range: 0.7–21 months). Median survival was not reached, with 14 patients (78%) alive 3.5–21 months after initiation of therapy. The main reason for discontinuation of therapy was bortezomib toxicity (mainly neurotoxicity), fatigue, peripheral edema, constipation, exacerbation of postural hypotension and thrombocytopenia. Dose reduction was necessary in seven patients (39%) and therapy had to be discontinued in one patient due to neurotoxicity [101]. In an extended multicenter study of 94 patients, of whom 81% had been pretreated, hematological response was 71%, including CR in 25%. Most patients had responded after the third cycle. With the median follow-up of 12 months (range: 0.5–48), estimated 1-year and 2-year survival rates were 78 and 71%, respectively [102]. In a study of 20 patients with active hematological disease, despite three lines of previous therapies including thalidomide, BDex was capable of achieving 15% complete and 65% partial hematological responses. In 40% of patients, bortezomib had to be discontinued owing to toxicity [103].

These studies demonstrate very promising results, with a high percentage of hematological response and a notable rapid response. Toxicity and unknown duration of response makes this regimen a less suitable first-line therapy. The main problem is the cumulative, dose-related (and reversible) neurotoxicity, which limits its applicability.

Other therapies

Several other therapeutic regimens have been described, mostly consisting of combinations of previously mentioned regimens.

A study of tandem cycles of intensive melphalan and PBSCT enrolled 62 patients with AL amyloidosis. Patients were treated with HDM and PBSCT. After 6 months, hematological response was evaluated. Patients with persistent plasma-cell dyscrasia and without grade 4 toxicity received a second cycle of IDM and PBSCT. A total of 25 patients (40%) had cardiac involvement. Nine patients (15%) had been pretreated. A total of 53 patients (85%) actually received HDM and PBSCT. Four patients died within 100 days of the first HDM and PBSCT treatment (TRM: 8%). Of the 49 assessable patients, 27 (55%) achieved a complete hematological response. Of the 22 patients without complete hematological response, 17 received a second cycle of IDM and PBSCT. The other five patients did not participate owing to patient choice (n = 4) and excessive nonhematological toxicity (n = 1). TRM of the second cycle was 6% (1/17). Overall TRM was 9% (5/53). A hematological response was achieved in five out of 16 patients (31%) after the second cycle. Overall hematological response rate was 67% (32/48) for surviving patients in whom treatment response could be assessed, and 60% (32/53) for all patients starting treatment in this trial. This is substantially higher than that observed in previous single-cycle HDM and PBSCT (42%) [21]. A higher percentage of pulmonary toxicity, culture-negative fever and septicemia were observed after the second cycle than after the first cycle. Overall median

survival for all 62 patients enrolled in the study was not reached, with a median follow-up being 43 months (range: 18–74). The survival compares favorably with a matched control cohort of 131 patients treated with a single cycle of HDM and PBSCT in earlier trials from 1994–2006 in the same center ($p = 0.042$). This survival difference is partly due to patient selection and improvement in supportive care, as TRM in this trial is relatively low [104].

Cohen *et al.* recently published a Phase II trial of risk-adaptive, intensive melphalan and PBSCT followed by adjuvant TDex for 9 months in patients with persisting clonal plasma-cell disease 3 months after PBSCT treatment. The authors treated 45 patients with risk-adaptive PBSCT using 100, 140, or 200 mg/m^2 melphalan in a highly selected patient group. The maximum number of organs involved was two (in 31% of patients) and cardiac involvement was seen in 24% of patients. TRM was low (4.4%) at the cost of a relatively low hematological response rate of 28%. Adjuvant therapy was started in 31 patients, of whom 16 (52%) completed 9 months of treatment. Improvement of hematological response was noticed in 13 patients. By intention-to-treat, overall hematological response rate was 71% (CR: 36%). With a median follow-up of 31 months, 2-year survival was 84%. These results of adjuvant TDex are promising, reducing TRM of melphalan and PBSCT. However, one might criticize this study based on the fact that patient selection was strict [105].

An extended study was recently described giving early (2–3 months after PBSCT) adjuvant treatment to patients with persisting hematological disease after HDM plus PBSCT (ten patients received Dex alone, 21 patients received TDex and 11 patients were given BDex). Patients with adjuvant treatment were more frequently hospitalized, particularly for infection and congestive heart failure, but an improved hematological response was demonstrated in 57%, with CR in 31% of patients. Of note, ten out of 11 patients receiving BDex demonstrated hematological response [106].

Schonland *et al.* described the results of allogeneic and syngeneic hematopoietic cell transplantation in 19 patients with AL amyloidosis. Indications for allo-transplantation were young age and relapsed or refractory disease. Previous therapy was HDM and PBSCT in seven patients. One patient died before engraftment. Overall TRM was 40%. Hematological response was seen in eight patients and after median observation of 31 months of surviving patients, only one relapse occurred. Limitations of this study are the retrospective nature, with inclusion of patients between 1991 and 2003 [107]. Given the high TRM and the availability of new treatment options such as thalidomide, lenalidomide and bortezomib, this treatment should only be considered as a rescue therapy after relapsed or refractory disease, after all current treatment options have been considered.

Chosing the optimal therapy in AL amyloidosis

Owing to the low incidence of AL amyloidosis, large studies are scarce and hardly any randomized, controlled studies comparing different therapeutic regimens have been conducted. Comparison of studies is difficult owing to differences in patient characteristics and pretreatment. Organ involvement can be very diverse and although there are internationally accepted criteria for organ involvement and treatment response, the various studies all have their own minor modifications. In studies with new treatment modalities, new, pretreated and relapsed patients are often included in the same study. Follow-up in some studies is relatively short and therefore, the duration of hematological and organ responses are not known. Therapy should ideally provide a quick, complete and longstanding response of FLC without side effects. Optimal therapy in an individual patient with AL amyloidosis has to be tailor-made. Treatment should be adjusted depending on type and severity of organ involvement, age, pretreatment and possible side effects of each individual therapy.

The best studied therapy, in terms of size and follow-up, is intensive chemotherapy with HDM or IDM followed by PBSCT. This treatment demonstrates high and durable hematological and organ response rates and substantial survival. It is the only therapy that has demonstrated a clinically relevant improvement in quality of life [63]. The main problem concerning this therapy is the relatively high TRM rate, and therefore, only a minority of patients are eligible to receive this therapy. Although the study by Jaccard *et al.* [75] demonstrates good outcome of MDex treatment, there are serious doubts as to whether this should become the first-line treatment of choice for patients who are eligible for HDM and PBSCT. Whether MDex is indeed an equal, or even better, first-line treatment must be confirmed in a second study conducted by one of the main centers. A disadvantage is that the use of MDex will reduce the potential number of

harvested peripheral stem cells. Therefore, at this time point we consider HDM and PBSCT to be the first-line therapy of choice for those patients eligible for HDM and PBSCT. However, this should be balanced against the risk of toxicity of this treatment. It is unknown whether induction therapy preceding HDM and PBSCT may have additional value. In contrast to MM, in most patients with AL amyloidosis there is a plasma-cell dyscrasia of very low grade without the malignant sheets of immature plasma cells as in MM. Studies with induction with VAD, followed by HDM and PBSCT, did not show superior overall response rates, although VAD was capable of inducing a quick and clear hematological response, and morbidiy and mortality were low in one study [38] but distinct in another [108]. A randomized trial using two cycles of MP as induction therapy showed no benefit, and even a survival disadvantage, in patients with cardiac involvement, which is not surprising considering that MP requires more time to be effective [68].

Induction therapy can be used as bridging therapy to HDM and PBSCT, and often has the advantage of being easy to perform while organizing HDM and PBSCT. Upfront use of newer treatment options such as TDex, LDex, BDex or combinations might increase the number of patients eligible for HDM and PBSCT. Melphalan- or cyclophosphamide-containing regimens are less attractive because they might influence the harvesting of stem cells. Therefore, patients who are not yet eligible for HDM and PBSCT at diagnosis but are reaching a hematological and organ response on upfront treatment might become eligible for HDM and PBSCT. Those patients who are definitely not eligible for PBSCT owing to clinical status (overt cardiac involvement, severe neuropathy and WHO performance score of 3 or higher) or age, are suitable candidates for melphalan- or cyclophosphamide-containing regimens.

Conclusion

Systemic amyloidosis has been a frightening and puzzling disease for patients and doctors for a very long time, particularly owing to the obscure nature of its appearance and progression. The chemical characterization of the different types, followed by the quantification of serum precursor proteins, especially SAA and free κ and λ light chains, provided a better understanding of the dynamic process of amyloid deposition and removal. Monitoring of the serum precursor protein SAA in AA amyloidosis is a good predictor of survival. SAA should play a more prominent role in the evaluation of new drugs for treating inflammatory diseases that may cause AA amyloidosis. Many new drugs, in particular the new class of biologicals, provide fascinating new tools that interfere deeply with the mechanisms of inflammation. Therefore, the prospects of patients with AA amyloidosis may improve in the near future.

Liver transplantation probably has a beneficial effect on survival and quality of life in patients with ATTR amyloidosis, although the long-term benefit is not yet known. Patients with the TTR-Met30 mutation seem to do better than patients with other TTR mutations. Therefore, new drugs interfering with amyloid deposition need to be developed and studied. Two trials are underway in which two such drugs, diflunisal and Fx-1006A, are currently being investigated.

The FLC assay has enabled monitoring of the serum precursor protein in patients with AL amyloidosis. Another major step forward is the development of the Tours consensus criteria for assessing organ involvement, hematological response and organ response. These criteria will enable the amyloid community to communicate better and compare the results of different studies. SAP scintigraphy is another useful way to monitor the course of amyloidosis in individual patients.

Figure 2 shows a flow diagram of a proposed treatment algorithm based on our opinion and on the studies of different regimens in AL amyloidosis. We feel that HDM/IDM plus PBSCT may be the best option for those patients eligible for this regimen, and therefore, we have chosen to give this option a central position in this flow diagram. Eligibility for this treatment has to be established using an algorithm as published by Skinner *et al.* [21], or using criteria of national or international boards. We also feel that, although evidence in AL amyloidosis is still lacking, three cycles of induction therapy upfront and PBSCT may be considered, especially in overt MM, to treat the underlying disease rapidly or possibly increase the number of patients eligible for HDM and PBSCT. The choice of induction therapy depends on patient characteristics and availability. For instance, in patients with neuropathy and autonomic dysfunction, TDex and BDex are less attractive and these patients should receive LDex. Patients with renal insufficiency can deteriorate using LDex, and for these patients, TDex or BDex may be a better choice. The dosage of thalidomide depends on tolerability, and in our experience, 100 mg is, in many patients, the highest dose tolerated. The starting dose of lenalidomide should not exceed 15 mg since higher doses

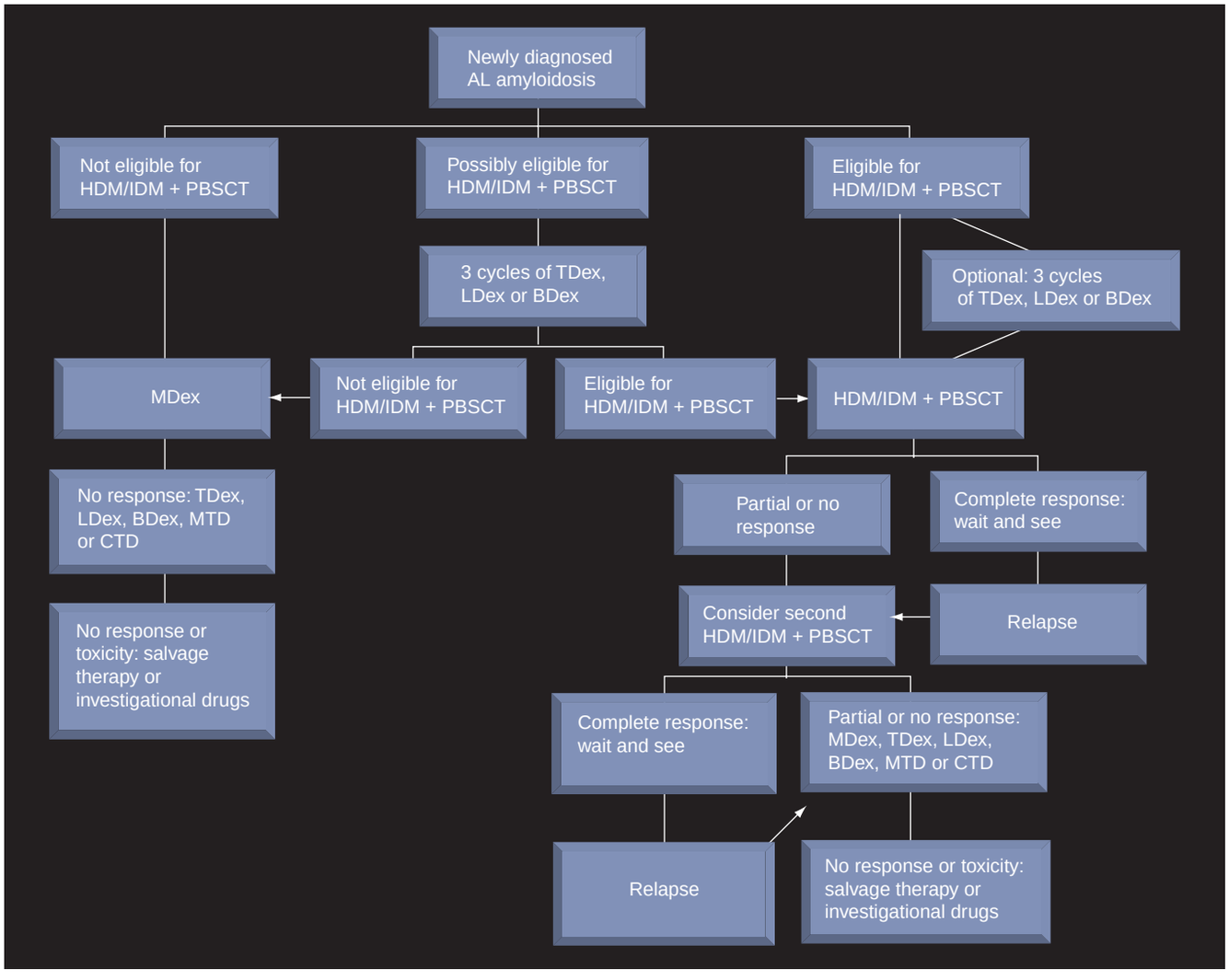

Figure 2. Proposed treatment algorithm based on the studies of different regimens in immunoglobulin light chain amyloidosis amyloidosis. In our opinion the primary aim in this schedule should be high-dose/intermediate-dose melphalan plus peripheral blood stem-cell transplantation if possible, and induction therapy may be considered upfront.
BDex: Bortezomib plus dexamethasone; CTD: Cyclophosphamide, thalidomide and dexamethasone; HDM: High-dose melphalan; IDM: Intermediate-dose melphalan; LDex: Lenalidomide and dexamethasone; MDex: Melphalan plus oral dexamethasone; MTD: Melphalan, thalidomide & dexamethasone; PBSCT: Peripheral blood stem-cell transplantation; TDex: Thalidomide plus dexamethasone.

were severely toxic in a majority of patients. The dosage of dexamethasone mainly depends on the severity of cardiac involvement. Dosage must be adjusted according to toxicity. Since the duration of response of these regimens is still unknown and toxicity is nearly always present and cumulative, we recommend HDM and PBSCT, even if CR is obtained. Stem-cell collection is obtained using G-CSF alone or, especially in cases of persistent MM after induction therapy, with cyclophosphamide (3 g/m^2) and G-CSF. Mobilizing chemotherapy might positively influence the number of collected stem cells. One should try to collect enough stem cells for a possible second PBSCT. Subsequently, HDM, or IDM in cases of creatinine clearance of less than 40 ml/min or severe liver disease, followed by peripheral stem-cell reinfusion is applied. If patients obtain a CR, no further therapy is given. If there is a partial response, no response or a relapse after reaching a CR, one might consider a tandem IDM and PBSCT, or change to primary salvage therapy. Which regimen is superior depends on patient characteristics and the effects of previously applied therapy. In this situation, the melphalan- or cyclophosphamide-containing regimen, such as MDex, should also be considered. Those patients ineligible for HDM/IDM and PBSCT will probably benefit most from MDex, since this regimen has been shown to be effective with durable response and

Executive summary

Prerequisite for adequate treatment is a proper diagnostic & prognostic work-up

- Diagnosis of amyloid in tissue using Congo-red dye:
  - Preferably use abdominal fat tissue aspirate.
- Typing the amyloid can be acheived using the following techniques:
  - Medical history and clinical picture.
  - Immunohistochemistry – highly specific for amyloid A amyloidosis (AA) only.
  - DNA analysis of *TTR* gene to detect or exclude hereditary transthyretin-related amyloidosis (ATTR).
  - Serum free light chain assay to detect low grade plasma-cell dyscrasia.
  - Mass spectrometry for unambiguous and reliable typing of amyloid.
- Assessment of organ function:
  - Using established international consensus criteria [18].
  - Using serum amyloid P component scintigraphy if available.
- Estimating prognosis and risk factors:
  - Consider the number, type and severity of organ involvement.
  - Cardiac involvement is an important negative prognostic factor.

New modalities for diagnosis & follow-up

- N-terminal probrain natriuretic peptide and troponin for assessing (severity of) cardiac involvement.
- Free light chain assay for detecting low-grade plasma-cell dyscrasia and for early monitoring of effect following therapy.

Current treatment modalities

- Primary treatment in the field of precursor-lowering therapy:
  - Chemotherapy in immunoglobulin light chain amyloidosis (AL).
  - Liver transplantation in ATTR amyloidosis.
  - Elimination of underlying inflammation in AA amyloidosis: aim is to achieve and maintain serum serum amyloid A protein below 4 mg/l.
    - Biologicals and DMARDs, either single drugs or combination therapy, can successfully control inflammation in chronic rheumatic and other inflammatory diseases.
    - Colchicine in familial Mediterranean fever.
    - Effective antibiotic and surgical treatment of chronic infections.
    - Surgical excision of Castleman's disease.

Novel treatment modalities

- Influencing stabilization and degradation of amyloid deposits:
  - Has a proven effect:
    - Eprodisate slowed the decline of renal function in AA amyloidosis.
  - Currently under study:
    - Diflunisal, FX-1006 and doxycycline in ATTR amyloidosis.
    - IDOX in AL amyloidosis?
    - CPHPC in all types of amyloidosis.
- Precursor-lowering regimens in AL amyloidosis:
  - High-flux hemofilters to remove free light chains in acute renal failure (experimental).
  - Owing to the low incidence of AL amyloidosis, studies are scarce. Therefore, only relatively small open-label studies are available for comparison. Recent placebo-controlled clinical trials are lacking.
  - High-dose/intermediate-dose melphalan and peripheral blood stem-cell transplantation is currently the best studied regimen with a high percentage of response, and it is the only regimen demonstrated to have a clinically relevant improvement in quality of life.
  - Melphalan plus oral dexamethasone:
    - High percentage of hematogical response.
    - Debate continues regarding its use as first-line treatment.
    - Negatively influences future stem-cell harvesting.
  - Thalidomide, lenalidomide and bortezomib:
    - Effective in combination with dexamethasone (thalidomide plus dexamethasone, lenalidomide and dexamethasone and bortezomib plus dexamethasone).
    - Applicability is limited by tolerability, particularly fluid retention and neuropathy by thalidomide and bortezomib, and renal insufficiency by lenalidomide.
  - Place of induction therapy before high-dose/intermediate-dose melphalan and peripheral blood stem-cell transplantation, adjuvant therapy thereafter and tandem cycles of intermediate-dose melphalan has to be determined.
  - The choice of therapy is based on individual patient characteristics and risk factors: therapy has to be tailor made.

less overt toxicity compared with TDex, LDex and BDex. If no response is obtained, other regimens should be tried, guided by organ involvement and individual patient characteristics.

Future perspective

The development of drugs that are able to interfere with amyloid deposition and enhance degradation might be the most promising goal for the future. However, new animal models and cell-culture models for amyloidogenesis of all three main types of amyloid will need to be developed first to facilitate thorough evaluation of such new drugs before introducing them into human studies. Until these new drugs become available, drugs lowering precursor protein will remain the backbone of therapy over the next few years. Therefore, the search for rapidly acting, long-lasting, safe and more potent precursor-lowering drugs will continue. In AA amyloidosis, new biologicals with diverse mechanisms of action will be studied. Owing to these diverse mechanisms of action, the chances of eliminating inflammation in the different underlying inflammatory diseases will increase. In AL amyloidosis, schedules with different time sequences and combinations of drugs will be compared for relevant outcome measures, such as time to hematological response, organ response, duration of responses, quality of life and survival. In ATTR amyloidosis early use of TTR tetramer-stabilizing drugs in carriers of the mutation who are still in a presymptomatic phase of the disease will be studied after proven effectiveness of these drugs in symptomatic patients.

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