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NEPHROLOGY FORUM

Emerging treatment approaches for the systemic amyloidoses

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CASE PRESENTATION

Patient 1

A 57-year-old man was diagnosed with AL amyloidosis after presenting with nephrotic syndrome. He had been well until 6 months earlier, when he noticed intermittent ankle swelling. He sought medical attention approximately 2 months later because of increased swelling of his legs and progressive fatigue.

At that time, his urinary protein excretion was 7.4 g/day. Serum creatinine was 0.9 mg/dL and serum albumin 3.1 g/dL. A renal biopsy disclosed amorphous material in the mesangium that was birefringent under polarized light when stained with Congo red dye. Immunofluorescence was positive for lambda light chain in the glomerular capillary walls, mesangium, and tubulointerstitium. Electron microscopy revealed randomly dispersed fibrils with a diameter of 10 nm throughout the mesangium and interstitium. A monoclonal IgG lambda protein and a monoclonal free lambda light chain were evident by immunofixation electrophoresis (IFE) of the serum and urine, respectively. Bone marrow biopsy had 5% plasma cells with lambda light chain predominance. Additional clinical evaluation was remarkable for orthostatic hypotension and hepatomegaly. Alkaline phosphatase was 480 U/L; other liver enzymes were normal. An electrocardiogram showed normal voltage, and ventricular wall thickness was normal by echocardiography.

Three months later, the patient underwent treatment with high-dose intravenous melphalan (200 mg/m^2) and autologous peripheral blood stem cell transplantation. Just prior to treatment, the urinary protein excretion was 10 g/day and serum creatinine 1.3 mg/dL. The peritransplant course was notable for anasarca, mucositis, a transient increase in serum creatinine to 3.2 mg/dL, and a neutropenic fever. Six months after treatment, a bone marrow biopsy showed fewer than 5% plasma cells with no light chain isotype predominance. The monoclonal immunoglobulin protein was no longer evident by serum or urine IFE. Together, these findings suggested a hematologic remission, and annual evaluations during the subsequent 4 years have shown no evidence of a recurrence of the plasma cell dyscrasia. Urinary protein excretion decreased progressively to 3.0 g/day, 1.1 g/day, 0.7 g/day, and 0.3 g/day at years 1, 2, 3, and 4, respectively. Serum creatinine concentration has fluctuated between 1.0 and 1.3 mg/dL during the 4 years following treatment. Serum alkaline phosphatase was 170 U/mL at the most recent visit, and hepatomegaly was no longer appreciable by physical examination.

Patient 2

A 42-year-old woman with a family history of amyloidosis was evaluated after amyloid was identified in a duodenal biopsy specimen. The patient's mother had systemic amyloidosis due to a mutation in transthyretin (TTR) that resulted in a substitution of methionine for valine at amino acid position 30 (Val30Met). The disease manifestations in the mother included sensorimotor neuropathy, autonomic neuropathy, cardiac disease, and subnephrotic-range proteinuria. The mother's symptoms began at approximately 40 years of age, and she died in 1980 at age 53.

The patient (that is, the daughter) was found to have subnephrotic-range proteinuria one year earlier and

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Key words: serum amyloid A, transthyretin, familial amyloidosis, serum amyoid P, GAG, AA amyloidosis, AL amyloidosis, stem cell transplant.

Table 1. Types of amyloidosis

Disease	Precursor protein	Amyloid protein	Organ involvement
Systemic forms			
AL amyloidosis	Immunoglobulin light chain	AL	Kidney, heart, liver, GI tract, spleen, nervous system, soft tissue, thyroid, adrenals
AA amyloidosis	Serum amyloid A (SAA)	AA	Kidney, liver, GI tract, spleen, autonomic nervous system, thyroid
Familial amyloidosis	Transthyretin, apolipoprotein AI, apolipoprotein AII, fibrinogen Aα chain, lysozyme, gelsolin, cystatin C	ATTR, AApoAI, AApoAII, AFibA, ALys, AGel, ACys	Varies with amyloid protein and mutation. Kidney affected in TTR, ApoAI, Apo AII, FibAα, and Lys disease
Senile systemic amyloidosis	Transthyretin (wild-type)	ATTR	Heart, soft tissue
Dialysis-related amyloidosis	Beta-2 microglobulin	Aβ2M	Periarticular tissue, bone
Localized forms			
Localized AL	Immunoglobulin light chain	AL	Tracheobronchial tree, bladder, ureter
Alzheimer's disease	Aβ protein precursor	Aβ	Brain
Creutzfeldt-Jakob disease	Prion protein	APrP	Brain
Type 2 diabetes mellitus	Islet amyloid polypeptide	AIAPP	Pancreas

underwent abdominal fat aspiration that was negative for amyloid by Congo red staining. Upper endoscopy was performed because of vitamin D deficiency, and amyloid was present in the duodenal biopsy specimen obtained during the procedure. The patient felt well and had no symptoms other than supraventricular tachycardia that began shortly after the birth of her fourth child and was controlled with atenolol. Physical examination that included a detailed evaluation by a neurologist was unremarkable. Electrocardiogram revealed left anterior fascicular block with normal voltage. Echocardiography disclosed that wall thickness, valves, and ventricular function all were normal. Serum creatinine was 1.0 mg/dL, and a 24-hour urine collection contained 1.8 g protein. Isoelectric focusing studies of the serum revealed abnormal migration of TTR protein, and subsequent DNA studies disclosed the presence of a Val30Met TTR mutation. At present, the patient is being followed closely in anticipation of orthotopic liver transplantation when the manifestations of amyloidosis become more pronounced.

DISCUSSION

DR. LAURA M. DEMBER (*Associate Professor of Medicine, Renal Section, and Renal Section and Amyloid Treatment and Research Program, Boston University School of Medicine, Boston, Massachusetts*): These cases describe two patients with systemic amyloidosis. Patient 1 has amyloid light chain (AL) amyloidosis, and Patient 2 has familial amyloidosis of the TTR type. The patient with AL amyloidosis underwent treatment with high-dose chemotherapy and autologous stem cell transplantation, and the patient with TTR amyloidosis is being followed closely in anticipation of future orthotopic liver transplantation. Although these treatment approaches appear to be quite different, they are similar in that they both target the source of the amyloidogenic protein. In AL amyloidosis, the source of the amyloidogenic protein is clonal plasma cells in the bone marrow, and in familial TTR amyloidosis, the source is the liver. In addition, both treatments are aggressive approaches that have substantial toxicity but offer the possibility of fully eliminating new amyloid production.

Targeting the source of the amyloidogenic protein has been the most widely employed and most successful treatment approach for the systemic amyloidoses to date. However, with advances in the understanding of the processes involved in amyloid fibril formation and tissue deposition, treatments directed at other targets are being developed, and some are being tested in clinical trials. This Nephrology Forum will focus on current and emerging approaches for the treatment of the systemic amyloidoses. My discussion of treatment will be organized around the therapeutic targets rather than the specific type of amyloidosis.

Classification and clinical features

The amyloidoses are a group of diseases in which proteins that are normally soluble deposit extracellularly in tissues as insoluble fibrils. The fibrils have a characteristic beta-pleated sheet configuration that renders them avid for Congo red dye. Classification of the amyloidoses is based on the precursor proteins that form the amyloid fibrils, and the distribution of amyloid deposition as either systemic or localized (Table 1) [1]. In systemic amyloidosis, the amyloidogenic protein is produced at a site distant from the sites of deposition. In contrast, in localized forms (for example, Alzheimer's disease), the amyloid deposition occurs at the site of production of the

amyloidogenic protein. Here we will focus on the systemic amyloidoses.

AL amyloidosis

AL amyloidosis, the most common of the systemic amyloidoses, reportedly affects 5 to 12 persons/million/year, although autopsy studies suggest that the actual incidence might be higher [2]. The amyloidogenic protein in AL amyloidosis is an immunoglobulin light chain or light chain fragment produced by clonal plasma cells in the bone marrow. The plasma cell burden is usually low and typically comprises 5% to 10% of the cells in the bone marrow [3]. However, approximately 10% to 15% of patients with AL amyloidosis have associated multiple myeloma [4].

In AL disease, amyloid deposition can occur in any organ except for the central nervous system. The organs most frequently involved are the kidney and the heart [4, 5]. Kidney involvement usually manifests as nephrotic syndrome and progressive impairment of renal function. Not all patients with renal involvement have proteinuria. Amyloid deposition that is restricted to the renal vasculature or tubulointerstitium reduces the glomerular filtration rate but causes minimal proteinuria. Amyloid deposition in the myocardium results in a restrictive cardiomyopathy. The left-ventricular wall is concentrically thickened with normal or reduced cavity size. The ventricular ejection fraction can be normal or only modestly decreased despite substantial amyloid infiltration, but impaired ventricular filling limits cardiac output [6]. Low voltage on the electrocardiogram is often present and reflects the infiltrative, rather than a hypertrophic, basis for the ventricular wall thickening. Liver involvement produces hepatomegaly that can be massive. Elevation in alkaline phosphatase with only a mild elevation in transaminases is characteristic of hepatic amyloidosis, in which infiltration of the sinusoids rather than direct hepatocyte injury occurs [7]. Autonomic nervous system disease can produce severe orthostatic hypotension and early satiety from delayed gastric emptying. Symmetric sensory neuropathy that progresses in a distal to proximal pattern is the usual manifestation of peripheral nervous system involvement. Soft tissue amyloid deposition produces carpal tunnel syndrome, skin nodules, periarticular infiltration, alopecia, nail dystrophy, macroglossia, submandibular gland enlargement, and hoarseness. Macroglossia is extremely rare in the non-AL amyloidoses. Other sites of amyloid deposition in AL disease include the lung, pleura, thyroid gland, and adrenal glands [4, 8].

The rate of disease progression is variable and depends somewhat on organ involvement. Overall survival in series published in the 1990s is approximately 12 to 24 months. Patients with clinically evident cardiac involvement have a median survival of approximately 6 months [4, 9–11].

AA amyloidosis

AA amyloidosis, also referred to as secondary amyloidosis, occurs in the setting of chronic inflammatory conditions. The amyloidogenic precursor protein is serum amyloid A (SAA), an acute-phase reactant synthesized by the liver. Several different SAA proteins can form amyloid deposits [12, 13]. The most common inflammatory diseases that underlie AA amyloidosis in developed countries are rheumatoid arthritis, inflammatory bowel disease, and familial Mediterranean fever (FMF), an autosomal-recessive disorder now known to be due to mutations in the gene encoding pyrin [14]. Chronic infections such as osteomyelitis, bronchiectasis, and tuberculosis still lead to the development of AA amyloidosis in some parts of the world.

The distribution of organ involvement is somewhat more restricted in AA amyloidosis than in AL disease. Most patients with AA amyloidosis have renal involvement. The liver, spleen, autonomic nervous system, and thyroid also can be involved, but in contrast to AL amyloidosis, cardiac involvement is rare. In general, AA amyloidosis progresses more slowly than does AL disease, but the course is variable and tends to parallel that of the underlying inflammatory condition [15, 16].

Familial amyloidosis

In the familial amyloidoses, a gene mutation inherited in an autosomal-dominant manner results in a single amino acid substitution that renders a plasma protein amyloidogenic. Mutations in the TTR gene are the most common cause of familial amyloidosis. Approximately 100 TTR mutations have been identified. Most of these mutations are amyloidogenic; however, several mutations appear to be non-pathogenic [17]. A protective role of some of these non-amyloidogenic mutations is suggested by the lack of amyloid disease in compound heterozygous individuals who have inherited one TTR allele with an amyloidogenic mutation and one allele with a non-amyloidogenic mutation [17, 18]. Amyloidogenic variants of apolipoprotein AI, apolipoprotein AII, fibrinogen A α–chain, lysozyme, gelsolin, and cystatin C underlie less common forms of familial systemic amyloidosis [1].

The clinical features of familial amyloidosis vary depending on the underlying amyloidogenic protein and the particular amino acid affected by the mutation. For example, peripheral and autonomic neuropathy are the typical manifestations in patients with the Val30Met TTR mutation, while cardiomyopathy is the predominant manifestation in individuals with the Val122Ile TTR mutation [19].

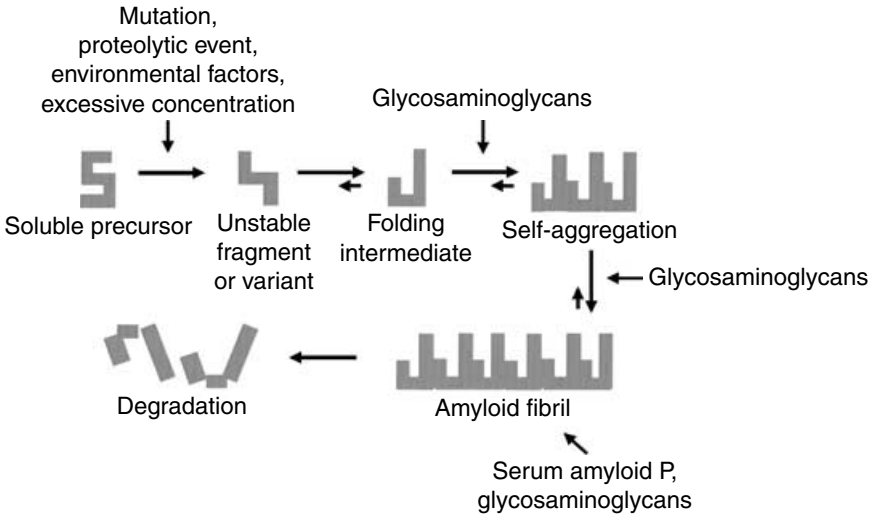

Fig. 1. Amyloid formation from precursor protein to tissue deposit. Amyloid formation begins with abnormal folding of a precursor protein. Self-aggregation of the folding intermediates leads to formation of protofilaments with high beta pleated sheet content. Amyloid fibrils consist of 4 to 6 protofilaments twisted around each other. Glycosaminoglycans and serum amyloid P component interact with amyloid precursors and/or intact fibrils promoting fibrillogenesis and stabilizing tissue deposits.

In senile systemic amyloidosis, wild-type TTR rather than a mutated form of the protein forms amyloid deposits predominantly, but not exclusively, in the heart. The disease tends to develop in elderly individuals and can result in cardiomyopathy that usually is not as severe or rapidly progressive as that associated with AL amyloidosis [20].

Dialysis-related amyloidosis

Dialysis-related amyloidosis occurs as a result of deposition of β-2 microglobulin amyloid in peri-articular tissue and bone [21]. It typically occurs in patients who have been dialysis-dependent for many years. β-2 microglobulin is amyloidogenic when present at high concentrations but not when present at physiologic concentrations. The serum concentration of β-2 microglobulin is markedly increased in patients with dialysis dependence because of reduced clearance by the kidneys, and probably also because of increased production due to dialysis-associated stimuli. Although a high concentration of β-2 microglobulin appears to be required for the development of dialysis-associated amyloidosis, other factors are likely important, as most patients with elevated serum β-2 microglobulin levels do not develop disease, and the concentration of β-2 microglobulin does not correlate with the severity of disease. Modification of β-2 microglobulin by advanced glycation end products, oxidation, or other constituents of the uremic environment might play a role in amyloid fibril formation, but evidence for this is inconclusive [21–25].

Carpal tunnel syndrome, tenosynovitis, destructive spondyloarthropathy, peri-articular soft tissue swelling, bone fractures, and arthralgias involving the shoulders, knees, wrists, and small joints of the hands are the typical clinical manifestations of dialysis-related amyloidosis. β-2 microglobulin amyloid deposits also have been identified in the gastrointestinal tract, heart, and blood vessels of patients with dialysis dependence, but the occurrence of clinically evident extra-articular disease is rare [26].

Fibril formation and tissue deposition

The process of amyloid fibril formation begins with abnormal folding of a protein that is normally soluble (Fig. 1). The abnormal folding can result from a single amino acid mutation, from a proteolytic cleavage event, or from intrinsic properties that only become pathogenetically significant when the protein is present in high concentrations or in the presence of specific local environmental factors [27, 28]. The abnormally folded intermediates aggregate into contiguous beta sheet polypeptide chains that form protofilaments with no appreciable likeness to the original precursor protein. An amyloid fibril is comprised of four to six of these protofilaments twisted around each other. One of the remarkable aspects of amyloidogenesis is that despite the marked diversity in structure and function of the amyloidogenic precursor proteins, amyloid fibrils are morphologically indistinguishable. The electron microscopic appearance and size of the fibrils, the x-ray diffraction pattern, the beta-pleated sheet configuration, and the ordered intercalation of Congo red dye that confers birefringence under polarized light are common to all amyloid fibrils [28]. In addition, all types of amyloid deposits, irrespective of the fibril type, contain constituents including serum amyloid P (SAP) protein, heparan sulfate proteoglycan, apolipoprotein E, laminin, and type IV collagen [29, 30].

Local factors influence both amyloid fibril formation and tissue deposition. The composition of basement membranes or extracellular matrix can contribute to targeting of amyloid to specific tissues. Glycosaminoglycan (GAG) moieties of proteoglycans appear to promote

fibrillogenesis by stabilizing or inducing conformational changes in amyloidogenic precursors that favor fibril formation, and by providing protection from proteolysis during fibril formation and after tissue deposition [31–35]. Local pH can affect the relative stabilities of the abnormal and normal conformations of the precursor protein and thus favor or retard fibrillogenesis. Experimental models suggest that amyloid fibrils themselves act as "seeds" within tissues, generating additional fibril formation by promoting amyloidogenic conformational changes in the soluble precursor protein [36].

Mechanisms underlying clinical disease

Two mechanisms have been proposed to explain organ dysfunction in amyloidosis. The first, sometimes referred to as the "amyloid hypothesis," is the direct disruption of tissue architecture and function by amyloid accumulation. That large quantities of deposited amyloid would have a deleterious impact on the surrounding tissue is easily appreciated from histologic examination of affected organs. In the kidney, the mesangium often appears to be essentially replaced with amyloid, and marked alterations in the components of the glomerular basement membrane are readily apparent by ultrastructural examination. In the heart, amyloid infiltration causes thickening and stiffening of the ventricular wall; these changes result in impaired diastolic filling and reduced stroke volume.

Several observations suggest that processes other than displacement of normal structures also are important contributors to the manifestations of amyloidosis [27]. Demonstrations of interactions between AA or TTR amyloid fibrils and the receptor for advanced glycosylation end products (RAGE), and induction of specific signal transduction pathways by these interactions, suggest that local cellular activation might contribute to the pathogenesis of amyloidosis [37, 38]. Additionally, discrepancies between structural findings and clinical outcomes indicate that the amount of amyloid accumulation does not fully account for disease manifestations. For example, despite indistinguishable echocardiographic features among patients with AL and TTR cardiac amyloidosis, the severity of heart failure and the cardiac mortality rate are substantially greater in AL compared with TTR disease [39]. Direct toxicity of amyloidogenic precursor proteins is suggested by findings that cellular oxidant stress is increased and that contractility and relaxation are impaired when cultured cardiomyocytes are exposed to amyloidogenic light chains [40]. Similarly, TTR folding intermediates and aggregates are cytotoxic in vitro [41, 42], and TTR aggregates have been detected in neuronal tissue in the absence of amyloid in patients with amyloidogenic TTR mutations [42]. The functional significance of the non-fibrillar TTR deposits in these patients is not clear. However, the possibility that precursor proteins and/or their folding intermediates are toxic has important implications for treatment strategies. Demonstrations that several amyloidogenic proteins are capable of forming ion-permeable channels have led to the hypothesis that channel formation in cell membranes underlies the cytotoxicity [43].

Table 2. Treatment targets for the systemic amyloidoses

Treatment target	Treatment approach	Treatment status
Precursor protein production or concentration	Anti-plasma cell chemotherapy	In use: AL
	Anti-inflammatory agents	In use: AA
	Liver transplantation	In use: Familial
	Renal transplantation	In use: DRA
	Hemofiltration or adsorption	In use: DRA
Fibril formation	GAG mimetics	Human studies: AA
	TTR stabilizers	Human studies: Familial
Amyloid deposits	GAG mimetics	Human studies: AA
	IDOX	Human studies: AL
	SAP depletion	Human studies: AL

Abbreviations are: AL, amyloid light chain disease; AA, amyloid A disease, DRA, dialysis-related amyloidosis; GAG, glycosaminoglycan; TTR, transthyretin; IDOX, 4′-iodo-4′-deoxy-doxorubicin; SAP, serum amyloid P.

Treatment targets

Each of the steps in the pathway from precursor protein to amyloid deposition is a potential target for treatment of the amyloidoses. Substantial progress has been made during the past several years in the design of new therapeutic approaches based on several of these targets (Table 2).

Target 1: Precursor protein production. Reducing the production of the amyloidogenic precursor protein is the mechanism of most treatments currently used in clinical practice. For AL amyloidosis, chemotherapeutic agents with activity against plasma cells, the source of the amyloidogenic light chains, have been used for the past several decades. The conventional approach, adopted from experience in treating multiple myeloma, is to administer multiple cycles of oral melphalan and prednisone. Although the efficacy of this treatment has been demonstrated in two randomized trials, the impact is modest. A hematologic complete response (CR), defined as normalization of the bone marrow and disappearance of monoclonal light chain from the blood and urine, occurs in fewer than 5% of individuals, and median survival is increased from 7 to 8 months to only 12 to 18 months [9, 10].

A more aggressive approach that also targets the source of the amyloidogenic protein is to administer melphalan intravenously at a myelo-ablative dose (140–200 mg/m^2) and follow the chemotherapy with autologous stem cell transplantation to facilitate bone marrow recovery [3]. The experience with this treatment has

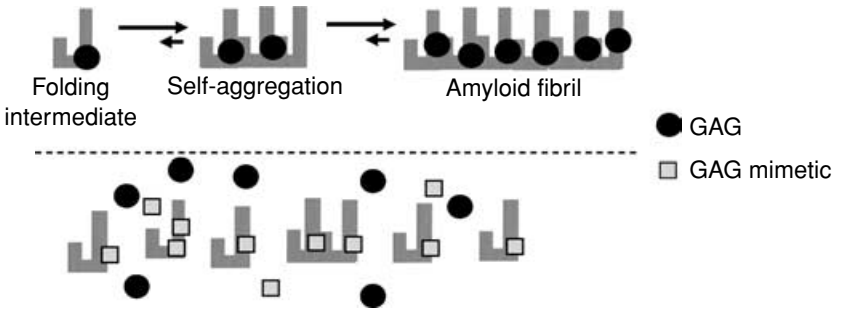

Fig. 2. Prevention of amyloid fibril formation by GAG mimetics. GAG mimetics are small molecules that compete with glycosaminoglycans (GAGs) for interaction with amyloidogenic precursors and fibrils and thereby inhibit fibril formation and tissue deposition.

been reported in nonrandomized studies from several centers [2, 44, 45]. In the largest of these series, treatment with high-dose melphalan and stem cell transplantation (HDM/SCT) was initiated in 312 patients [2]. The hematologic CR rate evaluated one year after treatment was 40%, and median survival was 4.6 years. Treatment-associated mortality, 13%, was higher than that typically seen when high-dose chemotherapy regimens are used for malignancies; this increased mortality rate reflects, in large part, the impact of underlying organ dysfunction on treatment-associated toxicity.

An important finding from the HDM/SCT experience is that improvements in function of affected organs can occur in AL amyloidosis, and that the improvements are much more likely in the patients who achieve a hematologic CR. For example, among patients with renal involvement, a hematologic CR was associated with a reduction in urinary protein excretion from a mean of 9.6 g/day prior to treatment to a mean of 1.6 g/day 12 months after treatment. In contrast, urinary protein excretion was unchanged in patients with persistence of plasma cell dyscrasia [46]. This observation, as well as similar findings in other organ systems [2], indicates that, despite pre-existing amyloid deposits, functional improvements are possible if new amyloid production is halted.

The current therapeutic approach for AA amyloidosis is treating the underlying inflammatory disease, thereby reducing production of SAA by the liver. In FMF, development of AA amyloidosis can be prevented in most patients by the administration of colchicine, which effectively inhibits FMF-associated inflammation, probably by inhibiting neutrophil chemotaxis and adhesion [47]. However, for many patients with other underlying diseases, adequate suppression of inflammation cannot be achieved with current anti-inflammatory and immunosuppressive agents, and SAA production and AA fibril formation persist.

Orthotopic liver transplantation has been used for approximately 15 years to eliminate the source of the amyloidogenic protein in familial TTR amyloidosis. This approach is now considered the definitive treatment for this disease [48–51]. Despite the synthesis of some TTR by the choroid plexus, mutant TTR disappears from the circulation after transplantation and neuropathy improves. Because wild-type TTR can deposit as amyloid at sites of pre-existing amyloid deposits, cardiac disease can continue to progress after liver transplantation, particularly if the pre-transplant amyloid burden is high [52]. Optimal timing of liver transplantation can be difficult to determine, but knowing the rate of progression of disease in other affected family members and the particular TTR mutation can help inform such decisions. Liver transplantation has been used to treat other types of familial amyloidosis as well, but the experience is largest for TTR disease [53].

Current treatments for dialysis-related amyloidosis aim to enhance β-2 microglobulin clearance and thereby decrease the concentration of the circulating amyloidogenic precursor protein. The most effective treatment for dialysis-associated amyloidosis is renal transplantation. Restoration of renal function increases β-2 microglobulin clearance and also might reduce β-2 microglobulin production. In the absence of renal transplantation, clearance of β-2 microglobulin can be enhanced with high-flux dialyzers or with hemodiafiltration. The use of a β-2 microglobulin adsorption column placed in series with a high-flux dialyzer has resulted in symptomatic improvement in small studies of patients with dialysis-related amyloidosis [54, 55].

Target 2: Fibril formation. Two approaches currently under investigation for inhibiting amyloid fibril formation will be reviewed. The first approach is the use of a GAG mimetic to prevent AA amyloid fibril formation. The second is the use of small molecules to stabilize TTR in its tetrameric conformation and thereby inhibit the formation of the folding intermediates that ultimately aggregate into amyloid fibrils.

Glycosaminoglycans such as heparan sulfate and chondroitin sulfate are constituents of amyloid deposits. A growing body of evidence suggests that these sulfated GAGs contribute to amyloidogenesis by binding to precursor proteins and inducing conformational changes required for fibril assembly, and by stabilizing amyloid fibrils (Fig. 2) [56–58]. Low-molecular-weight compounds that compete with sulfated GAGs for binding to the amyloidogenic protein have been identified as potential therapeutic agents [59–61]. A small, highly charged sulfonated molecule, NC-503 (1,3-propanedisulfonic acid,

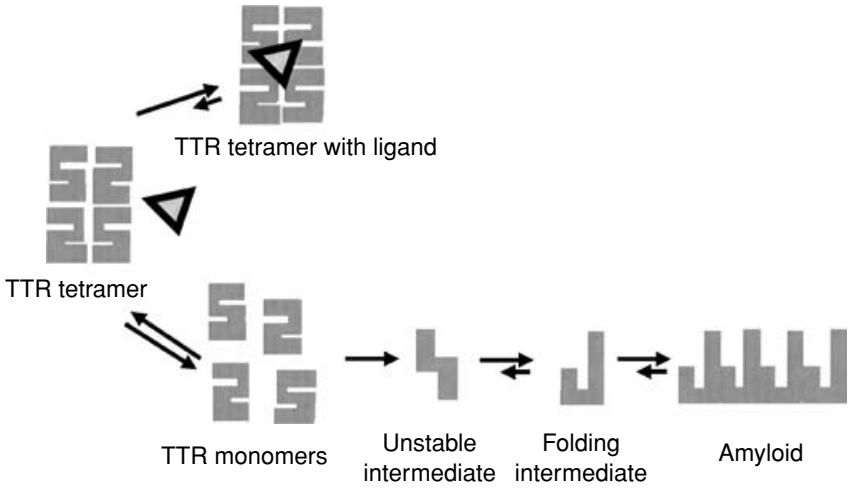

Fig. 3. Stabilization of TTR tetramer as a strategy to inhibit amyloid formation. TTR exists as a homo-tetramer with two ligand binding sites. Because amyloidogenic mutations favor monomer formation, and amyloid forms from monomers but not tetramers, molecules that stabilize TTR in its tetrameric configuration should prevent formation of folding intermediates and ultimately amyloid fibrils.

disodium salt), was designed specifically to inhibit the binding of sulfated GAG to amyloidogenic precursors. Anti-amyloidogenic activity of orally administered NC-503 has been demonstrated in murine models of AA amyloidosis. In these studies, NC-503 reduced splenic amyloid deposition in a dose-dependent fashion [62]. NC-503 is currently being studied as a treatment for AA amyloidosis in a multicenter international Phase II/III placebo-controlled trial. A second GAG mimetic that targets Aβ amyloid fibril formation is being studied in patients with Alzheimer's disease [63].

Human TTR functions as a transport protein for thyroxine and retinol-binding protein. The protein exists as a tetramer with four identical subunits and two ligand-binding sites located at the interface of the two dimers. TTR amyloid fibrils arise from the monomeric, but not the tetrameric, configuration. Amyloidogenic TTR mutations appear to destabilize the tetrameric configuration and facilitate monomer formation [64]. In contrast, binding of thyroid hormones and their derivatives stabilizes the tetrameric form of TTR. That is, occupation of the TTR binding site inhibits tetramer dissociation into monomers and thereby inhibits fibril formation by amyloidogenic TTR variants (Fig. 3) [65]. This tetramer-stabilizing effect of binding site occupation has led to efforts at identifying other tetramer-stabilizing molecules that could be administered therapeutically to prevent amyloid formation. Using functional screens and structure-based approaches, several such molecules have been identified including several nonsteroidal anti-inflammatory drugs (NSAIDs) [66–68]. Multicenter clinical trials using tetramer stabilizers are currently being planned. If effective at inhibiting amyloid fibril formation, treatment with stabilizers of the native TTR conformation could prevent the development of amyloid disease in individuals with known amyloidogenic mutations. In addition, stabilization of the TTR tetramer could be of value even after orthotopic liver transplantation because of the amyloidogenic potential of wild-type TTR in the presence of existing amyloid deposits. Although the TTR protein, with its ligand binding sites, might be ideally suited to the identification of "conformation stabilizers," this strategy of structure-based identification of stabilizing molecules also might be applicable to other amyloidogenic precursor proteins.

Target 3: Amyloid deposits. Amyloid deposits are relatively resistant to proteolysis. Evidence for regression of tissue amyloid by endogenous mechanisms comes primarily from demonstrations of functional improvements in affected organs and reductions in tissue uptake of radiolabeled SAP protein following treatment-induced cessation of amyloid precursor protein production [46, 69–72]. For example, uptake of radiolabeled SAP into affected organs is decreased in patients with AL amyloidosis following high-dose chemotherapy that has eradicated the plasma cell dyscrasia, and in patients with TTR familial amyloidosis following orthotopic liver transplantation. The clinical improvements and SAP scan results have been interpreted as evidence that amyloid deposits can be degraded once new amyloid deposition ceases [72, 73]. However, these demonstrations provide only indirect support for amyloid regression by endogenous mechanisms, and histologic confirmation is relatively scant.

Several interventions aimed at facilitating amyloid degradation are under investigation. The GAG mimetics are thought to not only inhibit amyloid fibril formation but also to destabilize tissue amyloid deposits and thereby promote their removal [59, 74]. Like GAG mimetics, inhibitors of heparan sulfate biosynthesis reduce amyloid deposition in vitro and in vivo and might have potential application as therapeutic agents [75]. An iodinated derivative of doxorubicin, 4′-iodo-4′-deoxydoxorubucin (IDOX), binds with high affinity to amyloid fibrils and promotes their disaggregation in vitro and in vivo in experimentally induced murine AA amyloidosis [76–78]. Administration of IDOX to patients with AL amyloidosis

showed promising results in a small, uncontrolled series, but its efficacy was not demonstrated in a larger multicenter trial [79, 80].

Disruption of the interaction between SAP and amyloid is another approach being investigated as a degradation-promoting treatment. Because SAP component is present in all types of amyloid deposits, targeting the SAP-amyloid interaction could have broad application [81]. SAP itself is highly resistant to proteolysis, and binding of SAP to amyloid fibrils protects them from proteolysis in vitro [82]. SAP exists in a dynamic equilibrium between the circulation, where it is unbound, and tissue, where it is bound to amyloid. Pepys et al hypothesized that removal of circulating SAP would drive SAP from tissue amyloid to the circulation and render the tissue amyloid less resistant to proteolysis [83]. R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC), a palindromic compound that binds with high affinity to SAP, cross-links two SAP molecules together in a manner that occludes the binding surface of SAP. CPHPC administration depleted SAP from the circulation and from amyloid deposits in murine models, and studies in humans demonstrated rapid SAP clearance from the circulation [83]. However, the impact of CPHPC administration on amyloid deposits in either animal models or in humans is not known.

Substantial progress in understanding the mechanisms of amyloid fibril formation and tissue deposition has enabled development of novel treatment approaches for the progressive, and often fatal, systemic amyloidoses. Despite the wide variety of amyloidogenic precursor proteins, the commonalities in the processes of fibril formation and biochemical composition of amyloid deposits should allow application of several of these treatment strategies to multiple types of amyloid disease. Although the systemic amyloidoses are rare diseases, the advances described in this Forum might have important implications for more common localized forms of amyloid deposition such as that occurring in Alzheimer's disease.

QUESTIONS AND ANSWERS

DR. NICOLAOS E. MADIAS (*Chairman, Department of Medicine, Caritas St. Elizabeth's Medical Center, Boston, Massachusetts*): How much do we know about the possible resorption of amyloid from the kidney, especially in the context of chronic kidney disease, in which factors that inhibit proteolysis also might be present? Does the decrease in proteinuria experienced by patients following high-dose melphalan and stem cell engraftment reflect decreased amyloid deposition and kidney reparation as determined by SAP scintigraphy or repeat kidney biopsy?

DR. DEMBER: Resistance to proteolysis is a feature of amyloid. Demonstrations of reductions in SAP uptake in the kidney and other organs when new amyloid production is halted by treatment have been interpreted as evidence for degradation of existing tissue amyloid deposits by endogenous mechanisms [70–72]. However, I would view the SAP scintigraphy findings as suggestive but not definitive evidence for amyloid degradation. It is not known whether amyloid that has been incorporated into tissue for long periods binds SAP in the same manner as newly deposited amyloid. It is possible that the changes observed in SAP scans before and after treatment reflect changes in interactions between SAP and amyloid rather than changes in tissue amyloid content. Very few histologic studies have addressed this issue, probably because of the invasive nature of the procedures required to obtain tissue. In a small case series, post-treatment renal biopsies from two patients with AL amyloidosis did not show reductions in tissue amyloid by Congo red staining compared with pretreatment biopsies despite near resolution of proteinuria [84]. Abdominal fat aspirates performed annually in our large cohort of patients with AL amyloidosis do not suggest that there is substantial amyloid resorption from fat tissue following eradication of the plasma cell dyscrasia. The marked reductions in proteinuria observed in AL amyloidosis after amyloid production is halted could reflect amyloid regression, but alternatively might be the result of elimination of a direct toxic effect on the glomerulus of fresh amyloid, amyloidogenic light chains, cytokines, or other mediators.

DR. MADIAS: Is any information available on relapse of the plasma cell clone in AL amyloidosis?

DR. DEMBER: The relapse rate for AL amyloidosis following high-dose chemotherapy appears to be substantially lower than that for multiple myeloma. We found that 6 of 73 patients (8%) with a complete hematologic response one year after treatment with high-dose melphalan and autologous stem cell transplant had a relapse of the plasma cell dyscrasia at two years. Relapses beyond two years were not observed [2]. In contrast, nearly all patients with multiple myeloma eventually relapse.

DR. MADIAS: Has this treatment been used for light-chain deposition disease?

DR. DEMBER: Yes. Bruno Royer and colleagues published a series of 11 patients with light- or heavy-chain deposition disease treated with high-dose chemotherapy and autologous stem cell transplantation [85]. The initial four patients received three to six monthly courses of cytoreductive chemotherapy (vincristine, doxorubicin, and methylprednisolone) prior to treatment with either combination chemotherapy (melphalan, cyclophosphamide, CCNU, VP16) and total-body irradiation, or melphalan and busulfan. The subsequent seven patients were treated with high-dose intravenous melphalan alone at doses similar to those used for AL amyloidosis. Six of the

11 patients achieved a complete hematologic response, and several patients had improvement in affected organ function. A marked reduction in proteinuria was observed in four of the patients following treatment.

At Boston University we are using high-dose melphalan with autologous stem cell transplantation for light-chain deposition disease (LCDD) as well. Although LCDD is less rapidly progressive and associated with a lower mortality rate compared with AL amyloidosis, extrarenal involvement is common, and thus aggressive treatment might be warranted, not only to prevent renal failure but also for cardiac, liver, or lung disease.

DR. JOHN T. HARRINGTON (*Dean Emeritus, Tufts University School of Medicine; Division of Nephrology, Tufts-New England Medical Center, Boston, Massachusetts*): When you spoke about the familial forms of amyloidosis and mentioned TTR as the most frequent type, you said that a number of other proteins could be similarly amyloidogenic. But I believe you also said that some of these proteins could be protective. Could you clarify that point?

DR. DEMBER: I was referring to what appears to be an anti-amyloidogenic effect of certain amino acid substitutions in the TTR protein. More than 100 mutations have been identified in the TTR gene. Most of these mutations render the TTR protein amyloidogenic. However, many of the mutations do not confer amylodogenic properties to the protein product. That is, under in vitro conditions, the TTR protein with a non-pathogenic mutation is no more likely to form amyloid fibrils than is wild-type TTR, and families that carry such mutations do not develop amyloidosis. Interestingly, a few of the mutations that have been identified appear to inhibit TTR amyloidogenesis. Under in vitro amyloid-promoting conditions, the TTR protein with these "protective" amino acid substitutions is less prone to amyloid formation than is wild-type TTR, and individuals who have inherited one allele with an amyloidogenic mutation and one allele with a "protective" mutation do not develop amyloidosis, unlike their family members who have one allele with the amyloidogenic mutation and one allele with the wild-type TTR gene. It appears from physicochemical studies that the protective amino acid substitution confers stability to the tetrameric configuration of TTR [18].

DR. RONALD D. PERRONE (*Division of Nephrology, Tufts-New England Medical Center*): Do you envision any new diagnostic tests? Are there any new serologic, imaging, or urine tests that might be helpful?

DR. DEMBER: The diagnosis of amyloidosis requires histologic demonstration of Congo red binding with birefringence under polarized light. At present, there are not alternatives to tissue examination with adequate specificity for making the diagnosis of amyloidosis. Determination of the type of amyloidosis requires the use of a variety of tests, including immunofixation electrophoresis of serum and urine, and bone marrow biopsy to look for evidence of a plasma cell dyscrasia; immunohistochemistry to identify specific proteins in tissue amyloid deposits; isoelectric focusing of serum proteins to identify abnormal TTR proteins; and DNA analysis to identify mutations in genes encoding TTR or other amyloidogenic proteins. For AL amyloidosis, the quantitative free light chain assay is a new test that appears to have utility both for diagnosis and in following the response to treatment directed against the clonal plasma cells [86]. This nephelometric assay measures free kappa and lambda light chains in the circulation or urine and provides a measure that is more quantitative than immunofixation electrophoresis. Because light chain excretion is dependent on GFR, it is the ratio of kappa and lambda light chains rather than their absolute concentrations that is the relevant measure in patients with renal impairment. As I mentioned earlier, radiolabeled SAP has been used to image tissue amyloid and follow disease progression. However, this methodology is not widely available for use in clinical practice.

DR. PERRONE: I have a patient, approximately 1.5 years post second deceased-donor kidney transplant, and 15 years post first deceased-donor transplant, who developed macroglossia and has AL amyloidosis. He is receiving standard immunosuppressive therapy for the kidney transplant with mycophenolate, cyclosporine, and prednisone, and he has excellent allograft function. Is he a candidate for high-dose chemotherapy with stem cell transplantation?

DR. DEMBER: I'd be interested in knowing whether AL amyloidosis was the cause of his native kidney failure. If so, his survival for 15 years without treatment of the plasma cell disease would be unusual.

DR. PERRONE: The cause is unknown, as the initial diagnosis was made in China.

DR. DEMBER: We have used high-dose melphalan with autologous stem cell transplant as treatment for two patients with AL amyloidosis and prior kidney transplants and one patient with LCDD and prior kidney transplant. In addition, there is a growing experience with the administration of high-dose chemotherapy and stem cell transplantation following heart transplant for patients with AL amyloidosis and cardiac involvement when the heart disease is severe enough to preclude initial aggressive anti-plasma cell therapy. Our approach in patients with renal allografts has been to continue giving calcineurin inhibitors and glucocorticoids throughout the peri-stem-cell-transplant period but to temporarily discontinue agents such as mycophenolate or azathioprine that have effects on the bone marrow and could interfere with marrow recovery. In addition, we have used cyclophosphamide rather than G-CSF as the stem cell mobilizing agent in these patients because of concern that the high doses of G-CSF required will trigger acute allograft

rejection. One of our three renal transplant patients had an episode of steroid-responsive acute renal allograft rejection that occurred approximately four months following stem cell infusion and might have been the consequence of immune cell recovery.

The patient you describe has macroglossia, which suggests soft tissue involvement. Is there any evidence of organ disease?

DR. PERRONE: He appears to have cardiac involvement with congestive heart failure.

DR. DEMBER: I asked this question because one could argue that soft tissue involvement alone is not an indication for treating with high-dose chemotherapy, but if cardiac or other organ involvement is present, aggressive treatment is warranted.

DR. MADIAS: Can you summarize any results of other organ function, namely liver, heart, and autonomic nervous system, following high-dose melphalan and autologous stem cell transplant and a hematologic response?

DR. DEMBER: Clinical improvements occur in all these organ systems following aggressive treatment and are more likely among patients who achieve a hematologic complete response. In our series of 312 patients with AL amyloidosis, clinical improvement in liver disease at 1 year, defined as a reduction in liver span of 2 cm or greater among those with hepatomegaly prior to treatment or a reduction in serum alkaline phosphatase concentration of 50% or greater, was seen in approximately 50% of patients with a hematologic complete response. Cardiac improvement at 1 year, defined as a reduction in intraventricular septal thickness of 2 mm or greater if it was abnormal prior to treatment or improvement in congestive heart failure based upon the New York Heart Association classification, was observed in 27% of patients with a hematologic complete response [2]. Autonomic nervous system dysfunction is more likely to reverse than is peripheral neuropathy; however marked improvement in sensory neuropathy has been observed following treatment in some patients. Also, in addition to clinical measures of organ function, improvements in both performance status as assessed by the Southwestern Oncology Group scale and quality-of-life as measured by the SF-36 questionnaire have been demonstrated among patients achieving a hematologic response [87].

DR. HARRINGTON: What is the normal role of SAP? When it is depleted by CPHPC, what happens to the patients?

DR. DEMBER: SAP is a member of the pentraxin family of proteins that includes C-reactive protein. Human SAP is synthesized by the liver and consists of five non-covalently associated identical subunits that form a disk-like structure. In addition to binding to all types of amyloid fibrils, SAP binds to DNA and chromatin in a calcium-dependent manner. The normal role of SAP is not fully elucidated, but it is believed to contribute to innate immunity, and recent studies suggest that SAP binds to apoptotic cells and facilitates phagocytosis of apoptotic bodies by macrophages [88]. Interestingly, the SAP knockout mouse develops an immune complex glomerulonephritis with auto-antibodies directed against DNA, chromatin, and histones. However, it is not clear that the development of auto-immunity in the SAP knockout mice is the result of SAP deficiency rather than an effect attributable to the genetic background of the mice, as the phenotype appears to be strain-dependent [89]. Of note, the SAP knockout mice have a normal lifespan. The impact of SAP depletion in humans is not known. The published experience with CPHPC administration is limited to small numbers of individuals, and the treatment duration was short.

DR. HARRINGTON: More than 30 years ago, Alan Cohen, John Mannick, and I published a paper about the first two patients with amyloidosis who received renal transplants [90]. Does your group have a series of long-term amyloid renal transplant survivors? What happens to them 15 to 20 years later?

DR. DEMBER: Most of the published literature on kidney transplantation in amyloidosis is in patients with AA amyloidosis. At most centers, patients with AL amyloidosis are not considered candidates for renal transplantation unless the underlying plasma cell dyscrasia is eradicated. Anthony Bleyer and colleagues reported renal allograft and patient survival according to the underlying renal disease using UNOS registry data and found that one-year and three-year allograft survival for patients with amyloidosis were 79.5% and 71.1%, respectively. Patient survival at one and three years was 87.1% and 79.3%, respectively. When compared with other causes of underlying renal failure, amyloidosis ranked 30/33 and 32/33 for allograft survival and patient survival, respectively, suggesting that outcomes are worse for this disease than for nearly all other underlying conditions leading to kidney transplantation [91]. Of note, fewer than 100 individuals with amyloidosis were identified in the UNOS registry during a 9-year period, reflecting the small numbers of patients with this disease who undergo renal transplantation. The published experience describing long-term outcomes is limited to case reports or very small case series. Because it is likely that most of the patients in existing databases received renal transplants while there was amyloid production ongoing, the findings of studies such as that by Bleyer et al probably are not applicable to patients who have treatment that fully eradicates new amyloid production.

DR. ANDREW S. LEVEY (*Division of Nephrology, Tufts-New England Medical Center*): What has been the experience in people with beta-2 microglobulin disease who receive a kidney transplant? Does the disease go away?

DR. DEMBER: Renal transplantation is the most effective treatment for beta-2 microglobulin amyloidosis. Substantial improvement in pain is typical very soon after transplantation, and presumably new amyloid deposition ceases with restoration of renal function. However, it is not clear whether there is regression of existing amyloid deposits. Uptake of radiolabeled SAP decreased in 8 of 9 patients who received transplants [92]; however, several studies found no evidence of radiographic resolution of bone cysts or histologic regression of amyloid many years after transplantation.

DR. ANNAMARIA KAUSZ (*Division of Nephrology, Tufts-New England Medical Center*): Were the dismal outcomes you presented in AL amyloidosis prior to routine stem cell transplantation? Is aggressive treatment with high-dose melphalan and stem cell transplantation becoming widely accepted as standard of care? Also, given that organ deposition of amyloid might not reverse following this treatment, do you envision a two-pronged approach in the future, whereby individuals undergo HDM/SCT, *and* receive a product to reduce fibril formation or degrade amyloid deposits?

DR. DEMBER: The use of high-dose melphalan and stem cell transplantation for AL amyloidosis has become more widespread over the past several years, and is becoming accepted as the best available treatment for the disease for selected patients. The recent decision by the Centers for Medicare and Medicaid Services (CMS) to expand Medicare coverage of autologous stem cell transplantation to include AL amyloidosis is a reflection of the acceptance of this treatment by CMS and professional societies. Challenges remain in modifying the treatment to reduce toxicity, which is substantial in these patients because of underlying organ dysfunction, in identifying the most appropriate candidates for treatment, and in developing anti-plasma cell therapies for patients who are too ill to undergo high-dose chemotherapy or who have persistent plasma cell disease after such treatment.

I believe that a multi-pronged approach will be applied in the future not only for AL amyloidosis but for the other systemic amyloidoses as well. In the example of AL amyloidosis, treatments directed at amyloid fibril formation or tissue amyloid deposits could be particularly valuable for patients who either do not achieve a hematologic complete response with high-dose chemotherapy or have persistent organ dysfunction despite a good hematologic response.

DR. MADIAS: As an extension to Annamaria's question, given the high mortality rate associated with the stem cell transplantation procedure, could you please expand on the timing of intervention? Also, what factors predict success?

DR. DEMBER: For AL amyloidosis, treating early in the course of the disease is desirable. While there is no evidence that early treatment is associated with greater chemo-responsiveness of the clonal plasma cells, there are advantages with respect to tolerance of the treatment and reversibility of organ dysfunction. In general, the toxicities associated with high-dose chemotherapy increase with the degree of organ dysfunction, and if organ dysfunction is of sufficient severity, treatment toxicities can preclude aggressive treatment. This is particularly relevant with cardiac involvement, as stem cell mobilization and collection are particularly challenging for patients with severe cardiac amyloid disease. The overall performance status of the patient also predicts treatment tolerance. And with respect to organ dysfunction, reversibility becomes less likely with greater degrees of organ dysfunction. Clear predictors of hematologic response to high-dose chemotherapy have not been identified.

DR. LEVEY: Why is AL amyloidosis so responsive to high-dose chemotherapy and transplantation when myeloma is not? I thought that hematologic malignancies with faster cell growth and a larger tumor burden have a better response to the chemotherapy.

DR. DEMBER: The difference in responsiveness between these two diseases is probably a function of the difference in plasma cell burden. As you point out, the plasma cell burden is substantially greater in multiple myeloma than in AL amyloidosis. Thus, there is a greater likelihood in multiple myeloma that not all clonal plasma cells will be eradicated by the chemotherapy. The increased plasma cell burden in multiple myeloma also increases the likelihood that the stem cells harvested prior to dose-intensive chemotherapy will be contaminated with clonal plasma cells that will ultimately engraft. To reduce the plasma cell burden prior to stem cell harvesting, cytoreductive therapy with multiple cycles of combination chemotherapy is generally administered to patients with multiple myeloma, and stem cell mobilization in such patients is usually performed with a cytotoxic agent such as cyclophosphamide rather than G-CSF. However, despite these measures, the number of clonal plasma cells at the time of stem cell harvesting and melphalan administration is still greater in multiple myeloma than in AL amyloidosis.

DR. KAUSZ: Is anything known about the potential toxicities of the investigational agents that reduce fibril formation or degrade amyloid deposits?

DR. DEMBER: NC-503, the GAG mimetic that inhibits AA amyloid fibril formation and promotes fibril degradation, is currently in clinical trials. Information should be available about both its effectiveness and toxicity within the next several months. In early-phase trials involving healthy individuals, the drug was well-tolerated without apparent toxicity, but the treatment duration was brief [62]. We all are familiar with the toxicities of NSAIDs, agents that are being investigated as TTR tetramer stabilizers. Whether reduced availability of TTR for binding to its natural ligands will have clinically important effects is

not known, but this is not a major concern because TTR functions as a "back-up" transporter of thyroxine and retinol-binding protein. The main toxicity of IDOX, the doxorubucin derivative that binds to all types of amyloid and is thought to promote clearance of deposits, is granulocytopenia. Cardiotoxicity, a concern with this agent as with other anthracyclines, was not apparent in small trials. In the report describing CHPHC administration to a small number of patients with amyloidosis, toxicity was not observed [83]. It must be noted that the published experience with each of these agents is limited to uncontrolled studies with small numbers of patients, and the findings regarding toxicity must be viewed as preliminary.

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