Amyloidosis and Waldenström's Macroglobulinemia

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Primary systemic amyloidosis is an immunoglobulin light chain disorder that is 1/5th as common as multiple myeloma. Amyloidosis is regularly seen in the practice of a hematologist and has recently undergone major advances in terms of the ability to evaluate responses as well as new therapeutic options that were not available when this topic was covered as an education session at the American Society of Hematology meeting 5 years ago. Waldenström macroglobulinemia (WM) is rarer than amyloidosis (1500 per year WM versus 3000 per year amyloid in the US), and recent consensus panels have established the definition of the disease, the diagnostic criteria, criteria for initiation of therapy and a new classification scheme. In this session, new developments in amyloid and macroglobulinemia, from suspicion of the diagnosis to treatment, are covered.

In Section I, Dr. Morie Gertz answers four specific questions: (1) When should amyloidosis be suspected? (2) How does one heighten ones index of suspicion for amyloid? (3) How is the diagnosis confirmed and the type classified as primary? (4) What is the prognosis and how is it accurately assessed? Recent findings on cardiac biomarkers, presenting features and use of the free light chain assay are reviewed. Staging for amyloid and recently proposed criteria of response and progression are covered.

In Section II, Dr. Giampaolo Merlini comprehensively reviews therapy of amyloidosis from the use of standard melphalan/prednisone to the recently described standard dose therapies including dexamethasone, thalidomide/dexamethasone, melphalan/dexamethasone and IV melphalan/dexamethasone. An extensive discussion of the role of high-dose therapy with stem cell reconstitution follows and includes patient selection, predictors of immediate morbidity and mortality, and survival expectation. Finally, a therapeuitc strategy is proposed.

In Section III, Drs. Steven Treon and Giampaolo Merlini review the most current information on WM. The consensus panel results and recommendations of the clinical pathologic definition of WM, the prognostic markers and the indications to initiate therapy in WM, the uniform response criteria in WM and available treatments for the disease are reviewed. Drs. Treon and Merlini cover recently published treatment protocols that use rituximab, purine nucleoside analogs, and alkylating agents. The current data on thalidomide, alpha interferon, and high-dose therapy are also covered.

I. Amyloidosis: Diagnosis and Prognosis

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Amyloidosis is a rare systemic disorder that results from tissue deposition of amyloid protein. Amyloid protein is defined by its resistance to proteolysis and its three-dimensional configuration as a beta pleated sheet. There are several subtypes of amyloidosis including primary amyloidosis, also known as light chain amyloidosis, secondary and familial amyloidosis.[1] The incidence of amyloidosis is 8 patients per million per year. The structural subunits of the amyloid protein in light chain (AL) amyloidosis are the fragments of monoclonal immunoglobulin heavy chains or light chains (**Table 1**). The symptoms of amyloidosis are vague and include fatigue, edema, and weight loss and are not helpful in formulating the correct differential diagnosis. Occasionally, patients are recognized because of their monoclonal protein and are diagnosed as atypical multiple myeloma because they have a light chain present but less than 10% bone marrow plasma cells. Since there is no diagnostic blood test, radiograph, or scan procedure, awareness of the diagnosis is essential to correctly identify patients early in the course. Below is a typical patient.

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Table 1. Nomenclature of amyloid.

Precursor Protein	Abbreviation	Clinical
Immunoglobulin protein	AL	Light chain amyloid
	AH	Heavy chain amyloid
	AL	Myeloma or macroglobulinemia (this is *not* secondary amyloid)
	AL	Localized bladder and bronchus
Amyloid A protein	AA	Secondary to infection, renal cell cancer and familial in familial Mediterranean fever (FMF)
Transthyretin	ATTR	Native transthyretin (TTR) in senile systemic amyloidosis, mutant in familial amyloidosis
Fibrinogen Aα	AFib	Hereditary renal amyloid
Apolipoprotein A	A Apo I	Cardiomyopathy neuropathy
Beta 2 microglobulin	Aβ2 M	Dialysis amyloid

Illustrative Case

A 79-year-old man had a 1-year history of dyspnea on exertion, lower extremity edema, and a 10-kg weight loss. An initial evaluation in a primary care setting including echocardiography and electrocardiography were interpreted as being nondiagnostic. The patient was referred for evaluation of noncardiac dyspnea to a pulmonologist. A computed tomography (CT) scan of the abdomen showed shoddy retroperitoneal lymphadenopathy, and laparoscopic biopsy of the nodes showed sinus histiocytosis. After the patient left Mayo Clinic, a monoclonal G-lambda protein was detected in the serum with a peak of only 0.5 g/dL. The 24-hour urine protein showed 330 mg but consisted of lambda light chain and no albumin. A repeat echocardiogram showed increased wall thickness and restrictive diastolic filling consistent with amyloid. A subcutaneous fat aspirate demonstrated amyloid. Amyloid stains subsequently performed on the abdominal lymph nodes showed vascular amyloid deposits.

In this section, four questions will be addressed: (1) When should amyloid be considered? (2) If the diagnosis is under consideration, what is an appropriate diagnostic evaluation? (3) If there is a very strong suspicion, how is the diagnosis confirmed? (4) What is the prognosis for patients with proven disease?

The physical findings of amyloidosis include enlargement of the tongue, periorbital purpura, and the shoulder pad sign. Although very specific for the diagnosis, these are easily overlooked and are seen in less than 20% of patients with AL. Reliance on symptoms and signs alone without being aware of the possibility of amyloid will inevitably result in an overlooked diagnosis.

Amyloidosis is a disease that infiltrates organs and causes their dysfunction. The four most common organs involved in amyloid include: heart, kidney, liver, and the peripheral nerve (**Figure 1**). Amyloidosis should be considered in the differential diagnosis of any patient with nephrotic syndrome without clear alternative explanation. Amyloidosis accounts for 10% of adult non-diabetic nephrotic syndrome. When adult patients are seen with nephrotic syndrome, amyloidosis, as well as nil disease, membranoproliferative glomerulonephritis, and membranous glomerulopathy must be considered in the differential diagnosis. One-half of patients presenting with amyloidosis have demonstrable cardiomyopathy. The symptoms can range from easy fatigability to overt congestive heart failure. This diagnosis must be entertained in any patient with cardiac symptoms of fatigue without a history of ischemia such as exertional angina or a previously documented myocardial infarction. The electrocardiogram (EKG) may show a pseudo-infarction pattern, and patients may be incorrectly diagnosed as having a silent ischemic syndrome. The findings on echocardiography, which include thickening of the heart walls, can be misinterpreted as ventricular hypertrophy as in our illustrative case. Any patient with unexplained cardiac symptoms without valvular disease, coronary artery disease, or long-standing hypertension should be considered for possible amyloidosis. We have seen patients referred to cardi-

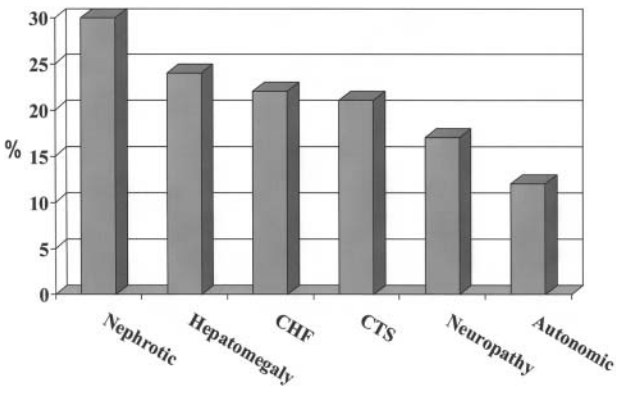

Figure 1. Clinical syndromes in amyloidosis.

Abbreviations: CHF, congestive heart failure; CTS, carpal tunnel syndrome

ologists with overt heart failure undergo cardiac catheterization, be found to have normal coronary arteries, and then be dismissed from further evaluation with no follow-up.

It is the hematologist's responsibility to educate specialists at their institution on the proper evaluation of a patient with an unexplained cardiac disorder or unexplained proteinuria. Immunofixation of the serum and of the urine is required to screen for light chain amyloidosis.

Amyloid involving the liver occurs in approximately one-sixth of patients and is characterized by palpable hepatomegaly, elevation of the serum alkaline phosphatase, and no imaging abnormalities by CT or magnetic resonance imaging (MRI). Symptoms may be limited to early satiety and weight loss. The clinician's responsibility is to obtain immunofixation of the serum and the urine in addition to the usual studies for hepatitis, primary biliary cirrhosis, and other infiltrative liver disorders.

One in 6 patients with amyloidosis presents with symptomatic sensorimotor peripheral neuropathy. The neuropathy can be both axonal and demyelinating. Symptoms occur primarily in the lower extremities, and sensory changes are greater than motor changes. There is often a 2-year delay between the onset of symptoms and the recognition of amyloid. Important clues include: half of the patients have associated carpal tunnel syndrome, and a number of them will have autonomic neuropathy. Autonomic failure manifests as alternating diarrhea and constipation, pseudo-obstruction with vomiting, orthostatic hypotension, and impotence. The neuropathy is frequently painful, requiring analgesics. Gabapentin and amytriptyline often fail to provide benefit. These patients may be recognized to have a monoclonal gammopathy but are often misdiagnosed as having monoclonal gammopathy of undetermined significance (MGUS)–associated neuropathy without proper diagnostic testing to exclude amyloid.

Amyloidosis should be suspected in any patient with nephrotic range proteinuria, infiltrative cardiomyopathy, peripheral neuropathy, hepatomegaly, symptoms of bowel pseudo-obstruction, or atypical multiple myeloma.

Screening for Amyloid

Amyloidosis is a plasma cell dyscrasia with a small monoclonal population of plasma cells in the bone marrow, and this knowledge can be used to advantage in screening for the disease. Since the amyloid deposits are composed of monoclonal light chains and heavy chains, most patients will have a detectable immunoglobulin abnormality either by immunofixation of serum, immunofixation of a 24-hour urine specimen, or a detection of an abnormal immunoglobulin-free light chain (Freelite®).[2] Screening electrophoresis is inadequate since 20% of patients with amyloidosis will not have an intact immunoglobulin protein in the serum or a level too low to demonstrate a spike on the electrophoretic pattern (**Figure 2**). It is mandatory that urine be screened in a patient with a compatible syndrome (**Figure 3**). When the serum and the urine are studied by immunofixation, nearly 90% will have a detectable monoclonal light chain. The immunoglobulin-free light chain nephelometric assay will be abnormal in three-quarters of the remaining patients in support of a tentative diagnosis of amyloidosis. When screening for amyloid, immunofixation has a higher sensitivity (90%) than amyloid stains performed on routine biopsy specimens such as fat (73%) or the bone marrow (72%).

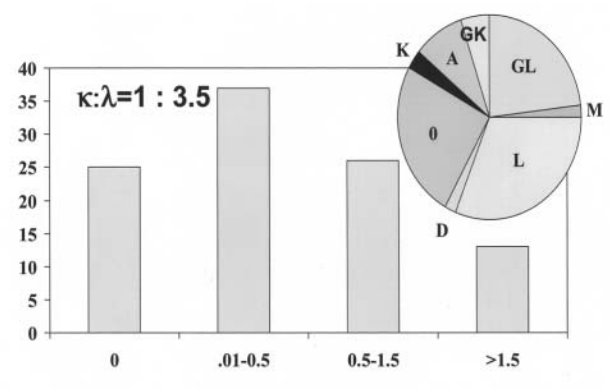

Figure 2. Serum M proteins in amyloidosis.

The pie chart gives the immunoglobulin heavy (A, G, D, M) and light chain seen at diagnosis (K = kappa, L = Lambda, 0 = none)

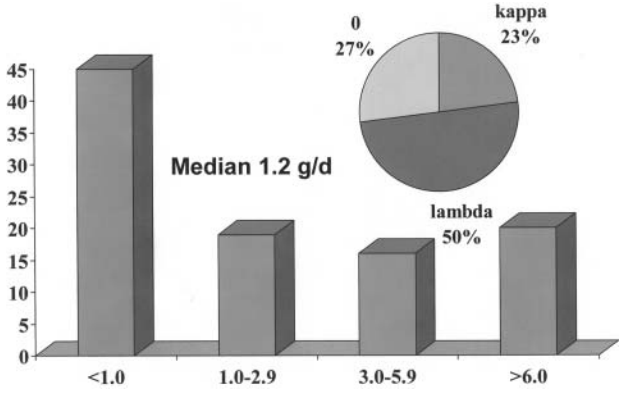

Figure 3. Urine M proteins in amyloidosis (g/24 hr).

How Is the Diagnosis of Amyloidosis Confirmed?

When a patient is seen with one of the clinical syndromes in **Figure 1** and is confirmed to have an immunoglobulin light chain abnormality by immunofixation or nephelometry, the index of suspicion for amyloid is high. As in all hematologic malignancies, biopsy verification of the diagnosis is required. All patients with amyloid nephrotic syndrome, amyloid cardiomyopathy, amyloid liver disease, or amyloid neuropathy can be confirmed with biopsy of the kidney, heart, liver, or sural nerve. Biopsies of the kidney and liver carry a risk of bleeding and often necessitate overnight hospitalization for the patient. Case reports exist of severe bleeding following liver biopsy and, rarely, hepatic rupture. These small risks can be avoided if the clinician is aware that amyloid is a likely diagnosis. Techniques exist that are easier and less expensive and that result in minimal risk to the patient. Congo red staining of a bone marrow biopsy will demonstrate amyloid in at least 60% of patients.[3] A marrow biopsy is required since amyloid deposits are rarely seen in the marrow aspirate. The subcutaneous fat aspirate demonstrates amyloid deposits in 70%–80% of patients (**Table 2**). Other centers do minor salivary gland biopsies and gingival biopsies.[4] The rectal biopsy still remains a sensitive diagnostic technique. Only 13% of amyloidosis patients have a negative bone marrow and fat aspirate. Because of the very low prevalence of amyloid, use of the fat aspirate as a screening tool in patients presenting with peripheral neuropathy in the absence of a monoclonal protein disorder has an extremely low yield.[5]

Once amyloidosis is proven by tissue biopsy, one must be certain that the amyloidosis is of the AL type.[6] When patients have a free light chain in the serum or urine, the likelihood of AL is high, but immunohistochemical staining of the biopsied amyloid deposits with kappa and lambda antisera to re-affirm the diagnosis is appropriate. It should also be kept in mind that nearly 3% of adults have MGUS. In patients who have intact immunoglobulin proteins in the serum with no detectable free light chain (FLC), the possibility of an incidental MGUS with a non-immunoglobulin form of amyloid must be kept in mind. Inherited forms of renal amyloidosis due to a mutant fibrinogen A-alpha chain have been described. This presentation is easily confused with nephrotic syndrome associated with light chain amyloid. Immunostains of available tissue for deposits of fibrinogen or transthyretin, which cause inherited amyloidosis, are important to reliably exclude a non-immunoglobulin form of amyloid.

Table 2. Noninvasive biopsy to diagnose amyloidosis (N = 151).

Fat+	Marrow+	62%
Fat+	Marrow–	11%
Fat–	Marrow+	15%
Fat–	Marrow–	13%

Amyloid cardiomyopathy occurs with high frequency in men over the age of 80, so-called senile cardiac (systemic) amyloid. There is an inherited form of amyloid specific to African-Americans. A mutation of transthyretin is carried by 3.9% of African-Americans, which would translate to 1.3 million adults in the United States. African-American men over the age of 70 with cardiac amyloidosis should be screened for mutant transthyretin (Ile 122). Immunohistochemical characterization of amyloid deposits is helpful in confirming the subunit protein comprising the amyloid.[7] All amyloid deposits contain P (pentagonal) component. P component is a glycoprotein comprising 20% of the amyloid fibril by weight. We routinely do amyloid typing with P component, as a positive control, as well as kappa and lambda to confirm the diagnosis. If the kappa and lambda results are negative, testing for transthyretin, fibrinogen, and occasionally lysozyme and apolipoprotein A is warranted.[8] Micromethods have been developed that permit mass spectroscopic screening of small samples to determine the subunit protein of amyloid.

The nephelometric analysis for serum immunoglobulin free light chains enhances one's ability to confirm the type of amyloid as AL. These antisera recognize epitopes of FLCs but do not detect light chains associated with an intact immunoglobulin molecule. When we applied this technique to 100 AL patients, the patients who had negative serum immunofixation showed an abnormal FLC ratio in 85% of kappa and 80% of lambda patients. When there was no monoclonal protein in the serum or in the urine by immunofixation, the FLC technique detected a kappa protein in 86% of kappa amyloid and a lambda in 30% of lambda amyloid. The detection of FLCs by the nephelometric system is particularly important in those patients who do not have light chains by immunofixation. We routinely measure the light chain in the serum of all patients, both to confirm its immunoglobulin light chain origin as well as to monitor therapy. In one study, nearly 10% of patients who were thought to have immunoglobulin light chain amyloid had amyloid due to other types, including 5% with fibrinogen amyloid and 4% with transthyretin mutations.[6] Inherited amyloidosis should be considered in all patients before therapy is initiated. The typing of the amyloid deposit is important because the different forms are clinically indistinguishable from each other. Renal amyloid due to long standing infection (AA) presents to the clinician identically to primary renal amy-

loid. Amyloid neuropathy due to a mutation of TTR presents with all the same clinical features of neuropathy seen in primary systemic amyloidosis. The tissues all appear the same by light and electron microscopy.[7,8]

Prognosis

The most common cause of death in amyloid is cardiac, either due to progressive congestive cardiomyopathy or sudden death due to ventricular fibrillation or asystole. Clinical outcome in patients and their likelihood of responding to treatment is, in large part, determined by the extent of cardiac involvement at diagnosis.[9] Previously, echocardiography with Doppler studies of diastolic function was critical in the assessment of patients newly diagnosed with AL. The recent introduction of strain echocardiography has added significant sensitivity in the assessment of cardiac function in AL.[10] Echocardiography is routinely done in all newly diagnosed patients and every 6 months during therapy. The presence of heart failure is associated with a median survival of only 6 months and is the most important clinical predictor of survival. Echocardiography allows measurement of both the ejection fraction and the interventricular septal thickness, both of which are important in predicting outcomes in patients with amyloid. Doppler echocardiography is used to measure diastolic performance and relaxation of the ventricle during diastole. If the deceleration time is 150 ms or less by Doppler, the 1-year survival is 49%. New measures of myocardial injury that are more reproducible than the echo have recently been introduced. Measurement of serum troponin T, a sensitive marker for ischemic cardiac injury,[11] has been shown to be a powerful predictor of survival in amyloidosis patients, both those treated conventionally[12] as well as those who become candidates for stem cell transplantation.[13] Serum troponin levels of less than 0.03, 0.03 to 0.1, and greater than 0.1 have permitted classification of AL patients into three groups of approximately equal size with differing survivals.

The N terminal fragment of pro-brain natriuretic peptide NT-Pro BNP is produced when the atria are dilated.[14] Elevation of the NT-Pro BNP has been shown to be predictive of survival following a diagnosis of amyloid. Combining the troponin with the NT-Pro BNP level has resulted in a new staging system. These two tests should be measured in all newly diagnosed patients with amyloidosis. Although a weaker prognostic indicator, the serum level of β2-microglobulin is valuable. Levels greater than 2.7 μg/mL predict shorter survival.

In conclusion, echocardiography, serum β2-microglobulin, troponin T, and NT-Pro BNP are important in assessing the prognosis in patients with amyloidosis.

Assessing the Response in Amyloidosis

Most centers define responses in amyloidosis based on suppression of the precursor immunoglobulin light chain. Unlike those with multiple myeloma, AL patients frequently do not have a quantifiable immunoglobulin protein in the serum, and serial measurement of the urine M protein can be fraught with difficulty, particularly in those patients who have albuminuria from renal amyloidosis. The nephelometric assay for immunoglobulin FLCs is an adjunct to assess response to therapy. Organ response parallels changes in the serum FLC assay. We serially evaluate the immunoglobulin serum free light chain and consider a 50% reduction to indicate a hematologic response and a normalization of the level to reflect a complete hematologic response. This technique has been incorporated into evaluation of response at most amyloidosis treatment centers.[15]

An accurate diagnosis of amyloidosis and its subtype classification is essential prior to treatment.[16] In AL, the median survival is approximately 2 years and is less than 6 months when there is significant cardiac disease. The early recognition of amyloidosis using the algorithm listed below and the careful distinction between immunoglobulin light chain amyloid and the non-immunoglobulin forms of amyloid is critical because systemic therapy[17] and transplantation[18,19] will not have any benefit in the other forms of amyloid (**Table 3**).[20,21]

Table 3. Key points—diagnostic pathway for amyloidosis.

1) Consider AL in differential if:
   - Nondiabetic nephrotic syndrome
   - Cardiomyopathy nonischemic: echo shows “left ventricular hypertrophy (LVH)”
   - Hepatomegaly with no scan defects
   - Chronic inflammatory demyelinating polyneuropathy
   - “Atypical myeloma” urine light chain + and marrow < 10% plasma cells
2) Perform immunofixation serum, urine, and immunoglobulin free light chain assay. If positive, amyloidosis becomes a likely explanation.
3) Biopsy bone marrow and subcutaneous fat. Do Congo red stains. Biopsy of kidney or liver are usually not required.
4) Assess prognosis. Echocardiography with Doppler. Serum troponin, brain nateiuretic peptide (BNP), β2-microglobuin.
5) Initiate therapy.

II. AL Amyloidosis: Therapeutic Strategies 2004

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The two keys to effective treatment of AL amyloidosis are early diagnosis and correct typing. Ideally, treatment should be started before irreversible organ damage has occurred. Localized or systemic deposition of protein fibrils with a beta-sheet structure is the lowest common denominator of a wide group of diseases with different causes, courses, treatments and prognoses.[1] Correctly typing the amyloid deposits (as outlined in Section I) is of paramount importance because this dictates both prognosis and treatment. Once the diagnosis of AL has been firmly established, the design of the therapeutic strategy depends on a fine balance between the efficacy of the chosen regimen and the individual patient's expected ability to bear the treatment's toxicity. The current therapeutic approach to systemic amyloidosis is based on the observation that amyloid deposits can be reabsorbed and organ function restored if the synthesis of the amyloidogenic protein precursor is shut down. Therefore, the aim of therapy in AL amyloidosis is to rapidly reduce the supply of amyloid-forming monoclonal free light chains by suppressing the underlying plasma cell dyscrasia while using supportive measures to sustain and possibly preserve organ functions.

Monitoring the Therapeutic Effect

The criteria for hematologic and organ response were reported in Section I; **Table 4** summarizes the criteria for hematologic response. Radiolabeled SAP is a specific tracer for amyloid and can monitor amyloid load serially, but its availability outside the UK is limited. Hematologic response usually translates into clinically improved organ function and is associated with a substantial survival advantage and improved quality of life. However, if the organ damage is advanced it may be irreversible despite suppression of the amyloid precursor. Most hematologically responding patients show a clinical response after 3–6 months although later responses up to 12 months have been recorded. Importantly, a complete clonal response is not a prerequisite for clinical response and clinical improvement may still occur in patients with a partial clonal response. However, the rate of clinical response is higher in patients with a complete hematologic response than in those with a partial one.

Effective Treatments

High-dose melphalan followed by peripheral blood autologous stem cell transplantation

High-dose melphalan (HDM) followed by peripheral blood autologous stem cell transplantation (PBSCT) is presently considered the most effective treatment for AL amyloidosis. Despite previous anecdotal reports, the paper by Comenzo and collaborators of the Boston University Amyloid Treatment and Research Program in 1996 ignited interest and introduced this procedure in the care of AL patients. The initial enthusiasm was

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Table 4. Criteria for hematologic response.

Complete response (CR)	• Serum and urine negative for a monoclonal protein by immunofixation • Free light chain ratio normal • Marrow contains < 5% plasma cells
Partial response (PR)	• If serum M component > 0.5 g/dL, a 50% reduction • If light chain in the urine with a visible peak and > 100 mg/day and 50% reduction • If free light chain > 10 mg/dL* and 50% reduction
Progression	• From CR, any detectable monoclonal protein or abnormal free light chain ratio (light chain must double) • From PR or stable response, 50% increase in serum M protein to > 0.5 g/dL or 50% increase in urine M protein to > 200 mg/day; a visible peak must be present • Free light chain increase of 50% to > 10 mg/dL
Stable	• No CR, no PR, no progression

* Given the assay imprecision, it is recommended that serum free light chain (FLC) values below 10 mg/dL, are not considered criteria for evaluation of hematologic response. In addition, the κ/λ ratio in patients with renal failure may be reduced by retention of healthy polyclonal free light chains. Caution should also be used in interpreting data between laboratories and if antisera batches vary over time.

soon tempered by the severe treatment-related mortality (TRM) caused by the toxic effects of the high-dose chemotherapy (melphalan) on organs severely compromised by amyloid disease. The peritransplant mortality was as high as 43% in the first multicenter survey reporting the outcome of transplantation (reviewed in [2]). The number of organs involved at the time of transplantation was prognostic, as confirmed in several subsequent studies. These findings strongly indicated the need for a risk-adapted approach with careful patient selection and attenuation of the dose of intravenous melphalan based on age and organ involvement. These aspects were thoroughly reviewed in 2002 by Comenzo and Gertz who analyzed the outcome of the single-center and multicenter trials.[2] The Boston Group[3] and the Mayo Clinic group[4] have recently reviewed their experience with stem cell transplantation for patients with amyloidosis. **Table 5** reports two proposed risk-adapted approaches for patient selection that present subtle differences. Only some AL patients are eligible for PBSCT, the percentage varying from approximately 16% to more than 50% depending on the patient population and possible pre-admission screening.

Blood stem cell mobilization and collection: Previous exposure to alkylating agents impairs hematopoietic stem cell collection. A total dose of melphalan exceeding 200 mg significantly reduces the ability to mobilize $CD34^+$ cells. Contrary to the common experience in multiple myeloma, deaths have been reported during mobilization and leukapheresis of AL patients with cardiac or multiorgan involvement.[2] Overall, the major complication rate is approximately 15% in AL patients. To minimize the risk of toxicity it is recommended that only granulocyte colony-stimulating factor (G-CSF) be used for mobilization since its use in combination with cyclophosphamide is associated with increased cardiac morbidity, a significantly higher number of aphereses required for CD34 harvesting, greater need of hospitalization, and increased toxicity.[4] The recommended G-CSF dose is 6 μg/kg every 12 hours for 5 days,[2] but 16 μg/kg given in a single dose or in 2 divided doses for 3 days has also been reported.[5] The recommended optimal dose of $CD34^+$ cells in AL patients is at least 5×10^6 $CD34^+$ cells/kg.[2] Contamination of the apheretic product with clonotypic immunoglobulin-positive plasma cells has been demonstrated, but CD34 selection is not presently recommended.

Conditioning: In most of the cases with AL, the clone size is modest: the median percentage in bone marrow is 5%–7%; therefore, debulking with VAD (vincristine, doxorubicin, dexamethasone [Dex]) or other regimens, as done in multiple myeloma, seems unnecessary. Possible benefits from VAD treatment before PBSCT have been claimed. Evidence from a ran-

Table 5. Proposed criteria for patient selection and dose adaptation for high-dose melphalan.

Good Risk	Intermediate Risk	Poor Risk/Ineligible	Ref.
Any age, all criteria met: • 1 or 2 organs involved • No cardiac involvement • Creatinine clearance ≥ 51 mL/min	Age < 71; either criteria • 1 or 2 organs involved (must include cardiac or renal with creatinine clearance < 51 mL/min) • Asymptomatic or compensated cardiac involvement	Either criteria • 3 organs involved • Advanced cardiac involvement	2
Melphalan dosing • 200 mg/m^2 if ≤60 y • 140 mg/m^2 if 61–70 y • 100 mg/m^2 if ≥ 71 y	**Melphalan dosing** • 140 mg/m^2 if ≤60 y • 100 mg/m^2 if 61–70 y	**Therapy** Melphalan and prednisone Clinical trials	
All of the following: • Age ≤65 y • Cardiac ejection fraction ≥ 0.45 • Lack of pleural effusion • Systolic blood pressure ≥ 90 mmHg • O_2 saturation ≥ 95%, room air • Performance status ≤2 unless due to neuropathy • Stem cell collection ≥ 2.5 x 10^6 cells/kg	All of the following: • Lack of pleural effusion • Systolic blood pressure ≥ 90 mmHg • O_2 saturation ≥ 95%, room air • Performance status ≤2 unless due to neuropathy And any of the following: • Age > 65 y ≤80 y • Cardiac ejection fraction ≥ 0.40 • Stem cell collection ≥ 2.0 x 10^6 cells/kg	Any of the following: • Age > 80 y • Uncompensated congestive heart failure • Cardiac ejection fraction < 0.40 • Persistent pleural effusions • Systolic blood pressure < 90 mmHg • O_2 saturation < 95%, room air • Performance status ≥ 3	3
Melphalan dosing • 200 mg/m^2	**Melphalan dosing** • 140 mg/m^2		

These criteria do not include powerful prognostic cardiac markers, troponins and the amino-terminal of the natriuretic peptide type B (NT-proBNP), which are useful in stratifying the risk in patients undergoing peripheral blood stem cell transplantation (PBSCT). It is expected that these markers will soon be integrated in the selection criteria.

domized clinical trial indicates that the delay associated with pretransplant cytoreduction, using oral melphalan and prednisone (MP), is likely to allow disease progression.[6] Conditioning is nowadays performed with intravenous melphalan using a risk-adapted dose modification (**Table 5**). Total body irradiation, (TBI; 550 cGy) followed by PBSCT was investigated in a small feasibility study: TRM was 15% in patients with poor- or intermediate-risk disease.[7] However, TBI is considered to produce significant cardiac toxicity. Tandem intermediate-dose (140 mg/m^2) melphalan is feasible in selected AL patients in whom a prior uneventful transplantation produced a partial hematologic response that did not translate into organ response. We successfully used this strategy in 2 patients who achieved a partial response at 3 months after the first transplant; both patients attained complete remission.

Transplant-related complications: PBSCT is associated with substantial morbidity and TRM (≤100 days after day 0) that are related to the dose of intravenous melphalan and the number of organs involved. The cause of death varies according to the eligibility criteria adopted by each center. Of the 277 patients who completed treatment in the Boston trials, 36 (13%) died within 100 days: 15 (42%) from cardiac-related causes (9 from sudden death or arrhythmia and 6 from heart failure), a further 9 (25%) from sepsis, and the remaining from various causes.[3] Of the 9 patients who died within day 100 at the Mayo Clinic, 4 died of gastrointestinal tract bleeding with multiorgan failure, and the other 5 died of cardiac arrhythmia, pulmonary embolism, disseminated aspergillosis, pneumonia and aspiration pneumonia.[4] Fatal cardiac arrhythmias and severe respiratory depression immediately after infusion of hematopoietic stem cells have been reported, suggesting dimethyl sulfoxide (DMSO) carries a much greater risk in the presence of amyloid cardiomyopathy. In addition, gastrointestinal bleeding is frequently seen in patients with AL, particularly in those with multiorgan involvement or on hemodialysis. Morbidity is also very high. Grade > 2 toxicities (National Cancer Institute Common Toxicity Criteria [NCI-CTC]) reported in a group of 152 patients were nausea and vomiting (46%), diarrhea (46%), mucositis (46%), peripheral edema (19%), renal toxicity (18%), sepsis (16%), hepatic toxicity (14%), pulmonary edema (13%), gastrointestinal bleeding (7%), and non-gastrointestinal bleeding (7%).[5] Acute renal failure is a frequent (up to 21%) but often, in approximately 50%, reversible complication. Factors predicting transplant-associated acute renal failure included creatinine clearance, proteinuria, cardiac amyloidosis, melphalan dose and sepsis. Opportunistic infections secondary to T cell depression can be seen at 3 months post-transplantation. The risk-adapted approach has contributed to reducing the TRM from the early 30%–40% to the current 13%–14%. As these criteria are further refined by experience, and as new prognostic markers became available and supportive therapy improves, it is expected that TRM will decrease further. Indeed, the TRM observed at the Mayo Clinic in 2003 was as low as 6%.

Hematologic and clinical response: **Table 6** reports the outcome of the main trials including 20 or more patients, reported after the review by Comenzo and

Table 6. Outcome of high (200 mg/m²)/modified (100–140 mg/m²) dose melphalan followed by peripheral blood stem cell transplantation (PBSCT).*

Patients Treated	HDM 200 mg/m^2	TRM#	Clonal Response (partial + complete)	Complete Response	Organ Response	Organ Resp./ Clonal Resp.	Center, Year, Reference
66	38	9/66 (14%)	33/66 (50%)	NR	32/66 (48%)	23/33 (70%)	Single center, 2002[4]
22	14	3/22 (14%)	13/22 (59%)	8/22 (36%)	10/22 (45%)	10/13 (77%)	Two-centers, 2004‡
20	9	7/20 (35%)†	56%	28%	§	NR	Single center, 2004[20]
277¥	155	36/277 (13%)	NR	73/238 (31%)**	80/238 (34%)	48/73 (66%)	Single center, 2004[3]

*Studies reporting 20 or more patients published in year 2000 or later are listed
#Treatment-related mortality within 100 days from melphalan administration
§ Organ response was listed according to organs and not to patients (renal 46%, cardiac 25%, liver 50%, neurologic 0%)
‡ Study performed at the Pavia Amyloidosis Centre and at the National Cancer Institute, Milan, data unpublished.
†After new selection criteria and prophylactic measures were introduced in January 1999, TRM decreased from 50% (5/10) to 20% (2/10)
¥Includes consecutive patients from 6 separate trials over 8 years, please note that 122 patients received an intermediate dose of melphalan (100–140 mg/m^2)
** Hematologic response was evaluated at 1 year: in 39 patients 1 year had not passed since treatment

Abbreviations: HDM, high-dose melphalan; TRM, treatment-related mortality; NR, not reported

Gertz.[2] The clonal (complete or partial) response rate is between 50% and 60% with a complete remission obtained in about one-third of the patients. Complete hematologic (clonal) response is positively associated with the dose of melphalan.[3] Clinical response rates vary from 34% to 55%, strongly depending on the time passed since the transplantation: renal response may require more than 1 year. Clinical response was seen in about 70% of those who had a hematologic response. Among 73 patients who achieved a complete hematologic response, organ responses were renal (29/46, 63%), gastrointestinal and liver (26/46, 57%), neuropathy (17/36, 47%), soft tissue (1/9, 11%), and most notably, more than one fourth (6/22, 27%) of patients with cardiac involvement showed improvement.[3] A complete hematologic response is associated with long-term survival and amelioration of organ dysfunction, which translates into improved quality of life.[3] This unsurpassed outcome might be biased by the patient selection. However, a case-matched control study comparing overall survival of 63 AL patients undergoing PBSCT with 63 patients not undergoing transplantation (52 received alkylating-based oral chemotherapy) showed a significantly prolonged survival in PBSCT patients.[8] The outcome of the ongoing randomized French trial comparing PBSCT versus oral melphalan and Dex will illuminate this very important point. More than 80 of the 100 planned patients have been enrolled as of April 30, 2004.

Maintenance therapy

At present there are no data on the utility of maintenance therapy with corticosteroids or interferon after PBSCT.

Allogeneic bone marrow transplantation

Allogeneic and syngeneic bone marrow transplants have been performed in sporadic cases with reported hematologic complete remission and improved proteinuria. However, selection criteria currently applied for candidates for allogeneic transplants and the toxicity of graft-versus-host disease severely limit their applicability. Nonmyeloablative allografting is still experimental in multiple myeloma and no data are available in AL.

Conventional melphalan and prednisone

Controlled studies indicate that patients given MP therapy benefited, compared to those treated with placebo or colchicine, showing that colchicine has no role in the treatment of AL. In the latest of these studies, a response assessed by organ function and monoclonal protein concentration was observed in 28% of patients treated with MP, and in 30% of them it was obtained after more than 1 year.[9] Patients who responded to MP survived longer than non-responders (50 months vs 36 months, $P = 0.03$). The retrospective review of the Mayo Clinic experience of melphalan-based therapy of AL showed that responses were never seen in patients whose serum creatinine was > 3 mg/dL or whose alkaline phosphatase concentration was more than four times the upper reference limit. Patients with amyloid cardiomyopathy can respond to MP and achieve long survival, while patients with neuropathy rarely benefit from this regimen. The median time to attain a response with melphalan was approximately 1 year, and among responders, 78% survived 5 years. Furthermore, all the 30 patients who survived for 10 years received melphalan-based therapy.

At the Pavia Amyloidosis Centre we have used MP to treat 207 consecutive patients with advanced disease unable to bear more-toxic regimens. According to the criteria outlined above, a response was observed in 40% of patients and translated into a significant survival advantage: median survival 18 months for non-responding versus 72 months in responding patients ($P < 0.001$). Ten percent of patients with heart involvement had a clinical improvement. The median time to achieve a response was 7 months. Although MP is the best-tolerated regimen, this slow response is an important disadvantage since many patients, especially those with rapidly progressive disease, may die due to inexorable amyloid deposition before they have had the chance to respond. The patients who are unable to tolerate prednisone due to advanced cardiac involvement may benefit from low-dose continuous oral melphalan. However, this regimen is not innocuous, since melphalan-based therapies carry the potential (actuarial risk 21%) for the development of late myelodysplasia or acute leukemia.

At the UK National Amyloidosis Centre 33 AL patients were treated with intravenous melphalan 25 mg/m^2 on day 1 associated with Dex 20 mg po daily on days 1–4 every 28 days, as first-line therapy. These patients were selected on the basis of not being fit enough to receive VAD, either due to age, poor performance status, severe amyloid cardiomyopathy or neuropathy. Clonal response, complete or partial, was observed in 46% of patients, with a TRM of 18%. Survival data are not available since the median follow-up was only 8 months.[10] This high TRM may reflect the poor-risk patient population treated and/or an excessive dose of melphalan.

The addition of multiple alkylating agents to MP is not indicated according to the data from a prospective randomized trial.[11]

High-dose dexamethasone-based regimens

A rapid response to therapy is essential in AL amyloidosis. In multiple myeloma VAD may induce a quick clonal response in patients with previously untreated or refractory disease. However, this regimen presents potential problems in AL patients: vincristine can severely exacerbate autonomic or peripheral neuropathy; due to its potential cardiac toxicity doxorubicin cannot be used in patients with overt heart failure; and the intensive high-dose Dex can cause severe fluid retention in patients with renal and cardiac amyloidosis or trigger severe, often fatal, ventricular arrhythmias. There are anecdotal reports of beneficial effects, especially in patients with nephrotic syndrome, although the regimen has not been assessed in a randomized controlled trial. At the UK National Amyloidosis Centre, 98 AL patients selected without symptomatic heart failure, autonomic neuropathy or severe peripheral neuropathy were treated with a median of 4 cycles of standard VAD or CVAMP (cyclophosphamide, vincristine, adriamycin, methyl-prednisolone) as first-line therapy. A clonal response was observed in 53 patients, as defined by a fall in the amyloidogenic class of serum FLC concentration by more than 50%, with improvement of the involved organ in half of patients. TRM was 7%, which is significant considering that these patients were selected for the lack of two important prognostic factors: symptomatic heart involvement and autonomic neuropathy. In 11 of the 53 responding patients (21%) there was subsequent clonal progression after a median time of 20 months (range 7–54).[10]

Results obtained in the treatment of multiple myeloma indicated that Dex accounted for most (80%) of the plasma cell reduction achieved with VAD and avoided the potential toxicity of vincristine and adriamycin. Pulsed high-dose Dex, as used in the VAD regimen, has been reported to benefit AL patients with varying response rates. The recently concluded Southwest Oncology Group (SWOG) trial (S9628) comprised 87 eligible and analyzable patients. Treatment consisted of pulse Dex as in the VAD regimen for 3 cycles followed by maintenance Dex (40 mg × 4 days/mo) and alpha interferon (5 million units thrice weekly).[12] The most common dose limiting toxicity (> grade 2) was increased edema/fluid overload in 12%, requiring dose reduction. Hematologic response was achieved in 53% patients with, notably, 24% complete response. This translated into 45% responses in any organ: renal 39%, soft tissue 25%, gastrointestinal 18%, hepatic 13%, cardiac 12%, and neurological 3%. The median progression-free survival was 27 months and overall survival 31 months. The toxicity of Dex used with the same schedule of the VAD regimen in AL patients is substantial (TRM 7%). A Dex-modified, milder, less toxic schedule (40 mg × 4 days every 21 days) induced organ response in 35% of patients in a median time of 4 months, without significant toxicity.[13] Most of the responses were observed in patients with kidney involvement and rarely in patients with heart involvement.

The association of melphalan to Dex (MDex) in 46 poor-risk patients who were ineligible for PBSCT (70% had severe, symptomatic heart involvement, and 52% had more than 2 organs involved) produced hematologic response in 67% (in a median time of 4.5 months) with 33% complete remission and functional improvement of the target organs in 48%.[14] Subsequent hematologic progression was observed in 3 of the 31 responding patients (10%) after 15, 26 and 41 months; 2 of these patients regained complete remission after 2 more courses of MDex. All other responsive patients maintained the response after a median time of 24 months (range 12–48). Five patients had severe adverse events, but none died. TRM was low (4%). The response rate observed in this poor risk series compares favorably with that achievable in unselected patients with MP and also with the results obtained with Dex plus interferon, with VAD or intermediate-dose melphalan/PBSCT. Despite advanced functional impairment, the hematologic response translated into improved function of the organs involved by the disease in almost half of the patients and resulted in a significant survival benefit. Most importantly, heart failure resolved in 6 of 32 cases (19%). These results indicate that this regimen may be a potential front-line therapy in selected patients.

Thalidomide

Thalidomide is poorly tolerated in AL amyloidosis, causing fatigue, progressive edema, cognitive difficulties, constipation, neuropathy, syncope due to bradycardia, and thromboembolic complications including some not frequently reported for other patient populations such as exacerbation of peripheral and pulmonary edema and worsening of renal function. Severe side effects impeded dose escalation above 200–300 mg/day and caused thalidomide withdrawal in 25%–50% of the patients.[15,16] We used a combination of intermediate-dose Dex (Dex 20 mg on days 1–4, every 21 days) with thalidomide given continuously (100 mg daily, with 100 mg increments up to 400 mg) to treat 31 AL patients who did not respond to or relapsed after first-line therapy. Only 11 patients (35%) tolerated 400 mg/day and received thalidomide for a median of 5.7 months (range: 4–14 months). The remaining 20 patients (65%) did not reach the target dose and received thalidomide (median dose 200 mg/day, range: 100–300 mg/day) for a median of 3 months (range: 0.5–13 months). Hema-

tologic and organ response were correlated with the dose of thalidomide, overall hematologic response was observed in 15 patients (48%), of whom 6 (19%) attained a complete response, and organ response in 8 patients (26%). Median time to response was 3.6 months (range: 2.5–8.0 months). Hematologic response to treatment resulted in a significant survival benefit ($P = 0.01$). Overall, 20 patients experienced severe (CTC grade ≥ 3) treatment-related toxicity: symptomatic bradycardia (8 patients; 26%), sedation/fatigue (4; 13%), constipation (2; 7%), acute dyspnea (2; 7%), deep venous thrombosis, skin lesions, epilepsy and renal failure (1 patient each; 3%, each). TRM was 3%.

A study recently presented by the UK National Amyloidosis Centre (H.J.B. Goodman, personal communication) included 80 patients with AL in whom cytotoxic therapy had either been ineffective or deemed too toxic to pursue. Thalidomide was taken for a median of 6 months (0.4–34), at a median dose of 100 mg/day (50–600). Thalidomide was used alone in 51 patients, with Dex in 8, and in combinations with alkylating agents in the remaining patients. Somnolence, constipation and/or neuropathy occurred in 50 patients (62%), symptomatic sinus bradycardia in 4 (5%), 3 had venous thromboses and 1 had a major arterial clot. Thalidomide was discontinued due to adverse effects in 25 patients (31%). There was no TRM. Partial hematologic response was observed in 24/44 (55%) patients treated with thalidomide alone and in 18/26 (69%) of those treated with any combination. No complete response was observed. Incidentally, the authors noticed that thalidomide appeared to increase all non-clonal light chains. Thus, data of serum FLC concentration need to be evaluated carefully. Organ function improved or remained stable in 26% of the 62 evaluable cases, and SAP scintigraphy showed regression of amyloid in 9 of the 50 evaluable patients. Seventeen patients on thalidomide died of progressive disease. This study indicates that lower doses of thalidomide are better tolerated but also produce fewer and incomplete hematologic responses. Overall, it seems that the combination of thalidomide and Dex may represent a valid option for refractory and relapsed patients. Due to the fragility of these patients, lower doses of thalidomide should be used and careful monitoring of the organ toxicity is necessary. At our center, we found monthly Holter monitoring helpful in detecting and treating bradycardia promptly.

Investigational Therapies

The thalidomide analog, Revlimid, and the proteasome inhibitor, Velcade, are both active in advanced and refractory multiple myeloma. The ability of these drugs, in combination with Dex, to rapidly reduce the level of the monoclonal protein makes them an attractive option also for AL patients, although more data on response duration and toxicity are needed.

The iodinated anthracycline, 4′ -iodo-4′ deoxydoxorubicin, used at a low, nonmyelosuppressive and nontoxic dosage, has produced responses in 6 of 40 (15%) patients in a multicenter trial. Strategies to combine 4′ -iodo-4′ -deoxydoxorubicin with chemotherapy to suppress precursor production and promote amyloid resorption would be a rational approach.

Along the same line, in an effort to promote amyloid resorption, small molecules targeting the common fibrillar architecture and common protective elements have been designed and tested in animal models and Phase II clinical trials. A compound able to crosslink serum SAP and clear it from circulation is under evaluation in patients with systemic amyloidoses, including a few patients with AL, at the UK National Amyloidosis Centre.

Antitumor necrosis factor alpha (TNFα) therapy, in the form of etanercept, in 16 patients with advanced AL produced symptomatic improvement in most of them, and half had objective responses, notably in those with macroglossia.[17] Larger trials are ongoing to evaluate the role and best dosage of etanercept in the management of AL.

Rituximab may be considered in patients with AL amyloidosis and IgM monoclonal protein.

Immunotherapy, both active and passive, is another challenging and promising approach. Dendritic cell-based idiotype vaccination has shown no side effects, but limited clinical activity. AL-amyloid burden can be markedly reduced in mice by passive immunization with an anti-light chain murine monoclonal antibody specific for an amyloid-related epitope.[18] A humanized antibody is being produced for a Phase I/II clinical trial in patients with AL.

Treatment of Localized AL Amyloidosis

Local production of amyloidogenic light chains and their deposition as amyloid fibrils can occur along the respiratory tract and in the bladder, urethra, head, neck and skin. Treatment is conservative and is based on excision and local therapy, although local relapses are possible with airway compromise. Amyloid deposits along the respiratory tract are best treated using endoscopic laser resection with possible stent implantation. Diffusely distributed tracheobronchial amyloidosis, considered unsuitable for bronchoscopic intervention, can obtain long-lasting benefit from external-beam radiation therapy. Amyloid of the bladder and urethra has been reported to benefit from local instillation of DMSO. Cutaneous amyloidosis can also be treated with topical application of DMSO.

Supportive Therapy

Supportive treatment aimed at improving or palliating organ function, maintaining quality of life, and prolonging survival whilst specific therapy has time to take effect has an important impact on survival. Supportive care should be considered a fundamental part of an integrated treatment approach to these patients and requires the coordinated expertise of several specialists who are familiar with this disease.

The mainstay of the treatment of amyloid cardiomyopathy is salt restriction and careful administration of diuretics, such as furosemide, scrupulously avoiding aggravation of intravascular volume contraction (due to concomitant nephrotic syndrome) and postural hypotension. If furosemide becomes ineffective in controlling edema, the addition of metolazone or spironolactone can be beneficial. Patients with reduced stroke volume can benefit from afterload reduction with angiotensin-converting enzyme inhibitors. However, these agents should be used with great caution, starting at the lowest effective dose, escalating carefully and withdrawing if postural hypotension develops. Digoxin is not generally helpful, with the possible exception in patients with atrial fibrillation and rapid ventricular response. Calcium channel blockers can aggravate the congestive heart failure. Patients with recurrent syncope may require permanent pacemaker implantation. Menacing ventricular arrhythmias benefit from treatment with amiodarone. In patients with end-stage heart failure, heart transplantation is the only life-saving procedure, which may allow subsequent treatment to control the amyloidogenic clone. Several patients have been transplanted at the Mayo Clinic and at the Boston University Amyloid Center. The main problem is recurrence of amyloid in the transplanted organ as well as progression in other organs. For this reason heart transplantation must be followed by anti-clone therapy. Although the long-term survival is statistically inferior to that of patients with non-amyloid heart disease, the actuarial 5-year survival appears to be 50%. Carefully selected patients, without other significant organ involvement, can benefit from this procedure.

Orthostatic hypotension is difficult to manage. The use of a waist-high, fitted elastic leotard is helpful. In our experience fluoricortisone is poorly tolerated because of aggravation of fluid retention. Midodrine can be helpful: the full dosage, 10 mg 3 times a day, should be reached gradually starting from the lower dose of 2.5 mg daily, and its renal excretion requires attention in patients with renal failure. Its main adverse effect is supine hypertension. Continuous noradrenaline infusion has been reported to be a successful treatment of severe hypotension refractory to conventional treatment.

Therapy of renal amyloidosis is limited to the control of the edema by diuretics. The main damaging mechanism is progressive tubular injury caused by glomerular protein loss. The use of angiotensin-converting enzyme inhibitors, in an attempt to reduce proteinuria, is reasonable, although their efficacy has not been proven. Treatment of hypercholesterolemia should be considered. Renal vein thrombosis is rarely, if ever, seen in these nephrotic patients and prophylactic anticoagulation is not recommended. End-stage renal failure is treated by dialysis. Both peritoneal dialysis and hemodialysis are equally effective. If the disease is not controlled by chemotherapy, extrarenal progression of amyloidosis is the main cause of death. Renal transplantation should be offered on a case-by-case basis to patients without symptomatic extrarenal involvement.

Diarrhea is a common and incapacitating problem. Octreotide decreases diarrhea both in its short-acting form (starting with 50 μg twice a day up to 100 μg thrice a day) and its long-acting depository form (10, 20 and 30 mg doses, administered every 4 weeks, adjusting the dosage to the response of diarrhea). The patient's social life can be improved by palliative diverting ostomies. Chronic intestinal pseudo-obstruction is usually refractory to treatment. Adequate oral or intravenous feeding is mandatory in patients with significant undernourishment. Patients who present with severe liver failure may be considered for liver transplantation. Successful sequential liver and stem cell transplantations have been reported.

Neuropathic pain is difficult to control. Gabapentin (starting with 300 mg daily and with daily increments up to 1800 mg), although well tolerated, often fails to relieve pain. Non-nephrotoxic analgesics may be used as adjuvant agents.

Bleeding in AL amyloidosis is frequent and multifactorial. Factor X deficiency dramatically improves following effective chemotherapy, including PBSCT, or after splenectomy.

Treatment Strategies

The availability of several effective regimens allows a better tailoring of treatment aimed at obtaining the most rapid and best suppression of the synthesis of the offending light chain at the minimum toxicity cost. In designing the therapeutic strategy, we must consider that although complete hematologic remission may seem the therapeutic target, reducing the amyloidogenic serum FLC concentration by 50%–75% is often sufficient to lead to stabilization or regression of amyloid deposits, with potential for improved organ function and extended survival.[19] However, the final outcome will be determined by changes in organ function, which

occur over a longer period. In order to minimize the toxicity associated with chemotherapy and gain precious time for possible alternative treatments, an aggressive follow-up with serial measurements of the monoclonal protein is recommended. In the case of PBSCT, monthly measurements could allow responses to be detected quickly, although it can take several months to reach the best response. In our experience, only 1 of the 7 patients who failed to show a hematologic response at +3 months obtained partial response at +12 months, suggesting that patients who do not respond by 3 months should be considered for alternative therapy, avoiding potentially harmful delay. Nonmyeloablative therapy, if tolerated, should be pursued to best response or plateau. It may be appropriate to discontinue chemotherapy if the monoclonal protein (a) is no longer detectable by high resolution immunofixation and with normal FLC ratio; (b) has fallen to a plateau level by 50% or more, for at least 3 months, and, despite signs of organ response, toxicity renders further chemotherapy undesirable; (c) has not fallen, or has increased after 2–3 courses of treatment, suggesting that an alternative regimen should be considered.

The main effective chemotherapy regimens for systemic AL amyloidosis have advantages and disadvantages, which are outlined in **Table 7**. Unfortunately, there are no data yet from prospective randomized trials to support the use of one agent over another, and the choice of strategy is mostly based on nonrandomized studies and personal experience. This accounts for discrepancies in the strategies proposed by different investigators. It is recommended that patients should be treated in the context of clinical trials whenever possible. Based on these considerations some suggestions can be made (**Box 1**).

Table 7. Primary treatment of AL: advantages and disadvantages of main regimens.

Regimen	Hematologic Response % (CR)	Organ Response %	Median Time to Hematologic Response (mo)	TRM %	Advantages	Disadvantages
PBSCT	45–60 (14–36)	34–55	3–4	13–14	•high response rate, •improved quality of life, •prolonged survival	• TRM and morbidity still significant • limited patient eligibility
MDex	67 (33)	48	4.5	4	•significant response rate, •low toxicity •applicable to most AL patients	• limited experience • depletion of stem cells
VAD	54 (NR)	50	NR	7	•significant response rate •no depletion of stem cells	• patient selection (vincristine is a poor choice in amyloid neuropathy and doxorubicin in amyloid cardiomyopathy) • significant TRM
HD-Dex	40–53† (16–24†)	12–45†	4	7	•significant response rate •no depletion of stem cells	• response rate improvable • low response rate in cardiac amyloid • significant heart-related TRM
MP	28–36 (uncommon)	25–30	7–11	low ~ 2	•low toxicity •well tolerated •can be applied in virtually all patients	• low response rate • unacceptably long time to achieve a response
Thalidomide*	25–69 (0–19)	25–30	4	low 0–3	•significant response rate •likely less marrow suppression	• limited tolerability due to severe toxicity

† Data from the SWOG study using intensive HD-Dex induction followed by maintenance with HD-Dex and interferon.
* In the study conducted at the Pavia Centre thalidomide was associated with intermediate dose dexamethasone; in the study conducted at the London Centre 51 patients were treated with thalidomide alone, and 29 patients were treated with thalidomide in association with dexamethasone and/or other alkylating agents.

Abbreviations: TRM, treatment-related mortality; NR, not reported; PBSCT, peripheral blood stem cell transplantation; MDex, melphalan and dexamethasone; VAD, vincristine, doxorubicin, dexamethasone; HD-DEX, high-dose dexamethasone; MP, melphalan and prednisone

Conclusions and Future Directions

AL amyloidosis is a treatable disease, and a substantial proportion of patients now attain a long survival: in our population, 22% of patients survive more than 10 years. There are several reasons for this improvement: early diagnosis, better treatment selection, more effective regimens and improved supportive therapy. New and sensitive biomarkers of cardiac dysfunction (BNP, troponins) and for monitoring the clone (by serial FLC quantification) are likely to improve patient selection, therapy management, and, possibly the outcome. Several investigational agents are under evaluation, and new effective drugs against the neoplastic plasma cells are already available. The outcome of ongoing randomized trials comparing PBSCT and other less toxic chemotherapy regimens will help greatly in optimizing the treatment of this difficult, but now manageable, disease.

Box 1. Strategy for AL treatment.

1. Patients who fulfill the criteria for high-dose melphalan (200 mg/m^2), with normal NT-proBNP and cardiac troponins serum concentration, may have stem cells harvested and may be offered the transplantation procedure. Patients who attain a partial hematologic response, not followed by organ response, can be considered for a second PBSCT or for any of the other regimens without melphalan. It is recommended that PBSCT be performed in units with expertise with AL amyloidosis.
2. Patients who are at intermediate risk (**Table 5**) should have stem cells harvested, and then may be treated with melphalan and high-dose dexamethasone (MDex). This regimen showed rapid action and hematologic complete response rates comparable to those obtained in PBSCT performed with modified melphalan (100–140 mg/m^2), but with very low toxicity. Alternatively, regimens not affecting the stem cell reservoir can be considered, keeping in mind that their toxicity is not negligible (7% TRM): a) VAD-like high-dose Dex induction followed by maintenance with Dex (and interferon)* b) patients < 70 years of age, without symptomatic heart involvement, autonomic neuropathy or polyneuropathy can be treated with VAD. Patients who attain a hematologic response and improvement of organ function and become eligible for PBSCT (melphalan 200 mg/m^2) can be considered for this procedure in case of hematologic relapse.
3. Patients who are considered at poor risk (**Table 5**) should be treated with MP or, preferably, included in investigational trials with the aim of improving the rate of response and its rapidity. In fact, these patients are those in most need of a rapidly effective treatment. For this reason at our center we offer these patients a trial with intermediate dose Dex (20 mg orally, days 1–4, every 4 weeks), melphalan (0.25 mg/kg, adjusted to moderate mid-cycle myelosuppression, days 1–4, every 4 weeks) and thalidomide (100–200 mg/day continuously).
4. Patients relapsing after alkylating-based chemotherapy may be offered intermediate-dose Dex and thalidomide.
5. Supportive therapy remains important in all patients. Sequential solid organ and stem cell transplantation should be considered in selected patients.

* Interferon might deplete stem cells

Abbreviations: PBSCT, peripheral blood stem cell transplantation; VAD, vincristine, doxorubicin, dexamethasone; Dex, dexamethasone; TRM, treatment-related mortality; MP, melphalan and prednisone

III. Waldenström's Macroglobulinemia

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Waldenström's macroglobulinemia (WM) is a distinct clinicopathological entity resulting from the proliferation of B lymphocytes that show maturation to plasma cells, constituting a pathognomonic bone marrow lymphoplasmacytic infiltrate, and that synthesize monoclonal IgM.[1] This condition is considered to correspond to the lymphoplasmacytic lymphoma as defined by the Revised European American Lymphoma (REAL) and World Health Organization classification systems.

Epidemiology and Etiology

WM is an uncommon disease, accounting for approximately 2% of all hematologic malignancies. The incidence rate for WM is higher among Caucasians, with African descendants representing only 5% of all patients. Genetic factors appear to be an important factor. There have been numerous reports of familial disease, including multigenerational clustering of WM and other B cell lymphoproliferative diseases. In a recent study, approximately 20% of 181 serial WM patients presenting to a tertiary referral had a first-degree relative with either WM or another B cell disorder **(Figure 4**; see Color Figures, page 516**).**[2] Frequent association with other immunological disorders in healthy relatives, including hypogammaglobulinemia and hypergammaglobulinemia (particularly polyclonal IgM), autoantibody (particularly to thyroid)

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production, and manifestation of hyperactive B cells have been reported. The role of environmental factors in WM is undetermined. There is no clear association with chronic antigenic stimulation from infections, autoimmune diseases, or allergy or with specific occupational exposure. The relevance of viral infection remains to be established. Data regarding a possible link between hepatitis C virus (HCV) and human herpesvirus-8 (HHV-8) and WM remain controversial.

Biology

Cytogenetic findings

Several studies, usually performed on limited series of patients, demonstrated a great variety of numerical and structural chromosome abnormalities. Numerical losses involving chromosomes 17, 18, 19, 20, 21, 22, X, and Y have been commonly observed, though gains in chromosomes 3, 4, and 12 have also been reported. Chromosome 6q deletions encompassing 6q21-22 have been observed in 40%–90% of WM patients, and at a comparable frequency among patients with and without a familial history.[3,4] Several candidate tumor suppressor genes in this region are under study, including BLIMP-1, a master regulatory gene implicated in lymphoplasmacytic differentiation. Notable, however, is the absence of IgH switch region rearrangements in WM, a finding that may be used to distinguish WM from cases of IgM myeloma where IgH switch region rearrangements are a predominant feature.[5]

Nature of the clonal cell

The WM bone marrow B cell clone shows intraclonal differentiation from small lymphocytes with large focal deposits of surface immunoglobulins, to lymphoplasmacytic cells, to mature plasma cells that contain intracytoplasmic immunoglobulins. Clonal B cells are sometimes detectable among blood B lymphocytes, and their number increases in patients who fail to respond to therapy or who progress.[6] These clonal blood cells present the peculiar capacity to differentiate spontaneously, in in vitro culture, to plasma cells. This is through an interleukin-6 (IL-6)–dependent process in IgM monoclonal gammopathy of undetermined significance (MGUS) and mostly an IL-6–independent process in WM patients. All these cells express the monoclonal IgM present in the blood, and a variable percentage of them also express surface IgD. The characteristic immunophenotypic profile of the lymphoplasmacytic cells in WM includes the expression of the pan-B cell markers CD19, CD20, CD22, CD79, and FMC7.2.[7,8] Expression of CD5, CD10, and CD23 may be found in 10%–20% of cases, and does not exclude the diagnosis of WM.[9] Moreover, expression of CD10 and CD23 may be clinically significant. A higher incidence of familial disease has been reported with CD10 expression, whereas more pronounced hypogammaglobulinemia was observed in patients with expression of either CD10 or CD23.[9]

The phenotype of lymphoplasmacytic cells in WM suggests that the clone is a post-germinal center B cell. This indication is further strengthened by the results of the analysis of the nature (silent or amino-acid replacing) and distribution (in framework or complementarity-determining regions [CDR]) of somatic mutations in Ig heavy- and light-chain variable regions performed in patients with WM.[10,11] This analysis showed a high rate of replacement mutations, compared with the closest germline genes, clustering in the CDR and without intraclonal variation. Subsequent studies showed a strong preferential usage of VH3/JH4 gene families, no intraclonal variation, and no evidence for any isotype-switched transcripts.[12,13] These data indicate that WM may originate from an IgM^+ and/or IgM^+ IgD^+ memory B cell. Normal IgM^+ memory B cells localize in bone marrow, where they mature to IgM-secreting cells.

Bone marrow microenvironment

Increased numbers of mast cells are found in the bone marrow of WM patients, wherein they are usually admixed with tumor aggregates **(Figure 5**; see Color Figures, page 516**)**.[14,15] Recent studies from Tournilhac et al demonstrated that mast cells induced WM cell proliferation and/or tumor colony formation—through, in part, constitutive expression of CD40 ligand (CD40L) (**Figure 5**; see Color Figures, page 516). Furthermore, it was shown by these investigators that WM cells may in part support mast cell expansion through elaboration of interleukin-3 (IL-3), a cytokine expressed by WM cells and found at significantly elevated levels in the sera of WM patients.[15]

Clinical Features

Waldenström's macroglobulinemia is a disease of the elderly, with a median age of 63 years (range 25–92), with a slight predominance of males over females.[16] The symptoms are usually vague and non-specific, the most common being weakness, anorexia, and weight loss. Raynaud's phenomenon and symptoms due to peripheral neuropathy may precede more serious manifestations by many years. Symptoms and physical findings at diagnosis are summarized in **Table 8**. Hepatosplenomegaly and lymphadenopathy are prominent in a minority of patients. Purpura is frequently associated with cryoglobulinemia and more rarely with AL amyloidosis, while hemorrhagic manifestations and neuropathies are multifactorial (see below). The morbidity

Table 8. Presenting features and physical findings at diagnosis in 215 patients with Waldenstrom's macroglobulinemia (reprinted from Merlini[39]).

	Frequency (%)
Symptoms	
Weakness	66
Anorexia	25
Peripheral neuropathy	24
Weight loss	17
Fever	15
Raynaud's phenomenon	11
Physical findings	
Hepatomegaly (> 2 cm from the costal margin)	20
Splenomegaly	19
Lymphadenopathy	15
Purpura	9
Hemorrhagic manifestations	7

associated with WM is caused by the concurrence of two main components: tissue infiltration by neoplastic cells and, more importantly, the physicochemical and immunological properties of the monoclonal IgM.

As shown in **Table 9**, the monoclonal IgM can produce clinical manifestations through several different mechanisms related to its physicochemical properties, nonspecific interactions with other proteins, antibody activity, and tendency to deposit in tissues.

Laboratory Investigations and Findings

Laboratory findings are summarized in **Table 10**.

Hematologic abnormalities

Anemia is the most common finding in patients with symptomatic WM and is caused by a combination of factors: mild decrease in red cell survival, impaired erythropoiesis, hemolysis, moderate plasma volume expansion, and blood loss from the gastrointestinal tract. Blood smears are usually normocytic and normochromic, and rouleaux formation is often pronounced. Electronically measured mean corpuscular volume may be elevated spuriously owing to erythrocyte aggregation. In addition, the hemoglobin estimate can be inaccurate, i.e., falsely high, because of interaction between the monoclonal protein and the diluent used in some automated analyzers. Leukocyte and platelet counts are usually within the reference range at presentation, although patients may occasionally present with severe thrombocytopenia. As reported above, monoclonal B-lymphocytes expressing surface IgM and late-differentiation B cell markers are uncommonly detected in blood by flow cytometry. A raised erythrocyte sedimentation rate is almost constantly observed in WM and may be the first clue to the presence of the macroglobulin. The clotting abnormality detected most frequently is prolongation of thrombin time. AL amyloidosis should be suspected in all patients with nephrotic syndrome, cardiomyopathy, hepatomegaly, or peripheral neuropathy. Diagnosis requires the demonstration of green birefringence under polarized light of amyloid deposits stained with Congo red.

Biochemical investigations

High-resolution electrophoresis combined with immunofixation of serum and urine are recommended for identification and characterization of the IgM monoclonal protein **(Figure 6**; see Color Figures, page 517**)**. The light chain of the monoclonal IgM is κ in 75%–80% of patients. A few WM patients have more than one M-component. The concentration of the serum monoclonal protein is very variable but in most cases lies within the range of 15–45 g/L. Densitometry should be considered to determine IgM levels for serial evaluations because nephelometry sometimes is unreliable and shows large intralaboratory as well as interlaboratory variation. The presence of cold agglutinins or cryoglobulins may affect determination of IgM levels and, therefore, testing for cold agglutinins and cryoglobulins should be performed at diagnosis. If present, subsequent serum samples should be analyzed under warm conditions for determination of serum monoclonal IgM level. Although Bence Jones proteinuria is frequently present, it exceeds 1 g/24 hours in only 3% of cases.[17] Serum β2-microglobulin was above the upper limit of the reference range (3 mg/L) in approximately 60% of WM patients at diagnosis (**Table 10**).

Table 10. Presenting laboratory findings for patients with Waldenström's macroglobulinemia.[39]

Parameter	Frequency (%)
Hemoglobin < 120 g/L	63
WBC < 3 x 10^9/L	4
Platelets < 100 x 10^9/L	16
IgM monoclonal component:	
kappa/lambda;	80/20
> 30g/L	35
Bence Jones proteinuria	38
Serum β_2-microglobulin > 3mg/L	62
Relative serum viscosity > 4	17

Table 9. Clinical manifestations caused by monoclonal IgM (From Merlini[39]).

Properties of the Monoclonal IgM	Resulting Condition	Clinical Manifestations
Physicochemical		
Intrinsic viscosity	Hyperviscosity syndrome	Fatigue, headache, blurred vision, easy mucosal bleeding, impaired mentation up to coma
Precipitation on cooling	Cryoglobulinemia type I	Raynaud's phenomenon, acrocyanosis, necrosis, ulcers, purpura, cold urticaria
Protein-protein interaction	Hemostatic abnormalities	Bleeding diathesis: bruising, purpura, mucosal bleeding; rarely, brain hemorrhages
Antibody activity versus:		
Nerve constituents	Polyneuropathies	Anti-MAG-related: symmetric, distal, progressive, sensorimotor neuropathy, ataxic gait, bilateral foot drop
		IgM with other specificities: • symmetric, distal, progressive painful sensory neuropathy • prominent motor neuropathy
IgG	Cryoglobulinemia type II	Weakness, purpura, arthralgias, proteinuria, renal failure, progressive, symmetric distal sensorimotor neuropathy combined with mononeuropathies (e.g., foot or wrist drop)
RBC antigens	Cold agglutinin hemolytic anemia	Mild, chronic hemolytic anemia exacerbated after cold exposure; Raynaud's phenomenon, acrocyanosis and livedo reticularis
Tendency to deposit into tissues		
As amorphous aggregates in skin, GI tract, kidney	Specific organ dysfunction	Skin: bullous skin disease, papules on extremities GI: diarrhea, malabsorption, bleeding Kidney: mild, reversible proteinuria, mostly asymptomatic
As amyloid fibrils (light chains)	AL amyloidosis	Fatigue, weight loss, periorbital purpura, edema, hepatomegaly, macroglossia Dysfunction of organs involved: kidneys, heart, liver, peripheral sensory and autonomic neuropathies

Abbreviations: GI, gastrointestinal; MAG, myelin-associated glycoprotein; RBC, red blood cell.

Blood viscosity

Blood viscosity should be measured if the patient has signs or symptoms of hyperviscosity syndrome. Measurement of viscosity in whole blood at low shear rates may be the best indicator of hemorheological changes in patients with WM. In practice, a correlation between level of M-protein and symptoms may be used to anticipate repeat plasma exchanges as the M-protein approaches the level associated with hyperviscosity. Fundoscopy remains an excellent indicator of clinically relevant hyperviscosity. Cryoglobulins should be searched for in the presence of suggestive clinical features. Rheumatoid factor activity and low C4 levels (< 8 mg/dL) are common findings in type II cryoglobulinemia.

Bone marrow findings

The bone marrow is always involved in WM. Bone marrow biopsy is necessary since aspiration frequently yields a "dry tap." Three cytological subtypes have been identified in conjunction with patterns of bone marrow infiltration: lymphoplasmacytoid, constituted by small lymphocytes and plasmacytoid cells characterized by a nodular pattern (47% of all patients); lymphoplasmacytic, in which small lymphocytes and mature plasma cells predominate and mast cells may be conspicuous, associated mainly with an interstitial/nodular pattern (42%); and polymorphous, with a packed marrow and characterized by a wide spectrum of cells, including small lymphocytes, plasmacytoid cells, plasma cells, large transformed cells, and immunoblasts with mitotic figures (11%).[18] "Intranuclear" periodic acid-Schiff (PAS)-positive inclusions (Dutcher-Fahey bodies) con-

Table 11. Classification of Waldenström's macroglobulinemia (WM) and related disorders.

Disorder	Laboratory Features	Clinical Features
WM	Lymphoplasmacytic infiltrate in marrow Specific immunophenotype[a] Serum monoclonal IgM	May be symptomatic or asymptomatic
IgM-related disorder	No marrow infiltration	Symptomatic, e.g., peripheral neuropathy, cryoglobulins, cold agglutinin disease, AL amyloidosis
IgM MGUS	No marrow infiltrate	Asymptomatic
Other B cell lymphoproliferative disorders[b]	Differentiate by immunophenotype and morphological characteristics	
μ-HCD	Heavy-chain fragment with no associated light chain	
IgM myeloma	IgM-producing plasma cells (cytoplasmic IgM^+, $CD20^{\pm}$ $CD138^+$, t(11;14))	Possibly associated with lytic skeletal lesions and hypercalcemia

[a] $sIg^+CD19^+CD20^+CD22^+CD79^+$

[b] Chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma, extranodal marginal-zone lymphoma, follicular lymphoma and mantle-cell lymphoma.

Abbreviations: HCD, heavy-chain disease; MGUS, monoclonal gammopathy of undetermined significance.

sisting of IgM deposits in the perinuclear space, and sometimes in intranuclear vacuoles, may be seen occasionally in lymphoid cells.

Treatment of Waldenström's Macroglobulinemia

As part of the 2nd International Workshop on Waldenström's Macroglobulinemia, a consensus panel was organized to recommend criteria for the initiation of therapy in patients with WM. The panel recommended that initiation of therapy should not be based on the IgM level per se, since this may not correlate with the clinical manifestations of WM. The consensus panel, however, agreed that initiation of therapy was appropriate for patients with constitutional symptoms, such as recurrent fever, night sweats, fatigue due to anemia, or weight loss. The presence of progressive symptomatic lymphadenopathy or splenomegaly provides additional reasons to begin therapy. The presence of anemia with a hemoglobin value of ≤10 g/dL or a platelet count $\leq 100 \times 10^9$/L owing to marrow infiltration also justifies treatment. Certain complications, such as hyperviscosity syndrome, symptomatic sensorimotor peripheral neuropathy, systemic amyloidosis, renal insufficiency, or symptomatic cryoglobulinemia, may also be indications for therapy (**Table 11**).[19]

Options for Initial Therapy

A precise therapeutic algorithm for the upfront treatment of WM remains to be defined given a paucity of randomized clinical trials in this uncommon disorder. The consensus panel of the 2nd International Workshop on Waldenström's Macroglobulinemia considered alkylator agents (e.g. chlorambucil), nucleoside analogs (cladribine or fludarabine), and the monoclonal antibody rituximab as reasonable choices for upfront therapy of WM.[20] Importantly, the panel felt that individual patient considerations, including the presence of cytopenias, need for more rapid disease control, age, and candidacy for autologous transplant therapy, should be taken into account in making the choice of a first-line agent. For patients who are candidates for autologous transplant therapy, and in whom such therapy is seriously considered, the panel recommended that exposure to alkylator or nucleoside analogue therapy be limited.

Alkylator-Based Therapy

Oral alkylating drugs, alone and in combination therapy with steroids, have been extensively evaluated in the upfront treatment of WM. The greatest experience with oral alkylator therapy has been with chlorambucil, which has been administered on both a continuous (i.e., daily dose schedule) as well as an intermittent schedule. Patients receiving chlorambucil on a continuous schedule typically receive 0.1 mg/kg per day, while on the intermittent schedule patients will typically receive 0.3 mg/kg for 7 days, every 6 weeks. In a prospective randomized study, Kyle et al (reviewed in Merlini and Treon[21]) reported no significant difference in the overall response rate between these schedules **(Table 12)**, although interestingly the median response duration was greater for patients receiving intermittent versus continuously dosed chlorambucil (46 vs 26 months). Despite the favorable median response duration in this study for use of the intermittent schedule, no difference in the me-

Table 12. Alkylator-based therapy in Waldenström's macroglobulinemia (reviewed in Merlini and Treon[21]).

Study	N	Setting	Regimen	Major RR[a]	Median Response Duration
Facon	110	UnRx	Chl (continuous)	31%	NA
Kyle	24	UnRx	Chl (continuous)	75%	26 months
	22	UnRx	Chl (intermittent)	64%	46 months
Dimopoulos	77	UnRx	Chl, P	72%	NA
Petrucci	31	UnRx	M,C,P → CP (continuous)	74%	66 months
Case	33	UnRx and Rx	M-2 (BCNU,V,M,P)	82%	43 months (CR), 39 months (PR)

[a] ≥ 50% reduction in serum IgM levels.

Abbreviations: CR, complete response; NA, not applicable; PR, partial response. Rx, previously treated; UnRx, previously untreated; Chl, chlorambucil; P, prednisone; M, melphalan; C, cyclophosphamide; V, Vincristine.

dian overall survival was observed. Moreover, an increased incidence for development of myelodysplasia and acute myelogenous leukemia with the intermittent (3 of 22 patients) versus the continuous (0 of 24 patients) chlorambucil schedule prompted the authors of this study to express preference for use of continuous chlorambucil dosing. The use of steroids in combination with alkylator therapy has also been explored. Dimopoulos and Alexanian (reviewed in[21]) evaluated chlorambucil (8 mg/m^2) along with prednisone (40 mg/m^2) given orally for 10 days, every 6 weeks, and reported a major response (i.e., reduction of IgM by greater than 50%) in 72% of patients. Non-chlorambucil-based alkylator regimens employing melphalan and cyclophosphamide in combination with steroids have also been examined by Petrucci et al and Case et al (reviewed in[21]) producing slightly higher overall response rates and response durations, although the benefit of these more complex regimens over chlorambucil remains to be demonstrated. Facon et al (reviewed in[21]) have evaluated parameters predicting for response to alkylator therapy. Their studies in patients receiving single-agent chlorambucil demonstrated that age > 60, male sex, symptomatic status, and cytopenias (but, interestingly, not high tumor burden and serum IgM levels) were associated with poor response to alkylator therapy. Additional factors to be taken into account in considering alkylator therapy for patients with WM include necessity for more rapid disease control given the slow nature of response to alkylator therapy, as well as consideration for preserving stem cells in patients who are candidates for autologous transplant therapy.

Nucleoside Analogue Therapy

Both cladribine and fludarabine have been extensively evaluated in untreated as well as previously treated WM patients. Cladribine administered as a single agent by continuous intravenous infusion, by 2-hour daily infusion, or by subcutaneous bolus injections for 5–7 days has resulted in major responses in 40%–90% of patients who received primary therapy, while in the salvage setting responses have ranged from 38% to 54% **(Tables 13** and **14**; reviewed in[21]**)**. Median time to achievement of response in responding patients following cladribine ranged from 1.2 to 5 months.[22,23] The overall response rate with daily infusional fludarabine therapy administered mainly on 5-day schedules in previously untreated and treated WM patients has ranged from 38% to 100% and 30% to 40%, respectively, which

Table 13. Nucleoside analogues in untreated Waldenström's macroglobulinemia (reviewed in Merlini and Treon[21]).

Study	Number of Patients	Median Number of Courses	Major RR[a]	Median Response Duration
Cladribine				
Dimopoulos	26	2	85%	2+–39+ months
Delannoy	5	2	40%	NA
Fridrik	10	4	90%	NA
Liu	7	3	57%	NA
Hellman	9	4	44%	NA
Fludarabine				
Dimopoulos	2	3	100%	NA
Foran	15	5.2[b]	79%	40 months
Thalhammer-Scherrer	7	6	85%	44+ months
Dhodapkar	118	4–8	38%	59 months

[a] ≥ 50% reduction in serum IgM levels.

[b] Mean number of infusions.

Abbreviations: RR, response rate; NA, not applicable.

Table 14. Nucleoside analogues in previously treated Waldenström's macroglobulinemia (reviewed in Merlini and Treon[21]).

Study	Number of Patients	Median Number of Courses	Major RR[a]	Median Response Duration
Cladribine				
Dimopoulos	46	2	43%	12 months
Delannoy	13	2	38%	NA
Betticher	25	3	40%	8 months
Liu	13	3	54%	NA
Hellman	13	4	38%	NA
Fludarabine				
Dimopoulos	26	3	31%	NA
Zinzani	12	6	41%	10+ months
Leblond	71	6	30%	32 months
Dhodapkar	64	4–8	33%	30 months

[a] ≥ 50% reduction in serum IgM levels.

Abbreviations: NA, not applicable; RR, response rate.

are on par with the response data for cladribine **(Tables 13** and **14**; reviewed in [21]). Median time to achievement of response for fludarabine was also on par with cladribine at 3–6 months. In general, response rates and durations of responses have been greater for patients receiving nucleoside analogs as first-line agents, although in four studies wherein both untreated and previously treated patients were enrolled, no substantial difference in the overall response rate was reported (reviewed in [21]). Myelosuppression commonly occurred following prolonged exposure to either of the nucleoside analogs, as did lymphopenia with sustained depletion of both $CD4^+$ and $CD8^+$ T lymphocytes observed in WM patients 1 year following initiation of therapy.[22] Treatment-related mortality due to myelosuppression and/or opportunistic infections attributable to immunosuppression occurred in up to 5% of all treated patients in some series with either nucleoside analogue. Factors predicting for response to nucleoside analogues in WM included age at start of treatment (< 70 years), pretreatment hemoglobin > 95 g/L, platelets > 75,000/mm^3, disease relapsing off therapy, patients with resistant disease within the first year of diagnosis, and a long interval between first-line therapy and initiation of a nucleoside analog in relapsing patients (reviewed in[21]). There are limited data on the use of an alternate nucleoside analogue to salvage patients whose disease relapsed or demonstrated resistance off cladribine or fludarabine therapy. Three of four patients (75%) responded to cladribine following relapse to fludarabine therapy, whereas only 1 of 10 (10%) with disease resistant to fludarabine responded to cladribine (reviewed in [21]). In a study by Lewandowski et al (reviewed in [21]), 2 of 6 patients (33%) responded to fludarabine, in spite of an inadequate response or progressive disease following cladribine therapy.

Monoclonal Antibody Therapy

Monoclonal antibody therapy has been extensively evaluated as both upfront and salvage therapy in patients with WM. Most of these efforts to date have centered on the use of rituximab, a chimeric monoclonal antibody that targets CD20, an antigen which is widely expressed on tumor cells in WM. Five years ago, case reports and small retrospective series indicated that treatment with single-agent rituximab may induce responses in patients with resistant WM. Subsequently several retrospective and prospective studies have indicated that rituximab, when used at standard doses (i.e., 4 weekly infusions at 375 mg/m^2) induced major responses in approximately 27%–35% of previously treated and untreated patients **(Table 15;** reviewed in [21]**)**. Furthermore, it was shown in some of these studies, that patients who achieved minor responses or even stable disease benefited from rituximab as evidenced by improved hemoglobin and platelet counts, and reduction of lymphadenopathy and/or splenomegaly. The median time to treatment failure in these studies was found to range from 8 to 27+ months. Studies evaluating an extended rituximab schedule consisting of 4 weekly courses at 375 mg/m^2/week, repeated 3 months later by another 4-

Table 15. Rituximab therapy in Waldenström's macroglobulinemia (reviewed in Merlini and Treon[21]).

Study	Number of Patients	Median Number of Courses	Major RR[a]	Median Response Duration
Byrd	6	4	57%	6.6+ months
Weber	7	4	75%	9.0 months
Foran	7	4	29%	NA
Treon	30	4	27%	8.0 months
Gertz	69	4	27%	27+ months
Dimopoulos	27	8	44%	16+ months
Treon	26	8	48%	29+ months

[a] ≥50% reduction in serum IgM levels.

Abbreviations: NA, not applicable; RR, response rate.

week course, have demonstrated major response rates of 44%–48%, with time to progression estimates of 16+ to 29+ months.[24,25]

Rituximab is a well-tolerated treatment. Approximately one-quarter of patients may develop grade 1 or 2 infusion-related symptoms consisting primarily of fever, chills and headache, while a cytokine-release syndrome has rarely occurred. Because myelosuppression is negligible, rituximab may represent the treatment of choice for patients who present with or develop cytopenias. Moreover, the lack of early or delayed myelosuppression makes rituximab an attractive treatment for patients who are candidates for stem cell collection and high-dose therapy.

In many patients, a transient increase of serum IgM may be noted immediately following initiation of treatment. The mechanism for this phenomenon is under investigation. Such an increase, however, does not herald treatment failure, and most patients return to their baseline serum IgM level by 12 weeks. Some patients do continue to show prolonged spiking despite demonstrating a reduction in their bone marrow tumor load.[26,27] However, patients with baseline serum IgM levels of > 50 g/dL or serum viscosity of > 3.5 cp may be particularly at risk for a hyperviscosity-related event, and in such patients plasmapheresis should be considered in advance of rituximab therapy.[27] Because of the decreased likelihood of response in patients with higher IgM levels, as well as the possibility that serum IgM and viscosity levels may abruptly rise, rituximab monotherapy should not be used as sole therapy for the treatment of patients at risk for hyperviscosity symptoms.

Time to response after rituximab is slow and exceeds 3 months on the average. Patients with baseline serum IgM levels of < 60 g/L are more likely to respond, irrespective of the underlying bone marrow involvement by tumor cells.[24,25] A recent analysis of 52 patients who were treated with single-agent rituximab has indicated that the objective response rate was significantly lower in patients who had either low serum albumin (< 35 g/L) or elevated serum monoclonal protein (> 40g/L M-spike). Furthermore, the presence of both adverse prognostic factors was related with a short time to progression (3.6 months). Moreover, patients who had normal serum albumin and relatively low serum monoclonal protein levels derived a substantial benefit from rituximab with a time to progression exceeding 40 months.[27]

The genetic background of patients may also be important for determining response to rituximab. In particular, a correlation between rituximab response and polymorphisms at position 158 in the Fcγ RIIIa receptor (CD16), an activating Fc receptor on important effector cells that mediate antibody-dependent cell-mediated cytotoxicity (ADCC), was observed in WM patients. Individuals may encode either the amino acid valine or phenylalanine at position 158 in the Fcγ RIIIa receptor. WM patients who carried the valine amino acid (in either a homozygous or heterozygous pattern) had a 4-fold higher major response rate (i.e., 50% decline in serum IgM levels) to rituximab versus those patients who expressed phenylalanine in a homozygous pattern.[28]

Because rituximab is an active and a nonmyelosuppressive agent, its combination with chemotherapy has a sound rationale. Weber et al[29] administered the combination of rituximab, cladribine and cyclophosphamide to 17 previously untreated patients with WM. A major response was documented in 94% of patients, with 18% of patients achieving a complete remission in this study. With a median follow-up of 21 months, no patient has relapsed in this study. Treon et al[30] have explored the use of rituximab and fludarabine in WM. In recently updated studies, 42 patients, including some previously untreated patients, have been treated. At least a minor response was noted in 90% (83% major response rate) of patients, and only 2 of 37 patients have progressed with a median follow-up of 15 months.

Alemtuzumab is a monoclonal antibody against CD52 antigen and is effective treatment for patients with chronic lymphocytic leukemia who have previously received a purine analog. Several studies have demonstrated wide expression of CD52 in WM. Preliminary clinical data on 7 heavily pretreated patients who received treatment with alemtuzumab showed 4 partial and 1 complete response, and a median response duration of 13 months.[31] Infectious complications were common in this study and included cytomegalovirus (CMV) reactivation, herpes simplex reactivation, aspergillosis and tuberculosis. In ongoing studies by the Waldenström's Macroglobulinemia Clinical Trials Group (WMCTG), 7 patients with 2 median prior therapies have received alemtuzumab. Six of 7 patients demonstrated a response, 3 minor and 3 major. Toxicities were mainly hematologic with reversible grade III/IV thrombocytopenia, neutropenia and prolonged pancytopenia in 1 patient.[32]

Treatment Options for Relapsed and Refractory Disease

A consensus panel on therapeutics for WM also considered options for patients with relapsed and refractory disease.[20] For patients in relapse or who have refractory disease, the use of an alternative first-line agent as defined above was considered as a reasonable choice, with the caveat that for those patients for whom autologous

transplantation was being considered seriously, further exposure to stem cell–damaging agents (i.e., many alkylator agents and nucleoside analogue drugs) should be avoided, and a non-stem cell–toxic agent such as rituximab should be considered if stem cells have not been previously harvested.

Thalidomide

Thalidomide, as a single agent and in combination with dexamethasone and clarithromycin, has also been examined in patients with WM, in view of the success of these regimens in patients with advanced multiple myeloma. Dimopoulos et al (reviewed in [21]) demonstrated a major response in 5 of 20 previously untreated and treated patients (25%) who received single-agent thalidomide. Dose escalation from the thalidomide start dose of 200 mg daily was hindered by development of side effects, including the development of peripheral neuropathy in 5 patients, obligating discontinuation or dose reduction. Low doses of thalidomide (50 mg orally daily) in combination with dexamethasone (40 mg orally once a week) and clarithromycin (250 mg orally twice a day) have also been examined, with 10 of 12 (83%) previously treated patients demonstrating at least a major response (reviewed in[21]). However, in a follow-up study by Dimopoulos et al[33] using a higher thalidomide dose (200 mg orally daily) along with dexamethasone (40 g orally once a week) and clarithromycin (500 mg orally twice a day), only 2 of 10 (20%) previously treated patients responded.

High-Dose Therapy and Stem Cell Transplantation

The use of transplant therapy has also been explored in patients with WM (reviewed in [21]). Eight previously treated WM patients between the ages of 45 and 69 years received either melphalan at 200 mg/m^2 (n = 7) or melphalan at 140 mg/m^2 along with total-body irradiation. Stem cells were successfully collected in all 8 patients, although a second collection procedure was required for 2 patients who had extensive previous nucleoside analogue exposure. There were no transplant-related mortalities and toxicities were manageable. All 8 patients responded, with 7 of 8 patients achieving a major response, and 1 patient achieving a complete response with durations of response raging from 5+ to 77+ months. Dreger et al (reviewed in [21]) investigated the use of the DEXA-BEAM (dexamethasone, BCNU, etoposide, cytarabine, melphalan) regimen followed by myeloablative therapy with cyclophosphamide, and total-body irradiation and autologous stem cell transplantation in 7 WM patients, including 4 untreated patients. Serum IgM levels declined by > 50% following DEXA-BEAM and myeloablative therapy for 6 of 7 patients, with progression-free survival ranging from 4+ to 30+ months. All 3 evaluable patients, who were previously treated, also attained a major response in a study by Anagnostopoulos et al (reviewed in [21]) in which WM patients received various preparative regimens and showed event-free survivals of 26+, 31, and 108+ months. Tournilhac et al (reviewed in [21]) recently reported the outcome of 18 WM patients in France who received high-dose chemotherapy followed by autologous stem cell transplantation. All patients were previously treated with a median of 3 (range 1–5) prior regimens. Therapy was well tolerated with an improvement in response status observed for 7 patients (6 PR to CR; 1 stable disease [SD] to PR), while only 1 patient demonstrated progressive disease. The median event-free survival for all nonprogressing patients was 12 months. Reports on the use of high-dose chemotherapy and allogeneic transplantation in WM are limited. Martino et al (reviewed in [21]) reported event-free survivals of 3 and 9 years for 2 young patients (ages 34 and 39) with progressive disease, including 1 patient who progressed after high-dose chemotherapy and autologous stem cell transplantation. Tournilhac et al (reviewed in [21]) reported the outcome of allogeneic transplantation in 10 previously treated WM patients (ages 35–46) who received a median of three prior therapies, including 3 patients with progressive disease despite therapy. Two of 3 patients with progressive disease responded, and an improvement in response status was observed in 5 patients. The median event-free survival for nonprogressing, evaluable patients was 31 months. Concerning in this series was the death of 3 patients owing to transplantation-related toxicity. Similarly, Anagnostopoulos and Giralt (reviewed in [21]) reported that 2 of 3 patients in their series who underwent allogeneic transplantation experienced an early death or death from complicating graft-versus-host disease. The third patient in this series did not respond to therapy. In view of the high rate of mortality associated with high-dose chemotherapy and allogeneic transplantation, Maloney et al (reviewed in [21]) have evaluated the use of nonmyeloablative allogeneic transplantation in 5 patients with refractory WM. In this series, 3 of 3 evaluable patients (all of whom had matched sibling donors) responded, with 2 CR and 1 in PR at 1–3 years post-transplant.

Response Criteria

Assessment of response to treatment of WM has been widely heterogeneous. As a consequence studies using the same regimen have reported significantly different response rates. During the 2nd International Workshop

on WM a consensus panel proposed guidelines for standardized response criteria that were subsequently discussed and modified and are summarized below.[34]

Complete response

Complete disappearance of serum and urine monoclonal protein by immunofixation, resolution of lymphadenopathy and of organomegaly, and no signs or symptoms that are directly attributable to WM. These findings must be confirmed 6 weeks later. Absence of malignant cells by bone marrow histologic evaluation is required.

Partial response

A ≥ 50% reduction of serum monoclonal protein concentration on electrophoresis and ≥ 50% reduction of lymphadenopathy and of organomegaly on physical examination or computed tomography. Symptoms and signs that are directly attributable to WM must resolve.

Relapse from CR

Reappearance of serum monoclonal protein as determined by immunofixation confirmed by a second measurement or reappearance of clinically significant symptoms and signs attributable to WM or development of any other clinically significant disease-related complication.

Progressive disease

A greater than 25% increase in serum monoclonal protein levels from the lowest attained response value as determined by serum electophoresis, confirmed by measurement 3 weeks later. For monoclonal protein nadirs ≤20 g/L, an absolute increase of 5 g/L is required to determine progressive disease (PD). PD may also be documented if there is worsening of anemia, thrombocytopenia, leukopenia, lymphocytosis, lymphadenopathy, or organomegaly directly attributable to WM or appearance of disease-related complications such as unexplained fever, night sweats, weight loss, neuropathy, nephropathy, symptomatic cryoglobulinemia, or amyloidosis. Serum monoclonal protein should ideally be measured by serum protein electrophoresis because in some settings the use of nephelometry to determine total serum IgM may be unreliable, particularly when the levels of the monoclonal protein are high. The presence of cryoglobulin or cold agglutinin may affect determination of IgM. Testing of these at baseline should be considered and if present serum samples should be re-evaluated at 37°C to ensure accurate and consistent determination of the monoclonal protein levels.

Prognosis

WM presents with a chronic, indolent course and a highly variable prognosis. The median survival reported in large series ranges from 5 to 7 years, although an observed survival of 9 years and a 10-year projected overall survival of 55% have been reported (reviewed in[21]). Because WM is a rare disease, relatively few studies on prognosis have been conducted on large patient populations. Advanced age, anemia, and thrombocytopenia were correlated by univariate analysis with a poorer outcome in virtually all studies. Neutropenia and male sex, weight loss and cryoglobulinemia, albumin level and blood cell counts, serum β2-microglobulin level and IgM level less than 40 g/L, and hyperviscosity and β2-microglobulin level were also significantly correlated with survival (reviewed in[21]). A few scoring systems have been proposed based on these analyses:

- age ≥ 70 years, hemoglobin < 90 g/L, weight loss, cryoglobulinemia[35]
- age ≥ 65 years, serum albumin < 40 g/L, hemoglobin < 120 g/L, cytopenias (platelets < 150×10^9/L, leukocytes < 4.0×10^9/L, neutrophils < 1.5×10^9/L)[36]
- serum β2-microglobulin ≥ 3 mg/L, hemoglobin < 120 g/L, serum IgM < 40 g/L[37]

An update of the study by Merlini et al,[16] which included 215 patients, indicated that serum β2-microglobulin, hemoglobin, albumin, and age defined prognosis of patients with WM thoroughly. In agreement with other studies, serum β2-microglobulin and hemoglobin level appeared to be the most consistent prognostic determinants. It is possible that with validation from future studies, both prognostic stratification and decision to start treatment may result from serum β2-microglobulin level and hemoglobin. Asymptomatic patients with low serum β2-microglobulin levels and preserved hemoglobin can be observed over long periods without therapy. Since WM is a disease of the elderly, up to 32% of patients die of unrelated causes[38] and the association with malignancy, both before therapy and during follow-up, is common (39% of patients in a series[37]). The most common causes of death in these patients are progression of the lymphoproliferative process (in about 50%), infections, and cardiac failure.[38] Few patients die of cerebrovascular accidents, renal failure, or gastrointestinal bleeding. In the pre-terminal stage of the disease, the development of aggressive large-cell lymphomas, usually of the immunoblastic type (Richter's syndrome) have been reported in 6% of patients treated for WM.[38] This transformation is characterized by unexplained fever, weight loss, rapidly enlarging lymph nodes, extranodal extension, and reduction of the level of monoclonal IgM. Rarely, WM may

be complicated by acute or chronic myeloid leukemia, in most cases after treatment with alkylating agents, although patients who had not been previously treated have also been reported.

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