High-Dose Cyclophosphamide Without Stem Cell Rescue in 207 Patients With Aplastic Anemia and Other Autoimmune Diseases

Amy E. DeZern, MD, Michelle Petri, MD, MPH, Daniel B. Drachman, MD, Doug Kerr, MD, PhD, Edward R. Hammond, MD, MPH, Jeanne Kowalski, PhD, Hua-Ling Tsai, ScM, David M. Loeb, MD, PhD, Grant Anhalt, MD, Fredrick Wigley, MD, Richard J. Jones, MD, and Robert A. Brodsky, MD

Abstract: High-dose cyclophosphamide has long been used as an anticancer agent, a conditioning regimen for hematopoietic stem cell transplantation, and a potent immunosuppressive agent in autoimmune diseases including aplastic anemia. High-dose cyclophosphamide is highly toxic to lymphocytes but spares hematopoietic stem cells because of their abundant levels of aldehyde dehydrogenase, the major mechanism of cyclophosphamide inactivation. High-dose cyclophosphamide therapy induces durable remissions in most patients with acquired aplastic anemia. Moreover, high-dose cyclophosphamide without hematopoietic stem cell rescue has shown activity in a variety of other severe autoimmune diseases.

Here we review the history of cyclophosphamide as it applies to aplastic anemia and other autoimmune diseases. We include historical data from early patients treated for aplastic anemia as well as data from 140 patients from an observational retrospective study in a single tertiary care hospital. This latter component was designed to assess the safety and efficacy of high-dose cyclophosphamide therapy without stem cell rescue in patients with refractory autoimmune diseases. We analyzed the 140 patients with severe, progressive autoimmune diseases treated. All patients discussed here received cyclophosphamide, 50 mg/kg per day for 4 consecutive days. Response, relapse, and overall survival were measured. Response was defined as a decrease in disease activity in conjunction with a decrease or elimination of immune-modulating drugs. Relapse was defined as worsening disease activity and/or a requirement for an increase in dose of, or administration of new, immunosuppressive medications.

Hematologic recovery occurred in all patients. The overall response rate was 94%, and 44% of those patients remained progression free with a median follow-up of 36 months (range, 1–120 mo) for the 140 patients analyzed together. The overall actuarial and event-free survival across all diseases at 60 months was 90.7% and 20.6%, respectively. High-dose cyclophosphamide without stem cell rescue is well tolerated and induces a high rate of remission in severe autoimmune diseases.

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Abbreviations: ATG = antithymocyte globulin, CIBMTR = Center for International Blood and Marrow Transplant Research, CsA = cyclosporine A, EBMT = European Group for Blood and Marrow Transplantation, EDSS = Expanded Disability Status Scale, EULAR = European League Against Rheumatism, HLA = human leukocyte antigen, HSCT = hematopoietic stem cell transplantation, MDS = myelodysplastic syndrome, PNH = paroxysmal nocturnal hemoglobinuria, SAA = severe aplastic anemia, SLE = systemic lupus erythematosus.

INTRODUCTION

Cyclophosphamide was synthesized in the late 1950s and remains one of the best characterized and most widely administered anticancer agents available; it is also a highly efficacious immunosuppressive agent.[16] The use of high dosages of cyclophosphamide (≥120 mg/kg [5000 mg/m^2] employed over 2–4 days) as conditioning for hematopoietic stem cell transplantation (HSCT) was developed by Santos and Owens in the late 1960s.[37] They were exploring a replacement for the dual anticancer and immunosuppressive properties of total body irradiation as an HSCT conditioning regimen, because of access issues and toxicity concerns. Animal studies showed cyclophosphamide to be a potent immunosuppressive; however, in contrast to total body irradiation, doses sufficient to allow allogeneic engraftment were not myeloablative.[37] In 1972, using high-dose cyclophosphamide as conditioning, Thomas et al[40a] reported the first successful human allogeneic HSCT in a patient with severe aplastic anemia (SAA). Later, reports of autologous hematopoietic recovery in SAA following HSCT with high-dose cyclophosphamide conditioning suggested that high-dose cyclophosphamide alone may be effective in treating SAA.[37a] Indeed, a small pilot study demonstrating durable remissions in 7 of 10 SAA patients following high-dose cyclophosphamide therapy without HSCT gave credibility to this hypothesis.[8]

Cyclophosphamide's unique pharmacology is responsible for its significant toxicity against the immune system without damage to hematopoietic stem cells at high doses. Cyclophosphamide is a prodrug that is converted by the liver to 4-hydroxycyclophosphamide and aldophosphamide. These compounds diffuse into cells and are converted into the active alkylating compound phosphoramide mustard through simple intracellular decomposition. The major mechanism of cyclophosphamide detoxification appears to be inactivation of aldophosphamide by cellular

From Division of Hematology (AED, RJJ, RAB), Division of Rheumatology (MP, FW), Division of Neurology (DBD, ERH), Division of Oncology Biostatistics (JK, HLT), Division of Dermatology (GA), Department of Medicine; and Department of Oncology and Department of Pediatrics (DML), The Johns Hopkins University School of Medicine, Baltimore, Maryland; and Biogen-Idec (DK), Cambridge, Massachusetts.

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Reprints: Dr. Robert A. Brodsky, MD, Division of Hematology, The Johns Hopkins University School of Medicine, 720 Rutland Avenue, Ross Research Building, Room 1025, Baltimore, MD 21205 (e-mail: rbrodsky@jhmi.edu).

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aldehyde dehydrogenase[38] to form the inert compound carboxyphosphamide. Cells with high proliferative potential, such as hematopoietic stem cells, are relatively resistant to cyclophosphamide because they express high levels of aldehyde dehydrogenase.[20,22,23] Lymphocytes have low levels of aldehyde dehydrogenase and are rapidly killed by high doses of cyclophosphamide; therefore, high-dose cyclophosphamide is highly immunosuppressive, but not myeloablative, allowing endogenous hematopoietic stem cells to reconstitute hematopoiesis.

Reasoning that high-dose cyclophosphamide without stem cell support can induce durable remissions in one autoimmune disease, SAA, we initiated trials of high-dose cyclophosphamide without HSCT for other severe refractory autoimmune diseases such as lupus, myasthenia gravis, multiple sclerosis, and others. In the current report we have compiled our single institutional experience using high-dose cyclophosphamide in 207 patients (67 patients with SAA and 140 patients with other autoimmune diseases). Long-term follow-up in our SAA patients has recently been reported[5]; hence, most new data in the current report will focus on the use of high-dose cyclophosphamide for autoimmune conditions other than SAA.

High-Dose Cyclophosphamide for Treating Severe Aplastic Anemia

Aplastic anemia is a rare bone marrow failure disorder characterized by pancytopenia and a hypocellular bone marrow.[6,45] Severe disease (SAA) is defined by a bone marrow cellularity of less than 25% and markedly decreased values of at least 2 of 3 hematopoietic lineages (neutrophil count <500/μL, platelet count <20,000/μL, and absolute reticulocyte count of <60,000/μL). Death may occur due to infection, hemorrhage, or evolution to clonal disease (myelodysplastic syndrome [MDS], leukemia, and paroxysmal nocturnal hemoglobinuria [PNH]). HSCT from a human leukocyte antigen (HLA)-matched sibling donor can cure most patients with SAA, but fewer than 30% of patients have a suitable HLA-matched sibling. Moreover, the best results with allogeneic HSCT are in children; adults do less well primarily due to complications from graft-versus-host disease. Alternative donor transplants[32] have the potential to cure SAA, but such transplants are usually reserved for second-line therapy because of their high rates of morbidity and mortality. Immunosuppressive therapy is also highly effective for treating SAA and is recommended for SAA patients who lack matched sibling donors or who are not suitable candidates for HSCT. The most commonly administered immunosuppressive regimen is antithymocyte globulin and cyclosporine A (ATG/CsA). ATG/CsA will achieve a hematopoietic response in 60%–70% of untreated SAA patients, and the probability of survival at 5 years ranges from 60% to 85%.[14,18,36] However, up to 40% of patients eventually relapse, and an additional 10%–40% develop a secondary clonal disease.[18,30,36,44]

To our knowledge, autoimmune impairment of hematopoiesis in aplastic anemia was first suggested by Mathe et al,[27] who described autologous hematopoietic improvement in aplastic anemia following partially mismatched HSCT using antilymphocyte globulin as a conditioning regimen. Shortly thereafter, several reports of autologous hematopoietic reconstitution in SAA patients after allogeneic HSCT following high-dose cyclophosphamide conditioning for SAA were reported, suggesting that high-dose cyclophosphamide alone was capable of treating the disease.[19a,37a,41] In 1976, a case report in *The New England Journal of Medicine* described a patient with aplastic anemia successfully treated with high-dose cyclophosphamide without HSCT.[2] In the late 1970s, Dr. Lyle Sensenbrenner initiated the first clinical trial of high-dose cyclophosphamide therapy for 10 patients with SAA who lacked an HLA-matched sibling donor during a time when ATG was unavailable.[8] Durable complete remissions were achieved by 7 patients. One of the complete responders died from the acquired immunodeficiency syndrome (AIDS) 44 months after treatment with high-dose cyclophosphamide. The 6 remaining patients were alive and in continuous complete remission, with a median follow-up of 10.8 years (range, 7.3–17.8 yr) at the time of publication. The median time to last platelet transfusion and time to 0.5×10^9 neutrophils/L were 85 and 95 days, respectively. None of the complete responders relapsed or developed a clonal disease.

Based on this encouraging data, a new trial of high-dose cyclophosphamide for SAA was initiated at Johns Hopkins in 1996. In contrast to the original trial, the new trial included patients who had failed to respond to standard immunosuppressive regimens (for example, ATG/CsA), and used granulocyte colony-stimulating factor to hasten neutrophil recovery. The results of this experience were recently reported.[5] Sixty-seven patients were enrolled (44 treatment naive and 23 refractory to immunosuppressive therapy). At 10 years, the overall actuarial survival was 88%, the response rate was 71% with the majority being complete, and the actuarial event-free survival (where death, relapse, MDS, HSCT, and PNH requiring treatment are defined as events) was 58% in 44 treatment-naive SAA patients. Patients with refractory SAA fared less well after high-dose cyclophosphamide therapy; at 10 years, overall actuarial survival, response, and actuarial event-free survival rates were 62%, 48%, and 27%, respectively. For treatment-naive patients, the median time to a neutrophil count of 0.5×10^9/L was 60 days (range, 28–104 d) and the median time to last platelet transfusion was 117 days (range, 24–640 d). For patients with refractory SAA, the median time to a neutrophil count of 0.5×10^9/L was 54 days (range, 35–119 d) and the median time to last platelet transfusion was 103 days (range, 51–751 d). The median time to complete remission was 20 months (range, 4–70 mo).

In summary, the majority of treatment-naive patients with SAA achieve durable remissions after high-dose cyclophosphamide therapy; however, patients who are refractory to standard immunosuppression do less well (Table 1).[1,2,5,8,21,26,42] Based on the success of using high-dose cyclophosphamide in treating SAA, we hypothesized that it may have activity in treating other severe autoimmune conditions.

High-Dose Cyclophosphamide for Treating Refractory Severe Autoimmune Diseases Other Than Aplastic Anemia

High-dose cytotoxic therapy followed by HSCT is an active treatment for severe autoimmune diseases.[9,10,28,43] Autologous HSCT has generally been preferred over allogeneic HSCT because of morbidity and mortality associated with graft-versus-host disease. The European Group for Blood and Marrow Transplantation (EBMT) and European League Against Rheumatism (EULAR) have established a registry to compile the results of phase I/II studies of autologous HSCT for the treatment of autoimmune disease.[43] In the United States, the results of using HSCT for the treatment of autoimmune disease are collected by the Center for International Blood and Marrow Transplant Research (CIBMTR).[24] Together, these groups have compiled data on more than 1000 patients. These data have been difficult to interpret because of diverse eligibility criteria among studies for the various autoimmune diseases, the heterogeneity of the conditioning regimens, and the differing stem cell products (bone marrow vs. peripheral blood and whether in vitro processing or purging was used to remove autoreactive

TABLE 1. Aplastic Anemia, Previous Reports

Author (Ref.)	No. of Patients	Disease Status	Survival (%)	Relapse	Clonal Disease	Duration of Follow-Up (mo) (range)
Baran[2]	1	Treatment refractory	100	0	0	7
Brodsky[9]	10	Treatment naive	70*	0	0	129.6 (87.6–213.6)
Li[26]	2	1 Treatment naive	Naive: 100	0	0	Naive: 8
		1 Treatment refractory	Refractory: 0			Refractory: died with treatment
Tisdale[42]	15	Treatment naive	80	0	1 PNH†	21.9 (1–33)
Jaime-Perez[21]	5‡	Treatment refractory	60	0	0	29 (0.3–40)
Audino[1]	5§	Treatment refractory	40	0	0	12 (0.5–12)
Brodsky[5]	67	44 Treatment naive	Naive: 88	2	Naive: 2 MDS	Naive: 58 (2–153)
		23 Treatment refractory	Refractory: 61.8	1	Refractory: 1 MDS 2 PNH	Refractory: 63 (1–141)
Total	105	70 Naive	Median	3	5	Median
		45 Refractory	Naive: 84			Naive: 40
			Refractory: 60			Refractory: 12

*One patient died of acquired immunodeficiency syndrome (AIDS) later.

†Present at onset.

‡Aged <16 yr.

§Aged <18 yr.

lymphocytes).[43] A study published in 2010 by the EBMT of 900 patients reported that the 5-year survival was 85% and the progression-free survival was 43%, although the rates varied widely according to the type of autoimmune disease.[17] The most common autoimmune diseases treated, accounting for roughly 50% of the cases, were multiple sclerosis and systemic sclerosis.

High-dose cyclophosphamide-based, nonmyeloablative conditioning regimens were used in over 50% of the HSCT cases reported by EBMT/EULAR and the CIBMTR.[10,17,43] A subset analysis between myeloablative and nonmyeloablative conditioning regimens demonstrated that myeloablative regimens were associated with an increase in treatment-related mortality and no clear advantage in terms of remission induction and relapse rate.[10,17,43] Hence, most investigators now favor nonmyeloablative, immunosuppressive conditioning regimens (usually high-dose cyclophosphamide ± other nonmyeloablative agents such as ATG) for HSCT in patients with autoimmune diseases.[10,43] Because high-dose cyclophosphamide spares hematopoietic stem cells, our group and others have shown it can be safely administered *without* stem cell support to patients with SAA and a variety of other severe autoimmune diseases.[4,5,7,19,29,35,40] Avoiding stem cell reinfusion has a number of potential advantages for treating autoimmune diseases. Not only do stem cell mobilization, collection, cryopreservation, and reinfusion add to the duration, cost, and toxicity of treatment,[12] but also the mobilized product contains numerous effector cells (autoreactive lymphocytes) that may contribute to relapse. Below, we summarize the results of high-dose cyclophosphamide without stem cell rescue in 140 patients with a variety of severe autoimmune diseases.

STUDY DESIGN AND METHODS

Patients

From August 1996 through December 2009, 140 patients with autoimmune diseases (excluding acquired SAA) were treated with high-dose cyclophosphamide. Some of the patients have been previously reported.[15,29,35,40] All protocols were approved by the Institutional Review Board of the Johns Hopkins University School of Medicine, and all participating patients (or their guardians) provided written informed consent. Patients were recruited from the hematologic, neurologic, dermatologic, gastrointestinal, or rheumatologic clinics of the Johns Hopkins Hospital. Inclusion criteria were defined in the original studies.[15,29,35,40] The heterogeneity of the patients can be seen in Table 2.

TABLE 2. Patients With Autoimmune Diseases Other Than Aplastic Anemia Treated With High-Dose Cyclophosphamide at the Johns Hopkins Hospital, August 1996–December 2009, by Disease Category

Disease	No.
Neurologic disorders	
Myasthenia gravis	14
Multiple sclerosis	47
CIDP/POEMS	5
Rheumatologic disorders	
Systemic sclerosis/scleroderma	7
Systemic lupus erythematosus	40
Vasculitidies	
Wegener granulomatosis	1
Gastrointestinal disorders	
Collagenous gastroenteritis	1
Autoimmune enteropathy	2
Hematologic immuncytopenias	
Autoimmune hemolytic anemia/Evan syndrome	9
Immune thrombocytopenia	4
Pure red cell aplasia	1
Dermatologic disorders	
Pemphigus vulgaris	9
Total	140

Abbreviations: CIDP = chronic inflammatory demyelinating polyneuropathy, POEMS = polyneuropathy, organomegaly, endocrinopathy, M-protein, skin abnormalities.

Patients with systemic lupus erythematosus (SLE) met ≥4 of the revised American College of Rheumatology classification criteria for SLE with moderate-to-severe activity. An additional requirement was lack of response or expected lack of response to moderate- to high-dose corticosteroids, to the equivalent degree of immunosuppression, or to appropriate other treatment.

Patients with scleroderma were considered eligible with a diagnosis of diffuse cutaneous scleroderma and evidence of clinically active disease. All patients had to have met the American College of Rheumatology definition of scleroderma. Active disease was defined as the patient having had 1) a history by examination or by the patient's self-report of worsening skin changes during the preceding 3 months; 2) diffuse scleroderma and evidence of other organ system disease progression described as a shift by 1 level in at least 1 other organ as defined by the Medsger severity score criteria; 3) diffuse scleroderma with evidence of active interstitial lung disease manifested by either >10% decline from the normal percentage predicted values or baseline values of the forced vital capacity or the diffusing capacity.

Patients with myasthenia gravis had to have a definite clinical diagnosis of myasthenia gravis, with typical physical findings, response to anticholinesterase agents, and at least some improvement with immunosuppressive or immunomodulatory agents. All patients treated here were selected because the myasthenia gravis was either refractory to multiple immunosuppressive agents or required toxic levels of these agents.

The eligible patients with multiple sclerosis were those who had failed or refused conventional therapy and had active disease.

Exclusion criteria included age >70 years, serum creatinine concentration >3.0 mg/dL, ejection fraction by echocardiogram <45%, or any underlying malignancy.

Treatment Schedule

High-dose cyclophosphamide (50 mg/kg) was administered intravenously for 4 consecutive days over 1 hour through a central venous catheter. The dose of cyclophosphamide was based on the lesser of actual or ideal body weight as determined by the Metropolitan Life table. Intravenous mesna (10 mg/kg) was administered 30 minutes before, and then 3, 6, and 8 hours after cyclophosphamide, as prophylaxis against hemorrhagic cystitis. Beginning 6 days after the last dose of cyclophosphamide, patients received granulocyte colony-stimulating factor (5 μg/kg per d) until the absolute neutrophil count exceeded 0.5×10^9/L.

Supportive Care

A serotonin 5-HT3 receptor antagonist such as ondansetron (32 mg) was administered intravenously before each dose of cyclophosphamide. Prophylactic antimicrobial support, consisting of fluconazole (400 mg/d), norfloxacin (400 mg/d), and valacyclovir (500 mg twice per d, if antibodies to herpes simplex virus were present), was given beginning the day after the last dose of cyclophosphamide and continued until the neutrophil count exceeded 0.5×10^9/L. *Pneumocystis* prophylaxis (usually trimethoprim-sulfamethoxazole or dapsone) was administered for 6 months. Packed red blood cell transfusions were administered to maintain a hematocrit level >25%. Platelet transfusions were administered to maintain a platelet count $>10 \times 10^9$/L, and for bleeding and procedures. All blood products were irradiated (>2000 rads) to prevent transfusion-associated graft-versus-host disease.

Outcomes and Analysis

Response was defined as a decrease in disease activity in conjunction with a decrease or elimination of immune-modulating drugs. Definitions of disease activity varied by disease and have been previously reported.[15,29,31,35,40] Relapse was defined as worsening disease activity and/or a requirement for an increase in dose of, or administration of new, immunosuppressive medications. Hospital days were determined from initial hospitalization for the cyclophosphamide dosing (if that occurred) and then any subsequent inpatient time for neutropenic fever or other complications. Inpatient stays were shortened, or in some cases not required, because patients had access to an intensive outpatient transplantation clinic at the Sidney Kimmel Comprehensive Cancer Center. Patients were seen in the outpatient transplantation

TABLE 3. Time Since Diagnosis, Number of Previous Treatments, and Disease Severity Before and After High-Dose Cyclophosphamide Treatment, Present Report

Disease	Time Since Diagnosis, yr	No. of Previous Treatments (Including Steroids)	Disease-Specific Severity Score at Time of Treatment	Disease-Specific Severity Score After Treatment, Even With Relapse
(No. of Patients)	Median (Range)	Median (Range)	Median (Range)	Median (Range)
Myasthenia gravis (14)	5.9 (0.8–16.8)	6 (4–8)	KPS* 70 (40–80)	KPS* 92.5 (50–100)
Multiple sclerosis (47)	6.1 (1.25–16)	3 (0–5)	EDSS† 4.5 (1.5–7)	EDSS† 3 (1–6.5)
Systemic sclerosis (7)	1.7 (0.3–6.8)	3 (2–5)	mRSS‡ 29.5 (17–48)	mRSS‡ 20 (2–40)
Autoimmune hemolytic anemia (9)	2.7 (0.2–9)	2 (1–4)	Median hemoglobin before treatment was 6.7 g/dL with transfusional support	Hemoglobin was at least 10 g/dL without transfusion support

*Severity score: KPS = Karnofsky Performance Scale. (Karnofsky DA, Burchenal JH: The clinical evaluation of chemotherapeutic agents in cancer. In: Macleod CM, ed. Evaluation of Chemotherapeutic Agents. New York: Columbia University Press; 1949: 199–205.)

†EDSS = Expanded Disability Status Scale. (Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale [EDSS]. Neurology. 1983;33:1444–1452.)

‡mRSS = modified Rodnan Skin Score. (Furst DE, Clements PJ, Steen VD, Medsger TA Jr, Masi AT, D'Angelo WA, Lachenbruch PA, Grau RG, Seibold JR. The modified Rodnan skin score is an accurate reflection of skin biopsy thickness in systemic sclerosis. J Rheumatol. 1998;25:84–88.)

TABLE 4. Review of 9 Deaths, Present Report

Patient	Cause of Death	Underlying Disease	Time-to-Death After Treatment (mo)
1	Suicide	SLE	3
2	Graft-versus-host disease after transplant*	Autoimmune hemolytic anemia/Hurler disease	36
3	Acute respiratory distress syndrome, sepsis	Autoimmune hemolytic anemia/ITP	12
4	ITP	ITP	12
5	ITP	ITP/Evan/autoimmune hemolytic anemia	12
6	Pneumonia	Systemic sclerosis	1.5
7	Infectious complications of original disease	Morphea	12
8	Narcotic overdose	Wegener granulomatosis	5
9	Congestive heart failure	Myasthenia gravis	16

Abbreviations: ITP = idiopathic thrombocytopenic purpura.

*Patient received transplant for Hurler disease before developing autoimmune hemolytic anemia.

clinic 7 days a week through the period of neutropenia, and antibiotics and blood products were administered as necessary.

Overall mortality was defined as death from any cause for a patient who had been treated with cyclophosphamide. Treatment-related mortality was defined as death within 90 days of treatment with high-dose cyclophosphamide. Event-free survival was calculated using the Kaplan-Meier estimate; events included death, relapse, or disease progression.

RESULTS

Patient Characteristics

From August 1996 through December 2009, 140 patients with severe autoimmune diseases were treated with high-dose cyclophosphamide. All but 1 patient with multiple sclerosis had received standard immunomodulatory therapy for their disease and were refractory to standard treatment. It is a heterogeneous population of diseases; some disease characteristics are shown in Tables 2 and 3. The median age at the time of treatment was 40 years (range, 1–70 yr). Most patients were white (75.7%) and female (62.1%.) The mean follow-up was 50.1 months (range, 1–127 mo).

Toxicity

There were few unexpected side effects.[15,25,29,34,35,40] Nausea with or without emesis during the treatment period was managed with intravenous and oral antiemetics. All patients experienced temporary alopecia and pancytopenia. Febrile neutropenia requiring admission to the hospital occurred in 53 (43%) patients. Of these 53 patients who developed fever, 33 had at least 1 readmission for the febrile episode. The mean duration of the hospitalization for febrile neutropenia was 4 days. Hemorrhagic cystitis requiring continuous bladder irrigation occurred in 3 (2%) patients (2 with lupus and 1 with scleroderma). There were no deaths before hematologic recovery, although 1 patient with scleroderma died on day 51 after cyclophosphamide of pneumonia that developed after hematologic recovery. A patient with lupus and comorbid depression committed suicide 3 months after treatment with high-dose cyclophosphamide, presumably unrelated to high-dose cyclophosphamide treatment. At last follow-up only 9 patients had died, most from their underlying disease (Table 4).

Hematopoietic Recovery

Hematopoietic recovery occurred in all patients. Patients achieved an absolute neutrophil count >500/μL at a median of 13 days (range, 8–22 d) after the last dose of cyclophosphamide. The median duration of neutropenia was 9 days (range, 4–23 d). The median time to last packed red blood cell transfusion after completion of high-dose cyclophosphamide was 13 days (range, 0–33 d), and the median time to last platelet transfusion was 12 days (range, 0–24 d). The median number of packed red blood cell transfusions was 2 (range, 0–27), and the median number of platelet transfusions was 2 (range, 0–18.)

Response

The overall response rate to high-dose cyclophosphamide was 94%. Of the 133 patients who responded, 59 remained progression free at last follow-up, with a median follow-up time of 36 months (range, 1–120 mo). (Figure 1) The overall mean duration of the response was 22 months (range, 3–120 mo) and varied by underlying disease. The response criteria were detailed for each disease and response was defined by the individual provider for the disease of interest.

In patients with SLE, 90% (36/40) had a response defined as partial or complete using the RIFLE scoring system.[3] The median duration of response was 12 months (range, 3–48 mo). Of the 40 patients with SLE, 16 had highly refractory disease and were treated on an open-label study; 24 were treated as part of a randomized trial comparing high-dose cyclophosphamide to monthly intravenous cyclophosphamide in a cohort of patients

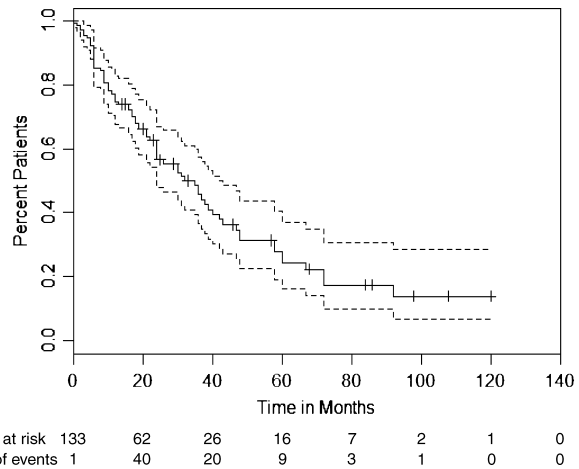

FIGURE 1. Progression-free survival for all patients receiving high-dose cyclophosphamide for all autoimmune diseases.

who were less heavily pretreated. In the randomized study, 6 of 7 patients with neurologic manifestations of SLE had a complete response; in the patients with renal manifestations only 4 of 10 patients responded to high-dose cyclophosphamide (2 complete and 2 partial responses). Patients in this randomized trial who did not respond to intravenous pulse-dose cyclophosphamide were eligible to cross over to the high-dose arm. It is noteworthy that of the 6 patients who crossed over to high-dose cyclophosphamide, 3 achieved a complete response.[34]

In patients with multiple sclerosis, 70.2% (33/47) had a response, defined as at least a 1-point improvement on the Expanded Disability Status Scale (EDSS).[13] The median duration of the response was 9 months (range, 6–42 mo). In the patients with multiple sclerosis treated with high-dose cyclophosphamide, 50% were female, mean age was 41.7 years, and mean duration of treatment of illness was 10.2 years (SD 7.1). The median number of failed medications before high-dose cyclophosphamide was 2.5 (range, 0–8). Relapses at the time of treatment ranged from 0 to 9 per year (median, 2 relapses per year). The annualized relapse rate during follow-up dropped to 0.27 compared to a rate of 1.37 in the 2 years before high-dose cyclophosphamide. With baseline disability ranging from EDSS 1.5 to 6.5, 20% of patients experienced sustained disability progression defined as an increased EDSS of 1 point or more when baseline EDSS <5.0, or an increase of 0.5 points or more when baseline EDSS ≥5.0. Patients with relapsing remitting multiple sclerosis had the most pronounced responses. No patient with a baseline EDSS score >6.5 had a sustained remission. Two patients had primary progressive disease, and neither patient responded to therapy. The 1 patient with secondary progressive disease achieved a transient 1-point EDSS improvement and has since relapsed.[13]

All 9 patients with autoimmune hemolytic anemia responded as defined by transfusion independence, and only 1 relapsed (at 22 mo). Two patients with autoimmune hemolytic anemia died in remission; 1 from complications of graft-versus-host disease following a matched unrelated donor bone marrow transplant that was performed before the development of the autoimmune hemolytic anemia, and 1 from idiopathic thrombocytopenic purpura that developed 14 months after high-dose cyclophosphamide therapy.

In the patients with pemphigus, all 9 patients had a response as defined by marked improvement in the number of blistering skin lesions and a reduction in prednisone dosage. The median duration of the response was 24 months (range, 6–60 mo).

In patients with myasthenia gravis, 13 of 14 (92.9%) responded as defined by improvement in myasthenia gravis disease activity score.[15] The median duration of the response was 13.5 months (range, 4–72 mo). Patients with anti-acetylcholine receptor antibody, anti-muscle specific kinase antibody, or no detectable antibody all responded equally well.[15]

Small numbers of patients with a variety of other severe autoimmune disorders were also treated (see Table 2). All of these miscellaneous autoimmune diseases responded to therapy, and 56% remained durable at last follow-up. All 3 patients with gastrointestinal disorders remained in remission with follow-ups of over 36 months. Of the patients with immune thrombocytopenic purpura, 3 of 4 remained disease free with a median follow-up of 40 months.

Overall and Event-Free Survival

The overall actuarial and event-free survival across all diseases at 60 months was 90.7% (95% confidence interval [CI],

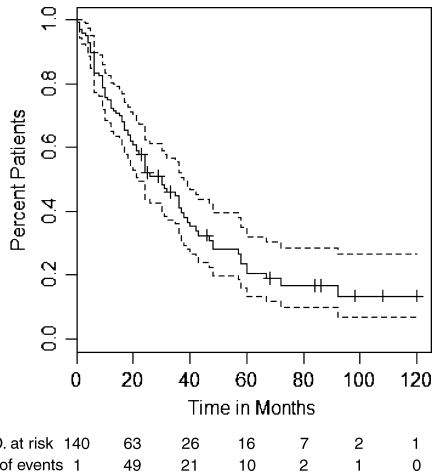

FIGURE 3. Event-free survival (patients experiencing death or relapse) for all autoimmune diseases.

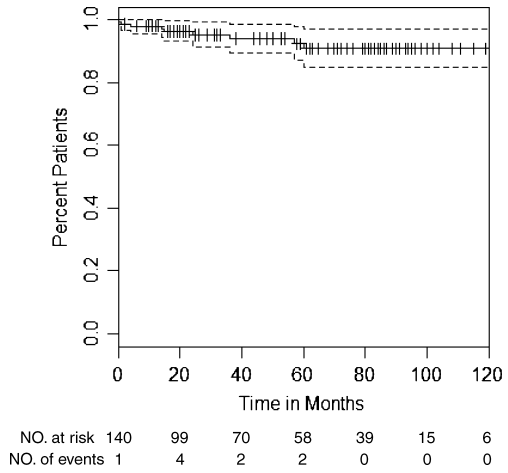

FIGURE 2. Overall survival for all patients receiving high-dose cyclophosphamide for all autoimmune diseases.

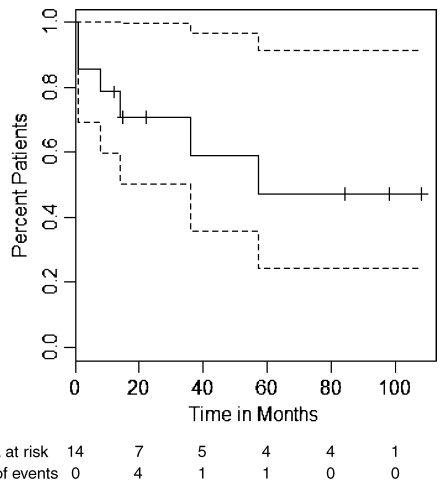

FIGURE 4. Autoimmune hemolytic anemia event-free survival.

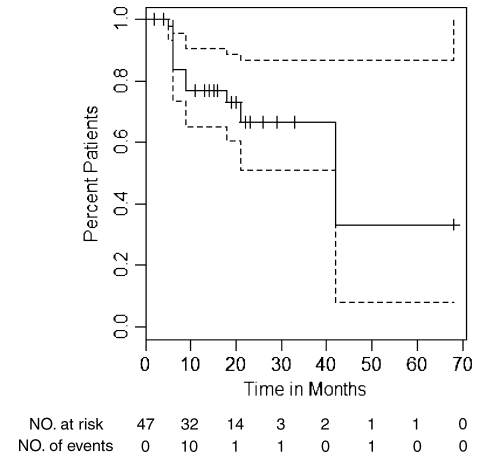

FIGURE 5. Multiple sclerosis event-free survival.

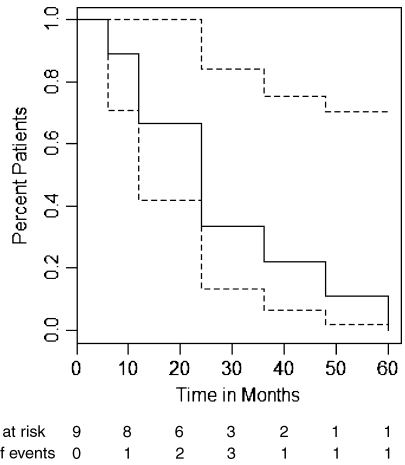

FIGURE 7. Pemphigus event-free survival.

84.8%–97.1%) and 20.6% (95% CI, 13.3%–32.0%), respectively (Figs. 1 and 2). Figures 3–9 demonstrate the event-free survival by disease category. The actuarial event-free survival at 60 months was 10.6% (4.2%–26.7%) for patients with SLE, 33.2% (8.1%–100%) for patients with multiple sclerosis, 32.1% (16.0%–64.2%) for patients with myasthenia gravis, 47.1% (24.3%–91.4%) for patients with autoimmune hemolytic anemia, 0% for patients with pemphigus, and 22.7% (4.9%–100%) for patients with the other autoimmune diseases.

DISCUSSION

High-dose cyclophosphamide leads to durable remission in the majority of patients with SAA. Results from over 100 SAA patients treated with high-dose cyclophosphamide have been reported in the medical literature, with roughly three-quarters of the patients reported from Johns Hopkins. According to these data, the response rate in treatment-naive patients is 70%, with the majority of responders achieving a complete remission (normalization of peripheral blood counts). Relapse and secondary clonal disorders (PNH and MDS) may occur; however, these appear to be rare events. Overall and event-free survival are less in patients with refractory SAA, and the risk of fungal infections is increased; however, durable remission has been described in roughly 25% of patients.

These data suggest that high-dose cyclophosphamide should be included along with the accepted standard of HSCT and ATG/CsA in treatment considerations for patients with SAA. Each therapy has inherent advantages and disadvantages (Table 5), yet the early mortality (within 6 mo of treatment) for all 3 is 10%–15%, and the 5-year survival is roughly 80%. The ideal therapy for SAA would be available to all patients, have a low toxicity profile, restore normal hematopoiesis without dependency on long-term medical therapy (drug-free remission), and eliminate the risk for late clonal disorders such as PNH and MDS. Treatment decisions must be based on both early toxicity and late complications from the disease. Allogeneic HSCT from a matched sibling remains the most appropriate therapy for children and young adults with SAA; it restores hematopoiesis in less than 4 weeks and cures the disease in more than 80% of cases.[33,39] Relapse and secondary clonal disorders are rare following allogeneic HSCT for SAA. For patients who are not suitable candidates for HSCT, both ATG/CsA and high-dose cyclophosphamide are reasonable options. Both have response rates of 70%. Our phase II data suggest that the relapse rate and the risk for secondary clonal disorders may be lower following high-dose cyclophosphamide than with ATG/CsA, but this needs to be tested in randomized controlled clinical trials.

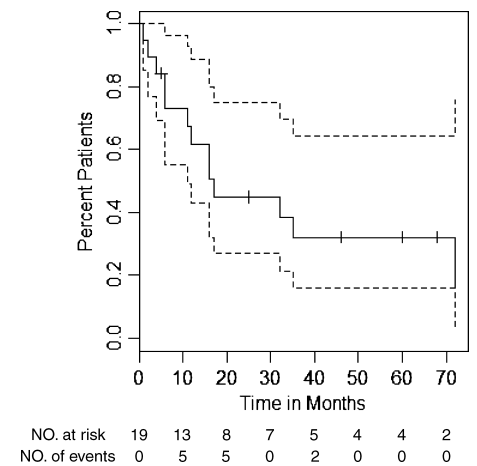

FIGURE 6. Myasthenia gravis event-free survival.

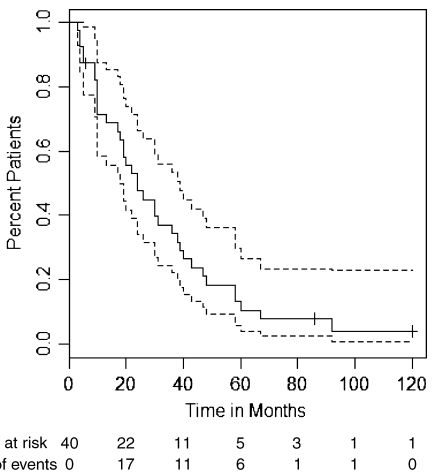

FIGURE 8. Systemic lupus erythematosus event-free survival.

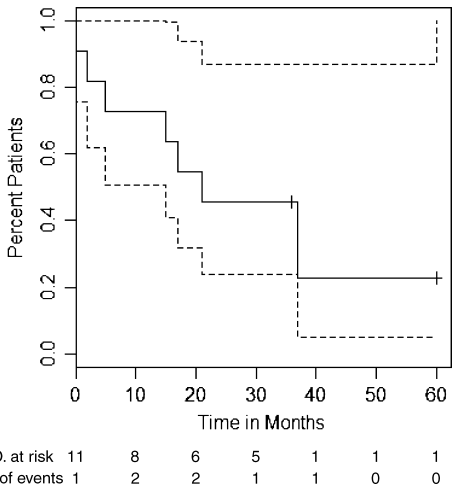

FIGURE 9. All other diseases event-free survival.

Our data also suggest that high-dose cyclophosphamide is effective in treating other severe refractory autoimmune diseases. Many investigators have questioned the safety of this approach, largely out of concern for the potential of protracted low blood counts that could lead to unacceptable morbidity and mortality.[10,43] In the current report of 140 patients receiving high-dose cyclophosphamide for autoimmune diseases, hematopoietic recovery was not delayed by the omission of autologous stem cell rescue. The median of 9 days with an absolute neutrophil count below 0.5×10^9/L and 13 days to transfusion independence is similar to that seen after autologous stem cell rescue. Febrile neutropenia occurred in less than 50% of patients, and the median number of days in the hospital was 6.

The treatment-related mortality of 0.7% in this group of heavily pretreated, refractory autoimmune disease patients compares favorably to the 5% and 2% transplant-related mortalities reported by the EBMT[17] and the Northwestern Group,[11] respectively. The EBMT found that 100-day transplant-related mortality varied by underlying disease. Specifically, that group reported a 2% transplant-related mortality in patients with multiple sclerosis, 6% in scleroderma, 11% in lupus, and 1% in patients with rheumatoid arthritis. We observed no treatment-related mortality in patients with lupus (n = 40) or multiple sclerosis (n = 47).

The overall response rate to high-dose cyclophosphamide was 94%, with 46% of patients still in remission at the time of the current report. The median duration of response was 15 months, but remissions lasting 5 years or more occurred in more than 20% of patients. These results are comparable to the 5-year overall survival of 85% and progression-free survival of 43% after HSCT reported by the EBMT. We used a rigorous definition of relapse—worsening disease activity from the point of improvement (not baseline) and/or a requirement of an increase in dose of, or administration of new, immunosuppressive medications. Thus, many of the patients classified in the current study as relapsed did not have progression of their disease from baseline. The durability of response after high-dose cyclophosphamide seemed to depend on the underlying autoimmune disease. More patients with autoimmune hemolytic anemia and multiple sclerosis achieved a durable response than did patients with pemphigus or lupus. However, since the number of patients with each individual disease was small, and most were refractory to other treatments, it is unclear whether this is truly a difference in activity against individual diseases or whether this reflects severity of the disease at the time of treatment.

The current series may help in the selection of patients with severe autoimmune diseases for high-dose cyclophosphamide treatment. Our data suggest that high-dose cyclophosphamide is not superior to monthly pulse dose cyclophosphamide, and we do not recommend the use of high-dose cyclophosphamide as front-line therapy in SLE. Nevertheless, high-dose cyclophosphamide can be effective salvage therapy for patients with refractory SLE, especially those with neurologic manifestations. Additional clinical trials of high-dose cyclophosphamide are needed to determine whether this therapy should be more widely used for multiple sclerosis. Our data suggest that high-dose cyclophosphamide is most effective for patients with relapsing-remitting disease and an EDSS of less than 6.5.

Eliminating stem cell rescue after high-dose cyclophosphamide shortens the duration of the procedure by several weeks, and this, coupled with no requirement for stem cell mobilization, collection, and cryopreservation, should also reduce the cost of the procedure. Moreover, avoiding stem cell infusion eliminates the potential of reinfusing autoreactive lymphocytes with the autograft. Mobilized peripheral blood products usually contain at least 10^9 CD3+ T cells, and current

TABLE 5. Treatment for Severe Aplastic Anemia

Treatment	Advantages	Disadvantages
Antithymocyte globulin/cyclosporine and prednisone	Response in 60%–80% of patients	Not curative
	Available immediately and without donor	Responses are often incomplete
	No graft-versus-host disease	High rate of relapse (10%–50%)
		Potential for secondary clonal diseases (10%–25%) such as MDS or PNH
High-dose cyclophosphamide	Response in 60%–80% of patients	Toxicities of cytotoxic chemotherapy with prolonged aplasias, risk of infection (fungal)
	Available immediately and without donor	
	No graft-versus-host disease	Low risk for relapse or secondary clonal disorders
Allogeneic bone marrow transplant	Response (cure) in 75%–85% of patients	Toxicities of cytotoxic chemotherapy
	Responses usually durable	Limited by age and donor availability
	Lowest risk for relapse or secondary clonal disorders	Risk of graft-versus-host disease

CD34 selection techniques deplete T cells by only 3–4 logs, which may be insufficient to eliminate autoreactive clones that could reestablish disease. Recently, it has been suggested that the process of HSCT induces fundamental changes to the immune system that induce long-term tolerance;[10] however, it is unclear if cryopreserved hematopoietic stem cells are superior to the endogenous stem cells that lead to hematologic recovery after high-dose cyclophosphamide at "resetting" the immune system.

Although both autologous HSCT and high-dose cyclophosphamide without stem cell rescue generate meaningful clinical benefits for patients with advanced or refractory autoimmune diseases, neither is curative for most such patients. The safety and relative ease of delivery of high-dose cyclophosphamide without stem cell rescue may allow this approach to be used earlier in patients with autoimmune disease, potentially improving effectiveness in these diseases. A randomized trial of high-dose cyclophosphamide compared with standard therapy in patients with relapsing-remitting multiple sclerosis is under development, and should help define the ultimate potential of this approach in autoimmunity.

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