Pulse Cyclophosphamide Therapy for Refractory Autoimmune Thrombocytopenic Purpura

By Alex Reiner, Terry Gernsheimer, and Sherrill J. Slichter

Autoimmune thrombocytopenic purpura (AITP) is generally a chronic disorder in affected adults. Twenty-five percent of these patients will become refractory to routine therapy (corticosteroids and splenectomy), as well as most other available agents. Intravenous pulse cyclophosphamide therapy was used to treat 20 patients with severe refractory AITP who had previously failed to achieve a sustained remission with a mean of 4.8 agents (range 2 to 8). Patients received 1 to 4 doses (mean 2.0) of 1.0 to 1.5 g/m² intravenous cyclophosphamide per course. Of the 20 patients treated with pulse cyclophosphamide therapy, 13 patients (65%) achieved a complete response (CR), four (20%) a partial response (PR), and three patients (15%) failed to respond. Of the 13 complete responders, eight have remained in remission with stable platelet counts during followup intervals of 7 months to 7 years (median 2.5 years). Five patients developed recurrent AITP 4 months to 3 years following a CR. Of these, two patients responded to subsequent courses of pulse cyclophosphamide therapy with current remissions of 1 and 4 years. Of the four patients who obtained a PR, two remain in partial remission after 10 months and 4 years; one relapsed after 18 months and, after retreatment, is still in remission at 6 months. Of the patient characteristics examined, duration of disease was most strongly associated with response to pulse cyclophosphamide. Side-effects of treatment included neutropenia (three patients, one of whom developed staphylococcal sepsis), acute deep venous thrombosis (two patients), and psorias abscess (one patient). Intravenous pulse cyclophosphamide should be strongly considered in the treatment of patients with refractory AITP. There is a relatively low incidence of side-effects, and it can be administered easily on an outpatient basis.

AUTOIMMUNE thrombocytopenic purpura (AITP) is a disorder characterized by thrombocytopenia with shortened platelet survival, normal to increased numbers of bone marrow megakaryocytes, and elevated levels of platelet-associated immunoglobulin G (IgG). Women are more commonly affected than men. Bleeding manifestations are variable, but are more likely to occur in older patients and those with severe thrombocytopenia. AITP is caused by autoantibodies directed against platelet membrane glycoproteins (eg, GPIb-IIIa, GPIb) that result in both removal of the antibody-coated platelets from the circulation by the reticuloendothelial system and defective platelet production and/or release of platelets from the bone marrow.

Initial therapy of AITP consists of corticosteroids, often combined with intravenous gammaglobulin (IV IgG) in severe episodes. There is a great predominance of the chronic form of the disease in the adult patient and most will ultimately require other forms of therapy, most commonly splenectomy. Together, corticosteroids and splenectomy induce long-term responses in about 75% of chronic AITP patients. The remaining 25% of patients who are refractory to standard therapy constitute a subgroup with a significantly higher mortality rate compared with those who respond to corticosteroids and/or splenectomy. Other treatment options are available for patients with refractory AITP, but response rates are generally low and unpredictable. The initial response rates for other agents such as vinca alkaloids, danazol, low-dose oral cyclophosphamide, azathioprine, and colchicine are less than 50%, with long-term response rates much lower. More recently, other agents such as α-interferon, cyclosporine, IV IgG, ascorbic acid, staph protein A immunoadsorption, anti-Fc receptor monoclonal antibody, and combination chemotherapy have been used in the treatment of refractory AITP, but the utility of these agents either has been unsatisfactory or remains to be confirmed in larger clinical trials.

Intermittent intravenous cyclophosphamide (pulse therapy) has been reported as an effective treatment for various autoimmune diseases including nephritis associated with systemic lupus erythematosus (SLE). In addition, an earlier study by Weinerman, et al indicated a beneficial effect of pulse cyclophosphamide in eight of 14 patients with AITP. Seven years ago, we treated our first patient—a 15 year old girl with severe refractory AITP who suffered an intracranial hemorrhage—with pulse cyclophosphamide, and she remains in long-term remission. We now report our experience using pulse cyclophosphamide to treat 19 additional severely-affected, highly-refractory AITP patients.

MATERIALS AND METHODS

Patient selection. Because of our interest in the care and management of patients with AITP, we are often consulted by community physicians to discuss treatment options for their patients with refractory disease. Based on our success with the initial patient (patient no. 1) treated with pulse cyclophosphamide, subsequent referrals were routinely advised to initiate a similar therapeutic regimen. The diagnosis of AITP in the study patients was made on the basis of thrombocytopenia unrelated to any underlying viral infection, collagen vascular disease, malignancy or medication, morphologically normal bone marrow aspirate with normal to increased numbers of megakaryocytes, and absence of splenomegaly. Patients were considered for therapy if either: (1) during an acute initial or recurrent episode of AITP, platelet counts could not be increased to a safe level despite treatment with multiple modalities; or (2) if chronic AITP could not be managed with acceptable doses of corticosteroids or other agents.
Pulse cyclophosphamide therapy. Each treatment course of high-dose cyclophosphamide therapy consisted of 1.0 to 1.5 g/m² of cyclophosphamide given by rapid intravenous infusion and repeated at four-week intervals. The number of doses administered per treatment course ranged from one to four, with a mean of 2.0. At the time of initiation of cyclophosphamide therapy, all patients were simultaneously receiving corticosteroids, and several were receiving other agents as well.

Treatment response criteria. Clinical response to cyclophosphamide was defined as follows: complete response (CR) = platelet count \( \geq 150 \times 10^{9}/L \); partial response (PR) = increase in platelet count to \( \geq 50 \times 10^{9}/L \); or no response (NR).

Platelet antibody assays. Platelet-associated and free circulating platelet antibodies were detected using both \(^{125}\)I-radioiodinated (RIA) and enzyme-linked (ELISA) platelet antiglobulin assays. The direct assay was performed using the patient's autologous platelets if the platelet count was above 10 to 20 \( \times 10^{9}/L \). In the indirect assays, patient serum was tested against platelets from one or more random normal group O blood donors. Data is not reported for the indirect assay unless the patient had never been previously pregnant or transfused so that an alloantibody reacting with donor platelets could be excluded as the basis for a positive test result.

Peripheral whole blood collected in 10% potassium-EDTA was centrifuged at 350g for 10 minutes to obtain platelet-rich plasma from patients or random donors. For the ELISA platelet antiglobulin assays, the platelets were washed three times with 0.01 mol/L Tris pH 7.5 containing 0.145 mol/L NaCl and 1 mmol/L EDTA, and the platelet concentration was adjusted to 100 to \( \times 10^{7}/L \). Five \( \times 10^{9} \) platelets were added to wells of a 96-well microtiter plate (NUNC, Roskilde, Denmark). The platelets were bound to the wells by centrifugation at 1000g for 10 minutes. Plates were washed three times with phosphate buffered saline (PBS), then blocked with 100 \( \mu \)L of 5% human albumin (New York Blood Center, McVile, NY) for 30 minutes at room temperature. Fifty \( \mu \)L of fresh (less than 24 hours old) patient or pooled normal serum diluted 1:2 and 1:4 in 5% human albumin was added to the microtiter wells of each platelet sample and incubated at 37°C for 60 minutes. Plates were then washed six times with PBS, and 50 \( \mu \)L of one of four different alkaline phosphatase-conjugated antiglobulin reagents diluted in 5% human albumin were added and incubated at room temperature for 30 minutes. The four detecting reagents were: (1) monoclonal antihuman IgG (ICN Biomedicals, Costa Mesa, CA) diluted 1:800; (2) monoclonal antihuman IgG (The Binding Site, Birmingham, UK) diluted 1:400; (3) goat antihuman IgM (Sigma, St Louis, MO) diluted 1:500; and (4) Staph Protein A (Boehringer, Mannheim, Germany) diluted 1:800. After washing six times with PBS, 50 \( \mu \)L of substrate (3.4 mg/mL p-nitrophenylphosphate in 0.05 mol/L carbonate 1 mmol/L MgCl₂, pH 9.8) was added to each well; the plates were sealed with plastic sealing tape and incubated at 37°C for 50 minutes. The reaction was stopped by the addition of 50 \( \mu \)L of 1 mol/L NaOH. Absorbance was measured using a Bio-Tek microtiter plate reader (Winooski, VT). A positive platelet antibody result is indicated if the OD is over two standard deviations above that of the pooled normal serum for any of the four detecting reagents. Serum pools were obtained from 20 to 30 previously untransfused male donors who were type A or AB. In our laboratory, the coefficient of variation of the OD for pooled normal serum is approximately 30%. Reference alloantiserum was used for standardization of the ELISA and as positive controls.

The platelet antiglobulin radioimmunoassay was performed using \(^{125}\)I-labeled anti-IgG and anti-C₃ as the detecting reagents as described previously. An elevated level of platelet antibody detected using any one (or more) of the six total antiglobulin reagents in either the ELISA or the RIA was considered a positive platelet antibody test.

RESULTS

Patient characteristics. The clinical characteristics of the 20 patients with refractory AITP treated with pulse cyclophosphamide are shown in Table 1. Fourteen patients were women, and six were men. Patient ages ranged from 15 to 79 years with a mean of 50 years. Concurrent diseases included diabetes mellitus in three patients (no. 8, 13, and 20), autoimmune hemolytic anemia in two patients (no. 14 and 16), hypothyroidism in two patients (no. 6 and 14), and multiple sclerosis in one patient (no. 2). One of nine patients tested had a positive antinuclear antibody (ANA; patient no. 10). The median duration of AITP before pulse cyclophosphamide therapy was six months and ranged from one month to 28 years. Bleeding manifestations were present in 16 patients. These mainly consisted of skin and mucosal bleeding, except for patient no. 1 who suffered an intracranial hemorrhage. Of the 20 patients, nine received pulse cyclophosphamide during their initial episode of AITP (patients no. 1 through 9), while 11 were treated during an AITP relapse (patients no. 10 through 20). Of the latter patients, four were in first relapse (no. 11 through 14), one was in third relapse (no. 10), and six patients had long-standing recurrent AITP with four or more relapses (no. 15 through 20). The 20 patients had been treated previously with a mean of 4.8 different agents (range, 2 to 8) before cyclophosphamide therapy. All 20 patients had received corticosteroids, and 19 had undergone splenectomy. Two patients had also undergone an accessory splenectomy (no. 18 and 20). Other previous therapies included vinca alkaloids (15 patients), IV IgG (15 patients), danazol (8 patients), plasmapheresis (4 patients), anti-D IgG (3 patients), low-dose oral cyclophosphamide (3 patients), azathioprine (3 patients), colchicine (2 patients), and ascorbic acid, Protein A immunoadsorption, and vinca-loaded platelets (1 patient each).

In addition to corticosteroid therapy, 13 courses of cyclophosphamide were administered concurrently with IV IgG, three courses with danazol, and one course with vincristine. In patients no. 10, 11, and 16, danazol had been administered for 3 weeks, 10 weeks, and 10 days before initiation of pulse cyclophosphamide therapy, respectively, without evidence of an increase in platelet count. Although patient no. 10 had transiently responded to vincristine, he had been receiving this agent for 1 month before initiation of pulse cyclophosphamide without effect.

The mean nadir platelet count precyclophosphamide for the 20 patients was \( 7 \times 10^{9}/L \) (range, 1 to \( 20 \times 10^{9}/L \) (Table 2). At the time of initiation of cyclophosphamide therapy, the mean platelet count was \( 68 \times 10^{9}/L \) (range, 1 to \( 534 \times 10^{9}/L \)). This elevation from baseline was due to the acute effect of IV IgG or high-dose corticosteroids in six of the patients.

Response to pulse cyclophosphamide therapy. Responses of the 20 refractory AITP patients to pulse cyclophosphamide are illustrated in Fig 1 and summarized in Table 2. The mean peak platelet count following the 27 treatment courses of pulse cyclophosphamide was \( 261 \times 10^{9}/L \) (range, 6 to \( 745 \times 10^{9}/L \)). Based on our response criteria, 13 patients (65%) achieved a CR, four (20%)
achieved a PR, and three (15%) had NR; thus, the overall response rate to the first treatment course with pulse cyclophosphamide was 85%. The mean time to response was 7 weeks and ranged from 1 to 16 weeks.

Of the 13 complete responders, eight have remained in remission with stable platelet counts during followup intervals of 6 months to 7 years (median, 2.5 years). This includes the index patient (no. 1), who fully recovered from an intracranial hemorrhage and has been well for 7 years. Five patients developed recurrent AITP 4 months to 3 years following a CR. Of these, two patients (nos. 13 and 16) responded to subsequent courses of pulse cyclophosphamide. Patient no. 13 relapsed after 9 months, then achieved a second CR with pulse cyclophosphamide that has persisted for 4 years. Patient no. 16 relapsed 3 years after her first CR with pulse cyclophosphamide, then achieved a second CR with pulse cyclophosphamide that lasted 10 months and finally a third CR, which has persisted for 1 year. Adding these two patients to the eight who remain in CR after their first treatment course with pulse cyclophosphamide gives a long-term CR rate of 10/20 or 50% with a median followup of 2.5 years. Five of these patients responded during their initial episode of AITP, and five of these were patients with a history of relapsed AITP.

The three other patients who relapsed after a first CR to pulse cyclophosphamide also received additional courses of pulse cyclophosphamide. Patient no. 15 relapsed 9 months after her CR, then responded very briefly to a second treatment course with pulse cyclophosphamide that lasted 5 months, then relapsed AITP.

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bleeding symptoms for the past years. Patient no. 17 relapsed 5 months after a first PR to cyclophosphamide therapy and died 1 month later with a platelet count of <10^9/L of unknown causes while in a nursing home with a platelet count of 500 to 750 mg weekly. The patient died two months later of complications of diabetes mellitus. Patient no. 18 has since achieved a CR with Protein A immunoadsorption that has persisted for two months. Patient no. 12 was refractory to corticosteroids, splenectomy, and IV IgG, then responded transiently to vincristine and danazol. He failed to respond to either low-dose oral or intravenous pulse cyclophosphamide therapy and died 3 months later with a platelet count of <10^9/L of uncontrolled hemorrhage and anemia after refusing further supportive transfusion therapy. Patient no. 19 died of complications following treatment with an anti-Fc receptor monoclonal antibody.

Platelet antibody data. Platelet antibody studies were performed for 12 of the 20 patients before pulse cyclophosphamide therapy. Platelet-associated antibodies were detected in 11 of these 12 patients. Ten of the patients had free, circulating platelet antibodies. Interestingly, the one patient who was negative for both platelet-bound and free antibody (no. 19) did not respond to pulse cyclophosphamide therapy.

Response versus patient characteristics. The relationship of several patient variables to their initial treatment

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>No. of Doses/ Treatment Program</th>
<th>Pre* Count × 10^9/L</th>
<th>Post† Count × 10^9/L</th>
<th>Response</th>
<th>Time to Response (wk)</th>
<th>Response Duration</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>&lt;1</td>
<td>530</td>
<td>CR</td>
<td>4</td>
<td>7 yr</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>215</td>
<td>CR</td>
<td>8</td>
<td>1 yr</td>
<td>Deceased (unknown cause)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>4</td>
<td>56</td>
<td>PR</td>
<td>10</td>
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<td>4</td>
<td>1</td>
<td>4</td>
<td>198</td>
<td>CR</td>
<td>1</td>
<td>8 mo</td>
<td>CR</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>6</td>
<td>389</td>
<td>CR</td>
<td>10</td>
<td>7 yr</td>
<td>CR</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>6</td>
<td>180</td>
<td>PR</td>
<td>6</td>
<td>10 mo</td>
<td>PR</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>9</td>
<td>79</td>
<td>PR</td>
<td>6</td>
<td>10 mo</td>
<td>PR</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>5</td>
<td>67</td>
<td>PR</td>
<td>16</td>
<td>5 mo</td>
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<tr>
<td>9</td>
<td>2</td>
<td>6</td>
<td>457</td>
<td>CR</td>
<td>8</td>
<td>2 yr</td>
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<td>10a</td>
<td>2</td>
<td>9</td>
<td>315</td>
<td>CR</td>
<td>8</td>
<td>4 mo</td>
<td></td>
</tr>
<tr>
<td>10b</td>
<td>2</td>
<td>14</td>
<td>377</td>
<td>CR</td>
<td>2</td>
<td>5 mo</td>
<td></td>
</tr>
<tr>
<td>10c</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>NR</td>
<td></td>
<td></td>
<td>PR to dz, ld cy, cs, IV IgG</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>9</td>
<td>260</td>
<td>CR</td>
<td>16</td>
<td>3 yr</td>
<td>CR</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>8</td>
<td>10</td>
<td>NR</td>
<td></td>
<td></td>
<td>Deceased (bleeding; refused additional therapy)</td>
</tr>
<tr>
<td>13a</td>
<td>1</td>
<td>2</td>
<td>146</td>
<td>CR</td>
<td>2</td>
<td>9 mo</td>
<td></td>
</tr>
<tr>
<td>13b</td>
<td>2</td>
<td>16</td>
<td>400</td>
<td>CR</td>
<td>8</td>
<td>4 yr</td>
<td>CR</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>6</td>
<td>440</td>
<td>CR</td>
<td>6</td>
<td>1 yr</td>
<td>CR</td>
</tr>
<tr>
<td>15a</td>
<td>2</td>
<td>3</td>
<td>373</td>
<td>CR</td>
<td>8</td>
<td>9 mo</td>
<td></td>
</tr>
<tr>
<td>15b</td>
<td>3</td>
<td>12</td>
<td>176</td>
<td>CR</td>
<td>6</td>
<td>2 mo</td>
<td>PR to IV IgG</td>
</tr>
<tr>
<td>16a</td>
<td>2</td>
<td>10</td>
<td>647</td>
<td>CR</td>
<td>4</td>
<td>3 yr</td>
<td></td>
</tr>
<tr>
<td>16b</td>
<td>1</td>
<td>9</td>
<td>745</td>
<td>CR</td>
<td>2</td>
<td>10 mo</td>
<td></td>
</tr>
<tr>
<td>16c</td>
<td>1</td>
<td>5</td>
<td>279</td>
<td>CR</td>
<td>2</td>
<td>1 yr</td>
<td>CR</td>
</tr>
<tr>
<td>17a</td>
<td>2</td>
<td>10</td>
<td>124</td>
<td>PR</td>
<td>10</td>
<td>18 mo</td>
<td></td>
</tr>
<tr>
<td>17b</td>
<td>2</td>
<td>20</td>
<td>63</td>
<td>PR</td>
<td>10</td>
<td>6 mo</td>
<td>PR</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>10</td>
<td>390</td>
<td>NR</td>
<td></td>
<td></td>
<td>CR to prot A</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>NR</td>
<td></td>
<td></td>
<td>Deceased (complications of another therapy)</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>5</td>
<td>234</td>
<td>CR</td>
<td>8</td>
<td>5 yr</td>
<td>CR</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete response; PR, partial response; NR, no response; dz, danazol; ld cy, low dose cytoxan; cs, corticosteroids; IV IgG, intravenous immunoglobulin; prot A, protein A immunoadsorption.

* Nadir platelet count pre-cyclophosphamide.
† Peak platelet count post-cyclophosphamide.
‡ Represents transient response to IV IgG.

Table 2. Response to Pulse Cyclophosphamide Therapy

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Complications of pulse cyclophosphamide therapy.

Side-effects occurred during six of the 27 treatment courses (22%). Neutropenia was the most frequent complication of pulse cyclophosphamide therapy and occurred during three of the 27 treatment courses (11%). In two patients (no. 3 and 15), the neutropenia was mild (nadir neutrophil count 1.0 and 1.5 \times 10^9/L, respectively). Patient no. 2, however, developed severe neutropenia (neutrophil count = 0.1 \times 10^9/L) complicated by an episode of staphylococcal sepsis. This patient eventually recovered and maintained a CR for one year, but subsequently died shortly after a second course of cyclophosphamide given at a lower dose (500 to 750 mg weekly). The cause of death is unknown.

Two patients (no. 10 and 16) developed acute deep venous thrombosis coincident with a rapid CR to therapy (platelet counts of 298 \times 10^9/L and 745 \times 10^9/L, respectively, at the time of the thrombosis). No known risk factors for venous thrombotic disease were present in either patient.

Finally, patient no. 17 developed a psoas abscess following the first dose of pulse cyclophosphamide, necessitating a delay of the second dose by 6 weeks. The psoas abscess was not accompanied by neutropenia in this patient, but he had been receiving prolonged therapy with corticosteroids. Following a relapse 1 year later, the patient received another two doses of pulse cyclophosphamide therapy without complications and again attained a PR.

Although 12 of the 27 cyclophosphamide treatment courses were initiated while patients were hospitalized because of their bleeding manifestations and low platelet counts, patients who were more stable could be treated as outpatients because of the low incidence of side-effects.

No long-term side-effects of pulse cyclophosphamide have been noted in any of our patients to date. In particular, none of the 20 patients have developed a secondary malignancy (median followup since first cyclophosphamide dose is 2 years, range 3 months to 7 years).

**DISCUSSION**

We report the responses of 20 clinically-severe and highly-refractory AITP patients to pulse cyclophosphamide. Bleeding symptoms were present at the time of treatment in 16 patients, including intracranial bleeding in the initial patient. More than half of the patients had recurrent AITP, including six patients with chronic, long-standing AITP marked by four or more relapses. Furthermore, all the patients had been heavily treated before receiving pulse cyclophosphamide. Each patient had failed to respond to an aver-

Table 3. Patient Characteristics Based on Response to Treatment

<table>
<thead>
<tr>
<th>CR (n = 13)</th>
<th>PR (n = 4)</th>
<th>NR (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>10/3</td>
<td>3/1</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td>AITP median duration</td>
<td>6 mo</td>
<td>5 mo</td>
</tr>
<tr>
<td>Primary/Recurrent</td>
<td>6/7</td>
<td>3/1</td>
</tr>
<tr>
<td>Mean no. doses cyclophosphamide</td>
<td>1.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Presence of antibody</td>
<td>7/7</td>
<td>3/3</td>
</tr>
</tbody>
</table>
rephosphamide (one to four doses of 1 to 1.5 g/m²) for an
and azathioprine. At best, these agents have response rates
are superior to those of standard secondary AITP agents
of 20 (65%) with a mean followup of 2.9 years. These results
ment course of pulse cyclophosphamide and are currently
patients who relapsed responded to a second and/or third treat-
phosphamide responses were associated with concurrent da-
doses reported to induce a long-term response. Four cyclo-
treatment. In addition, three of the initially-responsive pa-
therapy.
In this series of 20 highly refractory patients, 17 patients
had either a CR or PR to their initial course of pulse cyclo-
phosphamide (one to four doses of 1 to 1.5 g/m²) for an
overall response rate of 85%. Furthermore, 10 patients (50%) in
the series have had a sustained response to their initial
in addition, three of the initially-responsive patients
who relapsed responded to a second and/or third treat-
ment course of pulse cyclophosphamide and are currently
stable. Thus, the cumulative long-term response rate is 13
of 20 (65%) with a mean followup of 2.9 years. These results
are superior to those of standard secondary AITP agents
such as vinca alkaidoids, danazol, oral cyclophosphamide,
and azathioprine. At best, these agents have response rates
of approximately 50%, but sustained response rates are in
the range of only 5 to 25%.16

Our favorable results with pulse cyclophosphamide are comparable to three other studies. Figueras et al19 recently reported responses in eight (six complete, two partial) of 10 severely-affected, highly-refractory AITP patients treated with cyclophosphamide-based combination chemotherapy. Five of the responses (four complete and one partial) were sustained. These patients received between 3 and 8 monthly cycles of a chemotherapy regimen that included cyclophosphamide at total doses approximately the same as used in our study (≈0.5 g/m² given every 2 weeks) and corticoste-
roids along with either vincristine, procarbazine, and/or eto-
poside. Our data suggest that the most important component of
these combined treatment regimens was cyclophospha-
mide, and that the other agents may not be required to
achieve the desired result. Single-agent therapy would
clearly reduce costs, and possible complications, of therapy.
Secondly, Boumpas et al20 reported complete responses in
each of seven refractory patients with SLE-associated im-
mune thrombocytopenia treated with one to four doses of
cyclophosphamide at 0.75 to 1.0 g/m². Four of these patients
had sustained responses with a mean followup of 5.6 years. Thus, single-agent pulse cyclophosphamide appears to be
beneficial in both primary and SLE-associated AITP. Finally,
Weinerman et al20 reported responses in eight of 14 patients
with immune thrombocytopenia treated with varying sched-
ules of intermittent cyclophosphamide in doses ranging from
300 to 600 mg/m². These patients had previously failed corti-
costeroids and, in some cases, splenectomy. Four of the re-
ponders had disseminated SLE. Six of the responses were
sustained with followup ranging between 15 and 20 months.
The slightly lower response rate in this earlier report may
be due to the lower dose of pulse cyclophosphamide com-
pared with our study and those of Figuera et al21 and Boun-
pas et al22
The mechanism of action of pulse cyclophosphamide in
autoimmune disorders appears to be suppression of both T
and B lymphocyte numbers and function,26 which leads to
diminished autoantibody production.27,29 In previous studies
of AITP patients treated with cyclophosphamide or cyclo-
phosphamide-based combination chemotherapy, clinical re-
sponse was associated with reduction of platelet antibody
levels.23,31 In our series, postcyclophosphamide platelet anti-
body studies were performed in only three patients; two of
the three patients showed disappearance of platelet autoanti-
body following a complete, persistent response to pulse cy-
clophosphamide. In one patient (no. 1), platelet autoantibody
disappeared within 4 weeks of treatment. The other (no. 5)
had a more gradual decline in antibody level over a period
of several years. The third patient (no. 16) had persistent
platelet autoantibody 2 months after treatment that resulted
in a transient CR, but followup platelet antibody testing was
not performed.
Several patient characteristics were examined for an asso-
ciation with pulse cyclophosphamide treatment outcome.
Because of the small number of patients studied, none of the
associations reached statistical significance. Of those exam-
ined, shorter disease duration was most strongly associated
with response to pulse cyclophosphamide (P value = .10),
and patient age, sex, and phase of disease were all marginally
significant as well. The association of shorter disease dura-
tion has been noted for AITP response to oral low-dose
cyclophosphamide as well.32 Splenectomized patients have
also been reported to have a better response rate to oral
cyclophosphamide,33 but we are unable to confirm this asso-
ciation with pulse cyclophosphamide because all but one of
our patients had undergone splenectomy.
Acute side-effects occurred during six of 27 (22%) of
the pulse cyclophosphamide treatment courses. Neutropenia
occurred in three patients, but was severe in only one patient
in whom staphylococcal sepsis developed. The occurrence
of deep venous thrombosis in two patients was associated
with a rapid and complete response to pulse cyclophospha-
mide. Although cyclophosphamide itself is not associated
with hypercoagulability, it is possible that the rapid increase
in platelet count may have induced a transient hypercoag-
urable state due to the presence of circulating, immature hyper-
functional platelets that are thought to circulate in AITP
patients.34 However, the acute increase in platelet count
sometimes seen postsplenectomy for this and other disorders is not commonly associated with deep venous thrombosis unless other intrinsic abnormalities of the blood are present and associated with abnormal platelet function such as myeloproliferative disorders.\textsuperscript{35,36} Other known side-effects of cyclophosphamide such as nausea, vomiting, alopecia, and hemorrhagic cystitis were not noted in this series.

Although no long-term side-effects of pulse cyclophosphamide were noted in our patients, an association between cyclophosphamide and secondary cancers has been well-established. In patients with rheumatologic disorders treated with oral cyclophosphamide, the risk appears to be related to cumulative cyclophosphamide dose and duration of therapy.\textsuperscript{37} Krause\textsuperscript{38} reported three patients with AITP who developed acute myeloid leukemia 2 to 4 years after receiving low-dose oral cyclophosphamide. An increased risk of secondary malignancy has also been noted in patients treated with pulse cyclophosphamide for primary neoplasms\textsuperscript{39} and as a conditioning regimen preceding bone marrow transplantation for aplastic anemia.\textsuperscript{40} The incidence of secondary cancers in patients with autoimmune disease treated with pulse cyclophosphamide is unknown, but appears to be lower than with continuous oral cyclophosphamide.\textsuperscript{39} To date, there have been five cases reported of malignancy complicating pulse cyclophosphamide in SLE patients.\textsuperscript{40,41,42}

Another potential complication of pulse cyclophosphamide is sterility, although this side-effect is also less common than with continuous low-dose cyclophosphamide therapy. Nevertheless, infertility has been reported in over half of the women receiving pulse cyclophosphamide.\textsuperscript{43}

The role of pulse cyclophosphamide in the treatment of refractory AITP requires careful consideration of the relative benefits and risks. Our data suggest that pulse cyclophosphamide is an effective treatment for refractory AITP and should be strongly considered in severely-affected patients who do not respond to standard therapy. It may be particularly efficacious in elderly patients in whom the long-term risks of malignancy and sterility are less of a concern than in younger patients, as well as in patients for whom splenectomy is a high-risk procedure. Nevertheless, given the relatively high mortality rate of refractory AITP, treatment at any age may be justified. The optimum dose and treatment schedule of pulse cyclophosphamide remain to be determined.

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Pulse cyclophosphamide therapy for refractory autoimmune thrombocytopenic purpura [see comments]

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