Late Complications Following Treatment for Severe Aplastic Anemia (SAA) with High-Dose Cyclophosphamide (Cy): Follow up of a Randomized Trial

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Abstract

High dose cyclophosphamide (Cy) has been promoted as curative therapy for severe aplastic anemia (SAA). However, our randomized trial comparing antithymocyte globulin (ATG) and Cy was terminated early due to excess morbidity/early mortality in the Cy arm. We now report analysis of secondary endpoints at a median of 38 months. Relapse occurred in 6 of 13 (46%) responders in the ATG arm vs. 2 of 8 (25%) in the Cy arm (p=0.38). Five of 16 (31%) patients in the ATG arm and 4 of 15 (27%) patients in the Cy arm had evidence of PNH at diagnosis, with no significant change in the overall percentage of GPI-anchored protein-deficient neutrophils over extended follow-up in individual patients in either arm. Bone marrow cytogenetic abnormalities have been observed among surviving patients in both arms (2/14 ATG vs. 1/12 Cy, p=0.70). High dose Cy does not prevent relapse or clonal evolution in SAA.

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Introduction

Immunosuppression with antithymocyte globulin (ATG) and cyclosporine (CSA) results in long-term survival in patients with SAA comparable to that achieved by allogeneic bone marrow transplantation from a histocompatible sibling donor, yet problems including incomplete hematologic recovery, relapse, and the appearance of clonal hematopoietic disorders complicate long-term management. High dose cyclophosphamide has been proposed as an alternative immunosuppressive agent for the treatment of aplastic anemia based on encouraging results in two uncontrolled trials: in contrast to the experience with ATG-based treatments, neither relapse nor clonal disease were reported. We initiated a phase III randomized trial to compare response rates to immunosuppression with either ATG or high dose Cy, both combined with CSA. Secondary endpoints included relapse and clonal evolution. Although primary response rates were not significantly different at 6 months, our trial was terminated early due to excess morbidity and early mortality in the Cy arm. We now report analysis of secondary endpoints after extended follow-up at a median of 38 months.

Methods

The protocol was approved by the Institutional Scientific Review Committee and the Institutional Review Board of the National Heart, Lung, and Blood Institute, and all patients gave written informed consent. A sample size of 91 patients per arm was planned to allow comparison of the response proportions conducted at the 0.05 significance level, but the trial was terminated after 31 patients had accrued. Secondary endpoints included relapse, the appearance of clonal hematologic disorders, overall and event free survival. All endpoints were assessed at scheduled follow-up visits at 6 months, 12 months and yearly thereafter. Patients were considered responders if they experienced an improvement in blood counts sufficient to no longer meet criteria for severe disease, criteria which correlate with eventual transfusion independence. To better assess the quality of hematologic recovery, response was further classified with ordered, mutually exclusive criteria as partial response with transfusion dependence (PRd), partial response with transfusion independence (PRI) and complete response (normal or near normal blood counts; CR). Patients meeting criteria for sustained response of greater than 3 months who subsequently experienced a fall in counts sufficient to require reinstitution of immunosuppressive drugs were considered to have relapsed. Peripheral blood samples were analyzed by flow cytometry for the presence of GPI-anchored-protein-deficient granulocytes at presentation and at each scheduled follow-up; detection at 1.0% or greater on two or more evaluations was considered evidence for underlying PNH. Evolution to myelodysplastic syndrome (MDS) was defined by the characteristic marrow morphology or the presence of a consistent chromosomal abnormality; cytogenetic examination of bone marrow samples was performed at presentation, 6 month follow up and yearly thereafter.

Results and Discussion

Thirteen of 16 (81%) patients randomized to ATG and 8 of 15 (53%) patients randomized to Cy showed a hematologic response (Table 1). All responding patients, regardless of treatment allocation, eventually achieved transfusion independence (no remaining PRds), as predicted from previous results with standard immunosuppressive therapy. Complete responses were observed in 10 of 16 patients (63%) in the ATG arm and 6 of 15 patients...
Relapse is the most frequent long-term complication following immunosuppression with ATG-containing regimens, but was not observed in the early Cy treated patients. Indeed, relapse occurred in 6 of 13 (46%) responders in the ATG arm, but relapse was also observed in 2 of 8 (25%) responders in the Cy arm (p=0.38, Fisher’s exact). Four of 6 patients in the ATG arm and 1 of 2 patients in the Cy arm were retreated solely on a fall in the platelet count. Relapse in which blood counts again met criteria for severe disease was observed in 1 patient in the ATG arm and 1 in the Cy arm. Although the patient in the Cy arm presented initially with counts just satisfying severity criteria, relapse to super severe disease occurred more than two years after attaining a CR. All relapsed patients responded to reinstitution of immunosuppression with either CSA or ATG and CSA; 3 in the ATG arm and 1 in the Cy arm required the addition of ATG.

The association between PNH and aplastic anemia is well established; however, early studies suggesting evolution to PNH employed the now outdated Ham test. Patients with clinical PNH were excluded from our randomized trial; however, flow cytometric methods now allow more sensitive detection of such clones in not only erythroid but also myeloid cells, increasing both sensitivity and specificity, and the use of such techniques argues that PNH is a common and early event in SAA. The simultaneous absence of two GPI anchored proteins highly expressed on normal granulocytes, CD66b and CD16, at levels above our threshold of 1.0% was used as criteria for establishing PNH, such an expanded population was detected in 5 patients in the ATG arm and 4 patients in the Cy arm, at presentation. A GPI-anchored protein-deficient population just below our cutoff was detectable at diagnosis in one additional patient in the ATG arm; 1 subsequent determination was just above the cutoff, meeting our criterion for PNH. Regardless of treatment allocation, the overall percentage of GPI-anchored protein-deficient granulocytes has not changed significantly over extended follow-up (Table 2). Further, treatment of a single patient with clinical PNH with high dose Cy by compassionate exemption produced neither hematologic improvement nor a change in the percentage of GPI-anchored protein-deficient granulocytes over time. While detection of the PNH phenotype among blood cells of patients with aplastic anemia is relatively common, clinical PNH is less frequently observed and only one patient in each treatment arm subsequently has developed evidence of intravascular hemolysis.

The late occurrence of myelodysplastic syndrome is the most dire complication observed in patients with SAA and occurs in both successfully treated and persistently cytopenic patients. Evolution to myelodysplasia was not described in the two pilot series published to date. In the current protocol, marrow cytogenetic evaluation revealed the presence of abnormalities characteristic of MDS not only in 2 patients in the ATG arm (trisomy 8 9/20 metaphases at 6 month follow up increasing to 19/20 at 4 year follow up and 20q-, 9/50 metaphases at 3 year follow up with 0/6 at 4 year follow up), but also in 1 patient in the Cy arm (trisomy 8, 4/20 metaphases at 1 year follow up)(p=0.70, Fisher’s exact) despite a normal karyotype in all cases at presentation. These cytogenetic
abnormalities were noted, however, on routine marrow samples among patients who remain in clinical remission and underscore the importance of marrow sampling even among patients with stable, recovered blood counts. Further, such chromosomal abnormalities do not necessarily portend a poor prognosis in patients with SAA.\textsuperscript{13,14} While the early termination of our trial due to toxicity on the Cy arm does not permit a comparison of either response rates or long-term complications with adequate statistical power, the tempered enthusiasm for this approach we experienced due to the high degree of early toxicity is further dampened by our recent observations. Cy treatment does not prevent the familiar long-term complications experienced by patients treated with conventional immunosuppression for SAA and as such, the resulting early morbidity and mortality cannot be justified by their anticipated absence. The continued development of alternative immunosuppressive regimens which take into account both the late complications as well as early safety is thus warranted.
### Table 1. Results at median follow-up of 38 months

<table>
<thead>
<tr>
<th></th>
<th>ATG/CsA</th>
<th>Cy/CsA</th>
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<tbody>
<tr>
<td>Overall Response</td>
<td>13/16 (81%)</td>
<td>8/15 (53%)</td>
</tr>
<tr>
<td>CR</td>
<td>10 (63%)</td>
<td>6 (40%)</td>
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<tr>
<td>PRi</td>
<td>3 (18%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Relapse</td>
<td>6/13 (46%)</td>
<td>2/8 (25%)</td>
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<tr>
<td>Cytogenetic evolution</td>
<td>2/14 (14%)</td>
<td>1/12 (8%)</td>
</tr>
</tbody>
</table>

Overall responses are shown by type and overall percentage and do not differ between arms. Complete responses have been observed in 6 of 8 responders or 75% in the Cy arm and 10 of the 13 responders or 77% in the ATG arm (40% Cy and 63% ATG, overall complete response rates). No patients remain in the PRd response group. Relapse rates do not differ between arms (p=0.38) with 6 among 13 responders and 2 among 8 responders relapsing in the ATG and Cy arms, respectively.
<table>
<thead>
<tr>
<th></th>
<th>0 mo.</th>
<th>3 mo.</th>
<th>6 mo.</th>
<th>9 mo.</th>
<th>1 yr.</th>
<th>2 yr.</th>
<th>3 yr.</th>
<th>4 yr.</th>
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<tbody>
<tr>
<td>A1</td>
<td>15.3</td>
<td>8.8</td>
<td>21</td>
<td>32</td>
<td>37</td>
<td>43</td>
<td>69</td>
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<tr>
<td>A2</td>
<td>7.6</td>
<td>4.5</td>
<td>5.6</td>
<td>13</td>
<td>9.7</td>
<td>5.3</td>
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<tr>
<td>A3</td>
<td>7.6</td>
<td>1.5</td>
<td>2.7</td>
<td>ND</td>
<td>2.4</td>
<td>2.4</td>
<td></td>
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</tr>
<tr>
<td>A4</td>
<td>6.4</td>
<td>1.6</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>A5</td>
<td>8.9</td>
<td>15.8</td>
<td>11.6</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td></td>
<td>1.2**</td>
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<tr>
<td>C2</td>
<td>83</td>
<td>36</td>
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<td>C5*</td>
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The percentage of GPI-anchored protein-deficient granulocytes is shown over time in patients randomized to ATG (A1-5) or Cy (C1-4) in whom at least one value was above the threshold of 1%. Two patients (A1 and C2) have developed evidence of intravascular hemolysis. *Patient treated by compassionate exemption for primary PNH, (values at 1 mo 92% and 2 mo. 55.4%). ND not done. **Patient deceased prior to subsequent follow-up.
References


