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High-Dose Cyclophosphamide is Effective Therapy for Pediatric Severe Aplastic Anemia

Christopher J. Gamper, MD, PhD¹, Clifford M. Takemoto, MD², Allen R. Chen, MD, PhD¹, Heather J. Symons, MD¹, David M. Loeb, MD, PhD¹, James F. Casella, MD², Amy E. Dezern, MD³, Karen E. King, MD⁴, Andrea M. McGonigle, MD⁵, Richard J. Jones, MD⁶, and Robert A. Brodsky, MD³

¹Division of Pediatric Oncology, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

²Division of Hematology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA

³Division of Hematology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁴Division of Transfusion Medicine, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁵Division of Transfusion Medicine, Department of Pathology and Lab Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

⁶The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

Objective—Use of high-dose cyclophosphamide without hematopoietic stem cell transplant (HSCT) to treat severe aplastic anemia (SAA) has been controversial due to concern for increased infectious toxicity as compared to anti thymocyte globulin (ATG) and cyclosporine (CSA). As children often tolerate dose-intensive therapy better than adults, we sought to perform a detailed retrospective analysis of both treatment response and toxicity in 28 patients less than 22 years of age treated with 29 courses of high-dose cyclophosphamide as the sole form of immunosuppression.

Study Design—Children and adolescents with SAA who lacked an HLA-matched sibling donor were treated with cyclophosphamide 50 mg/kg/day for 4 consecutive days then received daily G-CSF until neutrophil recovery, transfusion support, and antimicrobial prophylaxis.

Results—Overall survival was 85%, with hematologic response of 79% and complete response of 66%. Cumulative incidences of bacterial infection (86%) and fungal infection (62%) were high

Correspondence: Christopher J. Gamper, Division of Pediatric Oncology, Department of Oncology, 1650 Orleans St., CRB I, Rm. 209, Baltimore, MD 21287; fax 410-502-7223; tel. 410-502-2342; cgamper1@jhmi.edu.

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but deaths due to infection were rare, as were clonal evolution (1/28), clinically relevant PNH (1/28), and relapse (2/28).

Conclusion—Response rates and survival following high-dose cyclophosphamide in pediatric patients with SAA exceed those seen in adults and compare favorably to ATG/CSA with manageable infectious toxicity.

Keywords

severe aplastic anemia; clinical trials; pediatric hematology/oncology

Introduction

Acquired severe aplastic anemia (SAA) is a rare and life-threatening form of bone marrow failure that has a bimodal age distribution with peaks in early adolescence and in late middle-age.^{1, 2} The majority of cases of SAA are believed to result from autoimmune depletion of the hematopoietic stem cell (HSC) compartment.³ While infections including cytomegalovirus, Epstein-Barr Virus, parvovirus, or non-ABC hepatitis may precede SAA, most cases have no clear inciting event and are characterized as idiopathic.³ Autoimmune depletion of the HSC compartment appears to be mediated by activated clonally expanded effector T cells, along with relative deficiency of immune suppressive regulatory CD4 T cells.^{4–8} Autoreactive effector T cells produce IFN- γ , TNF- α , and possibly IL-17 and possess the capacity to kill autologous hematopoietic progenitors. Immune-mediated HSC depletion results in transfusion dependence and neutropenia with a high risk of death from infection.⁹ Mortality in untreated patients may reach 80% by 2 years, so the standard of care for patients under age 40 is to proceed to human leukocyte antigen (HLA)-matched sibling hematopoietic stem cell transplant (HSCT) using cyclophosphamide 50 mg/kg/dose once daily for 4 doses, combined with anti-thymocyte globulin (ATG) as additional immune suppression. Typical graft versus host disease (GVHD) prophylaxis utilizes short course methotrexate and cyclosporine A (CSA). Outcomes are excellent in children and young adults with long-term overall survival of about 90%.^{10–14}

For patients who lack an HLA-matched sibling donor, the hypothesis that autoimmunity causes SAA led to therapeutic trials of immunosuppressive therapy (IST). In a randomized trial of ATG versus supportive care, over half of patients treated with ATG improved, while none receiving supportive care demonstrated a response.¹⁵ IST consisting of ATG together with CSA is even more effective, resulting in transfusion independence in 60–70% of patients by 6 months.^{13, 16–19} In patients who achieve a complete response to such IST, CSA is gradually tapered and discontinued. Unfortunately, up to 30% of responders relapse with aplastic anemia and require retreatment with extended courses of IST or allogeneic HSCT from an alternative donor, resulting in event or failure free survival rates of 33 to 56% in children and young adults.^{13, 14, 18, 20} Additionally, patients with poor responses to IST may develop clonal evolution or myelodysplastic syndrome (MDS) at incidences ranging from 3 to 20%.^{18, 19, 21}

High-dose chemotherapy can also have potent immuno-modulatory properties. A subset of patients undergoing HLA-matched sibling HSCT have transient donor engraftment but

demonstrate stable blood counts derived from recovery of autologous hematopoiesis.^{22, 23} Mechanistically, high-dose cyclophosphamide appears to be selective toxic to activated autoreactive T cells that possess low levels of the aldehyde dehydrogenase 1 enzyme family that comprise the major mechanism of cyclophosphamide inactivation and are expressed robustly by HSCs.^{24, 25} This observation led to development of a clinical trial at our institution using high-dose cyclophosphamide 50 mg/kg/dose $\times$ 4 doses without a stem cell rescue as immunosuppression for patients with SAA lacking an HLA-matched sibling donor.²⁶ This was demonstrated to be effective in a series of adult and pediatric patients with response rates of 71% for treatment naïve patients and 48% for patients with refractory SAA, with respective event free survivals of 58% and 27%.²⁷ However, concern for the potential of increased risk of infection following this approach has been raised based on increased rates of invasive fungal infection (4/15) and death (3/15) in a cohort of patients receiving high-dose cyclophosphamide with cyclosporine in a randomized comparison to ATG/CSA (0/16, and 1/16, respectively).²⁸ Such high rates of early treatment-related mortality differed from our institutional experience using high-dose cyclophosphamide in children and young adults, so we performed a retrospective analysis of treatment related complications and outcomes from the entire cohort of 28 pediatric patients treated with this approach, the largest series of pediatric patients reported in the literature treated with this protocol to date.^{29, 30} Response rates in children exceed those of adults and were not influenced by a history of non-response to prior treatment, with low rates of clonal evolution and relapse and without the requirement for prolonged immunosuppression. Although infections were frequent during the period before neutrophil recovery, mortality from infection was low.

Materials and Methods

Patient Selection

The Institutional Review Board of Johns Hopkins University approved retrospective chart analysis of patients under age 22 treated at our institution for SAA with high-dose cyclophosphamide. Patients were identified from a departmental database. Subjects over age 18 gave signed informed consent for treatment, and subjects less than age 18 were treated following their assent and signed informed consent from their parents or guardians. Between April 1997 and April 2013, 28 out of 29 consecutive patients with SAA referred to Johns Hopkins Hospital were offered treatment with high-dose cyclophosphamide. The one consecutive patient not offered treatment was deemed ineligible due to prior misdiagnosis and treatment with chemotherapy for pre-B acute lymphoblastic leukemia that was subsequently determined to be SAA instead. Six patients with relapsed or refractory SAA and 22 patients with new-onset SAA age 21 or younger were treated. Eighteen patients were enrolled and treated in an Institutional Review Board-approved institutional clinical trial conducted in accordance with the Declaration of Helsinki. (Trial registration: clinicaltrials.gov, identifier: NCT00004464.) Ten patients were treated following the protocol after it closed due to meeting its accrual goal, but all 10 met eligibility requirements for the protocol and received treatment as specified in the protocol. Eligibility required bone marrow cellularity less than 25% with 2 of the following laboratory abnormalities: absolute neutrophil count less than $0.5 \times 10^9/L$; platelet count less than $20 \times 10^9/L$; an absolute

reticulocyte count less than $60 \times 10^9/L$. Very severe aplastic anemia (VSAA) was defined as SAA with a neutrophil count less than $0.2 \times 10^9/L$. Only patients who lacked a suitable HLA-matched sibling donor were eligible for high-dose cyclophosphamide, while those with an HLA-matched sibling donor were referred for allogeneic transplantation as initial therapy.

Exclusion criteria included serum creatinine greater than $180 \mu\text{mol/L}$, cardiac ejection fraction less than 45%, diagnosis with an inherited bone marrow failure syndrome, or the presence of active malignancy. Pregnant females and females of childbearing age who were unwilling to use contraceptives were also excluded.

Treatment Plan

Cyclophosphamide was administered at 50 mg/kg of ideal body weight intravenously once daily on 4 consecutive days with intravenous 2-mercaptoethane sulfonate for prophylaxis of hemorrhagic cystitis either as 10 mg/kg 30 minutes before and 3, 6, and 8 hours after cyclophosphamide infusion or alternatively as 10 mg/kg mixed with the cyclophosphamide infusion and 30 mg/kg given as continuous infusion for 8 hours after completion of the cyclophosphamide. (Cyclophosphamide is not FDA approved for use in this indication, but this dose and schedule are widely used in the setting of HSCT preparative regimens.) Granulocyte colony stimulating factor at 5 mcg/kg daily was given to all patients after completion of cyclophosphamide until the neutrophil count was greater than 1.0×10^9 cells/L for 2 consecutive days and any infections had resolved. Packed red blood cells were transfused to maintain a hematocrit greater than 0.25. Platelets were transfused for bleeding or to maintain a count greater than $10 \times 10^9/L$. Antibiotic prophylaxis consisted of fluconazole (5 mg/kg/day, max 400 mg), norfloxacin (400 mg twice daily), and valacyclovir (250 mg/m²/dose, twice daily) beginning on the day after the last dose of cyclophosphamide and continuing until the neutrophil count exceeded 0.5×10^9 cells/L. *Pneumocystis jirovecii* prophylaxis with trimethoprim-sulfamethoxazole (2.5 mg/kg/dose based on trimethoprim, twice daily, max 160/800 mg) was given every Saturday and Sunday for 6 months after treatment. Broad-spectrum antibacterials were started with first fever and adjusted according to cultures. Antifungal coverage was broadened to amphotericin B liposome or voriconazole empirically for second fever. In cases of severe, uncontrolled infection and neutropenia, administration of granulocyte transfusions was permitted at the treating physician's discretion. Male patients who were post-pubertal were offered semen cryopreservation prior to treatment. Female patients who had reached menarche were offered menstrual suppression with leuprolide prior to treatment.

Statistical Analysis

The dataset was locked for analysis on June 18, 2015. Survival was defined as time until death or last follow-up. Complete response (CR) was defined as ANC $> 1 \times 10^9/L$, Hgb > 10 g/L, and platelets $> 100 \times 10^9/L$ without need for growth factor support, transfusion or alternative immunosuppressive drugs. Partial response (PR) was defined as no longer meeting criteria for SAA but insufficient to meet the criteria for CR. Death, relapse, clonal evolution, HSCT, and PNH causing hemolysis sufficient to require treatment were considered both competing risks for cumulative incidence analyses and events with regard to

event-free survival. Probabilities of overall and event-free survival were estimated according to Kaplan-Meier method using GraphPad Prism. Cumulative incidence of neutrophil recovery, platelet and red blood cell transfusion independence, and PR were calculated using competing risk analysis in the R software package with 95% confidence intervals (CIs) for probability estimates calculated using the method proposed by Choudhury and implemented in R by Scrucca.^{31, 32}

Results

Patient Characteristics

Twenty-eight patients under age 22 with SAA were treated with high-dose cyclophosphamide from April 1997 through March 2013. Eighteen were included in prior analysis of a cohort involving pediatric and adult patients and 4 of those 18 whose disease was associated with preceding hepatitis were described in a separate report.^{27, 33} Six patients had received at least 1 course of prior immunosuppressive therapy consisting of prednisone/ATG/CSA (n = 4), prednisone/IVIG (n = 1), or prednisone alone (n = 1), while 22 patients were treatment-naïve. One patient, who received high-dose cyclophosphamide as his initial therapy for SAA, was retreated with a second course after relapse associated with acute parvovirus infection 57 months after his initial treatment. He is counted once with respect to overall survival, but each of his separate courses of high-dose cyclophosphamide is analyzed independently with regard to treatment response, as he achieved a CR to his initial treatment. Demographic and disease characteristics of patients are shown in Table I. Median time from diagnosis to treatment among all patients was 29 days (range 5–1773), with median time in treatment-naïve patients being 27 days (range 5–349) and in previously treated patients being 400 days (range 25–1773). Nineteen patients had VSAA. Eight patients had preceding non-A, non-B, non-C hepatitis, and one each had acute Epstein-Barr virus and cytomegalovirus at the time of diagnosis. Eleven patients were being treated for one or more active bacterial or fungal infections within 30 days prior to starting treatment with cyclophosphamide and 4 were receiving empiric antibiotics for culture negative fever and neutropenia at the time of cyclophosphamide administration. Types of infection prior to treatment included: 3 cases of sinusitis, 3 cases of central line or surgical site cellulitis, 1 labial cellulitis, 2 cases of bacteremia, 1 case of neutropenic colitis, 2 cases of possible fungal pneumonia, 1 case of biopsy-proven pulmonary aspergillosis, and 2 cases of oral HSV reactivation. Twenty-seven patients had baseline screening for PNH clones in peripheral blood by flow cytometry with 19 having normal flow, 6 demonstrating either loss of expression of glycosylphosphatidylinositol anchored proteins or binding of fluorescent aerolysin on 0.1% of a target population (RBC, granulocytes, or monocytes), and 2 having inconclusive results due to absence of any granulocytes or monocytes for analysis. Red blood cells in these latter 2 patients had normal CD59 expression and they are counted as PNH negative.

Disease Response

The actuarial probability of survival at 10 years is 85% (95% CI, 64%–94%) with a median follow-up of 66 months (range, 2–165 months, Figure 1A). Actuarial event-free survival is 64% (95% CI, 37%–81%) at 10 years (Figure 1B). Hematologic response was observed in

79% with 12 patients reaching PR by 3 months (41%) and an additional 2 patients reaching PR by 6 months (cumulative response by 6 months of 48%). Nine additional patients ultimately reached PR, with some responses taking up to nearly 1 year (Figure 1C). Patients who failed to reach PR by 1 year never demonstrated any subsequent response. Ultimately 19 patients (66%) met criteria for CR as best response to treatment with 9 reaching CR by 1 year, 4 by 2 years, 4 taking greater than 2 years, and 2 with uncertain timing of CR due to incomplete records from referring physicians. Response rates did not differ between patients with VSAA (14 of 19 consisting of 11 CR, 3 PR) and SAA (9 of 10 consisting of 8 CR, 1 PR) ($P=0.6328$, Fishers Exact Test) or between patients receiving prior therapy (6 of 7 consisting of 3 CR, 3 PR) and those who were treatment naïve (17 of 22 consisting of 16 CR, 1 PR) ($P=1.0000$, Fishers Exact Test). The median time to ANC greater than 0.5×10^9 cells/L was 62 days (range, 33–328), to platelets greater than 50×10^9 /L without transfusion was 87 days (range, 45–859), and to last RBC transfusion was 262 days (range, 17–356) (Figure 1D). The median number of RBC units transfused was 21 (range, 4–110). The median number of platelet units transfused was 38 (range, 6–202).

Treatment failures are shown in Table II. Among 6 non-responders, 2 patients who remained severely neutropenic died of infection before receiving other therapy. Three non-responders proceeded to alternative donor HSCT, 2 for non-response and 1 for clonal evolution to MDS with monosomy 7, detected 19 months after treatment without a response. One is alive and well after mismatched unrelated donor HSCT, 1 died of pneumonitis after matched unrelated donor HSCT, and 1 died of infection following non-engraftment after reduced intensity HLA-haploidentical HSCT from a grandmother. One non-responder has required intermittent growth factor and transfusion support for over 8 years and is being evaluated for unrelated donor HSCT. One patient who presented with a large PNH clone at diagnosis, developed symptomatic PNH with hemolysis requiring treatment with eculizumab 7 years after her initial treatment.³⁴ Two patients who achieved a CR experienced late relapses of SAA after 38 and 57 months. One responded to retreatment with a second course of high-dose cyclophosphamide, transiently reaching CR but ultimately having a variable platelet count of $70\text{--}100 \times 10^9$ /L that is sustained over 6 years. The second proceeded to reduced-intensity HSCT from an HLA-haploidentical parent on an institutional clinical trial and is alive and well with full donor engraftment.

Of 19 patients with negative PNH testing prior to treatment, 2 developed detectable PNH clones $> 0.1\%$ after treatment without evidence of hemolysis. One had a PR with persistent thrombocytopenia and the other a CR. Of 6 patients with a detectable PNH clone before treatment, 2 improved or resolved, 1 was lost to follow-up before PNH could be reassessed, and 3 had increasing clones. One patient with a minute PNH clone at diagnosis (0.1% RBC, 0.1% granulocytes, 0.1% monocytes) was observed to have a rising PNH clone (1.5% RBC, 4% granulocytes, 8% monocytes) without hemolysis at the time of his late relapse with SAA after achieving a CR. Another patient who had a small PNH clone at diagnosis (0.1% RBC, 1% granulocytes, 0.9% monocytes) achieved a CR but has slightly higher persistent PNH clone 27 months after treatment (1% RBC, 2.2% granulocytes, 2% monocytes) without evidence of hemolysis and while retaining normal blood counts. The third patient, as noted above, was the only who developed symptomatic hemolysis requiring eculizumab.

Toxicity

All patients developed transient alopecia and most experienced nausea with or without vomiting that resolved shortly after the last dose of cyclophosphamide. Three patients (11%) experienced mucositis requiring intravenous narcotic analgesics. Hemorrhagic cystitis treated with aggressive platelet transfusion support and hydration occurred following 2 treatments (7%). Three patients were rehospitalized for vomiting and dehydration not related to cyclophosphamide or infection (11%) and one for severe constipation (3%). One patient was hospitalized overnight for observation after anaphylaxis to platelet transfusion (3%). Additional details regarding treatment related adverse events including infections are presented in Table III.

All patients experienced at least one episode of febrile neutropenia or an infection during their treatment, and some patients experienced more than one episode of infection. The number of episodes of infection, including documented baseline infection, versus time since treatment is depicted in Figure 2A. Infection was most likely to occur (33 infections in 22 of 29 patients at risk) in the first 30 days following high dose cyclophosphamide, encompassing the time from potential mucosal barrier injury through the beginning of ANC recovery, with risk of infection progressively falling through day 90. Only 6 of 28 evaluable patients (21%) experienced an episode of infection after 90 days. Three such patients were non-responders who experienced multiple episodes of infection or febrile neutropenia. Among the remaining 3 patients, one had sinusitis, one had a cellulitis at a Hickman catheter skin exit site and one had two episodes of Gram-negative bacteremia in the absence of mucositis and after resolution of neutropenia, suggesting that the infection was due to Hickman catheter contamination during care at home.

In total, 18 patients (62%) experienced at least 1 episode of bacteremia after treatment, with 4 episodes having concurrent hypotension consistent with sepsis. Some patients experienced several episodes of bacteremia with distinct organisms. Fifteen patients developed soft tissue infection distinct from episodes of bacteremia. While the cumulative incidence of patients experiencing at least 1 bacterial infection pre- or post-treatment was 25/29 (86%), only 1 bacterial infection was the cause of a patient death. Five patients developed yeast infections (candidemia in 2, pneumonia in 1, yeast UTI in 1, thrush requiring treatment in 1). Ten patients were diagnosed with possible invasive fungal infection and 3 diagnosed with proven fungal infection using EORTC/MSG consensus definitions.³⁵ Thus, the cumulative incidence of patients experiencing at least 1 yeast or fungal infection pre- or post-treatment was 18/29 (62%). Disseminated fungal infection was the cause of death in 1 patient. Four patients received granulocyte transfusions due to uncontrolled infection in the setting of severe neutropenia, with 3 ultimately clearing the infection. One patient developed significant HSV stomatitis following treatment (and 2 before treatment) that resolved upon treatment with IV acyclovir.

The median duration of initial hospitalization was 35 days (range, 5–135 days). The median number of hospitalizations was 3 (range, 1–22) (Figure 2B). The median number of total days of hospitalization was 50 days (range, 16–320 days) (Figure 2C). There have been 4 deaths (14%) that all occurred in non-responders. One early death (day 55) was due to persistent Gram-negative sepsis in a patient that never demonstrated ANC recovery. A

second patient with no ANC recovery died from disseminated fungal infection 534 days after treatment. Two non-responders died of complications after proceeding to HSCT as noted above.

With regard to potential late toxicities, 8 of 12 surviving female patients have had documentation of normal menstrual cycles or measurement of normal gonadotropin levels following treatment. Data are incomplete on 4 female patients who either have not reached menarche or returned to referring institutions and have not had data reported. Two patients have demonstrated spontaneous pregnancies. Five surviving post-pubertal male patients have normal gonadotropin and testosterone levels and 1 patient who was treated with 2 courses of high-dose cyclophosphamide developed gonadal failure requiring testosterone replacement. Gonadotropin and testosterone levels were not available for 6 surviving male patients, but all have demonstrated normal progression through puberty without symptoms of hypogonadism. One patient developed a small squamous cell carcinoma on a sun-exposed area that was treated with complete resection.

Discussion

This is the largest published series to date of the use of high-dose cyclophosphamide to treat children and young adults with SAA or VSAA who lack an HLA-matched sibling for up front treatment with HSCT. We provide detailed data on responses, treatment failures, and frequency and timing of toxicity to permit more accurate comparison with other treatment modalities used in children.

High-dose cyclophosphamide is an effective therapy in children and adolescents with a cumulative response rate of 79%. This compares quite favorably to the rates of 63–74% responses at 6 months reported in series of pediatric patients treated with ATG and CSA with or without additional immunosuppression or androgens, however the time to response appears longer than following ATG/CSA.^{13, 17, 18, 36} At 6 months the response rate to high-dose cyclophosphamide was 48%, but this rose over the next 6 months to 79%. Notably, the majority of patients treated (66%) eventually reached CR.

The reasons for the difference in timing of response between high-dose cyclophosphamide and ATG/CSA remain incompletely understood. Cells undergoing rapid proliferation, such as activated autoreactive T cell clones, should be sensitive to cyclophosphamide while cells with high levels of detoxifying enzymes, such as HSCs, should be resistant. This differential sensitivity is believed to play a significant role in cyclophosphamide's efficacy for SAA, even in the relapsed setting. Based on observed dynamics of count recovery, we speculate that a transient reduction in the number of HSCs or partially differentiated progeny is likely to occur following cyclophosphamide treatment but not after ATG/CSA based immunosuppression, resulting in a longer time to response. However, by a similar mechanism, ablation of autoreactive T cell responses by cyclophosphamide is also predicted to be more complete. Relapse after IST requiring retreatment or alternative donor HSCT is reported at rates ranging from 15.9 to almost 40% in series focused on pediatric patients with SAA.^{13, 17, 18, 20, 36, 37} The low estimated cumulative incidence of relapse we observed of 13% at a median follow up of over 5 years appears to support our hypothesis. While

dynamics of count recovery after high-dose cyclophosphamide appear to be slower, the net effect of improved stability of response is an event free survival of 64% for children and young adults treated with high-dose cyclophosphamide, with EFS ranges of 33–56% reported following IST in other pediatric series with similar duration of follow-up.^{13, 14, 20}

Possible concerns about a chemotherapy-based approach include the potential for higher acute and late toxicity.^{28, 38} Side effects such as nausea, vomiting, alopecia, mucositis, and hemorrhagic cystitis unique to cyclophosphamide were readily managed and produced no long lasting morbidity. Mucosal barrier injury in the setting of neutropenia would be predicted to increase the risk of invasive infection, and this could be a unique risk for patients receiving high-dose cyclophosphamide compared to ATG/CSA. For example, death due to invasive fungal infection was seen in 3 of 6 patients and 1 of 5 patients in small series of children with refractory SAA treated with high-dose cyclophosphamide and in 3 of 15 treatment-naïve adults receiving high-dose cyclophosphamide plus CSA.^{28–30} While our cohort did demonstrate a cumulative 86% of patients having a documented or suspected bacterial infection and 62% having a documented or suspected fungal infection, we did not observe a comparably high mortality rate from these complications. Our supportive care measures for prophylaxis against, monitoring for, and treatment of infection follow standard practice, but we believe one difference that may have contributed to good outcomes was the absence of concurrent CSA administration. CSA is included in IST for SAA due to its profound inhibition of T cell immune responses. However it has been shown to inhibit innate immunity as well, with corresponding increases in mortality from fungal infection in animal models.³⁹ In considering our reported incidence of infection, it is important to note that 53% of the patients (15/29) had documented bacterial or fungal infection or culture negative febrile neutropenia prior to initiation of treatment with high-dose cyclophosphamide. Furthermore, our observed cumulative incidence of infection is similar to the reported 81% incidence of any infection in patients with SAA treated with conventional IST over 6 years at a large tertiary care cancer center or the 33% incidence of culture negative febrile neutropenia and 48% incidence of bacteremia or soft tissue infection, totaling 81%, reported as supplementary data in a series comparing horse versus rabbit ATG given with CSA.^{19, 40} Despite the relatively high incidence of infection and that some patients had serial episodes of infection before count recovery, the vast majority of children responded to treatment with antibiotics and judicious use of granulocyte transfusions if infections occurred early prior to any ANC recovery. The net result was that death due to infection was uncommon, with 2 non-responders succumbing to infection and an additional 2 patients dying of presumed infection only after proceeding to alternative donor HSCT.

Additionally, we have not observed an accelerated rate of clonal evolution or malignant transformation despite chemotherapy exposure with median follow-up of over 5 years. One patient had a superficial squamous cell carcinoma that was completely resected, and one non-responder developed MDS with monosomy 7, similar to the reported incidence of clonal evolution and secondary neoplasms of 12 out of 113, 3 out of 77, and 7 out of 110 patients in pediatric series using ATG/CSA.^{18, 21, 36}

We have long routinely offered semen cryopreservation to males who will undergo exposure to gonadotoxic therapy, but recent advances in ovarian and unfertilized oocyte

cryopreservation have occurred since this study began, offering additional fertility preservation options to young women.⁴¹ None of our female patients had such therapy available at the time of their treatment, but gonadal function in our patient cohort appears to be good at the current duration of follow up. One male patient has developed premature gonadal failure following two courses of treatment, but 2 female patients have undergone spontaneous pregnancies. While gonadal toxicity from alkylator exposure is a concern, we expect our rates of premature gonadal failure to be similar to the low rate (8%) reported for women exposed to 200 mg/kg of cyclophosphamide without concurrent radiation treatment during bone marrow transplant by Sanders et al.⁴² Younger women fared particularly well in that cohort, with 27 of 27 women under age 26 retaining ovarian function after exposure to high-dose cyclophosphamide alone.

In considering other alternatives to initial treatment with high-dose cyclophosphamide or ATG/CSA for SAA for patients lacking an HLA-matched sibling donor, alternative donor HSCT for SAA has not historically been recommended due to increased risk of rejection, GVHD, and infection. This distinction between related and unrelated donors may be improving because of better HLA matching, new antimicrobial prophylaxis, and altered immunosuppressive strategies. Several studies have recently reported reduced intensity regimens for matched unrelated donor transplants with modest rates of acute and chronic GVHD that demonstrate similar efficacy to matched sibling donor transplants with 78% to 96% of patients surviving long-term, with better survival in patients treated with unrelated transplant as initial therapy before IST failure.^{11, 43–45} Additionally, post-transplant high-dose cyclophosphamide can be used to promote engraftment and alleviate GVHD. Such an approach has been used extensively at our center in the setting of hematologic malignancy and has been piloted in refractory and relapsed SAA in older adults with matched siblings.^{46, 47} This approach appears to make it safe and practical to use unmanipulated bone marrow or PBSC allografts from HLA-haploidentical related donors, increasing access to donors for patients without a match, and serves as the basis of several ongoing trials of reduced intensity conditioning HLA-haploidentical HSCT for relapsed and refractory SAA, along with an upcoming trial for initial treatment of SAA in patients that lack an HLA-matched sibling donor at our institution.^{48–50}

In summary, our data suggests high-dose cyclophosphamide can be a highly effective alternative strategy to traditional IST with ATG/CSA for children and young adults. It can avoid serum sickness, CSA-related nephrotoxicity, and the need for months of drug monitoring, if provided with appropriate supportive care including aggressive antibiotic prophylaxis and treatment of infection occurring during the early period with neutropenia. Alternatively, trials testing addition of novel agents such as eltrombopag to IST seek to improve upon sustained responses.⁵¹ There have also been continued advances in the safety of allogeneic HSCT from donors other than HLA-matched siblings. Survival of transplant patients with lower performance status is possible due to a better understanding of appropriate reduced intensity conditioning, and the use of post-transplant cyclophosphamide has expanded the donor pool to include HLA-haploidentical relatives. Thus, alternative donor transplants, which offer more rapid correction of neutropenia, may become a reasonable option for initial therapy of SAA for some patients. Ultimately, cooperative

multicenter randomized trials are needed to determine the optimal therapy to achieve long-term cure of children and young adults with SAA that lack a matched sibling donor.

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Abbreviations

ATG	anti-thymocyte globulin
CI	confidence interval
CR	complete response
CSA	cyclosporine A
GVHD	graft versus host disease
HLA	human leukocyte antigen
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplant
IST	immunosuppressive therapy
MDS	myelodysplastic syndrome
PNH	paroxysmal nocturnal hemoglobinuria
PR	partial response
SAA	severe aplastic anemia
VSAA	very severe aplastic anemia

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Figure 1.

Overall survival, event free survival, and responses after treatment of SAA with high-dose cyclophosphamide. A) Overall survival of 28 patients treated with 29 courses of high-dose cyclophosphamide. Dashed lines indicate 95% confidence intervals. Data are censored at the time of death or last follow-up. B) Event free survival following 29 courses of high-dose cyclophosphamide. Dashed lines indicate 95% confidence intervals. Death, relapse, clonal evolution, HSCT, and PNH requiring treatment are defined as events, and data are censored at the time of last follow-up. C) Probability of achieving Partial Response (PR) versus time after treatment (solid line) or competing risk (death, relapse, clonal evolution, HSCT, or PNH requiring treatment). Data for non-responders censored at time of last follow-up. D) Cumulative probability of recovery of peripheral blood counts over time after treatment with high-dose cyclophosphamide. Responses in recovery of ANC greater than $0.5 \times 10^9/\text{L}$, platelet count over $50 \times 10^9/\text{L}$ without transfusion, and RBC transfusion independence are shown with data censored at time of competing risk (death, relapse, clonal evolution, HSCT, or PNH requiring treatment) or time of last follow-up.

Figure 2.

Infection and hospitalization after treatment of SAA with high-dose cyclophosphamide. A) Number of patients experiencing an episode of infection during each 30 day period before or after treatment with high-dose cyclophosphamide following 29 courses of treatment. B) Number of patients experiencing an episode of hospitalization for any indication after treatment with 29 courses of high dose cyclophosphamide, including initial hospitalization for administration of treatment (median 3, range 1–22). C) Number of patients with total days of hospitalization within each 20 day range centered around indicated number of hospital days following treatment with 29 courses of high-dose cyclophosphamide (median 50, range 16–320).

Table I

Patient characteristics

	N=28
M/F, N	13/15
Median age at treatment, years (range)	12 (2–21)
Median time from diagnosis to treatment, days (range)	29 (5–1773)
Etiology, N	
Idiopathic	18
Hepatitis	8
EBV	1
CMV	1
Mean pretreatment ANC $\times 10^9$ cells/L (range)	0.145 (0–0.442)
Mean pretreatment Hgb g/L (range)	6.1 (2.7–7.9)
Mean pretreatment reticulocytes $\times 10^9$ cells/L (range)	18.0 (3.1–75.2)
Mean pretreatment platelets $\times 10^9$ cells/L (range)	8.4 (2.0–19.0)
Treatment Naïve	22
PNH Clone > 0.1% by Flow Cytometry	6

Abbreviations: M, male; F, female; y, year; EBV, Epstein Barr Virus; CMV, cytomegalovirus; ANC, absolute neutrophil count; PNH, paroxysmal nocturnal hemoglobinuria. Mean pretreatment Hgb, reticulocytes, and platelets reflect nadirs prior to transfusion therapy.

Table II

Treatment failures in 29 courses of therapy

Death (before response or other therapy)	2
Relapse (after prior response)	2
PNH (with clinical symptoms)	1
Need for continued G-CSF	1
HSCT	
Non response	2
Clonal evolution (e.g. Monosomy 7)	1

Abbreviations: PNH, paroxysmal nocturnal hemoglobinuria; G-CSF, granulocyte colony stimulating factor; HSCT, hematopoietic stem cell transplant.

Table III

Adverse Events

	Number
Mucositis	3
Hemorrhagic Cystitis	2
Vomiting/Dehydration, not chemotherapy related	3
Constipation	1
Anaphylaxis, transfusion related	1
Febrile Neutropenia	23
Bacteremia *	36
Sinusitis	2
Cellulitis	5
Myositis	1
Synovitis	1
Lymphadenitis	1
Parotitis	1
Pneumonia	
Bacterial	2
Viral	1
Candidal	1
Proven fungal	3
Possible fungal	10
Perineal cellulitis/proctitis	6
Periodontal or oral infection	2
Fungemia	2
Yeast UTI	1
Thrush	1
HSV Stomatitis	1
C. difficile colitis	2

* Organisms recovered (Number): Coagulase negative Staph (15), Strep viridans (7), Pseudomonas aeruginosa (4), Klebsiella spp (4), vancomycin resistant Enterococcus (3), Acinetobacter (3), Micrococcus luteus (2), Staph aureus (1), Enterococcus faecium (1), Rothia (1), Corynebacterium (1), Cupriavidis gilardii (1), Pantoea agglomerans (1), Fusibacterium (1), Achromobacter (1). Some blood cultures isolated multiple organisms during a single episode of bacteremia.