How I Treat Acquired Aplastic Anemia

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Abstract

Survival in severe aplastic anemia (SAA) has markedly improved in the past four decades due to advances in hematopoietic stem cell transplantation, immuno suppressive biologics and drugs, and supportive care. However, management of SAA patients remains challenging, both acutely in addressing the immediate consequences of pancytopenia and in the long term because of the disease’s natural history and the consequences of therapy. Recent insights into pathophysiology have practical implications. We review key aspects of differential diagnosis, considerations in the choice of first and second line therapies, and the management of patients after immuno suppression, based on both a critical review of the recent literature and our large personal and research protocol experience of bone marrow failure in the Hematology Branch of the National Heart, Lung, and Blood Institute.
Introduction

Until the 1970s severe aplastic anemia (SAA) was almost uniformly fatal, but in the early twenty first century most patients can be effectively treated and can expect long-term survival. Nevertheless, making a diagnosis and selecting among treatment options are not straightforward, and both physicians and patients face serious decision points at the outset of their disease to years after its presentation. We summarize our approach to SAA, with recommendations based on decades of experience in the clinic as well as a critical review of the literature. Unfortunately as a rare disease, there are few large trials of any kind and even fewer randomized controlled studies, which usually provide the best evidence to guide practice in the clinic.

Pathophysiology as Basis of Diagnosis and Treatment

The pathophysiology responsible for marrow cell destruction and peripheral blood pancytopenia has itself been inferred from the results of treatment in humans, with substantial in vitro and animal model support. The reader is referred to more didactic textbook chapters and formal reviews on these topics. The success of hematopoietic stem cell transplantation (HSCT) in restoring hematopoiesis in SAA patients implicated a deficiency of hematopoietic stem cells (HSC). Hematologic improvement following immunosuppressive therapy (IST), initially in the context of rejected allogeneic grafts and then in patients receiving only IST, implicated the immune system in destruction of marrow stem and progenitor cells. Immune mediated marrow failure can be modeled in the mouse by the “runt” version of
graft-versus-host disease (GVHD), with HSC depletion and hematopoietic failure induced by infusion of lymphocytes mismatched at major or minor histocompatibility loci.\textsuperscript{5,6}

Genetics influences both the immune response and its effects on the hematopoietic compartment. There are histocompatibility gene associations with SAA\textsuperscript{7}, and some cytokine genes may be more readily activated in patients due to differences in their regulation, as suggested by polymorphisms in promoter regions.\textsuperscript{8} An inability to repair telomeres and to maintain the marrow’s regenerative capacity, resulting from mutations in the complex of genes responsible for telomere elongation, has been linked to patients with familial or apparently acquired SAA, with or without the typical physical stigmata of constitutional aplastic anemia. These genetic factors are variable in their penetrance, ranging from highly determinant loss of function mutations to subtle polymorphisms.\textsuperscript{9}

About 5-10\% of patients with SAA have a preceding seronegative hepatitis.\textsuperscript{10} However, most patients do not have a history of identifiable chemical, infectious or medical drug exposure prior to onset of pancytopenia. The antigen(s) inciting the aberrant immune response have not been identified in SAA. Furthermore, the current simple mechanistic outline may be supplemented in the future with better understanding of now theoretical possibilities, suggested by provocative murine models: an active role of adipocytes in inhibition of hematopoiesis\textsuperscript{11,12}; interactions
among effector CD8 and CD4 cells and regulatory cells, and possible microenvironment “field effects” by stromal elements and niche cell interactions.

How We Diagnose SAA

Diagnosis and Differential Diagnosis

Patients with SAA usually have been previously well, with the exception of a preceding hepatitis, so constitutional symptoms and hepatosplenomegaly point to other diagnoses. For the consultant hematologist, the differential will mainly lie among hematologic syndromes, usually distinguishable on bone marrow examination. The reader is referred to general references for a complete list of diseases that can present with varying degrees of cytopenias. An elevated MCV is frequent in aplastic anemia at presentation. The “empty” marrow on histology of SAA is highly characteristic and a requisite for the diagnosis. Marked hemophagocytosis, obvious dysplasia, or increased blasts point to other diseases, although differentiation of hypocellular myelodysplastic syndrome (seen in about 20% of MDS cases) from aplastic anemia can be difficult. Megakaryocytes are the most reliable lineage to utilize in distinguishing MDS from SAA: small mononuclear or markedly aberrant megakaryocytes strongly suggest MDS, while megakaryocytes are markedly reduced or absent in SAA. In contrast, “megaloblastoid” and modest dysplastic erythropoiesis is not uncommon in an aplastic marrow, especially when a paroxysmal nocturnal hemoglobinuria (PNH) clone is present. Cytogenetics are helpful when typical of myelodysplasia (MDS), but some aberrations (such as trisomy 6, trisomy 8, 13q-) may appear in SAA that is responsive to
immunosuppressive therapy, according to some reports \textsuperscript{17,18}, but not confirmed by others \textsuperscript{19} (for our research protocols, abnormal chromosomes are an exclusion criterion).

Aplastic anemia and PNH overlap in about 40-50\% of cases (the AA/PNH syndrome)\textsuperscript{20}. At our institution, >1\% granulocytes deficient in glycosylphosphoinositol-linked proteins are detectable by flow cytometry and considered abnormal, but other methodologies can detect even smaller PNH clones. Such small clones do not result in significant hemolysis or risk of thrombosis, and whether the presence of a small PNH clone in the setting of hypocellular marrow failure has clinical significance or predicts response to treatment and outcomes is controversial\textsuperscript{21,22}. The presence of less than 50\% GPI-deficient circulating cells without evidence for thrombosis or significant hemolysis generally does not require PNH-specific therapy\textsuperscript{20}. Irrespective of PNH clone size, marrow failure in SAA should be treated promptly with immunosuppression or transplant, since complications of pancytopenia represent the more imminent cause of morbidity and mortality.

The appearance of the marrow in inherited and acquired aplastic anemia syndromes is identical, and the historical distinction between them is becoming blurred. Fanconi anemia is established by testing peripheral blood for increased chromosomal breakage after exposure to diepoxybutane or other clastogenic stress, but what is the upper age limit for performing this assay in patients that present with marrow failure, since Fanconi anemia manifestations can first appear in
adulthood? We use age 40 years as a threshold, but may perform testing on older patients if the family history is even minimally suggestive of inherited marrow failure, or there are potential consequences for an error in diagnosis, as for example exposure to chemotherapy. The diagnosis of Fanconi anemia is critical for treatment decisions and follow-up, since these patients do not respond to IST, require dose-reductions in transplant conditioning, and need careful follow-up for a range of non-hematologic malignancies.

The diagnosis and implications of documenting telomeropathies in patients with marrow failure are even more problematic, due to the range of clinical phenotypes: from classic X-linked dyskeratosis congenita kindreds with hemizygous DKC1 mutations, in which boys present early in life with pancytopenia and typical physical features (abnormal nails, leukoplakia, cutaneous eruptions), to older adults with heterozygous TERT or TERC mutations, in whom the family history can be negative or obscure and who lack pathognomonic physical findings. Blood leukocyte telomere length measurement is probably appropriate in all aplastic anemia patients but especially important in those who have a family history of aplastic anemia, isolated cytopenias, and leukemia—as well as pulmonary fibrosis or cirrhosis. We do not label such patients as dyskeratosis congenita (the most severe type linked to DKC1 mutations) but rather as telomere disease or telomeropathy, since the penetrance of the TERT and TERC gene mutations is much lower and the long-term clinical outcomes currently less clear but the focus of current investigations. Current clinical research protocols at NIH are investigating
the impact of telomere length and mutational status on aplastic anemia outcomes and the effects of androgens on modulating telomere attrition.

**Presentation and Patterns**

It is common for patients to seek medical attention due to symptoms of anemia or hemorrhage. Infection at presentation is uncommon, even with severe neutropenia. Pancytopenia can be discovered serendipitously at pre-operative evaluation, blood donation, or from screening testing. Curiously, there may be a prior history of a single lineage cytopenia, usually thrombocytopenia or anemia. For aplastic anemia patients who present with thrombocytopenia alone, standard therapies for immune thrombocytopenia are usually ineffective and eventually a diagnosis of marrow failure follows from the finding of a hypocellular marrow with reduced megakaryocytes. Macrocytosis and even mild anemia (or leucopenia) should suggest that ITP is not the correct diagnosis and stimulate an early marrow biopsy. Months of unsuccessful treatment with corticosteroids in these patients is unfortunate and should be avoided. A prior history of seronegative hepatitis in the months prior to pancytopenia defines post-hepatitis SAA. Chemical and medical drug exposures should be queried in the interview but these are notoriously difficult to evaluate quantitatively and the history is subject to recall bias. However, even with an exhaustive attempt to identify a putative trigger, confirmation of a causal relationship is difficult to ascertain and the management and outcomes not likely to differ. More important is a careful past medical history of earlier blood count abnormalities, macrocytosis, or relevant pulmonary or liver diseases in the patient
or the patient’s family, which may implicate an underlying telomere disorder. It is also prudent to rapidly assess whether matched sibling donors exist in the family for any patients younger than 40 (see below).

When a medical drug exposure is suspected, some physicians will monitor after its discontinuation; usually, by the time the patient has reached a tertiary care facility, some weeks of data can be assessed for any evidence of marrow recovery. However, the demographics, presenting symptoms, blood counts, marrow histology and response to therapy are not different between idiopathic and drug-associated aplastic anemia, and prolonged delay until initiation of primary treatment, in the hope of “spontaneous recovery”, is not generally desirable and can result in serious complications prior to definitive therapy.

How We Treat SAA

When and whom to treat

SAA almost always requires treatment, both immediate and definitive. For patients with moderate aplastic anemia, as defined by lack of blood count criteria for SAA, observation is often appropriate, especially when they do not require transfusion support. In our experience many of these patients may have stable blood counts for years, but in some pancytopenia may worsen over time. Those who progress to severe pancytopenia and meet criteria for SAA or become transfusion-dependent can then be treated according to current algorithms, as detailed below. Elderly, feeble, or patients suffering serious comorbidities may not benefit from the
aggressive approaches described below, especially if they are not bleeding and have neutrophil counts that protect from serious infections (generally > 200-400/ul). They may be stable and maintain quality of life with regular red blood cell transfusion, but age itself does not preclude IST.

**Immediate measures**

Symptoms related to anemia and thrombocytopenia can be readily corrected with transfusions, and infections must be addressed with broad-spectrum parenteral antibiotics when fever or documented infection occurs in the presence of severe neutropenia (<500/ul). Overuse of blood products should be avoided, but so also should inadequate transfusions; modern preparations of red cells and platelets are not likely to jeopardize graft acceptance at transplant, and they lead to alloimmunization in a minority of patients. We do not transfuse platelets prophylactically in SAA patients who have a platelet count >10,000/ul and who are not bleeding.

**What not to do**

Supportive measures alone, growth factors, androgens, or cyclosporine (CsA) are not definitive therapies. Patients should not be subject to trials of G-CSF or erythropoietin. Corticosteroids are of unproven benefit and inferior in efficacy to conventional immunosuppression regimens, but they are far more toxic and should not be used as therapy in SAA. It is very unfortunate when a patient with SAA presents for definitive transplant or IST, but already has a life-threatening fungal...
infection due to weeks or months of treatment with corticosteroids. Watchful waiting, especially if neutropenia is profound, can be harmful and is not indicated once a diagnosis of SAA is confirmed. If by the time the patient is referred to a hematologist after several weeks and the diagnosis confirmed, spontaneous recovery should be considered unlikely. Aplastic anemia is an unusual disease, and the practicing hematologist/oncologist should feel no hesitation in referring a patient rapidly to a specialized center or in seeking the advice of experts familiar with marrow failure syndromes.

**Choice of definitive treatment**

Hematopoiesis can be restored in SAA with HSCT or IST. While overall long-term survival is comparable with either treatment modality, transplant is preferred when feasible as it is curative.\(^1\) However, most patients are not suitable candidates for optimal initial HSCT due to lack of a matched sibling donor, lead-time to identify a suitable unrelated donor, age, comorbidities, or access to transplant. Therefore, IST is most commonly employed as first therapy in the US and worldwide.

**Transplant**

*Matched related HSCT*

The large experience with matched sibling HSCT from the 1970s to the 1990s defined the utility of this treatment modality in SAA.\(^1^2^9\) The historically high rate of graft rejection in SAA is now less problematic, likely due to patients moving faster to this treatment and thus avoiding heavy transfusion burdens, less immunogenic
blood products, and more efficacious conditioning regimens. The correlation of increasing age with the risk of GVHD and the significant morbidity and mortality of this transplant complication continue to impact on the decision to pursue HSCT versus IST as initial therapy in adults with SAA. In recent reporting by the Center for International Blood and Marrow Transplant Research (CIBMTR) of more than 1,300 SAA patients who were transplanted from 1991 to 2004, survival at 5 years for patients < 20 years of age was 82%, for those 20-40 years 72%, and for those over 40, closer to 50%. Rates of GVHD increased with age, accounting for much of the decreased survival in older patients, and much of the long-term morbidity. Thus, outcomes in the most favorable age group (children with matched sibling donor) resulted in long-term survival of approximate 80%. In the Seattle experience, most children who received HSCT from a histocompatible sibling with non-irradiation conditioning regimens were able to grow, develop normally and retain fertility. In this pediatric cohort, chronic GVHD associated to increased mortality, a finding similar to that of adult SAA patients. In a retrospective report from Seattle, the experience of matched related HSCT as first therapy in 23 older patients (> 40 years old) showed long-term survival of about 60%, in accordance with the CIBMTR data. Matched sibling transplant is always preferred in children with SAA, and it is an appropriate first choice for adults up through at least age 40, as proposed in several algorithms. Thus, as the risks associated to transplantation increase in patients > 40 years of age, we generally recommend IST first in this age group.
Stem cell source

In general, mobilized peripheral blood (PBSC) as a source of stem cells for transplantation has supplanted bone marrow because of higher stem cell doses and donor and physician preference due to ease of collection. Distinctively in SAA, PBSC results have been inferior to grafts of bone marrow origin. In a retrospective analysis, the rate of chronic GVHD was greater with peripheral blood (27%) compared to bone marrow stem cell grafts (12%) in patients less than 20. In a subsequent retrospective analysis, similar higher rates of chronic GVHD were observed for patients of all ages undergoing HSCT with peripheral blood compared to bone marrow derived stem cell grafts. For unrelated donor transplants, bone marrow source of stem cells was associated with lower rates of acute GVHD (31%) compared to peripheral blood derived CD34+ cells (48%), and better overall survival (76% vs 61%, respectively). In contrast to allogeneic transplantation undertaken for malignancies, where GVHD may offer graft-versus-tumor benefits, in SAA GVHD is unequivocally to be avoided, and its occurrence decreases survival and long-term quality of life. Thus, except in the experimental clinical research, bone marrow is preferred as the source of stem cells in SAA.

Alternative donor HSCT

Outcomes with unrelated donor (UD) HSCT have improved (Table 3), primarily due to more stringent donor selection facilitated by high-resolution molecular typing, less toxic and more effective conditioning regimens, and higher quality transfusion and anti-microbial supportive care. Small single institution studies with limited...
follow-up suggest that survival with UD HSCT in younger patients now approximates that of matched sibling HSCT (Table 3). However, experience from larger cohorts reported in the last 5 years from the US, Japan, Korea, and Europe suggests that the outcome with UD HSCT is still not as favorable as that of a matched sibling donor. In studies reported in recent years, the incidence of graft failure was about 10%, GVHD 30-40%, and survival in 3-5 years highly variable ranging from 42% to 94% (Table 3). One of the main difficulties in assessing outcomes with UD HSCT in SAA is the retrospective nature of most studies. Absent properly randomized or even large prospective studies, variability in patient selection and transplant regimens make general recommendations difficult. In a recent systematic review, great variability in reported outcomes was observed in UD HSCT studies in SAA. For example, overall survival with corresponding confidence intervals at 5 years were reported in about half the studies analyzed, and survival rates ranged from 28% to 94%. A meta-analysis revealed such heterogeneity between the studies of UD transplants in SAA, as to preclude a pooled analysis.

As of this writing, UD HSCT is not recommend as initial therapy, even in younger patients, for the following reasons: 1) the long-term survival among children who respond to horse anti-thymocyte globulin (ATG) plus CsA is excellent, approximating 90%; 2) optimal conditioning for UD HSCT is not yet defined; 3) graft rejection and GVHD remain problematic, especially in older patients; 4) chronic immunosuppression for GVHD increases mortality risk long-term; 4) more generalizable long-term data from larger cohorts suggest that long-term
survival is closer to 50-60%; 5) long-term effects of low dose total body irradiation and alkylating agents substituting for irradiation are not yet defined. To some extent, these considerations are moot: practically, identification of a matched unrelated donor and coordination with a transplant center usually takes several months, and delaying definitive IST while conducting a search for a non-family donor may be dangerous and, in our opinion, not indicated. A consequence of high resolution molecular matching is a more limited pool of ideal donors. Non-Caucasian ethnic groups are relatively poorly represented in bone marrow registries, and there are biologic limits due to the complex HLA genetics in African-Americans and children of mixed ethnicity. Of course, a donor search should be initiated soon after diagnosis for all younger patients in order to assess future options should immunosuppression be ineffective.

Prospective trials using umbilical cord (UC) HSCT in SAA are limited to smaller case series, which do show encouraging results.\textsuperscript{57,58} In contrast, experience from larger cohorts in retrospective analyses indicate that overall survival is not as favorable as in pilots, at about 40% at 2-3 years.\textsuperscript{59-61} Graft rejection and poor immune reconstitution remain problematic in UC HSCT.\textsuperscript{61} Parameters that have been associated with better outcomes are higher number of nucleated graft cells, certain conditioning regimens, and the degree of mismatch between the graft and recipient.\textsuperscript{59,61} As parameters associated with better outcomes are defined, results with UC HSCT should improve.
Immunosuppressive therapy

Standard initial immunosuppressive therapy is horse ATG and CsA, which produces hematologic recovery in 60-70% of cases and excellent long-term survival among responders, as shown in several large prospective studies in the US, Europe, and Japan.\textsuperscript{1,62-66} Despite the use of different horse ATG preparations, the rates, time course and patterns of hematologic recovery have been consistent across studies, which argues against significant lot variations affecting outcomes. As the addition of CsA to ATG increased the hematologic response rate, further attempts have been made to intensify immunosuppression and thus improve on this standard regimen. The addition of mycophenolate mofetil\textsuperscript{67} or sirolimus\textsuperscript{68} to horse ATG/CsA did not improve rates of response, relapse or clonal evolution. The use of tacrolimus as an alternative to CsA has not been systematically examined in SAA, and the experience is limited to case reports and small case series that suggest activity.\textsuperscript{69,70}

A more lymphocytotoxic agent, rabbit ATG, has been successful in salvaging patients with refractory or relapsed SAA following initial horse ATG\textsuperscript{71,72}, which stimulated its utilization as first-line therapy and the expectation that it would produce superior outcomes as compared to horse ATG.\textsuperscript{73-78} Several pilot or retrospective studies compared outcomes between the two ATGs (Table 2). However, in our recently reported large, randomized controlled study, hematologic response to rabbit ATG (37%) was about half that observed with standard horse ATG (68%), with inferior survival noted in the rabbit ATG arm.\textsuperscript{79} In the same study, an alemtuzumab only treatment arm (100 mg total) was discontinued early due to a low response rate and
an increase in early deaths, suggesting that more lymphocytotoxic regimens did not yield better outcomes in SAA. Thus, horse ATG/CsA remains the most effective regimen for first line therapy of SAA.

Cyclophosphamide was first reported to be active in SAA in the mid 1970s in a patient who had hematologic recovery after receiving 30 mg/kg per day over 4 days.\(^{80}\) This experience was expanded in the 1990s using higher doses (200 mg/kg total dose) at a single institution. Response rates were comparable to horse ATG, but with apparently fewer late events (relapse and clonal evolution) in historical comparison.\(^{81}\) However, cyclophosphamide was found to be excessively toxic due to fungal infections and deaths in a randomized study at the NIH, and relapse and clonal evolution were observed.\(^{82,83}\) Recently long-term follow-up of SAA patients treated with high dose cyclophosphamide showed that the cumulative incidence of invasive fungal infection was 21% in treatment-naïve and 39% in refractory SAA.\(^{84,85}\) These incidences of invasive fungal infections are higher compared to those observed with horse ATG, and represent the major toxicity of the high-dose cyclophosphamide regimen.\(^{79,82}\) Even patients presenting with neutrophil counts of 200-500/µl, who in our experience rarely develop serious fungal infections with ATG therapy, are rendered profoundly neutropenic for weeks to months by cyclophosphamide. Additionally, extended support for such patients, including long periods of hospitalizations, G-CSF, and antifungal prophylaxis, may be prohibitively costly. Therefore, in view of lack of reproducibility and significant toxicity concerns, this regimen is not recommended outside a clinical research protocol.
Immunosuppression administration

ATG

Because many hematologists may not be familiar with administration of polyclonal antibodies such as ATG, its immediate toxicities can be daunting for inexperienced nurses and physicians, and referral to hospitals with experience in treating SAA or enrollment into research trials is to be encouraged. We perform an ATG skin test to test for hypersensitivity to horse serum, and desensitize those reacting to the intradermal injection. We place a double lumen central line and maintain platelets > 20,000 /μL during the ATG administration period. In cases of platelet refractoriness, we test for alloantibodies to determine the need for best matched platelet products. We employ universal filtration of blood products to prevent alloantibody formation. There is no formal recommendation regarding the use of irradiated blood products following horse ATG in SAA, but our practice has been to apply universal irradiation in our protocols as more immunosuppressive regimens were studied, in accordance with recent recommendations from a European study survey.86 We do not require that patients be free of infection before initiating ATG, but prefer to establish responsiveness to antibiotic therapy at least for bacterial infections. However, prolonged attempts to clear fungal infections or extensive bacterial infections can delay definitive IST or HSCT therapies. We withhold β-blockers prior to ATG to avoid suppressing physiologic compensatory responses to anaphylaxis. Avoid starting ATG late in the day or on weekends when hospitals may be short-staffed.
ATG is usually administered at a dose of 40mg/kg over 4 hours, daily for 4 days. Prednisone 1 mg/kg is started on day 1 and continued for 2 weeks, as prophylaxis for serum sickness. Premedication before each ATG dose with acetaminophen and diphenhydramine is conventional, and common infusion reactions are managed symptomatically with meperidine (rigors), acetaminophen (fevers), diphenhydramine (rash), intravenous hydration (hypotension), and supplemental oxygen (hypoxemia). Occasionally hemodynamic and/or respiratory compromise can precipitate transfer to the intensive care unit, vasopressor support, and, rarely, intubation. In the presence of life-threatening reactions, the ATG infusion is slowed or held temporarily until alarming signs and symptoms subside. Depending on the severity of reactions we re-initiate ATG at the normal or slower the infusion rate (sometimes over 24 hours) in a monitored setting. Increased liver enzymes tend to normalize over several days and ATG may be infused despite mild to moderate elevation in transaminases. Changing ATG formulations (from horse to rabbit for example) should not be used as strategy to manage infusion related toxicities. For rising creatinine, CsA can be withheld temporarily until renal function improves. With this approach, a complete ATG course is accomplished in nearly all patients in our experience.

Cyclosporine

We initiate cyclosporine on day 1 to a target trough level between 200-400 ng/ml, starting at a dose of 10 mg/kg/day (in children, 15 mg/kg/day). Many patients develop hypertension during CsA treatment, and amlodipine is preferred due to
minimal overlap with CsA toxicities. Bothersome gingival hyperplasia can improve on a short course of azithromycin. Calcium channel blockers have been associated with worse gingival hyperplasia when combined with CsA. In general, we continue CsA in the setting of modest increases in creatinine, with careful monitoring of renal function and adjustment of dosing to achieve target CsA levels. Fine adjustment of the cyclosporine dose to the lower end of the therapeutic range, optimization of blood pressure control, adequate hydration, and avoidance of other nephrotoxic agents can improve the tolerability and allow for continued CsA use. More serious compromise of kidney function from baseline (> 2 mg/ml) may require temporarily cessation of cyclosporine with later re-introduction at lower doses with further increases as tolerated.

**G-CSF**

G-CSF has been extensively studied in combination with immunosuppression in prospective randomized trials, but these have consistently failed to show benefit in hematologic response or survival in SAA (Table 2). Of concern is that G-CSF has been associated with an increased risk of clonal evolution in some retrospective studies, but this observation has not been confirmed by others (the largest randomized study, of 192 patients conducted by the EBMT, has not had sufficient follow-up to definitely assess clonal evolution.) Therefore, due to the lack of benefit and the theoretical risk for potential harm, G-CSF is not recommended with ATG in our protocols. The decision to attempt to improve neutrophil with G-CSF is based on clinical grounds in selected patients who are actively infected and persistently
severely neutropenic (< 200 /μL), with reassessment and discontinuation after no
more than a few days or weeks if there is no significant response.

Antimicrobial prophylaxis

As anti- *Pneumocystis carinii* prophylaxis, we routinely use monthly aerosolized
pentamidine while patients are on therapeutic doses of CsA. This regimen was
introduced after we observed several cases of *Pneumocystic carinii* pneumonia at
our institution in the late 1980s in AA patients on CsA. Sulfa drugs are avoided due
to their myelosuppressive properties, and alternative regimens with dapsone or
atovaquone are sometimes used when aerosolized pentamidine cannot be tolerated
or in very small children. Antibacterial, antiviral and antifungal prophylaxes are not
routinely administered with standard horse ATG/CsA at our institution, but have
been used in the context of investigational regimens that are more
immunosuppressive.

**How we manage SAA after ATG**

We use a simple definition for hematologic response: no longer meeting blood count
criteria of SAA, which closely correlates with transfusion-independence and long-
term survival.63,99 Hematologic improvement is not to be expected for two to three
months after ATG, and therefore management of patients in this period requires
careful and prolonged attention. In our experience, the majority of responses (90%)
occur within the first 3 months, with fewer patients responding between 3 and 6
months or after.99 After ATG treatment, the threshold for prophylactic platelet
transfusion is reduced to >10,000 /μL (or for bleeding). Transfusion of red cells aims to alleviate symptoms of anemia, not simply to target a specific hemoglobin threshold. Sufficient red blood cell transfusions in symptomatic patients should not be avoided because of fear of iron accumulation or to reduce the risk of alloimmunization. In evaluating febrile neutropenic patients, in our experience simple chest x-ray is of limited value and we routinely pursue computerized tomography imaging of the sinus and chest followed by nasal endoscopy, bronchoscopy and biopsy for microbiologic confirmation when indicated. If fungal infection is suspected or neutropenic fever persists for more than several days despite broad spectrum antimicrobials, empiric antifungal therapy should include drugs active against Aspergillus sp, as this pathogen has remained the most common fungal isolate in SAA patients for the past 20 years.100

Management of responders to immunosuppression

Cyclosporine taper is common practice and seems logical, but adequate prospective comparative studies of such a strategy are lacking. Anecdotal and retrospective reports support a taper to decrease the rate of relapse.101 To 2003, we discontinued CsA at 6 months among responders.67,99 Since 2003, we have included a CsA taper in all patients who responded to horse ATG/CsA. Despite this change in practice, we have not observed a reduction in the rate of relapse in comparison to our large historical experience.
Fluctuations in blood counts are frequent in the weeks following immunosuppression, and too close scrutiny of small changes in counts for glimmers of a response is not helpful. We assess for response at 3- and 6-month landmark visits. Meaningful improvement may be evident earlier; neutrophils may rise within a few weeks of ATG administration. Complete normalization of blood counts is not seen in the majority of patients, although continued improvement may occur over time, sometimes over years. The long-term survival benefit of immunosuppression with horse ATG applies to all responders, partial and complete, and there is no rationale to pursue further immunosuppression (or HSCT!) in responding cases.

Refractory SAA

For protocol purposes, we define refractory SAA as blood counts still fulfilling criteria for severe pancytopenia 6 months following initiation of IST. Fortunately, in our experience, about half the patients classified as non-responders at 6 months will have had an improvement in neutrophil count. Even an increase in granulocytes to >200/ul generally avoids life-threatening infections, and provides time to carefully weigh options for further treatment; conversely, persistent severe neutropenia accelerates decision-making and may increase the desirability of more aggressive therapies, such as matched/mismatched unrelated transplants or matched sibling transplants in older patients.

Most marrow failures experts now agree that younger patients who have not responded to immunosuppression should consider an UD HSCT, especially if a high-
resolution genetic match has been identified (Figure 1). For patients who lack a
histocompatible donor or are not suitable for HSCT, a second course of
immunosuppression with rabbit ATG/CsA is efficacious in 30-70% of cases. We
found alemtuzumab monotherapy (without CsA) equivalently effective as was
rabbit ATG/CsA in a randomized study, with hematologic response observed in
about 30-40% of patients. Alemtuzumab may be an alternative to rabbit ATG in
refractory SAA and may appeal to older patients or those who experienced
significant toxicities with CsA (Figure 1).

Although androgens lacked efficacy in early randomized studies when combined
with ATG, anecdotal experience from uncontrolled studies suggest that these
agents can be beneficial in some patients, leading to sustained hematologic
recoveries. In patients who are refractory to IST and lack good HSCT options,
we offer a trial of androgen therapy for 3 months.

In a pilot trial at our institution, single agent oral eltrombopag produced
hematologic responses in 11 of 25 cases, with trilineage responses observed in
some suggesting a stimulatory effect of early myeloid progenitors.

When neutropenia is not severe, some persistently pancytopenic patients can be
supported for many years with transfusions, chelation, and growth factor
support.
Although 75-90% of patients will achieve hematologic recovery after 1 or 2 courses of IST, the mechanisms by which some patients persist with severe pancytopenia remains elusive. In some patients stem cell numbers may be too few to reconstitute adequate hematopoiesis, even after removal of an acute immune insult. Other possible explanations for failure to respond to ATG include a nonimmune etiology, inadequacy of current immunosuppressive agents, negative regulation of hematopoiesis by stromal elements, or an underlying telomeropathy. Interventions such as androgens, eltrombopag and novel immunosuppressants are being tested for their activity to circumvent these shortcomings.

How we follow SAA long-term

Responders should be followed for late complications of relapse and clonal evolution. We obtain a bone marrow for morphology and especially karyotype at 6 and 12 months after treatment, and then yearly to monitor for evolution. A hypocellular marrow should not be equated with persistent SAA or relapse in the setting of improving blood counts; marrow cellularity may not correlate with blood counts for months or years. Blood counts, not marrow cellularity, should guide management.

Hematologic relapse

There is no consensus on the definition of relapse. Pragmatically and in the clinical research setting, we have defined relapse when re-introduction of immunosuppression is required for decreasing blood counts, usually, but not always,
accompanying re-institution of transfusions. A trend, not a single blood count is preferable, and avoids over interpretation of oscillating numbers that can occur normally or in the setting of infection. In cases of frank recurrence of pancytopenia, the need for renewed therapy is obvious. A bone marrow examination should be performed at relapse to exclude clonal evolution.

Relapse is most simply treated with re-introduction (or dose increase) of CsA for 2-3 months. In responders, we then continue cyclosporine until counts have improved and stabilized, aiming to very gradually taper the drug as tolerated to the minimal dose (or to off) adequate to maintain counts, a process that may take years. When CsA alone is ineffective, a second course of rabbit ATG/CsA yields responses in about 50-60% of cases. Alemtuzumab monotherapy (without cyclosporine) may be similarly useful. We do not usually recommend UD HSCT on first relapse in younger patients, since most will respond to further immunosuppression. Relapse alone has not been correlated to worse survival in aplastic anemia.

**Clonal evolution**

The most concerning late event in SAA is clonal evolution to myelodysplasia and leukemia. This complication occurs in 10-15% of patients and usually manifests as worsening blood counts unresponsive to immunosuppression, prominent dysplastic findings in the bone marrow, and abnormal cytogenetics. Occasionally, a cytogenetic abnormality is reported in routine follow-up marrows despite good blood counts and without a dysplastic marrow. Interpretation of such an abnormality is not clear.
Repeating the bone marrow in several months is reasonable, and some clonal abnormalities appear to be transient and may not predict or precede worse blood counts. One exception is monosomy 7, which is almost always a dire finding,\textsuperscript{108} in these cases, we pursue HSCT as it represents the only definitive therapy.

**Durability of Response**

The goals of immunosuppression are improved life expectancy and a durable hematologic response that avoids relapse and clonal evolution. In our experience, hematologic relapse and clonal evolution usually occur within 2-4 years of IST.\textsuperscript{67,68,99} About 50\% of responders do neither relapse or evolve over the long term (Figure 3A), and they have excellent long-term survival (Figure 3B). Most patients who relapse can be rescued with further immunosuppression or by HSCT. In contrast, clonal evolution can confer a poor prognosis.\textsuperscript{108} Among NIH patients, high-risk evolution to monosomy 7, high-grade myelodysplasia, complex karyotype and leukemia are infrequent (Figure 3C), but greatly decreases survival compared to less high-risk forms of evolution (Figure 3D).

**Prospects for improved management of severe aplastic anemia**

Measurement of telomere length and blood counts offer the possibility of rational risk stratification of treatment in future protocols. In a recent report, pre-treatment telomere length correlated with relapse, clonal evolution, and survival.\textsuperscript{109} Patients with shorter telomeres in peripheral blood leukocytes were about twice as likely to relapse and 4-6-fold more likely to evolve to myelodysplasia or leukemia, with a
negative impact on survival. If confirmed in other series, this assay might also be useful in determining the level of risk and need for monitoring of patients after IST. Patients with normal telomere length and good reticulocyte numbers at diagnosis should do well long-term after minimal immunosuppression with horse ATG and CsA. Conversely, short telomeres and low reticulocytes might direct patients to therapies that offer better than 50% long-term survival. Other biomarkers or pathophysiological indicators should emerge from global assessments of the immune response and more sensitive measurements of stem cell reserve and function.

For HSCT, the critical issues are extending sibling transplant to older patients, essentially improving the prevention and management of GVHD, and providing alternative donor transplants to patients who lack family donors. In the latter circumstance, how much histocompatibility mismatch can be tolerated? How many patients are likely to find suitable donors in the registries in a timely manner? Is there an advantage to earlier transplant? Registry data will be critical to avoid the winner’s curse of single institution study reports.

For IST, the exact mechanism of ATG action on the immune (and hematopoietic?) system will be sought but may not be easily found. Further intensification of immunosuppression, by addition of agents beyond CsA or substitution of more potent ATG or cyclophosphamide, has not been successful. More appealing options are sequential approaches, for example repeated courses of ATG, and addition of
complementary drugs, such as androgens and novel growth factors (for example, eltrombopag), to assist in tissue regeneration and amplification of stem cell numbers. In the minority of patients with defined genetic lesions, especially of telomerase components, the special role of androgens in improving organ function and also stabilizing or even elongating telomeres should be studied in systematic protocols.

ACKNOWLEDGEMENTS

This research was supported by the Intramural Research Program of the NIH, National Heart, Lung and Blood Institute. Both PS and NSY wrote the manuscript. The authors had no conflict of interest. We would like to thank Dr. Colin Wu for assistance in generating the Kaplan-Meier curves.

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Table 1. Studies of alternative horse ATG/CsA regimens compared to standard horse ATG/CsA

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>N</th>
<th>Agent(s) added to horse ATG/CsA</th>
<th>Design</th>
<th>Outcome compared to standard horse ATG/CsA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kojima65 (2000)</td>
<td>119</td>
<td>G-CSF, danazol</td>
<td>Prospective, randomized</td>
<td>No difference in response, relapse, clonal evolution or survival</td>
</tr>
<tr>
<td>Gluckman89 (2002)</td>
<td>102</td>
<td>G-CSF</td>
<td>Prospective, randomized</td>
<td>No difference in response, relapse, clonal evolution or survival</td>
</tr>
<tr>
<td>Zheng77 (2006)</td>
<td>77</td>
<td>GM-CSF, EPO</td>
<td>Prospective, randomized</td>
<td>No difference in response or survival</td>
</tr>
<tr>
<td>Scheinberg67 (2006)</td>
<td>104</td>
<td>mycophenolate mofetil</td>
<td>Prospective</td>
<td>No difference in response, relapse, clonal evolution or survival compared to historical control</td>
</tr>
<tr>
<td>Teramura92 (2007)</td>
<td>101</td>
<td>G-CSF</td>
<td>Prospective, randomized</td>
<td>No difference in response, clonal evolution, and survival. Fewer relapses in G-CSF arm</td>
</tr>
<tr>
<td>Scheinberg68 (2009)</td>
<td>77</td>
<td>sirolimus</td>
<td>Prospective, randomized</td>
<td>No difference in response, relapse, clonal evolution or survival</td>
</tr>
<tr>
<td>Tichelli94 (2011)</td>
<td>192</td>
<td>G-CSF</td>
<td>Prospective, randomized</td>
<td>No difference in response, relapse, event-free and overall survival</td>
</tr>
</tbody>
</table>

*Only comparative studies that included cyclosporine with ATG are included.

ATG, anti-thymocyte globulin; CsA, cyclosporine; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; EPO, erythropoietin
Table 2. Studies comparing horse ATG/CsA and rabbit ATG/CsA as first therapy in SAA

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Horse ATG (N)</th>
<th>Rabbit ATG (N)</th>
<th>Horse ATG formulation</th>
<th>Rabbit ATG formulation</th>
<th>Horse ATG, response</th>
<th>Rabbit ATG, response</th>
<th>Design</th>
<th>Outcome comparison between ATGs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zheng</td>
<td>2006</td>
<td>47</td>
<td>32</td>
<td>Lymphoglobulin</td>
<td>Fresenius</td>
<td>79%</td>
<td>53%</td>
<td>Prospective, randomized</td>
<td>Comparative statistics not reported</td>
</tr>
<tr>
<td>Garg</td>
<td>2009</td>
<td>----</td>
<td>13</td>
<td>-----</td>
<td>Thymoglobulin</td>
<td>-----</td>
<td>92%</td>
<td>Retrospective</td>
<td>No comparative statistics, comparison with reported results with horse ATG in the literature</td>
</tr>
<tr>
<td>Atta</td>
<td>2010</td>
<td>42</td>
<td>29</td>
<td>Lymphoglobulin</td>
<td>Thymoglobulin</td>
<td>60%</td>
<td>35%</td>
<td>Retrospective</td>
<td>Difference at statistical significance (historical comparison)</td>
</tr>
<tr>
<td>Afable</td>
<td>2011</td>
<td>67</td>
<td>20</td>
<td>ATGAM</td>
<td>Thymoglobulin</td>
<td>58%</td>
<td>45%</td>
<td>Retrospective</td>
<td>Difference not statistically significant (historical comparison)</td>
</tr>
<tr>
<td>Scheinberg</td>
<td>2011</td>
<td>60</td>
<td>60</td>
<td>ATGAM</td>
<td>Thymoglobulin</td>
<td>68%</td>
<td>37%</td>
<td>Prospective, randomized</td>
<td>Statistically significant difference (direct comparison)</td>
</tr>
</tbody>
</table>

Other studies comparing horse and rabbit ATG as first therapy have been reported in abstract form only. A small Russian prospective randomized study (32 patients in total) showed superiority of horse ATG to rabbit ATG. A retrospective Spanish study (35 who received horse ATG and 75 rabbit ATG) showed no difference between the ATGs, however response rates to horse ATG were only 49%, much lower than the historical response rate for this regimen. A follow-up from the experience of Garg et al showed a reduction in the response rate of rabbit ATG to 62%, in a single arm study (N=21). The European Bone Marrow Transplantation Severe Aplastic Anemia Working Party conducted a matched pair analysis comparing horse and rabbit ATG: survival at 2 years was 56% for rabbit compared to 78% for horse ATG, and after censoring for stem cell transplantation, survival was 39% for rabbit and 72% for horse ATG. In the Polish and Korean pediatric experience (n=55 and 112, respectively), response rate to upfront rabbit ATG was about 50% at 6 months, which is lower than the historic response rate to horse ATG in this population (about 75%). For patients of all ages, the German and Korean experience (n=64 and 58, respectively) also show a 6-month response rate at 6 months, which is inferior to that of historical horse ATG as first therapy.

ATG, anti-thymocyte globulin; CsA, cyclosporine
Table 3. Studies of unrelated donor stem cell transplantation in SAA

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N</th>
<th>Design</th>
<th>Conditioning</th>
<th>Graft failure</th>
<th>Median age (years)</th>
<th>aGVHD grade II-IV</th>
<th>cGVHD</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim</td>
<td>2007</td>
<td>40</td>
<td>Prospective</td>
<td>Cy/TBI</td>
<td>5%</td>
<td>27</td>
<td>30%</td>
<td>38%</td>
<td>75% at 3 yrs</td>
</tr>
<tr>
<td>Maury</td>
<td>2007</td>
<td>89</td>
<td>Retrospective</td>
<td>Various</td>
<td>14%</td>
<td>17</td>
<td>50%</td>
<td>28%</td>
<td>42% at 5 yrs</td>
</tr>
<tr>
<td>Viollier</td>
<td>2008</td>
<td>349</td>
<td>Retrospective</td>
<td>Various</td>
<td>11%</td>
<td>18</td>
<td>28%</td>
<td>22%</td>
<td>57% at 5 yrs</td>
</tr>
<tr>
<td>Kosaka</td>
<td>2008</td>
<td>31</td>
<td>Prospective</td>
<td>Cy/ATG/TBI, Flu/Cy/ATG/TBI</td>
<td>16%</td>
<td>8</td>
<td>13%</td>
<td>13%</td>
<td>93% at 3 yrs</td>
</tr>
<tr>
<td>Perez-Albuerue</td>
<td>2008</td>
<td>195</td>
<td>Retrospective</td>
<td>Various</td>
<td>15%</td>
<td>10</td>
<td>43%</td>
<td>35%</td>
<td>51% at 5 yrs</td>
</tr>
<tr>
<td>Bacigalupo</td>
<td>2010</td>
<td>100</td>
<td>Retrospective</td>
<td>Flu/Cy/ATG, Flu/Cy/ATG-TBI</td>
<td>17%</td>
<td>20</td>
<td>18%</td>
<td>27% (no TBI) 50% (TBI group)</td>
<td>75% at 5 yrs</td>
</tr>
<tr>
<td>Kang</td>
<td>2010</td>
<td>28</td>
<td>Prospective</td>
<td>Flu/Cy/ATG</td>
<td>0%</td>
<td>13</td>
<td>46%</td>
<td>35%</td>
<td>68% at 3 yrs</td>
</tr>
<tr>
<td>Lee</td>
<td>2010</td>
<td>50</td>
<td>Prospective</td>
<td>Cy/TBI</td>
<td>0%</td>
<td>28</td>
<td>46%</td>
<td>50%</td>
<td>88% at 5 yrs</td>
</tr>
<tr>
<td>Yagasaki</td>
<td>2010</td>
<td>31</td>
<td>Retrospective</td>
<td>Various</td>
<td>3%</td>
<td>9</td>
<td>37%</td>
<td>27%</td>
<td>94% at 5 yrs</td>
</tr>
</tbody>
</table>

Outcomes shown are for the entire cohort reported in each study. Studies that include 4 or more conditioning regimens are reported as ‘various’. Only studies with greater than 20 patients reported in the past 5 years are depicted. Cy, cyclophosphamide; TBI, total body irradiation; ATG, anti-thymocyte globulin; Flu, fludarabine; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease. In Viollet et al, only the most recent cohort (after 1998) reported is shown.
Figure 1. Algorithm for initial management of SAA. In patients who are not candidates for a matched related HSCT, immunosuppression with horse ATG plus cyclosporine should be the initial therapy. We assess for response at 3 and 6 months, but usually wait 6 months before deciding on further interventions in case of non-responders. In patients who are doing poorly clinically with persistent neutrophil count < 200 /µL, we proceed to salvage therapies earlier between 3 and 6 months. Transplant options are reassessed at 6 months and donor availability, age, comorbidities, and neutrophil count become important considerations. We favor a matched unrelated HSCT in younger patients with a histocompatible donor, and repeat immunosuppression for all other patients. In patients with a persistently low neutrophil count in the very severe range, we may consider a matched unrelated donor HSCT in older patients. In patients who remain refractory after 2 cycles of immunosuppression, further management is then individualized taking into consideration suitability for a higher risk HSCT (mismatched unrelated, haploidentical or umbilical cord donor), age, comorbidities, neutrophil count, and overall clinical status. Some authorities in SAA consider 50 years of age as the cut-off for sibling HSCT as first line therapy.
In patients treated with immunosuppression, we follow for relapse (among responders) and clonal evolution in all patients. A gradual downtrend in blood counts may signify hematologic response, underscoring the important of routine monitoring in this setting. In cases of relapse, we usually re-introduce more immunosuppression in the form of oral cyclosporine and/or a repeat course with rabbit ATG/CsA or alemtuzumab. In those who are unresponsive to more immunosuppression, further management will depend on suitability for HSCT (age, donor availability, comorbidities). When only higher risk HSCT options are available (mismatched unrelated, haploidentical, umbilical cord) we consider non-immunosuppressive strategies such as androgens (12 week trial), combination growth factors (G-CSF + Epo for 12 weeks), or experimental therapies. In patients with a very low neutrophil count unresponsive to G-CSF associated to infections, we consider a higher risk HSCT in younger patients. We monitor for clonal evolution by repeated marrow karyotype assessment at 6 and 12 months, and then yearly thereafter. After 5 years, we tend to increase the interval between bone marrows. When faced with an abnormal karyotype such as del13q, trisomy 6, pericentric inversion of chromosome 1;9, del20q or trisomy 8, we assess for myelodysplasia by looking at blood counts, peripheral smear, and bone marrow morphology. On occasion these karyotypes may not equate to progression to myelodysplasia and not be detected on repeated marrow examination. In cases were there is worsening blood counts and/or more significant dysplastic changes in the marrow, our approach is to seek transplant options, therapies for myelodysplasia, or a clinical trial. Monosomy 7 is almost never a transient finding and commonly associates to a more rapid progression to myelodysplasia and leukemia. In these cases, our approach is to seek HSCT earlier.
Figure 3. Durability of Response after horse ATG. (A) Time to first late event among responders. The probability of a first late event (relapse or clonal evolution) among responders (N=243) is about 50%. (B) In those who do not experience a late event, long-term survival in 10 years is excellent at 95%; while in those who experience a late event survival is not as favorable, 65% in 10 years. (C) In our experience, high-risk evolution to monosomy 7, complex karyotype, high-grade myelodysplasia, or leukemia occurs in about 10% of responders long-term. (D) Among responders who clonally evolved (any cytogenetic abnormality), survival was worse in those with a high-risk clonal event (monosomy 7, high-grade myelodysplasia, complex karyotype, or leukemia) compared to responders who do not experience high-risk evolution (principal karyotype finding in this lower risk group were trisomy 8 and del13q). Of note, among the high-risk clonal evolutions in responders, all occurred in those who achieved a partial hematologic response at 6 months after immunosuppression. SD shown in Figures A and C, p=log-rank. Day 0 for all curves is the time of first horse ATG-based therapy. Data for other experimental immunosuppressive therapies as first line is not shown. A late event is defined as either relapse or clonal evolution, whichever occurred first. Patients with repeated relapse or cytogenetic abnormalities were counted once at the time of first event.
How I treat acquired aplastic anemia

Phillip Scheinberg and Neal S. Young