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Reviews

The Case for Upfront HLA-Matched Unrelated Donor Hematopoietic Stem Cell Transplantation as a Curative Option for Adult Acquired Severe Aplastic Anemia

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A B S T R A C T

The improved success of HLA-matched unrelated donor (MUD) hematopoietic stem cell transplantation (HSCT) for severe aplastic anemia (SAA) in recent decades has had an impact on the indications for and timing of this treatment modality. In the absence of a matched sibling donor (MSD), historically MUD HSCT was reserved as an option after failure to respond to at least 2 courses of immunosuppressive therapy (IST) in adults with SAA, but with improved outcomes over time, it is now considered following failure to respond to 1 course of IST. Recent national and international studies and guidelines now recommend upfront MUD HSCT as an option for children for whom an MUD is readily available, because outcomes are similar to those for MSD HSCT. Fludarabine-based conditioning and the use of in vivo T cell depletion with antithymocyte globulin or alemtuzumab has been associated with a reported overall survival (OS) of >85% in adult patients undergoing MUD HSCT. However, the recent introduction of eltrombopag for patients with SAA has transformed the treatment landscape, and there is currently much interest in its use with IST as upfront treatment, which showed a high response rate in an early-phase study. The risks of HSCT, especially graft-versus-host disease (GVHD), need to be carefully balanced against the concerns of IST, namely relapse and later clonal evolution to myelodysplastic syndrome (MDS)/acute myelogenous leukemia (AML). In the absence of a current prospective randomized trial comparing these 2 approaches, in this review we examine the evidence supporting consideration of early MUD HSCT in adults with SAA who would have been considered for MSD HSCT but who lack a MSD and for whom an MUD is readily available, especially using an irradiation-free conditioning regimen, with a low risk of GVHD, as another treatment option. This option may be offered to patients to provide them with an informed choice, with the aim of curing disease rather than achieving freedom from disease, relapse-free survival, or OS. Furthermore, understanding the immune signature for the response to IST and the immunologic responses to somatic mutations and clonal progression to MDS/AML may help define the future indications for upfront HSCT and a more precise medical approach to therapy.

CURRENT INDICATION FOR HLA-MATCHED UNRELATED DONOR HEMATOPOIETIC STEM CELL TRANSPLANTATION: HISTORICAL DEVELOPMENT OF TREATMENT OPTIONS

Allogeneic HLA-matched unrelated donor (MUD) hematopoietic stem cell transplantation (HSCT) for adults with acquired severe aplastic anemia (SAA) is currently recommended after failure to respond to a course of immunosuppressive therapy (IST) [1,2]. This guidance reflects the historical developments in HSCT and IST, as well as the improving outcomes of MUD HSCT over time. Although patients with SAA are surviving long term after both HSCT and IST, the long-term complications of clonal transformation to myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) and hemolytic paroxysmal nocturnal hemoglobinuria (PNH) after IST in up to 15% to 26% of patients at 10 years are notable [3-7]. The goals of HSCT for SAA are sustained hematologic (myeloid) engraftment, ideally with stable mixed T cell engraftment, absent or low levels of acute and chronic graft-versus-host disease (GVHD), low toxicity from the conditioning regimen, and avoidance of evolution to MDS/AML associated with nontransplantation therapies. With overall survival (OS) rates exceeding 80%, the composite endpoint of GVHD-free, relapse-free survival (GRFS) for assessment of post-transplantation outcomes for hematologic malignancies should now be used to evaluate morbidity as well as mortality [8].

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A landmark prospective randomized study published in 1976 by Camitta et al [9] from Seattle on behalf of the International Aplastic Anemia Study Group reported significantly improved survival of 67% after matched sibling donor (MSD) HSCT compared with androgens. That study highlighted the importance of early HSCT as a cure for SAA if HSCT is performed before alloimmunization of patients from multiple transfusions and before failure of conventional treatment. Allogeneic HSCT subsequently became the gold standard first-line treatment for patients with SAA with an MSD. Concurrently, antithymocyte globulin (ATG), with or without androgens, was being developed as an alternative therapeutic option for patients with SAA who lacked an MSD or who were ineligible for HSCT due to older age or the presence of nonsevere AA, resulting in similar OS as seen with MSD HSCT [10]. In a German prospective randomized study, the introduction of cyclosporine A (CSA) for use in combination with ATG significantly increased the response rate to 65%, compared with 35% with ATG alone [5,11]. This remains the current gold standard IST regimen, although the addition of the thrombopoietin receptor agonist eltrombopag to ATG and CSA is currently being explored in a European prospective randomized study following the high response rates of 85% reported in a Phase II trial from the US National Institutes of Health [12] (Figure 2).

Because only 20% to 30% of patients have an MSD, HSCT using MUDs was explored decades ago. However, outcomes of MUD HSCT performed in the 1980s and early 1990s were disappointing, with OS of only approximately 35% and high mortality from graft rejection, GVHD, and infections. Outcomes have improved steadily over time due to (1) improved HLA matching of donors using high-resolution DNA typing; (2) better conditioning regimens with the use of fludarabine, reduced cyclophosphamide doses, and avoidance of high-dose total body or total lymphoid irradiation; (3) the introduction of CSA as postgrafting immunosuppression not only to reduce GVHD, but also to aid engraftment; and (4) in vivo T cell depletion with ATG or alemtuzumab [13-17] and avoidance of peripheral blood stem cells (PBSCs) as a stem cell source when using ATG-based conditioning [18,19]. There also have been major improvements in supportive care over time, including treatment of infections, especially invasive fungal disease [20], and improved access to and quality of blood products, including HLA-matched platelet transfusions for HLA-alloimmunized patients [21] (Figure 1).

Consequently, the indication for MUD HSCT has advanced from failure to respond to at least 2 courses of IST to failure to respond to 1 course of IST. This necessarily means that when reviewing data to support a possible role of MUD HSCT as a first-line therapeutic option for adult patients with SAA, a comparison of MUD HSCT and MSD HSCT will involve first-line MSD versus second-line MUD HSCT. Previous treatment with IST results in a longer interval from diagnosis to HSCT, and time from diagnosis to HSCT of >3 months [13] or >12 months [17] has been identified as an independent risk factor for worse OS after HSCT. In contrast, in children with acquired SAA, data from first-line MUD HSCT and first-line MSD HSCT show similar outcomes [22]. Consequently, the use of MUD HSCT has advanced further in children than in adults, in that it is now accepted to consider first-line MUD HSCT for children in the absence of an MSD and the ready availability of an MUD. However, recent improvements in outcomes have also been reported after second-line MUD HSCT among adults, stimulating discussions among the adult transplantation community to consider upfront MUD HSCT as an option for adults with SAA as well.

HLA-MUD HSCT

A summary of outcomes after MUD HSCT using predominantly fludarabine-based conditioning with either ATG or alemtuzumab as in vivo T cell depletion is shown in Table 1.

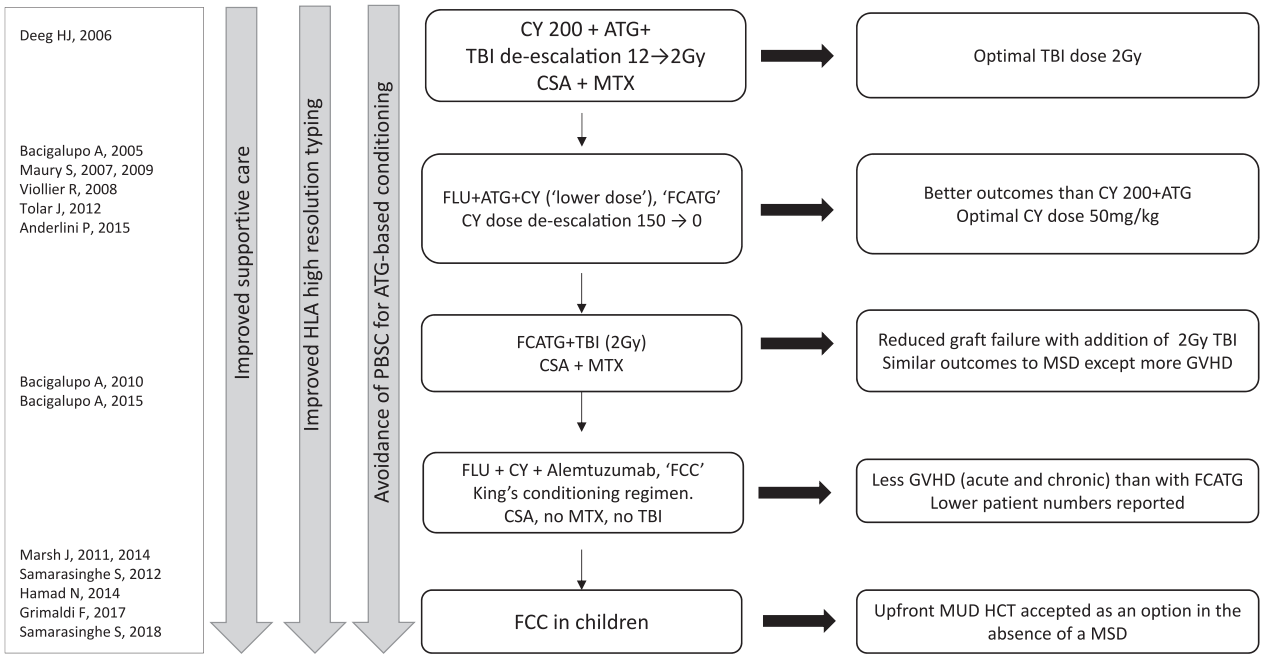

Figure 1. Development of conditioning regimens for MUD HSCT for acquired SAA. MTX, methotrexate.

Table 1
MUD HSCT for Adult Acquired SAA Using Predominantly Fludarabine-Based Conditioning in Patients Undergoing HSCT Since 1999

Study	Year of transplant	Patients, N	MUD patients, N	Med age (range)	% patients receiving FLU/CY	Graft failure	Acute GVHD	Chronic GVHD	Overall survival
Using Alemtuzumab as in vivo T cell depletion									
Marsh, 2011, UK + Toronto	1999-2009	50	29	35 (8-62)	100%	14.5%	13.5%	4%	83% at 2yr
Marsh, 2014, UK	1999-2009	100	55	20 (1.5-67.5)	60%	9%	24% (89% Gd I-II)	11%	88% at 5yr
Hamad, 2014, Toronto	2005-2013	41	12	37 (17-59)	71%	10%	27% (all Gd I-II)	15% (none severe)	85% at 3yr
Grimaldi, 2017, King's College Hospital	2007-2015	45	33	32 (15-63)	100%	2.2%	13%	13%	87% EFS at 5 yr
Samarasinghe, 2018, EBMT Registry	2000-2013	261	193	N/A	65% (MUD+MSD)	9.6%	6.7% Gd II-IV	16.9% at 5yr	81% at 5yr
Using ATG as in vivo T cell depletion									
Bacigalupo, 2015, EBMT Registry	2005-2009	1448	508	53% aged >20yr	62%	9%	25%	26%	Low risk 83%; Int risk 77%; High risk 64% (all at 5yr)
Anderlini, 2015, prospective phase I-II study, BMTCTN	2006-2013	79	79	24 (0.5-66)	100%	8% (CY50mg/kg) 15% (CY100mg/kg)	24% (CY50) 27% (CY100)	23% (CY50) 32% CY100)	97% (CY50), 81% (CY100) at 1 yr
Devillier, 2016	2000-2012	139	113	23 (1-66)	72%	7%	35% Gd II-IV	24& at 4yr	66% at 4yr
Samarasinghe, 2018, EBMT Registry	2000-2013	1283	431	N/A	20% (MUD+MSD)	11.3%	13.3% Gd II-IV	22% at 5yr	80% at 5yr

Improved Outcomes Using Fludarabine and ATG-Based Conditioning Regimens

The use of fludarabine-based conditioning regimens that used a lower dose of cyclophosphamide (CY; 300 mg/m^2× 4, compared with the standard dose of 200 mg/kg), along with in vivo T cell depletion (TCD) with fludarabine, cyclophosphamide, and ATG (FCATG) in older patients undergoing MSD HSCT, resulted in improved outcomes and reduced toxicity compared with high-dose CY (120 mg/kg) conditioning, which is now restricted to younger patients (age <30 years) undergoing MSD HSCT [23]. Although previous and more recent studies reported worse outcomes following HSCT in patients age >40 years [24-26], a study of 117 Korean MSD HSCT recipients treated with FCATG who underwent transplantation between 2002 and 2014 showed no difference in OS between patients age <40 years and >40 years and no differences in OS or failure-free survival (FFS) for the 4 subgroups age <30 years (97% and 76%), 31 to 40 years (91% and 70%), 41 to 50 years (88% and 77%), and >50 years (92% and 92%) [27].

The FCATG regimen was subsequently used for MUD HSCT. Although this resulted in improved survival, in 1 study graft rejection was 32% in patients age >15 years [28]. The addition of low-dose (2 Gy) total body irradiation (TBI) lowered the rate of graft rejection to 17% [29]. In a retrospective European Blood and Marrow Transplant Group (EBMT) study of 940 MSD and 508 MUD HSCTs performed between 2005 and 2009, using fludarabine-based conditioning in 62% of MUD transplant recipients, patients were stratified into low, intermediate, and high risk based on age <20 years or ≥20 years, time from diagnosis to HSCT <6 months or ≥6 months, CMV serostatus, use of bone marrow (BM) or PBSCs as the stem cell source, and ATG versus no ATG [14]. The use of PBSCs was the most significant negative predictive factor for OS. There was no significant difference in OS between MSD and MUD HSCT in the intermediate-risk and high-risk groups; for low-risk patients, OS was significantly better using upfront MSD, likely reflecting the smaller number of MUD transplants (n = 46) and possible selection bias (Figure 2). However, more acute and chronic GVHD w seen after MUD HSCT (25% and 26%, respectively, compared with 13% and 14%, respectively, after MSD HSCT) (Figure 3). A French/EBMT study of MUD HSCT showed a 4-year OS of 67% and worse outcomes for patients aged >30 years and patients who underwent transplantation between 2000 and 2012, using ATG as in vivo TCD in most cases. For the subgroup undergoing HSCT in more recent years (2006 to 2012), 4-year OS was 74%, the rate of acute GVHD was 35%, and that of 4-year chronic GVHD was 24%. A scoring system based on patient age (>30 years), HLA matching (use of 9/10 MUDs), and time to HSCT (>12 months) predicted worse OS in multivariate analysis [13].

The impact of reduced CY dose in combination with fludarabine, ATG, and 2 Gy TBI was evaluated in a prospective, multicenter phase I-II deescalation study in MUD HSCT recipients. The optimal CY dose was 50 mg/kg, associated with a 1-year OS of 97%. The incidence of acute GVHD was 23.7%; 2 patients had grade II and 3 had grade III acute GVHD (13%), none of whom died. Two patients (5%) died with chronic GVHD [30] (Table 1).

GVHD continues to impact mortality, morbidity and quality of life after HSCT for AA. Thus, a different approach is needed to reduce GVHD in HSCT for SAA.

Alemtuzumab-Based (FCC) Conditioning in HSCT for SAA: An Irradiation-Free Regimen

The so-called "King's FCC" regimen comprises fludarabine 30 mg/m^2, CY 300 mg/m^2× 4, and alemtuzumab (0.2 mg/kg on days -7 to-3). Key features of the FCC regimen compared with the FCATG regimen are as follows: (1) CSA is the sole postgraft immunosuppressive drug, in contrast to the requirement for both CSA and methotrexate following FCATG HSCT, thereby avoiding hepatotoxicity and mucositis associated with methotrexate; (2) FCC is an irradiation-free regimen for MUD HSCT, whereas low-dose TBI is required for FCATG MUD HSCT to reduce graft rejection; and (3) because alemtuzumab is highly effective in preventing GVHD, PBSCs can be safely used as the stem cell source instead of BM, to help ensure an adequate stem cell dose, an important factor in reducing the risk of graft rejection [15,31] (Table 2).

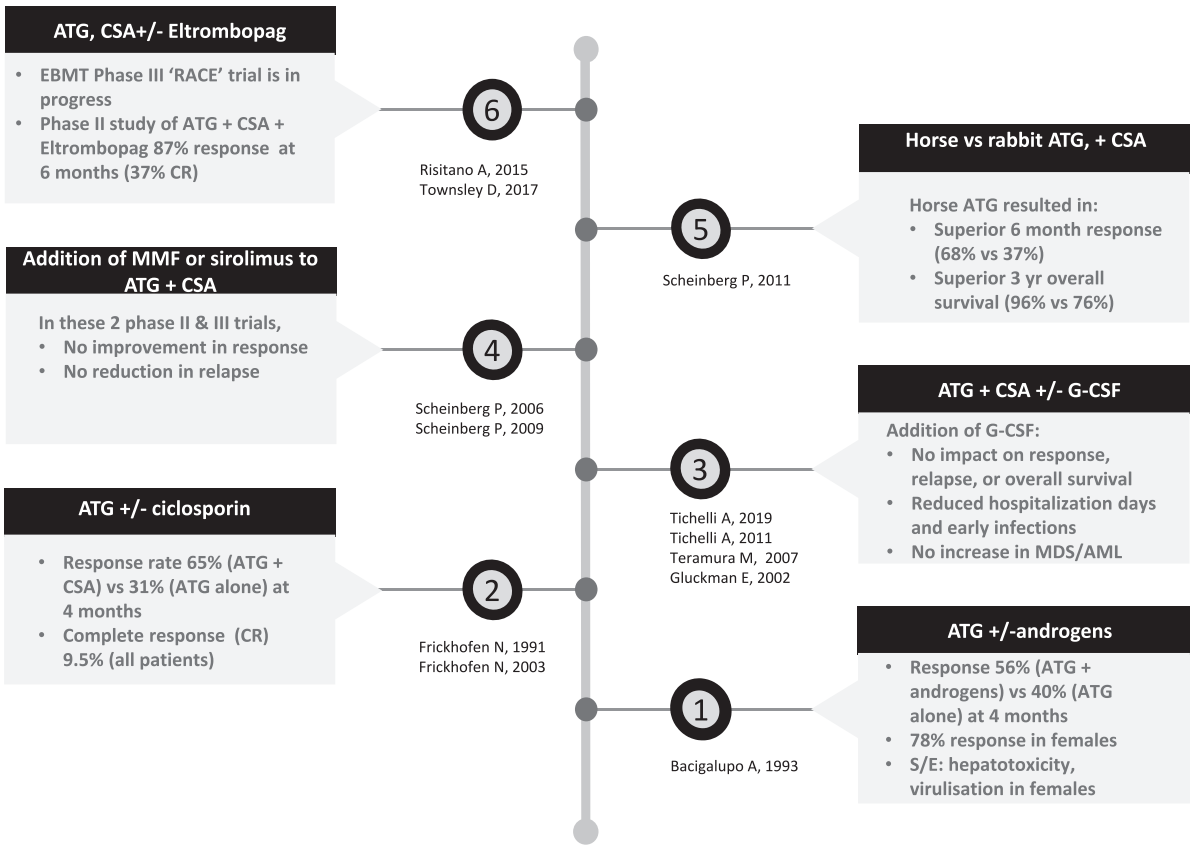

Figure 2. Prospective randomized trials of first line ATG-based IST to treat SAA. MMF, mycophenolate mofotil.

A multicenter, retrospective study of 50 patients reported a similar 2-year OS for MSD and MUD HSCT (95% versus 83%; $P = .34$); a cumulative incidence of graft failure of 9.5% and 14.5%, respectively; a low rate of acute GVHD (13.5%, all grade I-II and involving skin only), and chronic GVHD in only 2 patients (4%) [15]. This study showed for the first time the importance of the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) for outcomes in SAA, particularly in determining the suitability of older patients for HSCT. The remarkably low incidence of GVHD was associated with stable mixed T cell chimerism in the presence of full donor myeloid chimerism that persisted on withdrawal of postgraft CSA, suggesting a state of mutual donor-recipient tolerance. The basis of the mixed T cell chimerism was persistence of recipient CD8 T effectors; late graft failure rate was low but necessitated continuation of therapeutic CSA for at least 9 months post-HSCT.

A subsequent larger retrospective UK study of adults and children with SAA reported a 5-year OS of 88% for MUD HSCT using alemtuzumab-based conditioning, along with a lower risk of chronic GVHD compared with ATG-based conditioning [32]. An EBMT retrospective study analyzed patients who underwent HSCT between 2000 and 2013 with ATG (n = 1283), 259 of whom specifically received fludarabine, CY, and ATG; with alemtuzumab (n = 261), 171 of whom specifically received fludarabine, CY, and alemtuzumab; or with no serotherapy (n = 213). The risk of acute and chronic GVHD was significantly lower and OS was significantly better in both the ATG and alemtuzumab arms compared with the no serotherapy arm. Comparing ATG with alemtuzumab, the latter was associated with a lower risk of both acute and chronic GVHD [16].

Older patients (>50 years) have higher mortality after MUD HSCT compared with MSD HSCT and a low performance status <90%, as recently shown in a retrospective joint study of 499 patients from the EBMT and Center for International Blood and Marrow Transplant Research. Of note, in that study, an impact of alemtuzumab on the reduction of acute and chronic GVHD was noted in 60 patients who received alemtuzumab-based conditioning, who had an incidence of chronic GVHD of only 17% [33]. At King's College Hospital, 65 consecutive patients with SAA underwent HSCT uniformly with FCC conditioning, and outcomes were compared between patients age >50 years and those age ≤50 years. The majority of patients (78%) underwent transplantation from a MUD. GRFS was similar in the 2 groups (86% versus 96%, respectively), supporting the view that age alone is not a contraindication to HSCT. Instead, careful assessment of comorbidities before HSCT is important. In this cohort, OS was 98% with an HCT-CI score of <3, compared with 76% with an HCT-CI score of ≥3 (data not shown). Autoimmune cytopenias, excluding pure red cell aplasia associated with major ABO-mismatched transplants, have emerged as complications in up to 13% of patients and require further specific IST for some cases, most commonly warm-type autoimmune hemolytic anemia [31].

There are currently no data showing an increased risk of CMV infection with an FCC HSCT regimen compared with an ATG-based regimen. In fact, there is a paucity of data on the

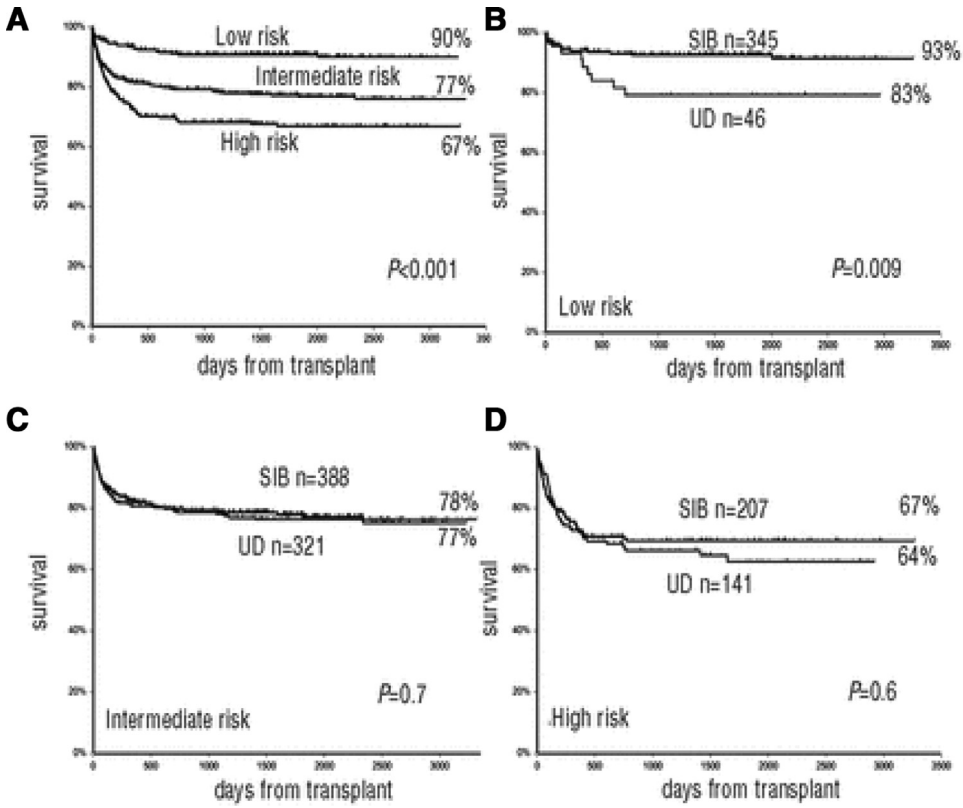

Figure 3. MSD versus MUD HSCT for SAA using predominantly ATG-based conditioning. In the EBMT retrospective study, out of 1448 patients, 508 were MUD recipients. Patients were stratified into 3 risk groups according to stem cell source, interval from diagnosis to HSCT, age, use of ATG, and CMV status. Of the MUD transplant recipients, 62% received fludarabine-based conditioning, and 61% received ATG. Modified with permission [14].

incidence of CMV reactivation in recently reported studies using ATG-based conditioned HSCT for SAA. In a study of 177 patients who underwent HSCT with FCATG, Shin et al [27] reported the need for preemptive CMV treatment in 59% of patients age 40 years and 44% of those age <40 years, compared with 42% of patients who underwent HSCT with FCC.

These results are in contrast to a recent retrospective study of ATG-based regimens that reported high mortality for patients aged >40 years even in the context of MSD HSCT [26], although a single-center study reported much lower mortality when a fludarabine-based ATG regimen was uniformly used in MSD HSCT [27].

The issue of fertility after fludarabine-based HSCT for SAA has not yet been systematically addressed. In a single-center study, 14 of 41 women age 16 to 45 who underwent HSCT after FCC conditioning (fludarabine 30 mg/m^2× 4, CY 10 mg/kg × 4, and alemtuzumab 60 mg) were evaluated for fertility. Of these, 13 had regular menses post- HSCT and 6 conceived (3 with healthy infants), 2 had medical termination, and 1 was predelivery. At a >1-year follow-up, 12 had normal estradiol levels [34].

Table 2
King's College Hospital FCC Protocol

F	Fludarabine 30 mg/m^2× 4, days -7 to -4
C	Cyclophosphamide 300 mg/m^2× 4, days -7 to -4
C	Alemtuzumab (Campath-1H) 0.2 mg/kg × 5, days -7 to -3
*	Postgraft immunosuppression: cyclosporin from day -1 to 12 months; no methotrexate
*	Irradiation-free regimen for MSD and MUD HSCT; FCC + 2 Gy TBI for 9/10 MUD HSCT

Outcomes of HSCT in Children with SAA Using an FCC Conditioning Regimen

FCC-conditioned HSCT in pediatric patients with SAA who had failed IST resulted in a 5-year FFS of 95%, absence of graft failure, low GVHD, and mixed T cell chimerism [35]. Because these results are identical to those seen with first-line MSD HSCT in children, this led to an EBMT retrospective matched comparison of 29 patients who underwent HSCT upfront from an MUD with matched historical controls undergoing upfront MSD HSCT (n = 87), 58 with upfront IST [22]. The 2-year OS was similar between MUD and MSD HSCT recipients (96% versus 91%) and in those with upfront IST (94%), but OS was only 74% among MUD HSCT recipients who had previously failed IST. The 2- year event-free survival (EFS) was 92% for upfront MUD HSCT, 87% for MSD HSCT, 40% for upfront IST, and 74% for MUD HSCT after failed IST, again showing better outcomes for first-line compared with second-line MUD HSCT and significantly worse EFS with upfront IST. The EBMT group concluded that upfront MUD HSCT for children with SAA may be considered as an option if an MUD is readily available. A similar recommendation for young adults has been proposed by the Seattle group [36,37].

Another important consideration in children is the speed of hematologic recovery, not only for neutrophils, but also for platelets. In a single-center study comparing outcomes of 11 children treated with upfront MSD HSCT and 12 children who received upfront IST, all children in the HSCT group achieved a normal platelet count and an unsupported neutrophil count of $\geq 1 \times 10^9$/L by day +60, whereas none of those treated with IST achieved a platelet count of $>40 \times 10^9$/L, and only 2 achieved a neutrophil count of $>1 \times 10^9$/L by day +60 [38].

ATG TREATMENT: THE CURRENT FIRST-LINE OPTION FOR SAA IN THE ABSENCE OF AN HLA-MSD

Current Standard IST Regimen

Prospective studies have identified horse ATG in combination with CSA as the current recommended IST regimen, with an overall response rate of 62% at 3 months and 68% at 6 months, OS of 96% at 3 years and 80% at 5 years, and a complete response rate of 10% [1,4,39]. In another study, FFS at 6 years was 44% for patient with SAA and 39% for those with very severe AA (VSAA) [40], and longer-term follow-up of the German prospective study reported an FFS after ATG and CSA of 39%, of whom 71% had SAA and the rest had nonsevere AA [5]. More recent longer-term data from the EBMT prospective randomized study of ATG and CSA with or without G-CSF show an EFS for all patients of 24% at 15 years, including 27% for patients age <20 years, 28% for those age 20 to 39 years, 30% for those age 40 to 59 years, and 12% for those age >60 years [40,41]. Predictors of better response include younger age, less severe disease, short duration (<6 months) from diagnosis to treatment, absolute reticulocyte count $\geq 20 \times 10^9$/L, absolute lymphocyte count $\geq 1.0 \times 10^9$/L, and the presence of PNH clones and *PIGA* and *BCOR*/*BCORL1* mutations [42-47].

Delayed Hematologic Recovery after ATG

Hematologic recovery following standard IST using ATG and CSA is delayed, with recovery starting at a median of 3 months, followed by continued gradual recovery thereafter, during which time they remain at risk of severe infection and bleeding. Mortality is approximately 20% for VSAA and 2% to 5% for SAA within the first 3 months after ATG treatment and approximately 25% for VSAA and 5% to 7% for SAA at 6 months after ATG [40]. Infection is the most common cause of death and may prompt early consideration of HSCT. Mortality after ATG treatment is approximately 10% at 3 months and 15% at 6 months for patients age 40 to 60 years and approximately 15% at 3 months and 30% at 6 months for patients age >60 years [40]. Severe cardiac events, including cardiac ischemia with sudden cardiac death, bradycardia, and congestive cardiac failure, occur in 20% of patients age >60 years following ATG treatment [48].

Novel Approach to IST: Addition of Eltrombopag to Horse ATG and CSA

The thrombopoietin receptor agonist eltrombopag has significantly changed outcomes for both refractory SAA and with IST as upfront treatment. It was first assessed in refractory SAA following lack of response to ATG/CSA in a Phase II and subsequent extension study [49,50]. Response occurs in 40% to 50% of patients [50,51], including not only platelet responses, but also bilineage and trilineage responses. Patients who achieve a "robust" response (defined as hemoglobin >10 g/dL, neutrophil count $>1.0 \times 10^9$/L, and platelet count $>50 \times 10^9$/L) maintained their response after discontinuation of the drug; however, clonal cytogenetic abnormalities occurred in 8 patients (19%; mostly nonresponders) at a median of only 3 months, with abnormalities of chromosome 7 (mostly monosomy 7) in 5 of these 8 patients [50,52]. Although BM dysplasia was reported infrequently, most patients proceeded to early HSCT. Early emergence of monosomy 7 is in contrast to the much later clonal evolution seen following standard IST with horse ATG and CSA.

These findings provided the rationale for the use of eltrombopag as upfront treatment of SAA in combination with ATG and CSA, to determine whether the addition of eltrombopag would further increase the response rate, particularly the rate of complete remission (CR), after ATG and CSA by comparison with historical data. A Phase I-II study of 92 patients reported a CR of 36% and an overall response rate (ORR) of 80% in the entire cohort, and 58% and 94%, respectively, in those patients receiving eltrombopag for 6 months starting on day +1, in contrast to 10% and 66%, respectively, among historical controls [12]. CR was defined not by normalization of the blood count, but rather by hemoglobin level >10 g/L, platelet count $>1.0 \times 10^9$/L, and neutrophil count $>1.0 \times 10^9$/L. Clonal cytogenetic evolution occurred in 7 patients (8%) at 2 years and in 8 patients (11%) at 3 years, with loss of chromosome 7 in 5 patients, and a similar frequency among the historical controls. However, a longer follow-up is needed to determine the full significance of early emergence of monosomy 7 and other clones. A smaller Phase II study of 38 patients from MD Anderson Cancer Center demonstrated no difference in overall response between ATG and CSA with or without eltrombopag (76% versus 71%) [53]. Nevertheless, based on the high response and CR rates observed, as of November 15, 2018, the Food and Drug Administration has fast-tracked the approval for eltrombopag as first-line treatment with standard IST using ATG and CSA for first-line treatment of SAA in both adults and children. Already in some US centers, the standard of care for patients receiving IST routinely includes eltrombopag, but we recognize the need for larger studies with longer follow-up.

A multicenter EBMT prospective randomized study comparing first-line ATG and CSA with or without eltrombopag (Randomized multicenter study comparing horse ATG, Cyclosporine ± Eltrombopag as front-line therapy for SAA [RACE]; Clinical Trials.gov identifier NCT02099747) is currently ongoing [54].

Late Events after ATG

Late events contribute to treatment failure and have an adverse impact on FFS. Nonresponse occurs in up to 35% of adults. Among responders, CSA dependency occurs in 26% of patients at 6 months and in 14% beyond 5 years [5]. Based on these data, maintenance CSA is continued for at least 12 months, but with careful monitoring for side effects of CSA [55]. Up to 50% of patients with AA at diagnosis have a detectable PNH (so-called "AA/PNH" syndrome with pancytopenia and hypocellular BM if associated with hemolysis, or "subclinical PNH" when hemolysis is not detectable) using sensitive, multicolored flow cytometry [7,42]. In 10% of patients, the PNH clone increases to such an extent, resulting in classic hemolytic PNH with hemolysis, with the potential for significant morbidity from thrombosis, necessitating evaluation of the patient for life-long (and expensive) treatment with complement-modulating agents (ie, C5-blocking monoclonal antibodies such as eculizumab or proximal inhibitors in clinical development).

Progression of SAA to MDS/AML after ATG/CSA represents the most feared late event, with the prevalence increasing with time to a maximum of 15% to 26% at 10 years [7]. The risk of solid tumors at 11 years is 11% [5]. Abnormal cytogenetic clones are detected in up to 20% of patients at diagnosis [44], and monosomy 7 is associated with a high risk of transformation to MDS and AML and poor outcomes [44]. Myeloid-specific somatic mutations occur in 20% to 25% of patients with AA at some time during the course of treatment [45,46]. In untreated patients, so-called "unfavorable" mutations *DNMT3A* and *ASXL1* were not predictive of later disease progression to MDS/AML but were associated with worse survival free from MDS/AML. Patients with "favorable" mutations involved *PIG-A* and *BCOR/BCORL1* and showed better response and survival after IST [45].

Predictive factors for clonal transformation include older age, short telomeres, and nonresponse to IST [3,47]. The presence of somatic mutations in genes most commonly mutated in myeloid malignancies such as *ASXL1*, *DNMT3A*, *RUNX1*, and splicing factor mutations with high variant allele frequency%, a high number of somatic mutations per patient, and longer duration of AA are predictive of later MDS/AML in patients following IST [7,46]. However, more robust predictive factors for later MDS/AML are needed, and explorations of potential predictive molecular and immunologic biomarkers are currently in progress [56]. The EBMT RACE study is also addressing the question of whether eltrombopag increases the risk of clonal evolution when used with ATG/CSA. Serial samples will be analyzed for somatic mutations and high-dimensional immunophenotyping using mass cytometry with CyTOF to develop an immune signature predictive not only for response to IST, but also for later clonal transformation [56].

In the meantime, this adds further weight to the consideration of upfront MUD HSCT using an FCC regimen as an option for treating SAA in the absence of an MSD prior to the development of MDS/AML. Once SAA has transformed to MDS/AML, the success of subsequent HSCT is inferior to that of MUD HSCT for SAA, with a 5-year OS of 45% to 49% [57,58].

CONCLUSIONS

With the recent data showing OS approaching 90% for both HSCT and IST, the goal of AA treatment is progressively shifting toward a curative aim. Upfront HLA-MUD HSCT is now accepted as an option for children with SAA who lack an MSD. MUD HSCT offers the possibility of long-term cure of SAA and of any accompanying PNH, and preventing the possible (albeit rare) later transformation into myeloid malignancies. The 1-year transplantation-related mortality (TRM) associated with FCC conditioned HSCT is low, at 5% to 14%, compared with early TRM with IST using ATG and CSA. The risk of GVHD using FCC is extremely low. Graft failure occurs in 2% to 15% of MUD transplants [15,31]. The risk of viral infection post-HSCT in general is of concern, but the most common cause of death after ATG therapy is infection. Organ toxicity post-HSCT in general is also an issue, but by avoiding the use of both methotrexate and irradiation with FCC, liver and lung toxicity in particular is very low. Autoimmune cytopenias have been observed after alemtuzumab-based conditioning for SAA. In addition to achieving restoration of hematopoiesis with normal hematopoietic stem cells, the speed of hematologic recovery after HSCT is much faster than that after standard IST, eventually preventing fungal infections and other complications that may affect long-term morbidity and mortality. Although the addition of eltrombopag to horse ATG and CSA as first-line treatment for SAA shows very promising results in terms of improved response, including C¯R, compared with historical data using ATG and CSA, the risk of later clonal transformation to MDS/AML remains a major concern meriting longer follow-up. Prospective comparisons of these 2 treatment strategies are desirable. A study of pediatric patients is currently ongoing in the US (ClinicalTrials.gov identifier NCT02845596); however, the design of such studies is not easy, given that a possible treatment delay may affect the outcome of SAA to an even greater degree than the treatment provided. The ideal study for adults would be a randomized trial of upfront transplantation with either an MSD or an MUD versus upfront IST before concluding that HSCT is the superior option, and both the FCC cohorts and the IST cohorts that have received elthrombopag upfront require larger patient numbers and longer follow-up to assess the impact on survival and possible “cure” of AA.

Development of a future personalized approach to treatment decision making in SAA is likely to be based on the molecular and immunologic signature that is predictive not only of response/lack of response to IST, but also, and more importantly, the risk of later clonal evolution to MDS/AML.

In the meantime, the proposal to consider upfront MUD HSCT in adults who would have been considered for MSD HSCT but who lack an MSD as an alternative to IST if an MUD is readily available is compelling and now warrants similar acceptance as for children with SAA. Patients should be carefully assessed for comorbidities before consideration for HSCT, especially older patients, but age per se should not be a contraindication because biological age is more relevant than chronological age, and by using the FCC regimen, HSCT can be safely delivered up to age 65 years. The level of HLA compatibility is important, in that a 10/10 (or at least 8/8) match is preferable to a 9/10 match.

Detailed discussions with each patient exploring individual values and risks should then permit the patient to make an informed decision. To deny a patient this option based on current guidelines is now difficult to justify, and patient choice also must be taken into consideration. A more urgent consideration for upfront MUD HSCT would be an acute presentation of SAA, and especially VSAA, with severe systemic infection. In this indication, as for all SAA, prospective collection of all upfront MUD HSCTs will tell us how this option needs to be positioned in the future algorithm of SAA treatment.

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