EWOG-SAA

Consensus for the treatment of

severe aplastic anaemia in children and adolescents

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Severe aplastic anaemia is a rare haematological disorder characterized by severe pancytopenia in the peripheral blood and a hypocellular bone marrow. Immunosuppressive therapy (IST) and haematopoietic stem cell transplantation (HSCT) are the main treatment modalities, and although the overall outcome is very good patients are at high risk of life threatening complications and should be treated by experienced paediatric haematologists in centres with access to stem cell transplantation facilities. Patients and their parents are to be instructed and informed carefully about the importance of specific precautionary measures (i.e. prevention of trauma, infections) and critical clinical signs.

For prevention of pneumonia by pneumocystis jiroveci a prophylaxis with trimethoprim/sulfamethoxazol (TMP/SMZ, Cotrimoxazol) is recommended. Antifungal prophylaxis covering molds (aspergillus etc.) is essential and should be performed according to local standards. Febrile episodes should be treated immediately with i.v. antibiotics and in case of suspected invasive fungal infection or persistent fever with appropriate empiric antifungal therapy according to local standards.

Transfusion support should be restrictive to reduce the risk of alloimmunization and other adverse events, but sufficient to avoid bleeding sequelae (refer to local standards or published guidelines). All blood products should be leukocyte depleted and irradiated. Single donor platelets harvested by apheresis are usually preferred.

In view of the excellent probabilities of overall survival with both treatment modalities the long-term outcome and the recognition, prevention and treatment of late effects are essential and should be discussed with patients and their parents. This includes, among others, renal insufficiency with long-term exposure to cyclosporine and the risk of clonal evolution in patients treated with IST, and the risk of chronic GvHD and possibly infertility/premature menopause in patients treated with HSCT.

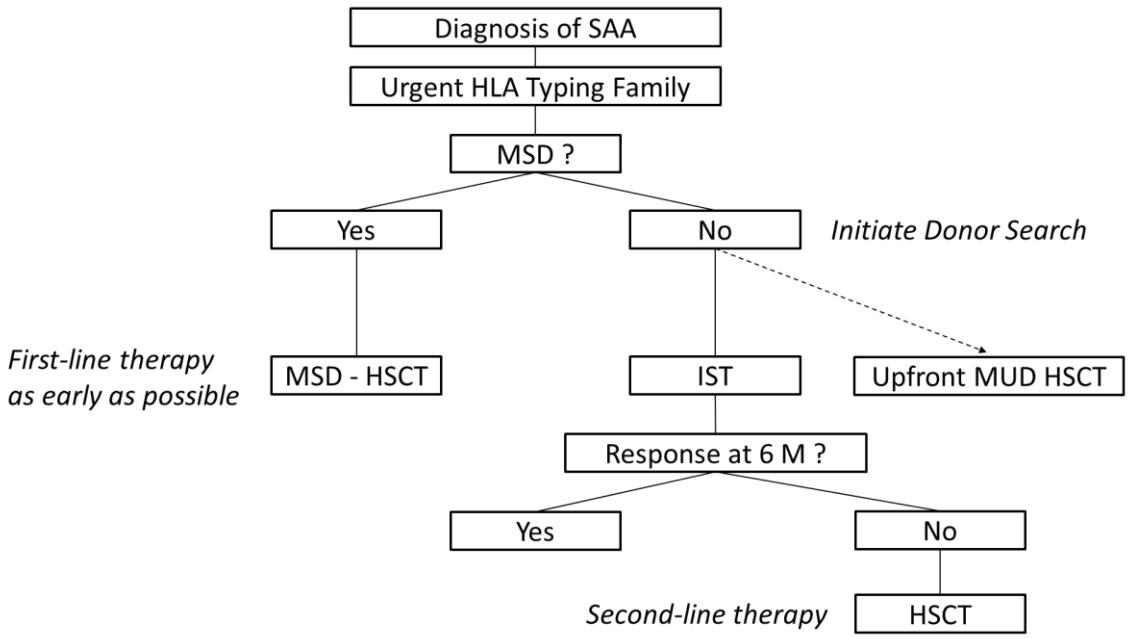


Figure 1: Treatment algorithm for children with SAA

The EWOG-SAA Consensus for Immunosuppressive Therapy in Aplastic Anaemia

1 Introduction

1.1 Immunosuppressive therapy in severe aplastic anaemia

Immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine A (CSA) has for decades been the standard therapy for adult and paediatric patients with severe aplastic anaemia (SAA), if a matched sibling donor is not available. In most previous studies, 50-70% of all cases with SAA respond to IST with horse-ATG and CSA.[1-5] The long-term survival after IST in historic cohorts of children with SAA was about 80–90%.[4-6] The interim analysis of EWOG-SAA-2010 showed an excellent survival of the responders at day 180 (responders, 100%, n=55 vs non-responders 90%, n=59, p=0.01).

1.2 Choice of anti-thymocyte globulin

Lymphoglobulin® (horse-ATG, Genzyme), which had been widely used for IST in Europe, was withdrawn from the market in 2007. In the absence of the availability of another licensed horse-ATG, it was replaced by rabbit-ATG (Thymoglobulin®) in most EWOG-SAA participating centers. However, in a randomized controlled trial, Scheinberg et al. reported the inferior response and the survival rate after rabbit-ATG (Thymoglobulin®, Genzyme) in comparison with horse-ATG (Atgam®, Pfizer) in adults with SAA.[7] The retrospective analysis in the GPOH-SAA 94 Study of German children comparing horse (Lymphoglobulin®) and rabbit-ATG (Thymoglobulin®) confirmed the inferior response rate in rabbit-ATG, although the survival rates were comparable between the two groups.[8] Therefore, Atgam®, which is currently the only available horse ATG on the market, was introduced in most European countries. Atgam® has been approved for the treatment of aplastic anaemia in January 2022 in Germany and many other European countries. The recent interim analysis of the EWOG-SAA-2010 has confirmed the previous findings and showed the lower response rate to IST in patients treated with rabbit ATG (Thymoglobulin®) (n=40) than those given horse ATG (Atgam®) (n=110) (22% vs. 42% at day 180, respectively, p=0.03) without difference in overall survival (88% vs. 93%). Several other nonrandomized studies showed inferior results for rabbit-ATG in adults and children with SAA.[9,10] No published series of SAA patients has shown the superiority of rabbit-ATG to horse-ATG. Based on these findings, the EWOG-SAA **recommends using horse-ATG** (Atgam®), whenever it is available. If horse-ATG is not available, rabbit-ATG (Thymoglobuline®) can be applied as an alternative therapy.

1.3 Granulocyte-colony-stimulating factor

The addition of granulocyte-colony-stimulating factor (G-CSF) may reduce the rate of early infectious episodes and days of hospitalization in very SAA (vSAA) patients, but has no effect on the response rate

to IST and survival.[11] Moreover, long-term (>300 days) administration of G-CSF can increase the risk of evolution to myelodysplastic syndrome (MDS).[12-14] According to these findings **G-CSF should only be applied in patients with severe neutropenia (absolute neutrophil count: ANC < 0.5 x 10^9/l) and long-term administration of G-CSF should be avoided (max. 60 days)**.

1.4 Eltrombopag

The NIH group first reported the efficacy of adding eltrombopag (EPAG) to standard IST (horse ATG + CSA) in previously untreated patients with SAA.[15] They showed that early start of EPAG (day 1 of IST) led to a remarkably higher response rate (94% overall and 58% complete response at 6 months) compared to a later start (day 14). The Severe Aplastic Anaemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT-SAA-WP) recently conducted a prospective multicentre randomized phase III trial in adults with SAA comparing the efficacy and safety between the groups with horse ATG + CSA only (group A, n=101) or combined with EPAG (group B, n=96) as a first line therapy.[16] The group B showed a significant higher rate of complete response (CR) at 3 months (A: 10% vs. B: 22%) and a higher rate of overall response (CR + partial response; PR) at 6 months (A: 41% vs B: 68%). The median time to the first response was shorter in the group B (A: 8.8 vs. B: 3.0 months). There was no difference in the incidence of severe adverse events and clonal evolution. These studies suggest the benefit of adding EPAG in adults with SAA.

However, the reports of efficacy of EPAG in children with SAA are limited. The NIH group compared the results of IST between children (aged <18 years) who received IST + EPAG (n=40) and a historical paediatric cohort (n=87) with IST alone.[17] They found no significant difference in either the overall response rate or CR rate at 4 months. Moreover, the trend towards relapse was higher and event free survival (EFS) was significantly lower in children who received additional EPAG compared to IST alone. Addition of EPAG to standard IST did not improve outcomes in children with treatment-naïve SAA in this study and the authors concluded that EPAG in children with SAA should not generally be considered standard of care. Goronkova, et al compared the efficacy and safety of IST with (n=49) or without (n=49) EPAG in children with SAA in a randomized study.[18] There was no difference in overall response at 4 months between IST with or without EPAG (53% vs. 65%, p=n.s.), while the CR rate (4 months) was higher in the IST+EPAG group (31% vs 12%, p=0.027). The overall response rate (4 months) was higher in the IST+EPAG group in SAA but not in vSAA group. There was no difference in survival between two groups. In summary, **current available data do not support the addition of EPAG to IST in children with treatment-naïve SAA as general standard of care.**

1.5 Monitoring of EBV

Reactivation of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) occurs very frequently in patients with SAA after IST, although it is usually subclinical and development of EBV-associated lymphoproliferative disease (EBV-LPD) is very rare. A NIH study showed that EBV activation occurred in 87% of patients and CMV reactivation in 33% of seropositive patients after IST, although there were no cases of EBV or CMV

disease.[19] Therefore, we recommend monitoring EBV levels by PCR in these patients regularly (e.g. every 2-4 weeks) and to watch out for signs of EBV-LPD, as localized leukoplakia, fever, enlargement of lymph nodes/liver/spleen, pneumonia and pharyngitis. EBV-LPD is diagnosed by clinical symptoms, histological examination and EBV detection. A higher EBV load is associated with a higher risk of EBV-LPD. Preemptive therapy with rituximab (anti CD20) may prevent the manifestation of EBV-LPD.

1.6 **Special consideration for hepatitis-associated aplastic anaemia**

Hepatitis associated aplastic anaemia (HAAA) is a well-recognized variant of acquired AA characterized by pancytopenia typically appearing 2-3 months (ranged 0 to 12 months) after acute hepatitis. HAAA occurs most frequently in young male children and usually no hepatitis virus can be detected. In the majority of the cases, cytopenia is fulminant and rapidly progressive, while some patients have only mild and stable cytopenia with histological features of refractory cytopenia of childhood. HAAA patients usually have lymphopenia (especially CD4-lymphopenia). There is no difference in response rate to IST between HAAA and idiopathic AA.[20,21] Rarely, patients have active hepatitis at diagnosis of SAA. In such cases, **patients may be benefit from early start of IST**, because IST may lead to the remission of hepatitis as well.[22]

1.7 **Second-line therapy for non-response, relapse and secondary myeloid malignancies**

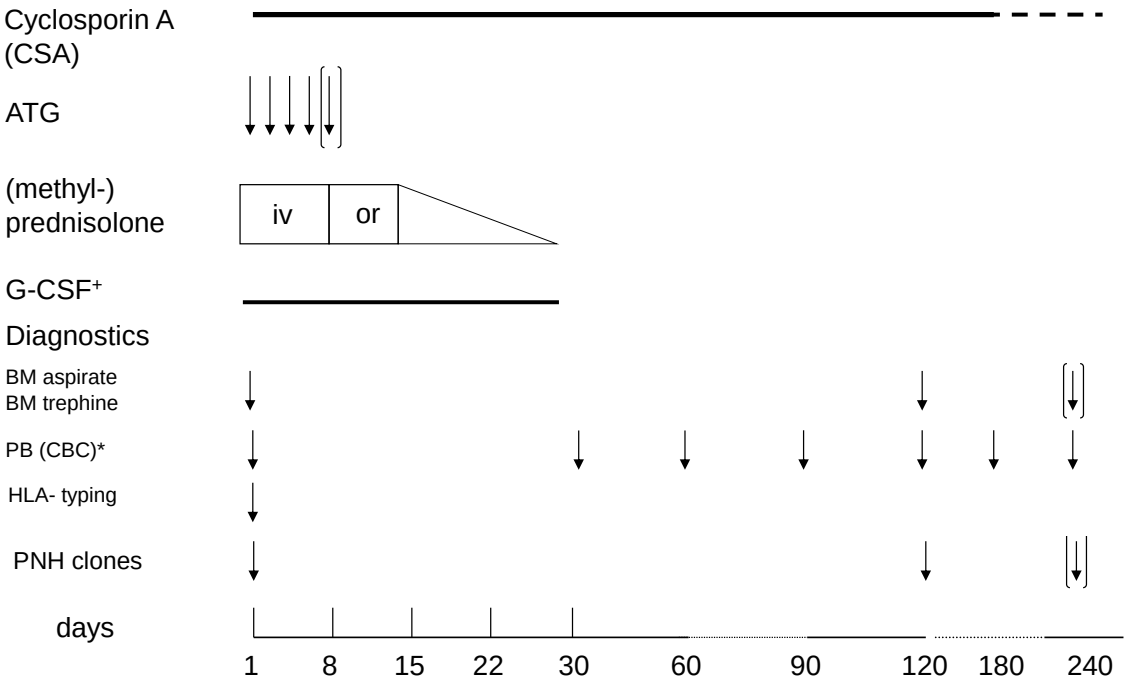
One-third of patients do not respond to IST. Relapse is observed in about 20-40% of patients in responders.[1,3,4,6] **2nd course of IST for non-responders or relapse is not recommended due to less efficacy and recent excellent results of HSCT in children**.[23-25] We recommend to proceed to HSCT in these patients.

Several studies showed that clonal evolution / secondary myeloid malignancy develops in 2–26% of patients with SAA following IST and a majority of patients have monosomy 7.[1,6,14,15,26,27] The incidence of clonal evolution was very low in the EWOG-SAA-2010 study (n=2/150), probably due to precise differential diagnosis between SAA and hypoplastic MDS at diagnosis and early HSCT in non-responders. Patients with secondary malignancy should be transplanted as soon as possible (see HSCT guidelines of EWOG-MDS for details). The overall survival at 5 years after HSCT of patients with secondary MDS following SAA was 41% in the GPOH-SAA 94 study.[28]

1.8 **Timeline of the start of IST after the diagnosis**

The precise diagnosis of SAA and exclusion of other diagnoses such as refractory cytopenia of childhood and inherited bone marrow failure syndromes are important. However, start of IST should not be delayed due to prolonged diagnostic procedures. **The interval between diagnosis and the therapy is strongly correlated with the outcomes of IST.** The response rates to IST in children with SAA were 66%, 60%, 51%, 41% and 35% in patients who received IST within <10 days, 11-20 days, 21-30 days, 31-90 days, >90 days after diagnosis, respectively (p=0.02) in a large paediatric study of IST.[29] Therefore, it is essential to start IST as early as possible after the diagnosis (ideally within two weeks, latest within 4 weeks).

2 Schedule of immunosuppressive therapy



+ G-CSF at day 1 for patients with an ANC < 500

* CBC for response evaluation: day 30, day 60, day 90, day 120, day 180, day 240, day 360, 1.5 yrs, 2 yrs, and thereafter yearly

Figure 2: Schedule for treatment with IST and response measurement of IST in SAA

CSA: Starts on the first day of the ATG administration at 5 mg/kg/day orally, divided in 2 doses. Levels (trough) should be aimed to be 100-200ng/ml. CSA should be continued at least until day 180.

ATG: Horse ATG (Atgam® Pfizer) 40 mg/kg/day administered in 6 - 8 hours i.v., day 1-4. The infusion time according to the manufacturer's instruction is 12 to 18 h with a lower limit of 4 h and centres should apply ATG according to their local standards.

If Atgam® is not available, rabbit ATG (Thymoglobulin® Genzyme) 3.75 mg/kg/day administered in 6-8 hours i.v., day 1-5.

Before administration of ATG antihistamines should be administered (in addition to prednisolone, see below) as prophylaxis. Hypersensitivity reactions should be treated with steroids, antihistamines and if appropriate also inhalations, i.v. fluid substitution and adrenaline.

(Methyl)prednisolone: (Methyl)prednisolone should be added iv or orally to prevent cytokine release and serum sickness, 1 mg/kg/day* starting as a bolus injection at least 30 minutes prior

to the first dose of ATG, this will be repeated for 5 days. Thereafter prednisolone (equivalent dose) will be administered in a dose of 1 mg/kg/day orally divided in 3 doses, until day 14. In the following 14 days prednisolone will be tapered by reducing the dose by 50% every 5 days until it stops at day 29.
* An additional dose of 1-2 mg/kg methylprednisolone may be necessary to treat severe adverse reactions during ATG therapy.

G-CSF: **G-CSF should only be given to patients with an ANC < 0.5 x 10^9/l at day 1 of IST** at a dose of 5 µg/kg/day sc or iv according to the direction of the manufacturer. The administration of pegylated G-CSF has not been evaluated in this setting and can therefore not be recommended. If there is a response (ANC > 0.5 x 10^9/l) at day 28 (or at any later time point) the dose is tapered by giving it every second day, every third day etc. If the WBC decreases again to 0.5 x 10^9/l, the original dose of 5 µg/kg is restarted. Increasing the G-CSF dose > 5 µg/kg/day is not recommended. **All patients should be off G-CSF therapy by day 60.** Patients with an ANC ≥ 0.5 x 10^9/l at day 1 who subsequently develop severe neutropenia should not receive G-CSF.

3 Measurement of response to immunosuppressive therapy

3.1 Time points

To evaluate (continuous) hematologic response:
Complete blood count (CBC) at day 30, 60, 90, 120,180, 240 and 360, 18 months, 2 years and later yearly after start of IST.
BM aspirate and biopsy at day 120 (4 months), and in case of relapse (= NR following PR or CR).

3.2 Definitions of response

The criteria for response are defined according to the International consensus on evaluation of IST in SAA. The criteria for red cell response were specified according to age adjusted normal ranges and their lower limits (-2SD). CR indicates blood values within the normal range. PR was subdivided into two groups, good PR (GPR) and poor PR (PPR) (Table 1.)

Complete remission (CR):	**All the criteria below have to be fulfilled:** • ANC ≥ 1.5 x 10^9/l • Haemoglobin ≥ age adjusted cut-off value 0.5 -2 years: 10.5 g/dl 2-14 years: 11.5 g/dl 15-18 years: 12.0 g/dl (girls), 13.0 g/dl (boys) • Platelet count ≥ 150 x 10^9/l.
Good partial response (GPR):	**All criteria below have to be fulfilled:** • No platelet or red cell transfusion • Self sustained haemoglobin ≥ 6.0 g/dl • Platelet count ≥ 50 x 10^9/l and ANC ≥ 1.0 x 10^9/l
Poor partial response (PPR)	**All criteria below have to be fulfilled:** • No platelet or red cell transfusion • Self sustained haemoglobin ≥ 6.0 g/dl • Platelet count ≥ 20 x 10^9/l and ANC ≥ 0.5 x 10^9/l
Non-response (NR):	**Neither PR nor CR is reached**

Table 1. Definitions of haematological response after IST

The dates of achieving haematological PR and CR following initiation of IST are reported. The date of PR is the date of first CBC indicating PR 28 days after the last platelet or red cell transfusion, the date of CR is reported accordingly. PR and CR should sustain for a minimum of 3 consecutive blood counts over a period of at least 28 days.

3 Continuation of therapy according to response status

Patients usually do not respond to IST immediately and it frequently takes several weeks until response to IST can be observed. However, the majority of responses occur before day 180.[30] Therefore, HSCT should generally not be performed before day 180 unless there is an urgent clinical need such as persistent very severe neutropenia, recurrent or severe infections, or persistent and unusually high transfusion requirement.

It should also be noted that patients with a fulminant disease and agranulocytosis (ANC 0) at diagnosis may have delayed response to IST (> day 180).[31] Indeed 41% of patients in the EWOG-SAA 2010 had ANC of 0 before IST and we observed a high incidence of late responses (> 180 days) among non-responders at day 180 (12/59=20%). In such cases with a fulminant disease, the timing of second-line

HSCT should be individually evaluated, if patients have a sign of response (e.g. increasing ANC) without severe neutropenia (<0.5 x 10^9/l), but do not fulfill the criteria of PR/CR at day 180.

Patients with persistent severe neutropenia (<0.2 x 10^9/l) at day 90 have a high risk of mortality. The internal analysis of the EWOG-SAA showed that overall survival was significantly lower in patients with less than 0.2 x 10^9/l neutrophils compared to those with 0.2 x 10^9/l or more neutrophils among non-responders at day 90 (78% vs 95%, p=0.01). Patients with severe neutropenia might benefit from early HSCT and therefore **patients with ANC of <200/µl at day 90 should proceed to HSCT.**

Time point of evaluation of response and procedures

Day 90 after initiation of IST:

- Neutrophil Non-Response (ANC < 0.2 x 10^9/l): patients should proceed to HSCT.
- Other NR (ANC $\geq$ 0.2 x 10^9/l), PPR or GPR: continue CSA

Day 120 after initiation of IST:

- Neutrophil Non-Response (ANC < 0.2 x 10^9/l): patients should proceed to HSCT
- Other NR (ANC ≥ 0.2 x 10^9/l) preparation of HSCT and proceed to HSCT if the patient is still in NR at day 180
- CR, PPR or GPR: continue CSA

Day 180 after initiation of IST:

- NR: patients generally qualify for HSCT. Individual decision whether HSCT may be postponed if patients show increasing neutrophils and ANC of >500/µl without fulfilling the criteria of PPR.
- PPR or GPR: continue CSA
- For patients in CR for ≥ 2 months, CSA should be tapered slowly (10% per month) under regular monitoring of blood counts (every other week).

CR at any time after day 180:

- For patients in CR for ≥ 2 months, CSA should be tapered slowly (10% per month) under regular monitoring of blood counts (every other week).

Day 240 after initiation of IST:

- PPR: proceed to HSCT when an alternative 9/10 identical donor (based on HR molecular typing) is available.
- GPR: continue CSA

Day 360 after initiation of IST:

- CSA should be slowly tapered (10% per month) regardless of response status.

4 Definition and Management of Relapse

Generally, falling blood counts should be documented in two consecutive CBCs within a 1-2 week time period. Other causes of decreasing blood counts are to be excluded. Relapse is defined for patients with CR, GPR or PPR irrespective whether they are on or off CSA. For these patients, relapse is defined as fulfilling again the definition of severe aplastic anaemia.

4.1 Patients on Cyclosporine

Patients with relapse on CSA qualify for HSCT.

4.2 Patients off Cyclosporine or during Tapering of Cyclosporine

Patients with relapse off CSA qualify for HSCT as soon as possible. Reintroduction of CSA can be considered until HSCT is available.

The EWOG-SAA Consensus for Hematopoietic Stem Cell Transplantation in Aplastic Anaemia

1 Introduction

Acquired SAA was one of the first diseases in which bone marrow transplantation has successfully been applied to cure bone marrow failure. HSCT from a matched sibling donor is still the treatment of choice in young patients with SAA. Rejection is the major cause of treatment failure and therefore the conditioning regimen has to be highly immunosuppressive.[32] To reduce long term sequelae,[26,33] irradiation based regimens have been replaced by cyclophosphamide based protocols.[34] In children, cyclophosphamide in combination with ATG is the commonly used regimen with an overall survival of 80-95%.[4,35] For GvHD prophylaxis the combination of CSA and methotrexate has resulted in superior outcome compared to single drug prophylaxis,[36] and there is sufficient evidence that the use of bone marrow as the stem cell source is associated with less chronic GvHD and better survival especially in children. [37,38] In case of non-engraftment or early rejection re-transplantation from the same or an alternative donor is successful in up to 60%.[39,40] Outcome after matched unrelated donor transplants in children with SAA has significantly improved in recent years resulting in probabilities of overall survival as high as 95%.[41,42] In view of these improvements upfront unrelated HSCT has been performed in an increasing number of patients resulting in outcomes comparable to the ones of patients being transplanted from MSD.[43,44] Given these excellent results and the relatively high incidence of early and late treatment failure of immunosuppressive therapy, leading to salvage stem cell transplantation, there is an ongoing debate whether upfront HSCT should be offered to young patients for whom a well matched unrelated donor is available in a reasonable time frame. HLA typing and the search for a suitable donor should therefore be initiated immediately after diagnosis of aplastic anemia.

1.1 Indication and Time Point

MSD-HSCT is the therapy of choice in young patients with SAA and in the presence of a MSD HSCT should be performed as soon as possible.

HSCT after failure of IST should generally not be performed before 6 months after start of IST unless there is an urgent clinical need such as persistent very severe neutropenia, recurrent or severe infections or persistent and unusually high transfusion requirement.

Upfront unrelated HSCT has been shown to result in a similar outcome compared to MSD-HSCT and possibly in a superior outcome compared to MUD-HSCT after failure of IST[43,44]. In an EWOG-SAA interim analysis (10/2022) 17/18 (94%) patients transplanted from a ≥ 9/10 HLA matched unrelated donor with no previous IST are alive with engraftment compared to 47/55 (85%) transplanted after failure of IST. Acute GvHD °II-IV and chronic GvHD was observed in 17% and 11% of patients, respectively. **Upfront MUD-HSCT therefore might be considered in cases where a 10/10 HLA matched donor is available, and HSCT can be performed within 2-3 months.**

1.2 Conditioning regimen

Although ATG has traditionally been part of the conditioning regimen in MSD-HSCT in SAA, it has failed to prove superiority in a randomized trial comparing cyclophosphamide vs cyclophosphamide/ATG[45]. The EWOG-SAA 2010 protocol therefore had recommended a cyclophosphamide only conditioning regimen for MSD-HSCT. However, the regimen was not well accepted and in an interim analysis, 29/49 patients transplanted from a MSD had received ATG. The outcome in both groups was comparable with an excellent overall survival (95 and 97%), good engraftment and a low rate of acute and chronic GvHD. As expected, there was a tendency to more viral infections in the ATG group. Acknowledging the limited acceptance of an ATG free conditioning regimen and the beneficial effects on engraftment and GvHD in published series the reintroduction of a **reduced dose of ATG to the conditioning regimen in MSD-HSCT** is now recommended. Recent data suggests that the efficacy of ATG and most importantly the effect on immune reconstitution is strongly correlated with the lymphocyte count at the first day of application indicating a role for individualized dosing.[46] The individualized dosing based on lymphocyte count, body weight and stem cell source has resulted in a better immune reconstitution without an increase in graft failure or GvHD in patients being transplanted for malignant as well as non-malignant disorders following a myeloablative regimen. Although it remains to be determined whether these observations can be confirmed in patients with SAA being transplanted with a reduced intensity regimen, we suggest that the strategy can be considered for MSD-HSCT given the low risk of graft failure and GvHD. Based on the higher risk of graft failure and GvHD for patients being transplanted from a MUD the strategy of individualized ATG dosing should only be applied in a controlled setting.

The addition of fludarabine to the conditioning regimen has resulted in better engraftment rates and the possibility to reduce the dose of cyclophosphamide.[47-50] EWOG-SAA has previously recommended a fludarabine containing regimen (Flu/Cy/ATG) for MUD-HSCT. With the aim of sparing short and long-term toxicity of high dose cyclophosphamide we now recommend the **same chemotherapy backbone (Flu 120 mg/m^2 / Cy 100 mg/kg) for patients being transplanted from a MSD.**

1.3 Immune reconstitution after HSCT

In vivo T-cell depletion with ATG or alemtuzumab has long been part of the conditioning regimen in patients transplanted for aplastic anemia. All ATG products and alemtuzumab have been shown to ensure engraftment and reduce the rate of acute and chronic GvHD. However, they are also associated with slow or impaired immune recovery and associated increase in viral infections. EWOG-SAA therefore aims to thoroughly **document immune reconstitution** and analyze the impact of immune reconstitution on standard outcomes.

2 Description of the Consensus on HSCT from an HLA-identical Sibling Donor

Donor and HLA Typing

HLA-identical sibling donor: High resolution (HR) molecular typing for both class I (i.e. A*, B* and C*) and class II loci (DRB1* and DQB1*) is recommended.

Stem cell source and graft composition

Unmanipulated bone marrow optimally with > $3.5x10^8$ NC/kg.

BM is the preferred stem cell source. In cases where PBSC are used in vivo T-cell depletion should be adapted (i.e. increase the dose of ATG Grafalon Neovii to 4 x 15 mg/kg).

Conditioning regimen

Preparation will consist of cyclophosphamide (25 mg/kg/day for 4 days from day –6 to day –3) and fludarabine (30 mg/m^2/day for 4 days from day –6 to day –3). In addition, rabbit ATG (ATG Grafalon Neovii 10 mg/kg/day for 4 days from day -6 to day –3) will be used during the conditioning regimen for *in vivo* T-cell depletion/modulation. Alternatively, Thymoglobulin® or an individualized dosing of ATG according to the publication of Admiraal et al might be considered.[46]

HSCT, MSD				
Time	Drug and Dosing			
Day -6	Flu 30 mg/m^2	Cy 25 mg/kg	BM:	ATG Grafalon® 10 mg/kg or Thymoglobulin® 2 mg/k
			PBSC:	ATG Grafalon® 15 mg/kg or Thymoglobulin® 2.5 mg/kg
				or individualised dosing (see Appendix)
Day -5	Flu 30 mg/m^2	Cy 25 mg/kg	BM:	ATG Grafalon® 10 mg/kg or Thymoglobulin® 2 mg/k
			PBSC:	ATG Grafalon® 15 mg/kg or Thymoglobulin® 2.5 mg/kg
				or individualised dosing (see Appendix)
Day -4	Flu 30 mg/m^2	Cy 25 mg/kg	BM:	ATG Grafalon® 10 mg/kg or Thymoglobulin® 2 mg/k
			PBSC:	ATG Grafalon® 15 mg/kg or Thymoglobulin® 2.5 mg/kg
				or individualised dosing (see Appendix)
Day -3	Flu 30 mg/m^2	Cy 25 mg/kg	BM:	ATG Grafalon® 10 mg/kg or Thymoglobulin® 2 mg/k
			PBSC:	ATG Grafalon® 15 mg/kg or Thymoglobulin® 2.5 mg/kg
				or individualised dosing (see Appendix)
Day -2	Rest			
Day -1				
Day 0	Allograft			

Strategy for GvHD prophylaxis and treatment

CSA 2.5 mg/Kg in 2-hour infusion twice a day (total dose 5 mg/Kg/day) starting from day –1 and with the objective of maintaining serum trough levels of approximately 200 ng/mL plus MTX: 3 doses on day +1, +3

and +6 at a dosage of 10 mg/m^2. It is essential to achieve a sufficient CSA level early in the course of transplant to avoid the risk of GvHD. In case of intolerance to i.v. CSA, switch to oral CSA or tacrolimus can be considered.

CSA tapering should be started around day +100 in the absence of acute GvHD and a stable donor chimerism. CSA discontinuation should take place around day +180.

Patients with acute GvHD should initially receive systemic steroids at a dosage of 2 mg/Kg. If regression occurs, steroids should be slowly tapered and discontinued. For persistent or progressive GvHD second line treatment, according to each center policy, is recommended.

Monitoring of chimerism

Monitoring of chimerism should start at time of neutrophil recovery. In general, these analyses are performed on day +30, +60, +100, +180 and +360 and at later time points according to the clinical requirements. Of note, patients with SAA might have complete haematological recovery in the presence of a mixed chimerism and there is no indication for performing DLI in patients with mixed chimerism.

Monitoring of immune reconstitution

Immune reconstitution should be monitored analysing the total lymphocyte count (TLC) and lymphocyte subsets. It is recommended that these analyses (TLC, CD3, CD4, CD8, CD19, CD56) are performed day +30, +60, +100, +180 and +360. In addition, measurements of CD4/CD45RA (day +100, +180 and +360) and the need for IVIG replacement should be documented.

3 Description of the Consensus on HSCT from a matched unrelated Donor

Donor and HLA typing

Unrelated BM/PBSC donor: identical or with a single antigen or allelic disparity (9-10/10) with the recipient using HR molecular typing for class I and II loci (i.e. A*, B*, C*, DRB1* and DQB1*).

Stem cell source and graft composition

Unmanipulated BM: > 3.5x10^8 NC/kg

Unmanipulated PBSC: > 4x10^6 CD34+ cells/kg and < 10x10^6 CD34+ cells/Kg. Although BM remains the preferred stem cell source, PBSC is frequently the only available stem cell product and the role of in-vivo and possibly in vitro T-cell depletion must carefully be considered in these cases.

Conditioning regimen

Preparation will consist of cyclophosphamide (25 mg/kg/day for 4 days from day –6 to day –3) and fludarabine (30 mg/m^2/day for 4 days from day –6 to day –3). In addition, rabbit ATG (ATG Grafalon Neovii 15 mg/kg/day or Thymoglobulin Genzyme 2.5 mg/kg/day for 4 days from day -6 to day –3) will be used during the conditioning regimen for *in vivo* T-cell depletion/modulation. The individualized dosing of thymoglobuline® according to the publication of Admiraal et al should only be considered in a controlled setting.[46]

HSCT, ≥ 9/10 HLA compatibel unrelated donor				
Time	Drug and Dosing			
Day -6	Flu 30 mg/m^2	Cy 25 mg/kg	BM/PBSC:	ATG Grafalon® 15 mg/kg or Thymoglobulin® 2.5 mg/kg or indiviualised dosing (see Appendix)
Day -5	Flu 30 mg/m^2	Cy 25 mg/kg	BM/PBSC:	ATG Grafalon® 15 mg/kg or Thymoglobulin® 2.5 mg/kg or indiviualised dosing (see Appendix)
Day -4	Flu 30 mg/m^2	Cy 25 mg/kg	BM/PBSC:	ATG Grafalon® 15 mg/kg or Thymoglobulin® 2.5 mg/kg or indiviualised dosing (see Appendix)
Day -3	Flu 30 mg/m^2	Cy 25 mg/kg	BM/PBSC:	ATG Grafalon® 15 mg/kg or Thymoglobulin® 2.5 mg/kg or indiviualised dosing (see Appendix)
Day -2				
Day -1				
Day 0	Allograft			

Strategy for GvHD prophylaxis and treatment

CSA 2.5 mg/Kg in 2-hour infusion twice a day (total dose 5 mg/Kg/day) starting from day –1 and with the objective of maintaining serum trough levels of approximately 200 ng/mL plus MTX: 3 doses on day +1, +3 and +6 at a dosage of 10 mg/m^2. It is essential to achieve a sufficient CSA level early in the course of transplant to avoid the risk of GvHD. In case of intolerance to i.v. CSA, switch to oral CSA or tacrolimus can be considered.

CSA tapering should be started around day +100 in the absence of acute GvHD and a stable donor chimerism. CSA discontinuation should take place around day +180.

Patients with acute GvHD should initially receive systemic steroids at a dosage of 2 mg/Kg. If regression occurs, steroids should be slowly tapered and discontinued. For persistent or progressive GvHD second line treatment, according to each center policy, is recommended.

Monitoring of chimerism

Monitoring of chimerism should start at time of neutrophil recovery. In general, these analyses are performed on day +30, +60, +100, +180 and +360 and at later time points according to the clinical requirements. Of note, patients with SAA might have complete haematological recovery in the presence of a mixed chimerism and there is no indication for performing DLI in patients with mixed chimerism.

Monitoring of immune reconstitution

Immune reconstitution should be monitored analysing the total lymphocyte count (TLC) and lymphocyte subsets. It is recommended that these analyses (TLC, CD3, CD4, CD8, CD19, CD56) are performed day +30, +60, +100, +180 and +360. In addition, measurements of CD4/CD45RA (day +100, +180 and +360) and the need for IVIG replacement should be documented.

4 HSCT from an alternative Donor

Although there is an increasing amount of data for alternative donor HSCT in aplastic anemia, there is no consensus on the best approach in the absence of a matched unrelated donor. Patients with indication for HSCT and no matched donor available might therefore be included in ongoing clinical trials of alternative donor HSCT in non-malignant disease. Indications include refractoriness to IST, relapse following IST and patients with severe infections and therefore the need for timely neutrophil recovery.

In the setting of haploidentical donors the most promising results were achieved with unmanipulated bone marrow and post-transplantation cyclophosphamide.[51-54] The conditioning regimen in a North American Phase II trial as well as a Brazilian case series consisted of fludarabine, cyclophosphamide and a single dose of 2 Gy TBI. The probability of overall survival was 81% at 1 year in the North American trial (n=32) and 79% at 2 years in the Brazilian case series (n=87). Both groups described a relevant risk of graft failure for the whole cohort ranging from 11 to 20% that was lower once the conditioning was intensified with a higher dose of irradiation (TBI 400 cGy). Although these are impressive results, the use of irradiation is a major concern in the pediatric population. In-vitro T-cell depletion has also been applied in patients with SAA included in larger series of haploidentical HSCT in non-malignant disease. Although the overall results of the whole cohort were impressive with a probability of overall survival of 91.4 % and an acceptable TRM (8.4%) the incidence of graft failure was rather high in patients with an increased risk of GF including SAA (CI of GF 55.5%).[55] It remains to be demonstrated whether more intensive conditioning regimens including alkylating agents such as thiotepa or treosulfan decrease the risk of graft failure in T-cell replete as well as deplete approaches.

Cord blood transplantation has rarely been applied in aplastic anemia. The most promising results were published by the French group. [56] They were able to demonstrate a probability of overall survival at 2 years of 88% in 26 patients using a fludarabine, cyclophosphamide, ATG and 2 Gy TBI regimen. The engraftment rate was high (88%), however the rate of grade II-IV acute and chronic GvHD were 45.8% and 36%, respectively.

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