Version 1

The complete treatment algorithm for SAA

An observational audit proposed by the WPSAA of the EBMT

Acquired Aplastic Anemia is a rare disease. Treatment consists of immunosuppressive treatment or stem cell transplantation, both of which have improved outcome greatly. There are, however, a substantial number of patients not responding to treatment or relapsing. Stem cell transplantation has been expanded to include donors other than identical siblings (unrelated donors, haploidentical donors) and cord blood as a stem cell source. The best use of these options remains unknown.

We here propose a complete treatment algorithm taking into account different sub protocols for different patient populations, age categories and availability of different types of donors to treat this disease.

Please be aware that some of this treatment recommendations are standard treatment approaches whereas others are less well established e.g. cord blood transplantation or IS with Campath and may be considered experimental. For such protocol the nature or the treatment needs to be discussed with the patient in detail with the alternative option of continuing supportive care clearly outlined.

If a specific center is following a local protocol patients may still be included. For centers not having their own protocol these recommendations as outlined here may be used as the groups' best possible treatment option.

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Patient registration:

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Every patient with newly diagnosed acquired aplastic anemia will be registered with the EBMT and will receive a UPN composed of CIC and aplastic anemia observational audit specific code

There will be an email registration with

Rosi.oneto@gitmo.net

With the following information

UPN:	_____
CIC (Center identification code):	_____
Physician	_____
Patient initials	_____
Patient birthdate	__/__/_____
Patient gender	_m_ / _f_
Diagnosis:	VSAA / SAA / moderate AA
Etiology	idiopathic / hepatitis / drugs / other
Diagnosis date	__/__/_____
Blood counts at diagnosis	Reticulocytes x10e9/l
	Platelets x 10e9/l (indicate if transfused)
	Neutrophils x 10e9/l
Planned initial treatment	_____

Patient consent

Dear patient

Data on your disease, aplastic anemia and the treatment will be collected in a non identifiable fashion (your name will not appear on the forms) by the EBMT the European Group for Blood and Marrow Transplantation. The purpose of this registration is to improve outcome for patients with this disease to treat them in the same way across Europe and to better understand which treatment works best. This registration will inform EBMT about the fact that you exist, that the diagnosis of aplastic anemia has been established as well as the severity of the disease and that you have made a decision as to the treatment together with your treating physician.

For this purpose registration is undertaken at the time of diagnosis. Later results of the treatment will be reported. The European Union has issued a directive (95/46/EC) regulating collection and storage of personal data. The EBMT database is held in the Netherlands.

In order to meet this regulation we ask for your consent.

I _____ / _____ have been informed to my satisfaction regarding reporting of my personal data to the EBMT and consent to the data being reported anonymously.

_____ Date, Signature

Diagnosis of acquired aplastic anemia:

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The diagnosis of Aplastic Anemia (AA) may be difficult and sometimes needs repeated marrow investigations. There are three diagnostic steps in AA.

Confirm the suspicion of AA and exclude other bone marrow failure diseases

Define the severity of the disease

Characterize the AA

Confirm the suspicion of AA and exclude other bone marrow failure diseases

For the diagnosis of AA the presence of pancytopenia and proof of an empty bone marrow is mandatory. Bone marrow hypocellularity in AA has been set as < 30% hematopoietic cells. However this definition has been established mainly for children and young adults. In healthy elderly patients bone marrow cellularity is physiologically decreased because of age. Therefore, cut-off <30% may not be applicable for elderly patients. Hematopoietic cellularity has to be assessed on trephine biopsy by examining 4-5 undistorted fields under 100 x magnifications. Plasma cells, lymphocytes sometimes forming follicles and mast cells are typical findings, but can be a confounding factor. They have to be excluded in the global evaluation of hematopoietic cellularity.

All congenital bone marrow failure syndromes as well as radiation- or chemotherapy induced aplasia are excluded from the acquired AA.

One of the most difficult diagnostic task is to distinguish AA from hypoplastic MDS. Both marrow failure syndromes show markedly hypocellular bone marrow with increased fat cells. The absence of dysplasia and/or blast cells as well as the lack of increased number of CD34-positive cells identified by immunohistochemistry are the most conspicuous elements supporting the diagnosis of AA. Nevertheless in many cases only the changes in the karyotype for instance the presence of a monosomy 7, are the only criterion in favor of a hypoplastic MDS. In the following table characteristics of both entities are summarized:

	AA	Hypoplastic MDS
Splenomegaly at diagnosis	absent	possible
Cytopenia	present	present
Dysplasia		

Erythropoiesis	possible	possible
Myelopoiesis	absent	possible
Megakaryopoiesis	absent	possible
Blasts	absent	variable
CD34+ immunohistochemistry	not increased	normal or increased
Marrow fibrosis	absent	possible
Karyotype	usually normal.	-7/del(7q) -5/del(5q)

*however transient clonal abnormality such as for instance trisomy 8, or some particular abnormality can be found in typical AA

Define the severity of the disease

Once the diagnosis of AA established, the severity of the disease has to be defined. The severity of the disease is exclusively based on values from the peripheral blood. There are three severity groups of AA

Severe AA (SAA)

For SAA at least two of the following three criteria have to be fulfilled:

Reticulocytes $<60x10^9$/L (using an automated analyzer) or $< 20 \times 10^9$/l (manual count)

Platelets $< 20x10^9$/L

Absolute neutrophils $<0.5 \times 10^9$/L

Very severe AA (vSAA)

For vSAA, the same criteria of SAA have to be fulfilled; however the absolute neutrophil count has to be $< 0.2 \times 10^9$/l

Moderate AA

Moderate AA is considered when the severity criteria of SAA are not fulfilled.

Characterize the AA

Aplastic anemia and PNH

There is a close interrelationship between AA and PNH. Patients with typical PNH can develop AA in the course of their disease and patients with AA often present a PNH clone. With flow cytometry it is now possible to detect very small clones of PNH. About 40-50% of the patients with acquired AA have a detectable PNH clone. Most clones are small and patients do not have symptoms related to PNH. However, in some patients the PNH clone can increase after immunosuppressive treatment and the patient presents the typical symptoms and complications of the disease. There are some data predicting a better response to immunosuppressive therapy in AA-patients with significant GPI-AP-deficiency.

Aplastic anemia and HLA-DR2 / HLA-DRB1*15

HLA-DR2 and particularly HLA-DRB1*15 (DRB1*1501 and DRB1*1502) has been shown to be involved in the development and the outcome of AA, AA patients possessing HLA-DR15 tend to be older; more than 50% of the patients with HLA-DRB1*1502 are older than 40 years of age. DRB1*1501 seems to be associated with the presence of a small population of PNH-type cells and a good response to the immunosuppressive therapy, in Japanese patients. In a recent study (Song et al, Hum Immunol 2010) of 37 Korean patients with severe AA responders to immunosuppressive treatment had a significant higher HLA-DR15 and lower DR4 frequency compared with non-responders. The response rates were the best in DR15+/DR4- patients), intermediate, and poor in DR15-/DR4+ patients were 88.9, 38.5, and 0%, respectively ($p = 0.00001$). At the allelic level, DRB1*1501 and closely linked DQB1*0602 were associated with a good response and DRB1*0405 and closely linked DQB1*0401 with a poor response to IST. HLA-DR typing might be useful for predicting a response to immunosuppression in AA patients.

Hepatitis associated Aplastic Anemia

Seronegative hepatitis is documented in 5-10% of patients with acquired AA (Young NS, BJH 1986; Mary JY, Blood 1990; Locasciulli 2010). An infectious agent however has not yet been identified. It typically occurs in young, healthy males with severe but self-limited liver inflammation. A common inciting infectious cause could be involved (Brown KE et al, NEJM 1997). Indeed, in hepatitis-associated AA, similar skewed T-cell repertoires have been detected in the liver and in the peripheral blood lymphocytes (Lu J et al, Blood 2004). Patients with post-hepatitis AA do not respond differently to immunosuppressive treatment compared to patients with idiopathic acquired AA.

Aplastic anemia associated with other autoimmune disorders (AID)

Associations of AA and other autoimmune disease (AID) have been shown in single case reports (Antic M et al, Hinterberger-Fischer M et al.). In a recent single centre report 5.3% of AA patient had an AID before the diagnosis of AA and 4.5% of them developed an AID after diagnosis and treatment for AA. AID can appear at any time before or after the AA. The frequency of a concomitant AID seems higher in older AA-patients; hence more than 25% of AA-patients diagnosed after 50 years of age presented a concomitant AID. In a large multicenter study of the SAAWP of the EBMT (data not yet published), 50 of 1251 AA-patients had a former diagnosis of AID. Whether the immunosuppression applied to treat the AA has an influence on the outcome of the AID remains a controversial topic. In

consideration of the high frequency of a concomitant AID in AA patients, it is unlikely that both diseases appear together just by chance.

Differential Diagnosis

Differential diagnosis includes any type of pancytopenia with hypoplastic bone marrow. They include

Congenital marrow failure syndromes

Fanconi Anemia (FA)
Dyskeratosis Congenita (DC)
Congenital Amegakaryocytic Thrombocytopenia (CAMT) in aplastic phase.
Schwachman–Diamond Syndrome.
Blackfan Diamond Anemia (Congenital hypoplastic anemia).

Hypocellular Refractory Cytopenia of unknown significance

Hypoplastic MDS or acute leukemia

Pure Erythroaplasia

Pure white cell aplasia

T-LGL-leukemia with pancytopenia

Suggested Diagnostic Workup for AA

Full blood count with reticulocyte count (automated or microscopic counting)

Peripheral blood film examination

PNH clone with a sensitive multicolor flow cytometry (for instance CD11b, 66b, CD55, CD59for granulocytes, CD 14/CD33/CD45 for monocytes, CD 59 and CD 55 for erythroid cells). In case of lack of monocytes and neutrophils, search of the PNH clone on lymphocytes should be performed.

Viral hepatitis studies (serological and DNA/RNA).

BM aspirate for morphology, cytogenetic, FISH-analysis (search for -7; +8), immunophenotyping, Pearls staining,

(Colony assay – research tool, not routine clinical diagnostic test in most centres), viral (HIV, CMV, EBV) and microbiological studies.

Marrow trephine biopsy assessing overall hematopoietic cellularity, single lineage cellularity, ALIP, blasts (CD34, CD117) and fibrosis.

DEB or MMC sensitivity test. Cell cycle analysis by flow cytometry may be accepted for differential diagnosis with FA.

Mutation analysis of TERC/TERT (for differential diagnosis with hidden forms of autosomal dominant Dyskeratosis Congenita), cMPL (for differential diagnosis with Congenital Amegakaryocytic Thrombocytopenia), Schwachman–Diamond genes. These studies apply particularly to younger patients. These analyses are not yet available as routine clinical diagnostic tests – they are currently only performed by a small number of research laboratories.

HLA-typing (search for HLA-DRB1*15) and family typing when patients eligible for HSCT

Auxiliary tests include:

-Vertebral MRI (uniformly fatty marrow in AA *vs.* a spotty mixture of hypo and hyper cellular marrow in MDS).

-HbF, often increased as expression of marrow stress.

-Serum bilirubin and LDH possibly increased due to minor degree of dyserythropoiesis, haptoglobin decreased due to minor degree of dyserythropoiesis.

. Vitamin B12, folic acid, ferritin, fibrinogen.

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Supportive Care in Aplastic Anemia:

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General medical management of hospitalized aplastic anemia patients

Patients with aplastic anemia should be managed by a multidisciplinary team. Patients with low neutrophil counts are at risk for severe infections and management of these patients should therefore be restricted to experienced centers.

If the patient's condition allows (ECOG 0-2), the evaluation of patients with the possible diagnosis of aplastic anemia (AA) can be done as an out-patient.

In case of severe or very severe aplastic anemia the usual rules have to be observed: Flowers and plants as potential source of fungal spores and Pseudomonas should be avoided. Low microbial food is recommended (fruits and vegetables have to be boiled or peeled, nuts or dried fruits should be avoided, milk products and meat have to be well boiled or pasteurized). In case of severe mucositis parenteral nutrition may be necessary. Hospitalization should preferably be in one or two beds rooms with en-suite facilities. Isolated and laminar air flow facilities are not imperative but should be used if available.

Staff should follow local guidelines regarding clothing and hygienic routines. As a minimum, hand washing and rubbing with alcohol based disinfection solutions must be used before and after handling the patient. Protective clothing should be used when a staff member comes in direct contact with the patient but this is not necessary for casual contact.

There should be procedures in place for management of central and peripheral venous catheters. Staff handling venous lines should wash their hands, use alcohol based disinfectant, and use gloves and suitable protective clothing depending on the procedure. Dressings covering central venous lines should be changed regularly.

Alcohol-based solutions for hand disinfection should be provided

Individual hygiene rules should be explained and applied to the patient and visitors. The use of toothbrushes could be limited in patients with severe thrombocytopenia; vigorous brushing is to be avoided and may be replaced by antiseptic medical mouth rinse.

Bed sores must be avoided. Additionally passive mobilization and breathing exercises by a physical therapist may be helpful. Physical exercises are recommended if the patient is well enough.

Daily physical examination (especially infection and bleeding signs) and monitoring of vital parameters (blood pressure, heart frequency, temperature) as well as physical parameters like weight, respiration, stools and diuresis have to be evaluated.

Blood counts should be monitored regularly, usually 3 times/week. Other blood tests will be taken as required depending on the patient's status and given treatment.

General medical management of non- severe aplastic anemia patients

The non-severe AA patient can be followed as an outpatient. Recommendations vary depending on blood counts and the personal risk of the patient. In case of a reduced neutrophil count measures to avoid infections should be taken. In mainly thrombocytopenic patients the focus will be the prevention of bleeding complications. For example hormone therapy for women to control menorrhagia.

The blood counts should be followed in all patients with non-severe aplastic anemia regularly to permit early detection of disease progression.

Prevention and treatment of infections

Prevention of infections

Patients with aplastic anemia are at risk for bacterial and fungal infections. The individual risk of a patient is mainly determined by the neutrophil, and monocyte counts.

Severe aplastic anemia patients with prolonged periods of severe neutropenia have a high mortality by fungal (Aspergillus) infections. Therefore prophylactic antifungals are often used but there are no uniform recommendations. In our opinion for patients with very severe aplastic anemia (neutrophils $< 0.2 \times 10^9$/l) prophylactic antifungals should be used in general. Itraconazole, voriconazole or posaconazole appear to be more effective than fluconazole, as they have activity against Aspergillus whereas fluconazole does not. In patients with a higher neutrophil count use of antifungal prophylaxis should be decided based on local experience with fungal infections.

Prophylactic antibiotics may prevent Gram-negative sepsis in severe aplastic anemia patients. It is not known if quinolone antibiotics (such as ciprofloxacin) or a combination of non-absorbable antibiotics (such as neomycin and colistin) are most effective. Thus the choice of antibiotic prophylaxis should be taken by the treating centre and should depend on the local microbiological flora and rates of resistance.

There are no general recommendations for antiviral prophylaxis and Pneumocystis jirovecii pneumonia prophylaxis. In transplanted patients antiviral prophylaxis with aciclovir and PJP-prophylaxis is routinely given. For patients after immunosuppressive therapy with ATG the usage of antiviral and PJP-prophylaxis depends on the individual centre but many use a combination of aciclovir and cotrimoxazole.

Vaccination strategies for aplastic anemia patients are not well defined except for patients undergoing transplantation where recommendations are well established. Some centre adopt the recommendations for pneumococcal and influenza vaccination for patients with AA not undergoing transplantation, but other do not because of concern about relapse of aplastic

anaemia following vaccinations in general. There is , however, no evidence to support that vaccinations are a risk factor for aplastic anemia relapse.

Treatment of infections

Fever with neutropenia is an indication for immediate hospitalization. Diagnostic procedures should include careful physical examination, blood cultures and cultures from other relevant sites while chest X-ray is optional. However, treatment of infection must be started at once without waiting for the. Culture results . Therefore, local hospital guidelines for treatment of febrile neutropenia should be followed. In many centers this includes a combination of a broad spectrum ß-lactam antibiotic and an aminoglycoside. In addition patient history and recent medication should also be taken into consideration.

In cases of persisting fever or previous or suspected fungal infection, systemic antifungal therapy should be used early in the therapy of fever in neutropenic aplastic anemia patients. In these cases CT scanning of the chest should be performed.

In the case of suspected viral infections, antiviral therapy should be included in the therapeutic regimen.

The use of G-CSF (5µg/kg per day) may result in a temporary increase of neutrophils and may therefore be beneficial. Even though there are no guidelines it is clear from several studies that prophylactic growth factors do not improve overall results but growth factors are regularly given when treating infectious complications of AA.

In life threatening infections during neutropenia the use of irradiated granulocyte transfusions should be discussed with the awareness of limited data to support this procedure and possible side effects.

Hematopoietic growth factors

There are currently no data to support the routine use of growth factors in aplastic anemia. G-CSF is often used in neutropenic infections.

Transfusion therapy

Platelet and red blood cell transfusions should be given to reduce the risk of bleeding complications and anaemia and to maintain quality of life.

Platelet transfusions: Hospitalized aplastic anemia patients should receive prophylactic platelet transfusions in case of platelets < 10 x 10^9/l without fever, bleeding signs or history of major bleeding events. Hospitalized aplastic anemia patients with fever, or bleeding signs or history of relevant bleeding (for example cerebral bleeding) should receive prophylactic platelet transfusions in case of platelets < 20 x 10^9/l. For invasive procedures platelet transfusions must be given to achieve the recommended levels. There are no data that the use

of non-HLA-matched apheresis platelet concentrates are superior to pooled platelet concentrates. Transfusions are often withheld of aplastic anemia patients for the fear of alloimmunization and an increased risk of graft rejection after allogeneic SCT. But corresponding data are old and from a period before leukocyte depleted blood products were routinely used. It is likely that universal leukoreduction of blood products has reduced patient alloimmunization although further studies are needed to confirm this.

In the case of inadequate platelet count increment after platelet transfusion, screening for HLA-antibodies should be performed. If HLA-antibodies are detected, HLA-matched platelets should be used for the further transfusions.

Red blood cell (RBC) transfusions

A safe hemoglobin level depends on co-morbidities and the physical state of the patient. Therefore the decision to transfuse RBCs depends on clinical symptoms, Hb-value and quality of life. In this context the transfusion trigger in the most aplastic anemia patients range between 80-85 g /L. But patients with cardiac, pulmonary or cerebral co-morbidities may require a higher transfusion trigger.

Fresh frozen plasma and granulocyte transfusions

The indications for FFP in aplastic anemia patients are the same as in the universal guidelines. Granulocyte transfusions may be considered in life threatening infections with neutropenia.

Irradiation and CMV-testing

There is lack of an evidence base for using irradiated blood products in aplastic anemia patients, especially since introduction of the universal leukoreduction. Nevertheless, following a recent EBMT SAAWP survey, is it recommended to irradiate all blood products for aplastic anemia patients during and after IST whether with ATG or Campath, and to continue for at least as long as patients are immunosuppressed with a reduced CD4/CD8 ratio or a minimum of 6 months after IST. Patients undergoing HSCT must receive only irradiated blood products, as for all other allogeneic HSCT patients.

Granulocyte transfusions must be irradiated in every case as well as HLA-matched platelets, or blood products from family donors.

In general, there is no need for CMV-negative blood products given universal leukodepletion, but some centers give only CMV negative blood products for patients undergoing HSCT where both the patient and donor are CMV negative.

Iron chelation therapy

Recently iron overload has been recognized as a poor prognosis factor for SCT as well as a potential factor for worsening of bone marrow function. Therefore iron chelation therapy should be taken in account in aplastic anemia with serum ferritin level > 1000 µg/l. On the other hand due to potential side effects like renal, hepatic, cardiac function impairment and

case reports of associated cytopenias a risk –benefit –analysis for every individual patient should be performed. Nevertheless for patients in remission after SCT or immunosuppressive therapy and with iron overload, phlebotomy is the method of choice with the fewest side effects.

Androgens

Androgens have been widely used in the past for the treatment of aplastic anemia, (Blood 1990; 76:2222), but had been largely abandoned due to side effects (hirsutism and hepatic toxicity), with the advent of cyclosporine in the management of SAA and with the lack of a significant efficacy. A prospective randomized EBMT study had shown that response was improved in females given androgens up front, together with anti-lymphocyte globulin (BJH, 1993; 83:145). More recently marrow CD34+ cells exposed to androgens, have been shown to have increased telomerase activity and higher TERT mRNA levels (Blood 2009; 114: 2236): these data argue for a possible role of androgen in the treatment of patients with AA, which needs to be tested prospectively. A proportion of non responders to IS therapy, may experience hematologic recovery with a course of androgen therapy, sometimes combined with cyclosporine.

Psychological support

As aplastic anemia is a rare disease at time of initial diagnosis, a careful explanation about the nature of the disease, treatment, prognostic and social impact is important for the patients and their families. The treating physician should allow time for a comprehensive dialogue regarding all possible situations that may occur during the treatment with a special focus on the chronic nature and the potential slow response of the disease. As the diagnosis of aplastic anemia is a life changing experience some patients will need professional psychological support.

For some patients it is helpful to be in contact with other aplastic anemia patients. Details of patient support groups should be provided.

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Standard Treatment: Immunosuppression

Front Line Immunosuppressive Treatment of Acquired Aplastic Anemia with Rabbit ATG, Cyclosporine A and on demand G-CSF.

C. Dufour, J Svahn, A. Bacigalupo

Background and Rationale

Standard front line treatment for Acquired Aplastic Anemia (SAA) for patients, who lack an HLA identical family donor, is combined immunosuppression (IS).

Historical data have shown that combination therapy with ATG and CSA provides a response rate of 70 - 90 % (1, 2) and does significantly better than either agent alone (1).

CSA at 4-6 mg/kg/day given for 12 months, in combination with ATG, was shown to be associated with a response rate of 71% (4). Slow tapering of CSA (0.3-0.7 mg/kg/month) resulted in a significantly reduced incidence of relapse (8%) *vs.* rapid (> 0.8 mg/kg/month) tapering (60%) (4). All these data support the concept that the more intense and prolonged the immunosuppression the higher the success rate.

Infections during combined IS, as first cause of death (30%) (1), adversely impact on survival. Studies on pediatric populations attribute a mortality rate of 9 % to infections (3). Most infections tend to occur during the first month from diagnosis (3) and are associated to disease-related neutropenia. Since the early nineties, G-CSF has been added to ATG and CSA with the aim to reduce infection related mortality.

Data regarding the increased risk of late clonal diseases after IS regimens containing G-CSF are not unequivocal. One study indicates a significantly higher hazard (1.9) of MDS/AML associated with use of G-CSF and a significantly worse outcome of relapse in G-CSF treated patients (5). On the other hands, prospective randomized trials showed that G-GCSF in combination with ATG and CSA reduces the risk of relapse after IS and does not increase the occurrence of MDS/AML during the study follow up period (6, 7).

It is the recommendation of this treatment algorithm to use G-CSF "on demand" during infectious episodes in neutropenic patients. This strategy might contribute to minimize infectious risks and, given the short term use, minimize the potential risk of late clonal disease.

Aims

Primary aim is to evaluate the response rate (partial* and complete**) of newly diagnosed SAA, VSAA, Non Severe AA patients treated with a combination IS therapy consisting of ATG and CSA plus "on demand" G-CSF.

* Partial Response: Platelets > $20x10^9$/L, Hb >80g/L without transfusions, PMN > $0.5x10^9$/L

** Complete Response: Platelets > $150x10^9$/L, Hb >120g/L, PMN > $1.5x10^9$/L

The following groups of patients shall be treated according to this protocol

Patients aged from 0 to 60 years with newly diagnosed VSAA, SAA or Non Severe AA

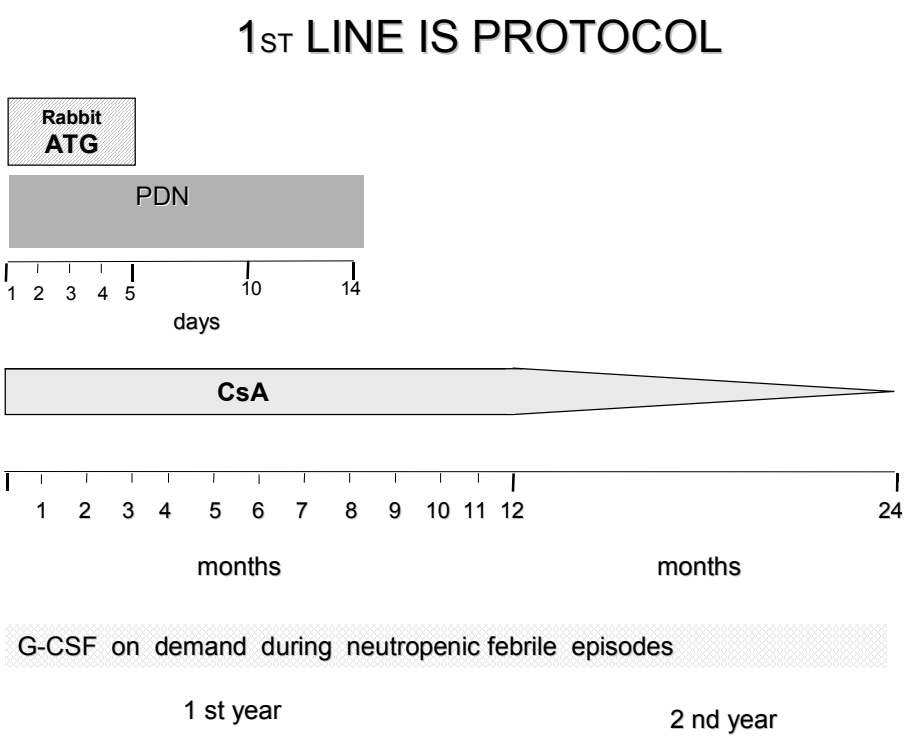
Treatment protocol:

Rabbit ATG (Thymoglobulin 3.75 mg/kg body weight/day) to be given in a 12-18h infusion, for 5 days (d 1-5).

CSA 5 mg/kg/ day per os from day 1-5 to day 365. Trough whole blood CSA levels 150-250 ng/ml (watch for toxicity, e.g. renal insufficiency, hypertension etc). Then tailing by 5-10% dose/month up to month +24 (watch carefully for dropping blood counts while reducing CSA). Maintain for longer if no CR has been reached.

Methylprednisolone (or prednisone) 1-2 mg/kg/day from d 1 to d 5 as a 30 mi i.v. bolus 30 min prior to Rabbit ATG. Oral Methylprednisolone (or prednisone) 1 mg/kg/day from d 6 to d 14 then tapering off over the next 14 days. (Do not taper if serum sickness)

G-CSF (lenograstim or filgrastim) 5 μg/g/day during febrile or infectious episodes when ANC < 0.5x109/L. Stop when infection cleared or ANC > 1.0 x109/L.



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Standard Treatment: SAA in the adult patient 18-40 with an HLA identical sibling donor, standard transplant protocol

Maria Beatrice Pinazzi, Anna Locasciulli, Maria Teresa van Lint

For a young adult patient newly diagnosed with SAA with an HLA identical sibling the standard treatment is hematopoietic stem cell transplantation. The definition of "young" is not entirely straightforward, and is probably best placed around age 30-40 but performance status and disease severity play a role. Obviously response to HSCT may be more rapid as compared to IS and may be preferable in a situation of an infected or transfusion refractory patient.

Several studies have shown that

Conditioning is Cyclophosphamide 200mg/kg in combination with ATG even though the randomized trial by the CIBMTR had not shown a significant difference between Cy+ATG and Cy alone.

GvHD prophylaxis is CSA + short course MTX (Study by GIMEMA and EBMT comparing CSA+MTX vs. CSA alone)

Stem cell source is marrow rather than peripheral blood (observational study by CIBMTR and EBMT showing less chronic GvHD and superior survival for BM vs. PB.

Conditioning	Cy 200mg/kg + ATG (Thymoglobulin 2 x 3.75 mg/kg)
Stem Cell Source	Unmanipulated Bone Marrow
GvHD Prophylaxis	CSA + short course of 5 mg/m2 MTX on days +1,+ 3,+6

Peripheral blood may be used if the donor is not willing to donate marrow; however patient and donor have to be informed that this is second choice

If several donors are available preference should be given for a male donor for a male patient (less GvHD) and a female donor for a female patient (less graft failure). Other than that, age, CMV serostatus and blood group matching have to be taken into account.

A modification to the above regimen employed by some centers is to use alemtuzumab instead of ATG and CSA alone without MTX, as this may result in less acute and chronic GVHD (Ismail 2010; Siegal, 2008).

(Islam MS, Anoop P, Datta-Nemdharry P, Sage D, Gordon-Smith EC, Turner D, Wiltshire S, O'Regan L, Marsh JCW. Implications for CD34+ cell dose on clinical and haematological outcome of allo-SCT for acquired aplastic anaemia. **Bone Marrow Transplantation** (12 October 2009) doi:10.1038/bmt.2009.267 Original Article

Adult patients older than 40 years refractory to at least one immunosuppressive course: HLA-identical sibling HSCT using fludarabine-based conditioning

Sébastien Maury, Mahmoud Aljurf

Allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-identical sibling donor is the first-line treatment of choice for newly diagnosed patients with severe acquired idiopathic aplastic anemia (SAA) if they are younger than 30-40 years. However, for older patients aged 40 years or over, the decision whether to treat with immunosuppressive therapy (IST), namely antithymocyte globulin (ATG) and cyclosporine, or to transplant upfront with an HLA-identical sibling donor remains a key question. Two large studies from Seattle and the European Group for Blood and Marrow Transplantation (EBMT) SAA Working Party that examined survival by age group did not show an advantage for either HSCT or IST in older patients. This was notably related to a lower long-term survival after HSCT for older patients, i.e. over 40 years in the Seattle cohort and over 20 years in the European study. The negative impact of older age at transplant has been confirmed in several other studies.

The standard conditioning regimen for HLA identical sibling HSCT relies on cyclophosphamide (CY) combined or not with ATG. In order to improve survival in patients older than 40 years, the use of less cytotoxic but more immunosuppressive regimens including low dose cyclophosphamide (below the standard dose of 200 mg/kg) in combination with ATG, while adding fludarabine (Flu), might be an option to explore with the aim of reducing transplant-related mortality. Such Flu-based regimens have been explored in various non-malignant diseases without conferring a significant survival advantage when compared to conventional myeloablative regimens. In patients with SAA specifically, several previous non-comparative studies have reported encouraging results in the setting of HLA-matched related HSCT. A recent report from the EBMT SAA working party analyzed retrospectively 30 patients older than 30 years receiving such reduced-intensity conditioning HSCT (using Fludarabine 30mg/m2 x 4, Cyclophosphamide 300mg/m2 x 4 and most with ATG) and compared their outcome to a control group receiving the standard regimen (cyclophosphamide+/-antithymocyte globulin) over the same study-period (1998-2007). Patients conditioned with Flu had a higher probability of age-adjusted overall survival than the control group (p=0.04).

HLA-identical sibling HSCT using fludarabine-based conditioning for patients >40 years:

Acquired aplastic anemia refractory to at least one course of ATG-based immunosuppressive treatment

First or second HSCT (if the first HSCT did not use Flu in the conditioning regimen)

Conditioning regimen:

Fludarabine: 30 mg/m2/d from d-5 to d-2 = 4 days (total dose = 120 mg/m2)

Cyclophosphamide: 30 mg/kg from d-5 to d-2 = 4 days (total dose = 120 mg/kg)

Rabbit ATG (Thymoglobulin): 3.75mg/kg from d-3 to d-2 = 2 days

Source of stem cells: Bone marrow preferred over PBSC

GvHD prophylaxis: Cyclosporine (from day -2) and mini-dose Methotrexate (5 mg/m2 at days +1, +3, +6)

Centers not willing to use the Fludarabine based regimen shall use the standard Cyclophosphamide 200 mg/kg + ATG regimen. Outcome will be compared in a retrospective analysis.

A modification to the above regimen employed by some centers is to use alemtuzumab instead of ATG and CSA alone without MTX, as this may result in less acute and chronic GVHD (Ismail 2010; Siegal, 2008).

Infectious disease prophylaxis will be done according to centre policy. Specific attention will be given to Epstein-Barr virus (EBV) reactivation during the transplant by bi-weekly monitoring of EBV load and pre-emptive treatment with Rituximab if needed.

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Unrelated donor search and unrelated donor transplantation in the adult patient 18-40 without an HLA identical sibling, failing immunosuppression.

A Bacigalupo, J Marsh

The outcome of unrelated donor transplants for patients with AA, has improved in the last decade (1, 2): better selection of HLA matched donors has probably played a major role, but also significant changes in the conditioning regimen have occurred (3-5). The first of these studies tested de-escalating doses of radiation, from 6 Gy down to 2 Gy, and concluded for best results for patients receiving 2Gy, with 8/13 patients surviving (4). A Japanese study reported 154 SAA patients undergoing an UD transplant, the majority receiving 3Gy TBI (3): unfavorable factors for survival were older age (>20 yrs), conditioning without anti-thymocyte globulin (ATG) and a long (>3 years) interval from diagnosis to transplant. An EBMT study tested a non-radiation based program (5): results were overall encouraging with 70% surviving, although rejection was high in young adults over the age of 14. The EBMT is currently testing a conditioning regimen which is very similar to the Japanese regimen: FLU-CY-ATG and low dose TBI (2 Gy) (unpublished).

As a consequence of less toxic conditioning regimens and improved donor/recipient matching, survival has almost doubled in the past decade (1) from 38% in 1991-1996 to 65% in the period 1997-2002 (6), and in the latter period survival after UD transplants in children is 75% vs. 63% for adults >16 years of age (1). Results of UD transplants have improved to such an extent, that treatment recommendations should be adapted: in children without a matched sibling donor, an UD search should be started at diagnosis, and transplantation should be seriously considered after failure of one course of immunosuppression in the presence of a suitable donor. In young adults between 20 and 30 the same may be true. Whether this should be modified according to the likelihood to rapidly identify a 10/10 UD based on the initial high resolution HLA typing will be subject to further research. Adults over the age of 30 should be entered on a prospective trial. Alternative donor transplant is an option for second line treatment in patients failing 1 or 2 courses of immunosuppressive treatment.

Donor selection: suitable donors are considered to be 10/10 matched, i.e. high resolution matched for HLA –A, -B, -C, -DRB1, -DQ. There are currently no recommendations as when to accept unrelated donors with mismatches.

Cord Blood Transplants are discussed briefly here as these are part of a separate chapter. A proportion of patients will not have a matched donor in the family, and will not find a suitable unrelated donor in the world wide network (bone marrow donors worldwide, BMDWW). The percentage of these patients, who lack a donor, will vary between 5% and 40%, according to the ethnic origin of the patient. Cord blood transplantation (CBT) is an alternative which has been successfully explored in patients with hematologic malignancies (7). Due to the high rate of rejection in AA patients and the low cell numbers of CB Units, transplants of unrelated cord blood has usually been discouraged in this setting. However, a recent study from the Japanese group (8) reports 31 CBTs with an overall survival of 42%, but a more encouraging 80% survival for patients receiving the FLU-CY-TBI 2 Gy combination as a conditioning

regimen. Thus cord blood may not be the first option in AA patients lacking a family or unrelated donor, but some investigators are exploring this stem cell source, and results may be encouraging with appropriate cell dosing, double units, alternative routes of administration, and new conditioning regimens.

Current ongoing protocol within the EBMT

Regimen A is a radiation free protocol including fludarabine 30 mg/m2 x 4, cyclophosphamide 300 mg/m2 x 4 and ATG 3.75 mg/kg x 4 (FCA):

We currently restrict this program to children under the age of 14 years. The reason to add TBI in adults was based on a high rejection rate with FCA in patients over the age of 14.

Regimen B is an FCA with the addition of low dose radiation (FCA-TBI): fludarabine 30 mg/m2 x 4, cyclophosphamide 300 mg/m2 x 4 and ATG 3.75 mg/kg x 2, TBI 2 Gy, which is open for patients above the age of 14 years, up to the age of 55 years.

A modification to the above regimen employed by some centers is to use alemtuzumab instead of ATG and CSA alone without MTX, as this may result in less acute and chronic GVHD (Gupta, 2005; Siegal, 2008).

These 2 protocols have currently enrolled 100 patients, grafted from unrelated or family mismatched donors in EBMT centers.

Table 1. Clinical data of patients.

	FLU-CY- ATG 15	FLU-CY-TBI 200- ATG 7.5
patients	52	48
Median age	13 (3-51)	27 (7-53)
Median year Tx	2003	2006
Unrelated donors	46	41
Family mismatched	6	7
Interval Dx-TX	530 days	461 days
GvHD III-IV	1	1
Rejection	9 (17%)	8 (17%)
Surviving	39 (75%)	38 (79%)
Median FU (days)	762 (100-2349)	1665 (39-3532)

The actuarial survival of these 2 regimens is depicted

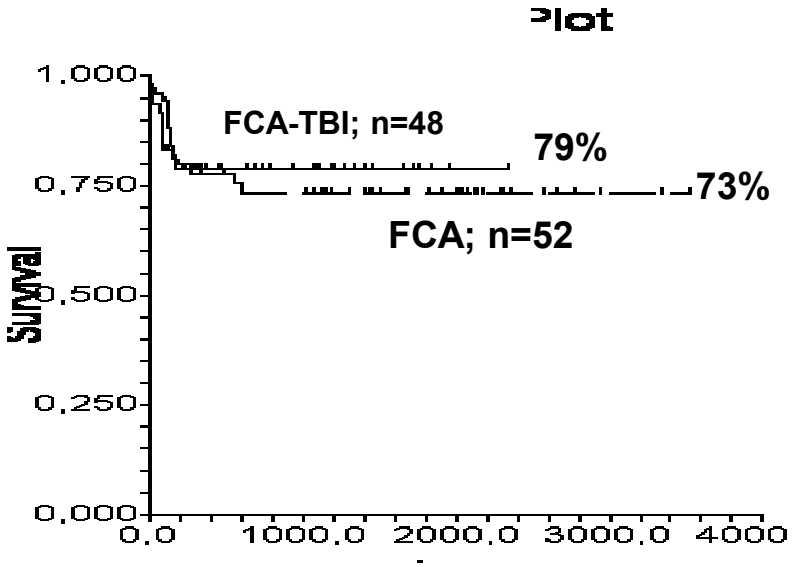


Fig.1

Causes of death are outlined

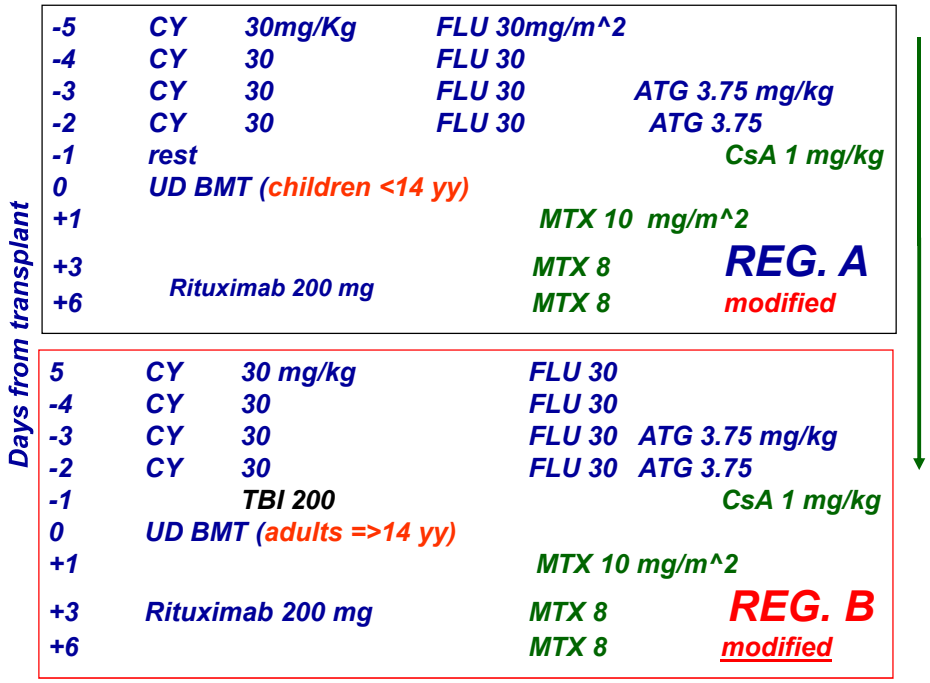
CAUSE OF DEATH

median age 14		*median age 29*	
REGIMEN A		***REGIMEN B***	
		GVHD	*2*
Graft Failure	*3*	*Graft Failure*	*4*
EBV LPD	*2*	*EBV LPD*	*2*
Infection	*2*	*Infection*	*1*
Hemorrhage	*0*	*Hemorrhage*	*1*
Other	*6*		

Problems include EBV reactivation and possible LPD, which calls for prophylactic rituximab (see schedule A and B). Graft failure is still seen also with TBI 2 Gy.

The proposed protocols included increased dose CY (from 300 mg/m^2x4 to 30 mg/kgx4), reduced ATG (from 15 mg/kg to 7.5 mg/kg) and prophylactic rituximab 200 mg on day+5. The use of rituximab for this purpose will be optional as practice will differ among centers

Outlined below are the proposed treatment regimens within the WPSAA treatment algorithm for children < 14 y and children > 14 y and adults with UD donors



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Pediatric issues

Elisabeth Korthof, Albert Bekassy, Valérie Mialou, Monika Fuehrer, Ayad Ahmed Hussein

The group of pediatricians within the WPSAA recommends the following:

For patients < 18y with SAA with an HLA identical sibling

Use the same protocol as for adult patients 18-40 years of age with an HLA-identical sibling, i.e. Cy200 + ATG conditioning, CSA+MTX for GvHD prophylaxis, marrow as a stem cell source

For patients < 18y patient without an HLA identical sibling

Use the same protocol of immunosuppression as for adult patients 18-40 years old without an HLA-identical sibling. Start unrelated donor search upon diagnosis; proceed to matched unrelated donor transplantation after failure of 1 course of immunosuppressive treatment.

Unrelated donor search and transplantation in pediatric patients < 18y

URD search should be initiated at primary work up and decision making for IS. If a search prognosis indicates that a MUD will be found easily, it is reasonable to wait with a complete search until it is clear that a transplant has to be done at evaluation at 3 months after the start of ATG+CSA. Recent studies found a highly significant improvement of survival in patients transplanted after 1998 as compared to earlier transplants, and 4 year survival data for unrelated transplants equal those of identical sibling transplant (Kennedy-Nasser 2006). Improvement in survival is associated with less graft failure (primary and secondary) less acute GvHD and less chronic GvHD. The causes of this improvement are not clear but improvement in unrelated donor transplantation is likely to be due to better donor/recipient HLA-matching. Another factor may be the increasing size of the donor registries with currently 14 million donors to choose from, a pool that was smaller in the 1990s. The number of unrelated donor transplants per year since 1998 has increased as well, probably reflecting the expectation of better outcome with the availability of better typing technology.

Hierarchy of donor preferences should be as follows: 1. MUD, 2. 1 Ag MMUD, 3. depending on the centers experience: matched or minimally mismatched cord blood, or haploidentical donor (see specific chapters). Where it is possible to choose between donors who are equal with regard to HLA matching, sex matching should be taken into account (equal sex better than different sex when no ATG in the conditioning).

The group recommends using the WPSAA EBMT protocols as outlined in the chapter “Adult patient 18-40 without an HLA identical sibling, failing Immunosuppression. Unrelated donor search and unrelated donor transplantation” and to use the TBI free regimen for children < 14 years and the regimen with 2 Gy of TBI for adolescents > 14 years.

For centers not participating in the EBMT protocol the following recommendations

Source of stem cells should preferentially be bone marrow.

Conditioning: Fludarabine 4 or 5 x 30 mg/m2 (-7/-6 -3); Cyclophosphamide 4 x 50 mg/kg (-5 -2); ATG rabbit 4 x 2.5 mg/kg (-5 -2).

Graft versus Host prophylaxis: Cyclosporine A 2 mg/kg/day in 2 doses iv, starting at day -1, changing to 6 mg/kg/day in 2 dosages orally. Full dose according to trough levels of 100-200 ng/ml, up to nine months, tapering off in three months. Methotrexate 10 mg/m2 i.v. at d+1, day 3 and 6. In case of a T cell depleted graft no MTX; in case of a CD34+ selected graft no GVHD prophylaxis at all.

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The elderderly: Patient with SAA, AA >60 years of age

A Tichelli, J Marsh

Older age is not per se a reason for withholding specific treatment in elderly patients (>60 years, even older than 80 years) with aplastic anemia. There is no place for allogeneic HSCT as first-line treatment in patients >60 years. The treatment decision in the elderly and the type of treatment to choose should be based on

Severity of the disease and mainly severity of neutropenia and its clinical complications (infections).

The presence of comorbidities

The willingness of the patient and his family to be treated

For patients eligible for immunosuppressive treatment, the choice of first-line immunosuppression should be based on the risk of severe infections and requirement of a rapid response versus stable condition and non-severe disease. The principle is to treat patients with severe disease and/or requiring rapid response more intensely than those with less severe disease or with a condition allowing for time until response achievement.

It has been shown that ATG+CSA is significantly better than CSA alone in respect of response rate and disease free survival. Patients treated with CSA alone needed more often to be retreated with a second course of immunosuppression using ATG + CSA

However, there was no difference in survival, because CSA refractory patients responded to second line treatment with ATG+CSA

This means that the patients who respond to first-line CSA will benefit, since they can be treated as outpatients; in addition, non-responders are not exposed to a high immunosuppressive load and treatment related toxicity.

Elderly patient (>60) in the inpatient setting

Patients with very high risk for severe infections (neutrophils < 0.2 $x10^9$/L) or presenting at diagnosis with severe infection needing hospitalization should be treated upfront with ATG-+CSA as this treatment gives the highest chance of a rapid response. The use of CSA alone is associated with delayed response and a reduced response rate compared with the combination of ATG and CSA. Therefore, in these high risk patients any delay in response by using a less efficient immunosuppression (CSA alone) would present in increased risk.

Elderly patient (>60) in the outpatient setting

In patients who are not at immediate risk for severe infections and who are therefore managed as out-patients, receiving supportive care until response, first-line treatment with CSA alone is recommended.

This definition includes mainly patients with non-severe AA, and particularly with neutrophil counts > 0.5 x10^9/L without infection. Severe thrombocytopenia is usually not an indication for hospitalization. SAA with mainly severe thrombocytopenia and anemia but not severe neutropenia can be included into this group. The use of androgens, particularly in men can be considered in case of CSA intolerance (for instance renal impairment)

Elderly patient refusing or not able to receive treatment

Such patients should receive best supportive care alone. Outcome of these patients should be recorded as well

Transplant in the elderly

There is little data on patients >60 years with allogeneic HSCT as first-line treatment, although there is some data in patients >60 years receiving HSCT after having failed immunosuppression. This does not apply to patients with a syngeneic donor in whom transplant should be first choice.

HSCT is an option in elderly patients refractory to immunosuppression using for instance a reduced conditioning regimen with Fludarabine, low dose cyclophosphamide and ATG (Maury et al Haematologica 2009). Even though it is impossible to extrapolate across different disease entities it is of interest that in patients with MDS transplanted using RIC HSCT, there was no significant difference in non-relapse mortality for patients aged > 60 compared with 50-60 years (Lim et al, JCO 2009).

Haploidentical Transplantation with or without added MSC for Patients without a Sibling or Unrelated Donor failing 2 courses of Immunosuppression

Fabio Ciceri, Elisabeth Korthof

Haploidentical transplants have been carried out in aplastic anemia with varying results (Woodard et al, 2004). In 2009 we started a survey based on EBMT ProMISe data to sort out the current practice in order to be able to set up a prospective study for haploidentical transplantation in SAA.

Currently, there are insufficient data to make a definite recommendation regarding graft composition and conditioning regimen. However, a profound T-cell depletion both in-vivo and ex-vivo, is recommended to realize a maximal GvHD prevention. Our current proposals for haploidentical-SCT in SAA are as follows. The donor should be choosen based on an extended family search by the following hierarchy of criteria: 1. HLA typing, 2. viral status of donor and recipient, 3. donor/recipient ABO blood group matching.

Stimulation of donor with G-CSF 10 µg/kg/day for 5 or 6 days. CD34+ selection by Clinimacs (Isolex or Cellpro may be used instead) to reach 6-8 x 10e6 CD34+ cells/kg patient body weight, with a maximum of CD3+ cells of 5 x 10e4/kg patient body weight.

Conditioning regimen proposed is fludarabine 5 x 30 mg/m2 (-7 to -3), cyclophosphamide 4 x 50 mg/kg (-5 to -2), ATG 4 x 2.5 mg/kg (-4 to -1). No post-transplant graft versus host disease prophylaxis.

Conditioning regimens with a T-cell repleted graft that include the use of high dose cyclophosphamide post HSCT to delete alloreactive T-cells, or rapamycin as GvHD prophylaxis are being used for haploidentical HSCT for haematological malignancies, and have also been reported anecdotally in PNH. At present, a T-repleted strategy is not recommended for patients wuth SAA.

Co transplantation of mesenchymal stem cells (MSCs) to enhance engraftment is of interest and may be studied in this situation. There is now some experience with MSCs to advice giving them in patients at risk for non-engraftment of their transplant, like a haploidentical one in SAA. Centers which are using MSCs should cooperate to share a common protocol, which could be as mentioned above, with the addition of MSCs. The MSC donor could be the same as the HSCT donor.

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Single or double Cord Blood Transplantation for Patients without a Sibling or Unrelated Donor, failing 2 Courses of Immunosuppression

R Peffault de Latour, J Marsh, G Socié

Synopsis of the Study of the French Society for Stem Cell Transplantation (SFGM) Severe Aplastic Anemia and Cord Blood Transplantation

Background

The outcome of patients with Severe Aplastic Anemia (SAA) who failed or relapsed after immunosuppressive therapy (IST) has greatly improved over the time but is still poor. The outcome of UD transplants for patients with SAA has improved in the last decade (1, 2). Results of UD transplants have improved to such an extent that treatment strategy may be affected. In children without a matched sibling donor, current guidelines recommend proceeding to HSCT after failing one course of IST provided a fully matched donor at allele-level for MHC class I and II antigen is available (3). In adults, alternative donor transplant is an option for second line treatment in patients failing 1 or 2 courses of IST (2, 4). Unfortunately, many patients, especially those from ethnic minority groups or less homogeneous populations, do not have a suitable UD (4). Unrelated cord blood transplantation (UCBT) is an alternative option which has been successfully explored in patients with hematologic malignancies (5, 6). To date, there are only a few reports on UCBT in patients with SAA. Primary reports showed poor outcome and high incidence of graft failure (5, 7) while few small series and case reports of successful UCBT for SAA have recently been reported (8, 9, 10, 11). The largest cohort of 31 patients has been published by the Japanese group with a 2-year overall survival of 41%, suggesting that UCBT can be an alternative treatment for SAA patients who failed IST and have no suitable bone marrow donor (12).

Retrospective study on 71 patients diagnosed with SAA (9 PNH) who received an UCBT in 32 centers (23 EBMT centers): A study by EUROCORD and the Aplastic Anemia WorkingParty of the EBMT (13)

We conducted a retrospective analysis on 71 patients (33 male) diagnosed with SAA (9 with PNH) who received a single UCBT (n=57, 79%) or double UCBT (n=14, 19%) from January 1996 to January 2009 in 32 centers (23 EBMT centers). The median age was 13 years (range 2-68 years; 28 adults). Median disease duration before UCBT was 14 months (2-140). Fifty five patients (89%) received immunosuppressive therapy before transplantation and most patients were highly transfused prior to UCBT. Seven percent of cord blood units were identical to recipients (antigen level for HLA-A and B and allelic level for DRB1), 28% of units had 1 HLA mismatch and 65% had 2 or 3 HLA disparities. Median infused cell dose was 4.3 x107 TNC/Kg (2.1-34.9) and 2.1 x105 CD34 cells/Kg (0.4-19) for single UCBT and 7.4 x107 TNC/Kg (5-14.7) and 3.5 x105 CD34 cells/Kg (1-8.7) for double UCBT. Forty six patients (69%) received a reduced intensity conditioning regimen, most of which were fludarabine-based. Twenty three patients received a total body irradiation (2 Gray, n= 11) and antithymoglobulin was given to 53 patients (79%). Graft-versus host disease (GVHD) prophylaxis consisted mainly of cyclosporin+steroids (70% of patients). Cumulative incidence (CI) of neutrophil recovery(>500mm^3) at day 60 was 51±6% with a median time of 25 days (6-91). In multivariate analysis, the only factor associated with shorter time to engraftment and higher probability of engraftment was pre-freezing TNC dose (>3.9 107/Kg, HR: 1.5, 95%CI: 1-2.2, p=0.05).Chimerism analysis for patients who engrafted (n=37)

showed full donor chimerism in 82%. The CI of grade II-IV acute GVHD was 20±5% (10 grade II, 5 grade III, 2 grade IV). Eleven patients of 34 at risk developed chronic GVHD leading to a CI of 18±5% at 3 years. With a median follow-up of 35 months (3 - 83), the estimated probability of 3-years overall survival (OS) was 38±6%. The main cause of death was graft failure associated with infections (n=14, 32%). In multivariate analysis, the only factor associated with survival was pre-freezing TNC dose (>3.9 107/Kg, RR: 0.4, 95%CI: 0.2-0.8, p=0.007). The estimated probability of 3-year overall survival (OS) for patients who received more than 3.9 107/Kg TNC was 45% compared to 18% for those who received less (Figure 1). Other factors such as number of HLA disparities or use of single or double CB unit were not associated with any outcome. However, the 3-year OS after single CBT was 37% and 43% after double CBT. In conclusion, this study highlights the fundamental role of the TNC dose (>3.9 x 107/kg TNC/Kg) on both engraftment and overall survival using cord blood as stem cell source in SAA. It could justify the use of double cord blood transplant if necessary for this indication. Graft failure remains a major issue in this particularly high risk population. Those results need to be confirmed in a prospective study to warrant the inclusion of dCBT in the treatment strategy of diseases with high risk of rejection.

It is the proposal of the group to recommend the SFGM protocol for centers who transplant patients outside the protocol. It is obvious that including patients into the protocol is the preferred option.

Protocol Title: Cord Blood Transplantation in acquired Severe aplastic anemia (SAA). A Non randomized phase II study

Indication: SAA in relapse after immunosuppressive therapy in the absence of an HLA identical donor.

Objectives: Overall survival at one year. Efficacy $\geq$50%, rejection rate $\leq$20%.

Inclusion criteria:

-Age: 3 - 55 years old

-Acquired aplastic anemia (with severe aplastic anemia criteria without clonal evolution) in relapse or treatment failure after immunosuppressive therapy

-Karnovsky Index ≥ 60%

Stem cell source: 2 cord Blood units both containing more than $5x10^7$ frozen nucleated cells/Kg with no more than 2 mismatches between them and with the patients. The use of one cord blood unit is possible if the compatibility and the cell dose is respected. Matching is based on low resolution matching in class I (HLA-A and –B) and high resolution matching on class II (HLA-DRB1)

Informed consent.

Number of patients to included in the protocol: 26 to detect an overall survival rate of $\geq$50% at one year (power at 90%). An overall survival of less than 20% indicates the non efficacy of Bone Marrow Transplantation (according to a one step Fleming Scheme with a unilateral test of 2.5%).

Treatment: Conditioning regimen: Fludarabine 30mg/m2 from d-6 to d-3, Cyclophosphamide 30mg/Kg from d-6 to d-3, ATG (thymoglobulin) 3.75mg/Kg from d-3 to d-2, Total Body Irradiation (2 Grays) on d-2.

Transplantation (d 0): if 2 units, they are injected with 6 hours difference

GvHD prophylaxis: Cyclosporine (from day -3)±steroids (1mg/Kg 28 days) in case of engraftment syndrome.

Infectious prophylaxis during transplant: 1 injection at day 5 of anti-CD20 (Rituximab®) 150mg/m2 to prevent EBV reactivation, the fungal empiric treatment consisted in Caspofungine (Cancidas®). The use of growth factors are recommended at day 5 (Neupogen®). Other transplant procedures and clinical care are not different from the other transplants (JACIE recommendations).

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Alternative immunosuppression in patients failing immunosuppression with ATG who are not transplant candidates: Campath (alemtuzumab)

A Risitano, H Schrezenmeier

Title: alemtuzumab and low-dose cyclosporine-A as alternative immunosuppressive treatment for severe aplastic anemia (SAA) and single-lineage aplastic patients.

Indication: acquired aplastic anemia (severe and moderate) failing after at least 1 course of ATG-based immunosuppression and single lineage marrow failures (PRCA, PWCA, AMT). The presence of a PNH clone is NOT an exclusion criterion. No indication and/or no donor available for allogeneic stem cell transplant

Background: Campath 1H (alemtuzumab) is a humanized anti-CD52 monoclonal antibody which kills all CD52-bearing cells (including both T and B lymphocytes) via both antibody-dependent cellular cytotoxicity and complement-mediated lysis. Its strong lymphotoxic effect results in a powerful IS activity, which may be beneficial in aplastic anemia and single lineage cytopenia patients. Thus, Campath is a good candidate agent for experimental immunosuppressive regimens to be investigated in the treatment of bone marrow failure patients.

Centers are encouraged to participate in the study summarized below. For non participating centers it is recommended to follow the treatment protocol.

Endpoints: to investigate safety and efficacy of the proposed treatment. Safety will be evaluated as occurrence of adverse effects, while efficacy as overall survival, hematological response (partial and complete, including time to response) and failure free survival.

Centers: all WPSAA centers; Naples as coordinator centre.

Study design: phase II, open label, not randomized study.

EudraCT number 2008-001151-22

Treatment:

Investigational treatment. Patients will receive Alemtuzumab (MabCampath) for 5 days, starting with a dose of 3 mg on day 1, followed by 10 mg on day 2 and 30 mg on days 3 and 4 (and 5, only for SAA patients). The drug is available as 30 mg vials; it will be administered as subcutaneous injections. As prevention of Campath-related side effects, including first dose reaction, 30 minutes before alemtuzumab a premedication will be administered, which includes steroids, an anti-histamine and paracetamol. Patients suffering from single lineage marrow failure will receive only 73 mg of alemtuzumab (3, 10, 30 and 30 mg in 4 consecutive days).

Concomitant treatments. The patients will also receive oral cyclosporine, starting on day 7 at the dose of 1 mg/kg, and then adjusted on blood levels for at least 180 days, and then tapered according to clinical conditions. Anti-infectious prophylactic measures will be adopted to

prevent opportunistic infections. Anti-bacterial and anti-fungal prophylaxis will be administered to all patients with ANC <500. Anti-CMV (only in seropositive patients) and anti-pneumocystis prophylaxis is recommended as long as CD4+ lymphocytes <0.1 G/L or for at least 3 months after alemtuzumab. Additional supportive therapies (including blood cell component transfusions) will be administered according to standard practice, as well as any additional medication needed as a result of concomitant morbidities. Irradiated blood products (>25 Gy) are to be used.

Duration of the study: subjects will remain on study for a minimum of 180 days. As long-term effects of investigational treatments are an objective of the study, the follow-up of patients will cover a minimum of 24 months from treatment starting.

Preliminary data (Risitano et al, BJH 2010). We have investigated this alemtuzumab-based experimental immunosuppressive treatment (IST) regimen in 35 patients with aplastic anaemia, pure red cell or pure white cell aplasia (of whom 25 included in a prospective clinical trial). Treatment was administered on an outpatient basis, with the exception of patients requiring hospitalization for clinical reasons (e.g. symptomatic thrombocytopenia). No serious toxicity due to the investigational treatment was observed (the most frequent AE was injection-related fever and/or cutaneous rash, which occurred 28% of patients),. Adverse events were clinically irrelevant, and infectious events were rare. The total response rate was 58%, 84% and 100% in SAA, PRCA and PWCA, respectively; responses were faster in PRCA and PWCA patients (range 1-4 months), compared with SAA (3-10 months). Survival and cumulative incidence of response analyses in the 25 patients in the prospective trial were 73% and 89%, respectively. Relapses were not infrequent, but re-treatment by further courses of alemtuzumab was easy and effective. This is the largest cohort of marrow failure patients receiving alemtuzumab, which confirms data from other smaller series (Willis et al, 2001; Kim et al, 2009, Gomez-Almaguer et al, 2009). These data provide evidence that alemtuzumab-based IST is feasible and manageable in patients suffering from immune-mediated bone marrow failure syndromes, with response rates which seem non-inferior to standard IST regimens.

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