EWOG-MDS

Guidelines for Hematopoietic Stem Cell Transplantation (HSCT)

in Childhood MDS and JMML for Patients enrolled

in EWOG-MDS Studies

EWOG-MDS Consensus Conference

Freiburg, October 25/26, 2016

These guidelines were established and agreed upon by the EWOG-MDS HSCT Board during a Consensus Conference on October 25/26th 2016 in Freiburg, Germany. They were modified for presence of constitutional syndromes in August 2017. The previous consensus is the Version 1.2, 01.09.2013.

Version 1.3, 15.08.2017

Abbreviations

AML:	acute myeloid leukaemia
ANC:	absolute neutrophil count
ATG:	anti-thymocyte globuline
BM:	bone marrow
BU:	busulfan
CB:	cord blood
CSA:	cyclosporine A
CY:	cyclophosphamide
DLI:	donor leukocyte infusion
EFS:	event free survival
GVHD:	graft-versus-host disease (GVHD)
HR:	high resolution
HSCT:	hematopoietic stem cell transplantation
IST:	immunosuppressive therapy
JMML:	Juvenile myelomonocytic leukaemia
MDS:	myelodysplastic syndrome
MDS-EB:	MDS with excess of blasts
Mel:	melphalan
MMF:	mycophenolatmofetil
MSD:	matched sibling donor
MTX:	methotrexate
MUD:	matched unrelated donor
NC:	nucleated cells
PB:	peripheral blood
PBSC:	PB stem cell
RAEB:	refractory anaemia with excess blasts
RAEB-T:	RAEB in transformation
RBC:	red blood cells
RCC:	refractory cytopenia of childhood
RIC:	reduced intensity conditioning
OS:	overall survival
SAA:	severe aplastic anaemia
STR:	short tandem repeats
Treo:	treosulfan

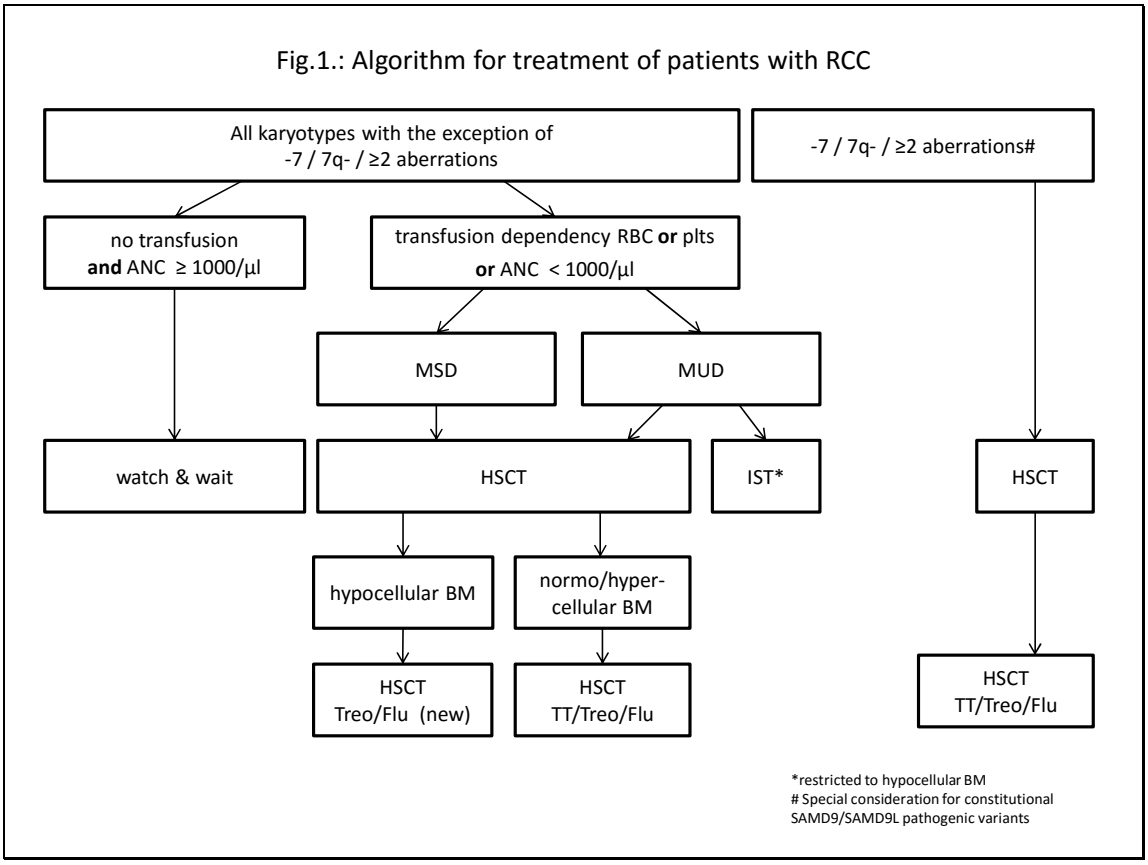
1. Refractory Cytopenia of Childhood (RCC)

Major change from the previous consensus (version 1.2, 01.09.2013):
Patients with hypocellular RCC without karyotypic abnormalities are transplanted with a regimen consisting of treosulfan and fludarabine aiming for an improved rate of engraftment

1.2 Algorithm of therapy, modifications from previous guidelines

Refractory cytopenia of childhood (RCC) is the most common subtype of paediatric myelodysplastic syndrome (MDS).[1] RCC is frequently characterized by hypocellular bone marrow (BM), absence of chromosomal aberrations, and low risk of progression to advanced MDS. However, about 10-15% of patients with RCC display an abnormal karyotype with monosomy 7, del(7q) or ≥ 2 aberrations generally associated with a high risk of progression to MDS with increased blast count.[2] Thus, patients with RCC can be stratified for therapy according to karyotype (see Fig. 1).

Fig.1.: Algorithm for treatment of patients with RCC



It recently became evident that approximately half of all paediatric patients with primary MDS and monosomy 7 or del(7q) have an underlying constitutional disorder with pathogenic variants in the genes for *GATA2* or *SAMD9/SAMD9L*. [3,4] (Wlodarski, unpublished). Most of these patients present as RCC. MDS with monosomy 7/del(7q) in GATA2 deficiency is a

progressing neoplasia which can successfully be transplanted according to EWOG-MDS recommendations [3]. Patients with SAMD9/SAMD9L constitutional disorder and a diagnosis of MDS with monosomy 7/del(7q) require special considerations which in some instances may include an observant strategy (Wlodarski, unpublished).

In the absence of monosomy 7, del(7q) or ≥ 2 aberrations, RCC patients with mild cytopenia (no transfusion dependency for red blood cells [RBC] or platelets and an absolute neutrophil count [ANC] ≥ 1000/µl) may have a stable course of disease and therefore qualify for a watch-and-wait strategy. For patients with more pronounced cytopenia (transfusion dependency for RBC and/or platelets and/or ANC<1000/µl) treatment is outlined according to cellularity

In patients with hypocellular BM, dyskeratosis congenita should be excluded by telomere length/molecular studies. HSCT with a reduced intensity conditioning (RIC) has been the treatment of choice for hypocellular RCC and normal karyotype if a matched sibling donor (MSD) was available; in the absence of a MSD options for therapy include HSCT from a matched unrelated donor (MUD) following RIC, or for selected patients immunosuppressive therapy (IST) with anti-thymocyte globuline (ATG) and cyclosporine A (CSA).[5,6] For IST, the use of horse ATG is recommended.[6]

HSCT following the previously recommended RIC preparative regimen with thiotepa, fluadarbine in 169 patients with hypocellular RCC without karyotypic abnormalities resulted in a probability of overall survival (OS) and event free survival (EFS) of 0.94 (0.90-0.98) and 0.83 (0.76-0.89), respectively.[7] However, about 10% of patients experienced primary or secondary graft failure and 12% received a 2nd procedure such as a stem cell boost and/or 2nd HSCT.

In contrast, all patients with RCC who have been transplanted following a treosulfan based regimen (TT/Treo/Flu) based on cellularity and/or karyotypic abnormalities experienced a prompt initial engraftment with a very low incidence of secondary graft failure (2/44 patients) and had a comparable overall outcome (pOS 0.92 (0.83-1.00), pEFS 0.86 (0.73-0.99). In view of these results it is consensus to transplant patients with hypocellular RCC without karyotypic abnormalities following a regimen consisting of treosulfan and fludarabine aiming for an improved rate of engraftment and maintain the recommendation for RCC with normo-/hypercellular BM and the specified chromosomal aberrations.

In patients with non-response or relapse after IST, BM aspirate and biopsy should be repeated prior to HSCT and the transplant procedure should be performed according to BM cellularity and karyotype at this time point. Patients who progressed to advanced MDS following IST are to be transplanted according to the recommendations for secondary MDS following severe aplastic anaemia (SAA) (see 4).

1.2 Details of HSCT specifics for RCC subtypes

1.2.1 RCC with hypocellular BM and normal karyotype, unsuccessful karyotype studies, or karyotype other than monosomy 7, del(7q) or ≥ 2 aberrations

1.2.1.1 Investigations to be performed/repeated at time of HSCT within 6 weeks before the allograft

- BM aspirate to evaluate blast percentage
- Karyotype of BM cells to detect abnormalities
- BM trephine to evaluate cellularity, fibrosis and blast infiltration
- Skin biopsy for cryopreservation of cultured fibroblasts
- Cryopreservation of viable BM and peripheral blood (PB) cells
- Storage of DNA

1.2.1.2 Criteria for donor selection

- HLA-identical sibling BM/PB stem cell (PBSC)/cord blood (CB) donor: molecular typing for class I loci (i.e. A, B and C) and high resolution (HR) molecular typing for DRB1 and DQB1 locus if parents typing is available. If not, HR molecular typing for both class I and II loci is recommended
- Phenotypically identical relative: HR molecular typing for class I loci (i.e. A, B and C) and for DRB1 and DQB1 locus
- Unrelated BM/PBSC donor: identical or with a single disparity in class I or II loci with the recipient using HR molecular typing for class I loci (i.e. A, B and C) and for class II loci (i.e. DRB1, DQB1). Preferential choice of DRB1 matched donor is recommended.

1.2.1.3 Stem cell source

- BM
- PBSC, only if the donor refuses to donate BM
- CB, only for HLA-identical sibling allograft

1.2.1.4 Graft composition

- Unmanipulated BM cells: > $3.5x10^8$ nucleated cells (NC)/kg
- Unmanipulated PBSC: > $4x10^6$ CD34+ cells/kg and < $10x10^6$ CD34+ cells/kg or < $5x10^7$ CD3+ cells/kg
- CB: at least $3.5x10^7$ NC/kg before thawing

1.2.1.5 Conditioning regimen

- ATG Novii (Grafalon®) will be used in patients transplanted from unrelated donors during conditioning regimen for *in vivo* T-cell depletion/modulation: 15 mg/kg/day for 3 consecutive days (-4 to -2). If alternative types of ATG are employed, equivalent doses of the product should be given.
- Preparation will consist of Fludarabine (40 mg/m^2/day from day -6 to day -3) and Treosulfan (14 g/m^2/day from day -6 to day -4).

1.2.1.6 Strategy for graft-versus-host disease (GVHD) prophylaxis and treatment

- HLA-identical sibling BM/PBSC donor: CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) starting from day -1 and with the objective of maintaining serum levels between 100-200 ng/mL (continuous infusion over 24 hours is also acceptable), and methotrexate (MTX): 3 doses on days +1, +3 and +6 at a dosage of 10 mg/m^2.
- HLA-identical sibling CB donor: CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) with the objective of maintaining serum levels between 100-200 ng/mL. Continuous infusion over 24 hours is also acceptable.
- Unrelated donor recipients: CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) starting from day -1 and with the objective of maintaining serum levels between 100-200 ng/mL (continuous infusion over 24 hours is also acceptable), and MTX: 3 doses on days +1, +3 and +6 at a dosage of 10 mg/m^2.
- CSA tapering should be started around day +100 in the absence of grade II-IV acute GVHD and steroid treatment. CSA discontinuation should take place around day + 180.
- Patients with grade II-IV 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 centre policy, is recommended.

1.2.1.7 Monitoring of chimerism

Monitoring of chimerism through short tandem repeats (STR) is recommended to start at the time of neutrophil recovery and should be performed on day +30, +60, +100 and +180. In case of re-growth of recipient cells, a closer monitoring is recommended in order to obtain information on the evolution of this situation of mixed chimerism. There is no indication for performing donor leukocyte infusion (DLI) in these patients in consideration of the negative risk/benefit ratio of GVHD in these patients.

1.2.1.8 Special considerations

In patients experiencing either primary or secondary graft failure, a second transplant option should be offered as soon as possible, even considering a T-cell depleted allograft from an HLA-disparate relative if the unrelated volunteer is not willing to donate for the second time.

1.2.2 RCC with normo- or hypercellular bone marrow and normal karyotype, unsuccessful karyotype studies, or karyotype other than monosomy 7, del(7q) or ≥ 2 aberrations

These patients should be treated as recommended for hypocellular RCC with the exception of an additional dose of thiotepa added to the preparative regimen.

1.2.2.1 Conditioning regimen

- ATG Novii (Grafalon®) will be used in patients transplanted from unrelated donors during conditioning regimen for *in vivo* T-cell depletion/modulation: 15 mg/kg/day for 3 consecutive days (-4 to -2). If alternative types of ATG are employed, equivalent doses of the product should be given.
- Preparation will consist of Thiotepa 8 mg/kg/d on day -7, Fludarabine (40 mg/m^2/day from day -6 to day -3) and Treosulfan (14 g/m^2/day from day -6 to day -4).

1.2.3 RCC patients with monosomy 7, del(7q) or ≥ 2 aberrations (and any cellularity)

1.2.3.1 Investigations to be performed/repeated at the time of HSCT within 6 weeks before the allograftBM aspirate to evaluate blast percentage

- Karyotype of BM cells to detect abnormalities
- BM trephine to evaluate cellularity, fibrosis and blast infiltration
- Skin biopsy for cryopreservation of cultured fibroblasts
- Cryopreservation of BM and PB cells
- Storage of DNA

1.2.3.2 Criteria for donor selection

- HLA-identical sibling BM/PBSC/CB donor: molecular typing for class I loci (i.e. A, B and C) and HR molecular typing for DRB1and DQB1 locus if parents typing are available. If not, HR molecular typing for both class I and II loci is recommended
- Phenotypically identical relative: HR molecular typing for class I loci (i.e. A, B and C) and for DRB1 and DQB1 locus
- Unrelated BM/PBSC donor: identical or with a single disparity in class I or II loci with the recipient using HR molecular typing for class I loci (i.e. A, B and C) and for class II loci (DRB1, DQB1).
- CB donor: 6/6 or 5/6 matched donor using HR molecular typing for class I loci (i.e. A and B) and DRB1 locus. Preferential choice of DRB1 matched donor is recommended.

1.2.3.3 Stem cell source

- BM
- PBSC, only if the donor refuses to donate BM
- CB

1.2.3.4 Graft composition

- Unmanipulated BM cells: > $3.5x10^8$ NC/kg
- Unmanipulated PBSC: > $4x10^6$ CD34+ cells/kg and < $10x10^6$ CD34+ cells/kg or < $5x10^7$ CD3+ cells/Kg
- CB: units containing more cells have to be preferentially selected. In any case, a patient must receive at least $3.5x10^7$ NC/kg before thawing.

1.2.3.5 Conditioning regimen

- ATG Novii (Grafalon®) will be used in patients transplanted from unrelated donors or relatives other than an HLA-identical sibling during conditioning regimen for *in vivo* T-cell depletion/modulation: 10 mg/kg/day for 3 consecutive days (-4 to –2). If alternative types of ATG are employed, equivalent doses of the product should be given.
- Preparation will consist of Thiotepa 8 mg/kg/d on day -7, Treosulfan 14 g/m^2/day for 3 consecutive days (-6 to -4) and Fludarabine 40 mg/m^2/d for 4 consecutive days (-6 to -3).

1.2.3.6 Strategy for GVHD prophylaxis and treatment

- HLA-identical sibling BM/PBSC donor and patient at HSCT < 12 years: CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/Kg/day) starting from day -1 and with the objective of maintaining serum levels between 100-200 ng/mL (continuous infusion over 24 hours is also acceptable), HLA-identical sibling BM/PBSC donor and patient at HSCT ≥ 12 years: CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) and short course MTX (3 doses on days +1, +3 and +6 at a dosage of 10 mg/m^2).
- CSA tapering should be started around day +60 in the absence of grade II-IV acute GVHD and steroid treatment. CSA discontinuation should take place around day + 100.
- HLA-identical sibling CB donor: CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/Kg/day) with the objective of maintaining serum levels between 100-200 ng/mL (continuous infusion over 24 hours is also acceptable).
- Unrelated BM/PBSC donor recipients: CSA 1.5 mg/Kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) starting from day –1 and with the objective of maintaining serum levels between 100-200 ng/mL (continuous infusion over 24 hours is also acceptable), and MTX: 3 doses on days +1, +3 and +6 at a dosage of 10 mg/m^2.
- Unrelated CB donor recipients: the recommended GVHD prophylaxis is CSA (at the same dose indicated for BM/PBSC allografts), and steroids (methylprednisolone 1.5 mg/kg/day starting from day -4 for 3 weeks with a rapid tapering in the absence of GVHD in 2 more weeks). Alternatively, steroids can be replaced by mycophenolatmofetil (MMF).

- CSA tapering should be started around day +100 in the absence of grade II-IV acute GVHD and steroid treatment. CSA discontinuation should take place around day +140.
- Patients with grade II-IV 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 c entre policy, is recommended.

2 Primary MDS with excess blasts (MDS-EB) and MDR-AML

2.1 Algorithm of therapy, modifications from previous guidelines

Major change from the previous consensus (version 1.2, 01.09.2013):
All patients with MDS-EB/MDR-AML are transplanted with a conditioning regimen consisting of Bu/Cy/Mel independent of the patient's age

Patients with MDS with excess of blasts (MDS-EB) including the subtype refractory anaemia with excess blasts (RAEB) in transformation (RAEB-T) of the former FAB-classification, and acute myeloid leukaemia (AML) evolving from MDS (MDR-AML) have a poor prognosis when treated with chemotherapy only. Conventional AML-type chemotherapy without HSCT resulted in survival rates below 30% [8,9], thus, all patients are transplant candidates. Previous analysis from the EWOG-MDS study showed that the pOS at 5 years for 97 patients with MDS-EB, who received HSCT with the conditioning regimen of busulfan, cyclophosphamide, and melphalan (Bu/Cy/Mel), was 0.63 [10]. The subtype of MDR-AML had an inferior outcome with a pOS of 0.32 The presence of a structurally complex karyotype is a strong prognostic marker predicting poor outcome [11]. Because patients ≧ 12 years of age had a high risk of transplantation-related mortality in this study, in 2013 EWOG-MDS recommended an intensified GVHD prophylaxis for patients transplanted from a MSD (CSA/MTX) and a treosulfan based conditioning regimen in this age group. In the 2016 analysis, patients ≧ 12 years with MDS-EB transplanted following a conditioning regimen consisting of Bu/Cy/Mel from a MSD receiving GVHD prophylaxis with CSA only had a worse outcome compared to the ones receiving CSA/MTX justifying the intensified GVHD prophylaxis for the older patients. In contrast, the preliminary analysis of patients with MDS-EB transplanted following the previously recommended treosulfan based regimen experienced a cumulative incidence of relapse of approx. 40% resulting in EFS at 4 years of only 51%. Therefore, the 2016 consensus is to transplant all patients with MDS-EB with a conditioning regimen consisting of Bu/Cy/Mel independent of the patient's age.

2.2 Details of HSCT specifics in MDS-EB and MDR-AML

2.2.1 Investigations to be performed/repeated at time of HSCT within 6 weeks before the allograft

- BM aspirate to evaluate blast percentage
- Karyotype of BM cells to detect abnormalities
- BM trephine to evaluate cellularity, fibrosis and blast infiltration
- Skin biopsy for cryopreservation of cultured fibroblasts
- Cryopreservation of viable BM and PB cells
- Storage of DNA

2.2.2 Criteria for donor selection

- HLA-identical sibling BM/PBSC/CB donor: molecular typing for class I loci (i.e. A, B and C) and HR molecular typing for DRB1 locus if parents typing available. If not, HR molecular typing for both class I and II loci is recommended
- Phenotypically identical relative: HR molecular typing for class I loci (i.e. A, B and C) and for DRB1 locus
- Unrelated BM/PBSC donor: identical or with a single disparity in class I or II loci with the recipient using HR molecular typing for class I loci (i.e. A, B and C) and for DRB1 locus
- CB donor: 6/6 or 5/6 matched donor using HR molecular typing for class I loci (i.e. A and B) and DRB1 locus. Preferential choice of DRB1 matched donor is recommended

2.2.3 Stem cell source

- BM
- PBSC
- CB

2.2.4 Graft composition

- Unmanipulated BM cells: > $3.5x10^8$ NC/kg
- Unmanipulated PBSC: > $4x10^6$ CD34+ cells/kg and < $10x10^6$ CD34+ cells/kg or < $5x10^7$ CD3+ cells/kg
- CB: units containing more cells have to be preferentially selected. In any case, a patient must receive at least $3.5x10^7$ NC/kg before thawing.

2.2.5 Conditioning regimen

- ATG Novii (Grafalon®) will be used in patients transplanted from unrelated donors or relatives other than an HLA-identical sibling during conditioning regimen for *in vivo* T-cell depletion/modulation: 10 mg/Kg/day for 3 consecutive days (-4 to –2). If alternative types of ATG are employed, equivalent doses of the product should be given.
- Preparation will consist of busulfan i.v. in 4 doses per day for 4 consecutive days (-7 to -4) (according to the manufacturers recommendation 0.8 - 1.2 mg/kg/dose) (or orally when the i.v. preparation is not available), cyclophosphamide 60 mg/kg/d on 2 consecutive days (-3 to -2) and melphalan 140 mg/m^2/d on day -1.
- Busulfan i.v. with an adjustment of the dose according to recipients body weight is recommended. PK study on the first dose of busulfan (especially if administered orally) is recommended and plasmatic levels of the drug should be comprised in the range between 700 - 950 ng/ml.
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2.2.6 Discussion on strategy for GVHD prophylaxis and treatment

HLA-identical sibling BM/PBSC donor and patient at HSCT < 12 years CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) starting from day –1 and with

the objective of maintaining serum levels between 100-200 ng/mL(continuous infusion over 24 hours is also acceptable); HLA-identical sibling BM/PBSC donor and patient at HSCT ≥ 12 years CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) and short course MTX (3 doses on days +1, +3 and +6 at a dosage of 10 mg/m^2).

- CSA tapering should be started around day +60 in the absence of grade II-IV acute GVHD and steroid treatment. CSA discontinuation should take place around day + 100.
- HLA-identical sibling CB donor: CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) with the objective of maintaining serum levels between 100-200 ng/mL (continuous infusion over 24 hours is also acceptable);
- Unrelated BM/PBSC donor: CSA 1.5 mg/kg in 2-hour infusion twice a day (total dose 3 mg/kg/day) and short course MTX (3 doses on days +1, +3 and +6 at a dosage of 10 mg/m^2).
- Unrelated CB donor recipients: the recommended GVHD prophylaxis is CSA (at the same dose indicated for BM/PBSC allografts) plus steroids (methylprednisolone 1.5 mg/kg/day starting from day - 4 for 3 weeks with a rapid tapering in the absence of GVHD in 2 more weeks). Alternatively, steroids can be replaced by MMF.
- CSA tapering should be started around day +100 in the absence of grade II-IV acute GVHD and steroid treatment. CSA discontinuation should take place around day + 140.
- Patients with grade II-IV 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 centre policy, is recommended.

2.2.7 Special considerations in regards to therapy prior to HSCT

The role of intensive chemotherapy prior to HSCT has been a matter of debate for decades and there are no solid data to make clear evidence based recommendations. However, patients with MDR-AML may have a benefit from AML like therapy prior to HSCT as reflected by a tendency for a better event free survival (EFS) [10]. Furthermore, our recent analysis of the 159 patient with advanced MDS showed that patients with more than 20% blasts at HSCT have a significantly worse probability of event-free survival due to a slightly higher rate of TRM and a significantly higher risk of relapse.

Based on these observations patients with advanced disease (≥ 20% BM blasts) are eligible to receive chemotherapy prior to HSCT. It is not possible to clearly define the best type of intensive chemotherapy and the pre-transplant regimen has to be adapted to the individual case. However, there is an overall agreement that patients with MDR-AML should currently be treated with one cycle of cytoreductive chemotherapy with the aim of reducing blast count (not an induction of remission) and then be transplanted. It is proposed to base the pre-transplant cytoreductive chemotherapy on the international AML-Relapse protocol from 2002 (using eg. daunorubicin/cytarabine/fludarabine). There is currently no consensus on the use of cytoreductive therapy in patients with RAEB-t (≥20, <30% BM blasts) and application of chemotherapy should be based on an individual decision taking the clinical

condition, the rate of progression and the karyotype into account.
Similar to intensive cytoreductive therapy, there is insufficient data to propose guidelines on the use of hypomethylating agents like azacitidine in paediatric MDS [12] [13]. In adult MDS, responses to hypomethlyating agents have preferentially been described in MDS with monosomy 7 or TP53 mutations [14] [15].

2.2.8 Special consideration for relapsed MDS-EB

Patients with relapse of MDS-EB following HSCT generally have a poor prognosis. However, analysis of patients included in the EWOG-MDS 98 and 2006 studies revealed a pOS and pEFS of 0.29 and 0.27 at 5 yrs for patients who had received a second allograft following relapsed MDS-EB. The most important predictive factor was time from 1st HSCT to relapse with lower EFS for patients relapsing earlier than 9/12 months after 1st HSCT. Furthermore, the incidence of relapse following 2nd HSCT was lower for patients transplanted with PBSC and patients experiencing chronic GVHD indicating the relevance of graft versus leukaemia effect.

Although data on 2nd HSCT are frequently associated with a considerable bias as not all relapsed patients are eligible for 2nd HSCT, the data presented allow at least some conclusions:

- Eligibility: Patients with a relapse earlier than 9 months following HSCT should not be offered 2nd HSCT unless they are stable enough to delay 2nd HSCT until at least 12 months following 1st HSCT.
- Therapy prior to 2nd HSCT: consider azacytidine, consider induction in patients with > 20% blast with or without monosomy 7
- Donor: if the donor in the 1st HSCT was a MSD, 2nd HSCT should be performed with a MUD. According to the data presented there is no indication to change from one MUD to another MUD. HSCT from a haplo-identical donor should be performed within a clinical trial whenever possible.
- Stem cell source: PBSC should be the preferred stem cell source
- Preparative regimen: no specific regimen can be recommended.
- GVHD-Prophylaxis: restrictive GVHD Prophylaxis should be employed i.e. CSA/MTX or CSA/MMF without ATG for MUD

3 Secondary MDS following treatment for a first malignancy

Patients with secondary MDS following chemotherapy +/- radiation have a poor prognosis. Preliminary results of the EWOG MDS study showed that the outcome of patients with secondary MDS following haematological malignancies was superior compared to patients with secondary MDS following solid tumors. In addition, a structural complex karyotype was associated with very poor prognosis. In patients with less than 5% of blasts in BM and/or PB, all treatment failures were associated with transplant related complications and no relapse was observed in this group. Based on these observations it is recommended to treat patients with secondary MDS and less than 5% of blast in BM and/or PB with the

thiotepa/treosulfan/fludarabine regimen described for patients with RCC and monosomy 7, del(7q) or ≥ 2 abnormalities (see 1.2.3). Patients with secondary MDS and an increase in blasts following haematological malignancies should be transplanted according to the recommendations for advanced MDS (see 2). Based on the current data it is not possible to give clear recommendations for 2nd MDS after solid tumour or with complex karyotype.

4 Secondary MDS following SAA

Outcome of patients with secondary MDS following IST for severe aplastic anaemia is (SAA) unfavourable. The major problem after HSCT in these patients is the high rate of TRM. It is recommended to transplant patients with 2nd MDS after SAA without increase of blasts according to the recommendations for RCC and monosomy 7, del(7q) or ≥ 2 abnormalities (thiotepa/treosulfan/fludarabine) (see 1.3), whereas patients with an increase in blasts should be treated according to the recommendations for primary advanced MDS (see 2).

5 Juvenile myelomonocytic leukaemia (JMML)

5.1 Algorithm of therapy, modifications from previous guidelines

JMML is, at least in part, due to aberrant signal transduction of the RAS signalling pathway, and up to 85% of patients harbour driving molecular alteration in 1 of 5 genes (*PTPN11*, *NRAS*, *KRAS*, *CBL*, and *NF1*). The curative treatment for patients with JMML is HSCT and the EWOG-MDS group has successfully used a busulfan-based regimen in this cohort of patients[7]. However, the clinical course and the outcome of HSCT in patients with JMML are variable and at least partially depend on the molecular genotype.

It has been recognized for some time that patients with heterozygous germline mutations in *PTPN11*, *NRAS* or *KRAS* can develop a myeloproliferative disorder which is transient in nature and does not require HSCT[16-19]. Some of these patients with Noonan syndrome benefit from cytoreduction (i.e. 6-mercaptopurine: 6-MP), and up to 10% acquire clonal events and develop JMML.

More recently it became evident that JMML in patients with germline CBL and acquired loss of heterozygosity or biallelic *CBL* mutations [20] may have a self-limiting course as well. Thus, many of these patients may not require HSCT for JMML and may be followed like Noonan patients with TMD.
In contrast, patients with JMML and (clinical diagnosis of) NF1, somatic PTPN11 mutation, somatic KRAS mutation, and those without known molecular lesion have no chance of survival without HSCT and, thus, have a clear indication for HSCT.

Patients with NRAS mutation (somatic) show various clinical courses and some patients survive without HSCT. In these patients decision to proceed with HSCT should be made depending on the severity of disease

The preparative regimen with busulfan, cyclophosphamide and melphalan has provided an acceptable HSCT outcome for all JMML subtypes. Interestingly patients with JMML and mutation in KRAS as well as patients with none of the known molecular lesions have a very low risk of relapse and therefore might benefit from a less intensive conditioning regimen.

5.2 Details in HSCT specifics in JMML and its molecular subtypes

5.2.1 Investigations to be performed/repeated at time of HSCT, that is within 6 weeks before the allograft

- BM aspirate to evaluate blast percentage
- Karyotype of BM cells to detect abnormalities
- Skin biopsy for cryopreservation of cultured fibroblasts
- Cryopreservation of viable BM and PB cells
- Storage of DNA

5.2.2 Criteria for donor selection

- HLA-identical sibling BM/PBSC/CB donor: molecular typing for class I loci (i.e. A, B and C) and HR molecular typing for DRB1 locus if parents typing available. If not, HR molecular typing for both class I and II loci is recommended
- Phenotypically identical relative: HR molecular typing for class I loci (i.e. A, B and C) and for DRB1 locus;
- Unrelated BM/PBSC donor: identical or with a single disparity in class I or II loci with the recipient using HR molecular typing for class I loci (i.e. A, B and C) and for DRB1 locus.
- CB donor: 6/6 or 5/6 matched donor using HR molecular typing for class I loci (i.e. A and B) and DRB1 locus. Preferential choice of DRB1 matched donor is recommended.

5.2.3 Stem cell source

- BM
- PBSC
- CB

5.2.4 Graft composition

- Unmanipulated BM cells: > $3.5x10^8$ NC/kg
- Unmanipulated PBSC: > $4x10^6$ CD34+ cells/kg and < $10x10^6$ CD34+ cells/kg or < $5x10^7$ CD3+ cells/kg
- CB: units containing more cells have to be preferentially selected. In any case, a patient must receive at least 3.5 $x10^7$ NC/kg before thawing.

5.2.5 Conditioning regimen for patients with NF1, somatic PTPN11 mutation, somatic NRAS mutation (excluding the selected patients that do not proceed to HSCT see 5.1.)

- ATG Novii (Grafalon®) will be used in patients transplanted from unrelated donors or relatives other than an HLA-identical sibling during conditioning regimen for *in vivo* T-cell depletion/modulation: 10 mg/kg/day for 3 consecutive days (-4 to –2). If alternative types of ATG are employed, equivalent doses of the product should be given.
- Preparation will consist of busulfan i.v. in 4 doses per day for 4 consecutive days (-7 to -4) (according to the manufacturers recommendation 0.8 - 1.2 mg/kg/dose) (or orally when the i.v. preparation is not available), cyclophosphamide 60 mg/kg/day on 2 consecutive days (-3 to -2) and melphalan 140 mg/m^2/d on day -1.
- Busulfan i.v. with an adjustment of the dose according to recipients body weight is recommended. PK study on the first dose of busulfan (especially if administered orally) is recommended and plasmatic levels of the drug should be comprised in the range between 700 - 950 ng/ml.

Conditioning regimen for patients with somatic KRAS mutation or no mutation in one of the known genes

- ATG Novii (Grafalon®) will be used in patients transplanted from unrelated donors or relatives other than an HLA-identical sibling during conditioning regimen for *in vivo* T-cell depletion/modulation: 10 mg/kg/day for 3 consecutive days (-4 to –2). If alternative types of ATG are employed, equivalent doses of the product should be given.
    - Preparation will consist of thiotepa 8 mg/kg/d on day -7, fludarabine (40 mg/m^2/day from day -6 to day -3) and treosulfan (14 g/m^2/day from day -6 to day -4).

5.2.6 Strategy for GVHD prophylaxis and treatment

- HLA-identical sibling BM/CB/PBSC donor and patient at HSCT younger than 4 years and with less than 20% BM blasts: CSA 1 mg/kg in 2-hour infusion twice a day (total dose 2 mg/kg/day) starting from day –1 and with the objective of maintaining serum levels between 80-150 ng/mL
- HLA-identical sibling BM/CB/PBSC donor and patient at HSCT older than 4 years or with more than 20% BM blasts: CSA 0.5 mg/kg in 2-hour infusion twice a day (total dose 1 mg/kg/day)
- Unrelated donor recipients or children transplanted from a relative other than an HLA-identical sibling: CSA 1 mg/kg in 2-hour infusion twice a day (total dose 2 mg/kg/day) starting from day –1 and with the objective of maintaining serum levels between 80-150 ng/mL
- Unrelated BM/PBSC donor recipients or children transplanted from a relative other than an HLA-identical sibling will receive MTX: 3 doses on days +1, +3 and +6 at a dosage of 10 mg/m^2
- For children given an unrelated donor CBT, the recommended GVHD prophylaxis is CSA (at the same dose indicated for BM/PBSC allografts) plus steroids (methylprednisolone 1.5 mg/Kg/day starting from day –4 for 3 weeks with a rapid tapering in the absence of GVHD in 2 more weeks). Alternatively, steroids can be

replaced by MMF.

- CSA tapering should be started around day +40 in the absence of grade II-IV acute GVHD and steroid treatment. CSA discontinuation should take place between day + 60 and +90.
- Patients with grade I (namely only limited skin involvement) acute GVHD should not receive systemic steroid treatment.
- Patients with grade II-IV should initially receive systemic steroids at a dosage of 2 mg/kg. If regression occurs steroids should be rapidly tapered and discontinued (e.g. within 2 weeks). For persistent or progressive GVHD second line treatment, according to each center's policy, is recommended.

5.2.7 Monitoring of chimerism/MRD

Monitoring of chimerism through STR or SNP-STR should start on day +15 after the allograft and it is recommended to be performed weekly on PB until day +90 and then at least monthly until the patient is off immune-suppressive therapy. In case of re-growth of recipient cells (e.g. first positivity of mixed chimerism or reappearance of MRD) immune-suppressive therapy must be discontinued immediately. There is no indication for performing DLI in these patients.

5.2.8 Therapy prior to HSCT

There is increasing evidence that azacitidine can induce clinical,haematological, cytogenetic and molecular remission in some children with JMML [13 21]. It is currently unknown, whether therapy with azacitidine prior to HSCT can improve outcome.

5.2.9 Special considerations for relapsed JMML

In case of progression to frank relapse or if the patient experiencing mixed chimerism is not receiving any immune suppressive therapy a second HSCT must be early considered. Preliminary data demonstrate promising results for 2nd HSCT in patients with relapsed JMML using the previously described thiotepa/treosulfan/fludarabine regimen and, thus, the use of this regimen is recommended.

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