EBMT/ESID GUIDELINES FOR
HAEMATOPOIETIC STEM CELL
TRANSPLANTATION FOR PRIMARY
IMMUNODEFICIENCIES
A. Introduction

Primary immunodeficiencies are rare heterogeneous disorders. Patients present with a variety of clinical symptoms and a wide range of infections and other complications. Treatment by bone marrow transplantation is increasingly successful (reference: Antoine C, et al. *The Lancet* 2003;361:553-60; Gennery et al., *JACI* 2010;126:602-610) and the joint EBMT/ESID Working Party has played a pivotal role designing and developing the guidelines which have led to this success.

The clinical heterogeneity of the patients, together with the fact that outcome data are based on observational studies, means that it is not yet possible to recommend tightly defined clinical protocols for transplanting these conditions. Each case needs to be carefully evaluated in a centre which has significant ongoing experience of performing these procedures. The exact transplant protocol will be devised using these guidelines, but sometimes modified according to the particular variant of the primary immunodeficiency and/or the patient’s clinical condition. For all these reasons the Working Party strongly recommends that all patients with primary immunodeficiency are transplanted in a centre that regularly transplants such cases, and also actively participates in the Working Party, as only in this way can optimum results be obtained.

The guidelines are reviewed on an annual basis and sub-groups of Working Party members revise some of the guidelines for specific conditions each year.

B. Conditioning Regimens

Over the years a number of different conditioning regimens have evolved as newer, less toxic conditioning agents have been made available. For these and other reasons, it has been difficult to gather data on the use of a particular conditioning protocol for any one disease such that a strong recommendation can be made. In most cases, groups of primary immunodeficiencies have been transplanted using certain generic protocols often with modifications (e.g Flu/Melph/Campath or ATG). It is also important to note that specific conditioning regimens are not risk factors for survival in the SCETIDE data.

To address these issues and to simplify matters, the IEWP decided that rather than to recommend specific protocols for specific conditions, one approach would be to make a list of protocols available. For disease groups, a recommendation would be made to choose from the protocol list e.g for Wiskott-Aldrich syndrome with a MUD use protocol A, B or D. The aim of this approach is that:

1) By limiting the number of protocols available, there will be less variation between centres

2) If centres use specific protocols as defined, then we will be able to gather data on the success or otherwise of a specific protocol in treating these conditions

3) We also recognise that for smaller or less experienced centres this guidance is important and by making these guidelines available on the EBMT and ESID websites, the information is readily available

We have therefore made a list of four protocols A-D which are outlined and are recommended for the majority of diseases. Specific details/examples of these protocols are made available in the appendix. Exceptions to these recommendations are SCID, where some transplants can be undertaken without any conditioning and severe immunodeficiencies associated with radiosensitivity which require specialised protocols.

We ask that if protocols are used, then they are adhered to in terms of dosing and schedule as much as possible since only then can meaningful data be accrued over time.
Protocol A is aimed at PID (inc HLH) patients with standard risk and where a greater degree of myeloablation is required to promote increased donor engraftment than protocol B (for haplo-identical T cell depleted grafts Thiotepa needs to be added in NON-SCID patients to achieve engraftment).

Protocol B is aimed at PID (inc HLH) patients with organ toxicity / reduced performance scale and CGD patients (see CGD specific guidelines).

<table>
<thead>
<tr>
<th>PROTOCOL</th>
<th>CHEMOTHERAPY</th>
<th>SEROTHERAPY</th>
<th>GVHD PROPHYLAXIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Busulfan (iv) (wt or AUC dosing)&lt;sup&gt;1&lt;/sup&gt; Fludarabine 160 mg/m2</td>
<td>&lt;sup&gt;†&lt;/sup&gt;Campath 1H (TD 0.6-1mg/kg) OR &lt;sup&gt;††&lt;/sup&gt;ATG (TD 10mg/kg)</td>
<td>CyA or CyA + MMF or MTX (as 2&lt;sup&gt;nd&lt;/sup&gt; agent)</td>
</tr>
</tbody>
</table>

- <sup>1</sup>AUC dosing for iv Bu = 90+/− 5 mg*h/L. (see appendix for specific protocols for different donor sources and dosing)
- <sup>†</sup>Campath 1H – Alemtuzumab
- <sup>††</sup>ATG – Genzyme rabbit ATG
- Busulfan/Cyclophosphamide conditioning is no longer recommended by the IEWP because of the increased risk of VOD.
## Reduced Intensity Conditioning

<table>
<thead>
<tr>
<th>PROTOCOL</th>
<th>CHEMOTHERAPY</th>
<th>SEROTHERAPY</th>
<th>GVHD PROPHYLAXIS</th>
</tr>
</thead>
</table>
| **B**    | Busulfan (iv) (AUC dosing)<sup>2</sup>  
  Fludarabine 180 mg/m² | †Campath 1H (TD 0.6-1mg/kg)  
  OR  
  ††ATG (TD 7.5-10mg/kg) | CyA  
  or  
  CyA + MMF or MTX (as 2<sup>nd</sup> agent) |
| **C**    | Fludarabine 150 mg/m²  
  Melphalan 140 mg/m² | Campath 1H (TD 0.6-1mg/kg) | CyA  
  or  
  CyA/MMF |
| **D**    | Treosulphan 42 g/m²  
  Fludarabine 150 mg/m² | None  
  or  
  Campath 1H(0.6-1mg/kg) | CyA  
  or  
  CyA/MMF |

- <sup>2</sup>AUC dosing for iv Bu = 60+/- 5 mg*h/L. (see appendix for specific protocols for different donor sources and dosing)
- Avoid Melphalan 140mg/m² < 1 year of age unless HLH
- Treosulphan 36g/m² < 1 year of age (see appendix for specific protocols)
- If using ATG with protocols C or D – be aware of increased incidence of EBV-PTLD
- For these protocols if using matched UD or MFD – PBSCs are stem cell source of choice
- If using BM consider decrease in Campath 1H dose to 0.6mg/kg esp if condition requires full donor chimaerism as in WAS or MHC class II deficiency
1 Severe Combined Immunodeficiency (SCID)  
(arising from all molecular defects but for the purposes of conditioning regimens defined immunologically by profound T cell lymphopaenia OR by oligoclonal non-functional T cells as in Omenn’s syndrome)

I Genotypically identical donor (and phenotypically identical donor esp in SCID-X1/ADA SCID)

- conditioning: no
- T-cell depletion: no
- GvHD prophylaxis: no

(*applies also for ADA’, Omenn S. and other “leaky” SCID, SCID with maternal GvHD)

NB consider conditioning in
  a) Omenn’s syndrome with autoreactive T cells
  b) SCID with maternal GvHD
  c) in those with failure of primary engraftment

Consider 2nd transplant if there is failure of T cell recovery 1yr after initial transplant

II matched unrelated donor (MUD) OR phenotypically identical family donor (BM or PBSCs):

- Protocol A, B or D
- PBSCs are preferred stem cell source for matched (10/10) MUD and MFD with protocol D
- Serotherapy
- Use CyA (+ MMF if using PBSCs as stem source due to increased T cell dose)

III UCB

- Protocol A, B or D
- Serotherapy
- Consider omitting serotherapy if well matched (6/6 or 5/6) donor and/or concern of viral infection
- CyA (+ MMF or steroids if increased concern of GvHD or if omitting serotherapy)

IV HLA- nonidentical (haplo) family donor

- Protocol A
- Use T depleted graft (CD34 + selection)
- CyA or none
  (in case of primary GvHD from maternal-fetal transfusion or Omenn Syndrome, therapy / prophylaxis of GvHD is usually needed and should be continued for 3 months)

Alternative protocol for SCID with haplo donor (esp T-B+ SCID)

(These transplants are most successful in T-B+ SCID and show the best results in patients under 3mths of age. In these transplants B cell engraftment is only seen in ~30% of cases and long term Ig replacement may be necessary)

- conditioning: no
- T-cell depletion: yes (CD34+ selection)
- GvHD prophylaxis: no (unless CD3+ cell dose >5 x 10e4kg)

(See appendix 1 for algorithms of treatment for SCID-X1 and ADA SCID)
2 Radiosensitivity Disorders (DNA Ligase 4, Cernunnos-XLF, NBS)

Patients with combined immunodeficiencies due to radiosensitive disorders such as DNA ligase 4 deficiency or Cernunnos deficiency are increasingly being identified and being offered for haematopoietic stem cell transplant. Patients with Nijmegen breakage syndrome may present with evidence of immunodeficiency (Ref) or more often with malignancy, particularly leukaemia or lymphoma, requiring transplantation. As many of the conditioning regimens are particularly damaging to DNA less toxic regimens are required to successfully treat these patients. It should be noted that in particular the long term outcome of patients with Nijmegen breakage syndrome following BMT has yet to be determined and transplanting these patients should be taken only on a case by case basis after careful consideration of risks and benefits and possibly in consultation with other centres that have transplanted these patients.

DNA Ligase 4 Deficiency

Few transplants have been performed for this. Radiation should be avoided as these patients respond very badly (Riballo 1999). Full conventional conditioning with high dose Busulfan and Cyclophosphamide is associated with an adverse outcome (Van der Burg M, 2006, Buck D et al, 2006). Conditioning regimens containing low intensity conditioning agents such as Fludarabine, Thiotepa and low dose Cyclophosphamide (5 mg/kg for 4 days) has resulted in successful outcomes (Gruhn 2007, Enders A 2006, Cale C personal communication).

Nijmegen Breakage Syndrome

It has been recognised for a long time that these patients have significant radiosensitivity. 5 patients with Nijmegen breakage syndrome who had undergone transplant were presented at the European Society of Immunodeficiency meeting in Hertogenbosch in October 2008 (Albert MH, 2008). Of the 5 patients, 1 receiving moderate dose Busulfan (10 mg/kg) and Cyclophosphamide (120 mg/kg) died. All other received Fludarabine with or without Melphalan, Thiotepa, ATG, OKT3 or Campath with or without Cyclophosphamide 20 mg/kg. One received low dose (5 Gy) irradiation. All 4 patients survived, engrafted and are doing well.

Cernunnos Deficiency

No published reports have described successful transplant for this condition. Transplantation has been successful using modified Fanconi syndrome regimens (Slatter M, personal communication).
Suggested protocol for transplanting patients with DNA breakage repair disorders is as follows:

Day -9  Fludarabine 30 mg/m²  
Alemtuzumab* 0.2 mg/kg  

Day -8  Fludarabine 30 mg/m²  
Alemtuzumab* 0.2 mg/kg  

Day -7  Fludarabine 30 mg/m²  
Alemtuzumab* 0.2 mg/kg  

Day -6  Fludarabine 30 mg/m²  
Alemtuzumab* 0.2 mg/kg  

Day -6  Cyclophosphamide 5 mg/kg  
Fludarabine 30 mg/m²  
Alemtuzumab* 0.2 mg/kg  

Day -4  Cyclophosphamide 5 mg/kg  

Day -3  Cyclophosphamide 5 mg/kg  

Day -2  Cyclophosphamide 5 mg/kg  

Day -0  MMF 15 mg/kg tds – wean by 25% per week over 4 weeks from day +28  
Cyclosporin from day – 1  
GCSF 5 micrograms/kg from day +8  

*Suggest removal of Alemtuzumab in MSD transplants

References


3 Combined immunodeficiencies inc:
Wiskott-Aldrich Syndrome, CD40L deficiency, PNP, XLP, Undefined T cell disorders, MHC class II def, LAD, Osteopetrosis

I Genotypically identical donor (inc 1 Ag mismatch)
- Protocol A or B or D
- Use serotherapy if 1 Ag mismatch
- CyA/MMF or CyA/MTX

II MUD or phenotypical matched donor (NOT for WAS – see below)
- Protocol A, B, C or D
- PBSCs are preferred stem cell source for matched (10/10) MUD and MFD with RIC protocols C and D
- Serotherapy
- CyA
- MTX or MMF as second agent (CyA/MMF if using PBSCs as stem cell source)

III UCB
- Protocol A, B or D
- Serotherapy
- Consider omitting serotherapy if well matched (6/6 or 5/6) donor and/or concern of viral infection
- CyA (+ MMF or steroids if increased concern of GvHD or if omitting serotherapy)

IV Haploidentical donor
- Protocol A plus Thiotepa as specified in the SUMMARY-Table: Myelo-Ablative Conditioning in Inborn Errors, page 30
- Use T depleted graft (CD34+ selection)
- Serotherapy (ATG)
- None or CyA

For Wiskott Aldrich Syndrome with MUD/MFD
- Protocol A, B or D
- PBSCs are preferred stem cell source for matched (10/10) MUD and MFD with protocol D
- Serotherapy
- Use CyA
- Add MTX or MMF as a second agent (use CyA/MMF if using PBSCs as stem cell source)
4 Osteopetrosis

Matched Sibling Donor

Inclusion Criteria
- HLA-genoidentical Donor

Exclusion Criteria
- Neuronopathic form (MRI, genetics: OSTM1+) contact one of the Authors
- Osteoclast poor form (bone biopsy evaluation or genetics: TCIRG1-, CLCN7-, RANK-, RANKL+) contact one of the authors
- CLCN7+: neuronopathic forms should be excluded contact one of the authors

Conditioning
- **Standard Protocol, Busulfan-based:**
  - Busulfex (weight adapted, kinetics recommended): day -8 to day -5
  - Fludarabine (150 mg/m²): 30 mg/m²/day, day -7 to day -3
- **Pilot Protocol, Treosulfan-based:**
  - Treosulfan (> 1 y: 42 g/m², < 1 y 36 g/m²): 14 g/m²/day or 12 g/m²/day, day -7 to day -5
  - Fludarabine (150 mg/m²): 30 mg/m²/day, day -7 to day -3
  - Thiotepa (10 mg/kg): 2 x 5 mg/kg at day -4

Transplant
- BM (1st choice): > 5 x 10⁸ NC / kg BW
- PBSC (2nd choice): >10 x 10⁶ CD34+ / kg BW

Boost
- (Not regular)

GvHD prophylaxis
- CSA (3 mg/kg/day): start i.v. at day -5, serum level 100 to 150 day 0 to day 100, then tapering 20% every two weeks
- If donor is >14 years old and/or PBSC were used: additional MMF 1200 mg/m² start i.v. at day 0, stop at day 30

* The pilot protocol may be used in high risk situations (significant extramedullary haematopoiesis, significant hepatosplenomegaly, hydrocephalus, pulmonary hypertension, infection, patients > 1 year of age, retransplant) according to an international Treosulfan trial by Sykora and Wachowiak.
Matched Unrelated Donor

Inclusion Criteria
- HLA-matched unrelated donor (10/10 matched, 4 digits; single HLA-C or HLA-DQ mismatch are allowed)

Exclusion Criteria
- Neuronopathic form (MRI, genetics: OSTM1+) contact one of the Authors
- Osteoclast poor form (bone biopsy evaluation or genetics: TCIRG1, ClCN7-, RANK-, RANKL+) contact one of the authors
- CLCN7+: neuropathic forms should be excluded contact one of the authors
- HLA-genoidentical donor available

Conditioning
- **Standard Protocol, Busulfan-based:**
  - Busulfex (weight adapted, kinetics recommended): day -8 to day -5
  - Fludarabine (150 mg/m²): 30 mg/m²/day, day -7 to day -3
  - Thiotepa (10 mg/kg): 2 x 5 mg/kg at day -4
  - Serotherapy, ATG-Thymoglobulin (Genzyme) suggested (10 mg/kg): 1 mg/kg day -3, 3 mg/kg/day day -2 to day 0
- **Pilot Protocol, Treosulfan-based:**
  - Treosulfan (> 1 y: 42 g/m², < 1y 36 g/m²): 14 g/m²/day or, 12 g/m²/day, day -7 to day -5
  - Fludarabine (150 mg/m²): 30 mg/m²/day, day -7 to day -3
  - Thiotepa (10 mg/kg): 2 x 5 mg/kg at day -4
  - Serotherapy, ATG-Thymoglobulin (Genzyme) suggested (10 mg/kg): 1 mg/kg day -3, 3 mg/kg/day day -2 to day 0

Transplant
- BM (1st choice): > 5 x 10^8 NC / kg BW
- PBSC (2nd choice): >10 x 10^6 CD34+ / kg BW;
  - T-cells may be reduced in vitro to 10-50 x 10^6 CD3+/kg BW

Boost
- (Not regular)

GvHD prophylaxis
- CSA (3 mg/kg/day): start i.v. at day -5, serum level 100 to 150 day 0 to day 100, then tapering 20% every two weeks
- MMF 1200 mg/m² start i.v. at day 0, stop at day 30

* The pilot protocol may be used in high risk situations (significant extramedullary haematopoiesis, significant hepatosplenomegaly, hydrocephalus, pulmonary hypertension, infection, patients > 1 year of age, retransplant) according to an international Treosulfan trial by Sykora and Wachowiak.
HLA-Haploidentical Donor

Inclusion Criteria
• Haematopoietic insufficiency (transfusion dependent)

Exclusion Criteria
• Neuronepatic form (MRI, genetics: OSTM1+) contact one of the Authors
• Osteoclast poor form (bone biopsy evaluation or genetics: TCIRG1-, ClCN7-, RANK-, RANKL+) contact one of the Authors
• CLCN7+: neuropatic forms should be excluded contact one of the Authors
• HLA-matched donor available

Conditioning
• **Standard Protocol, Busulfan-based:**
  o Busulfex (weight adapted kinetics recommended): day -8 to day -5
  o Fludarabine (150 mg/m²): 30 mg/m²/day, day -7 to day -3
  o Thiotepa (15 mg/kg): 2 x 5 mg/kg at day -4, 1 x 5 mg/kg at day -3
  o Serotherapy, ATG-Thymoglobulin (Genzyme) suggested (10 mg/kg): 1 mg/kg day -3, 3 mg/kg/day day -2 to day 0

• **Pilot Protocol, Treosulfan-based** (cave: this protocol has not been explored so far – contact the coordinator, if considered):
  o Treosulfan (> 5 kg: 42 g/m²): 14 g/m², day -7 to day -5
  o Fludarabine (150 mg/m²): 30 mg/m²/day, day -7 to day -3
  o Thiotepa (15 mg/kg): 2 x 5 mg/kg at day -4, 1 x 5 mg/kg at day -3
  o Serotherapy, ATG-Thymoglobulin (Genzyme) suggested (10 mg/kg): 1 mg/kg day -3, 3 mg/kg/day day -2 to day 0

Transplant
• T cell depleted PBSC (method according to local protocols):
  o stem cells: >10 x 10⁶ CD34+ / kg BW;
  o T-cells: < 2 x 10⁶ CD3+ / kg BW

Boost
• Part of stem cells collected at transplant should be stored and pre-emptively given, if necessary, as a boost at day +28; cumulative T-cell dose in transplant and boost should be < 4 x 10⁶ CD3+ / kg BW

GvHD prophylaxis
• T-cell depletion

Remarks:
• **HLA-haploidentical transplantation in OP is associated with high risks such as graft rejection, graft failure, toxic and infectious complications. This procedure should be performed in experienced centres only!**
• A **standard and optimal conditioning regimen for HLA-haploidentical HSCT in OP has not been established. The proposed protocols have been explored in very few patients so far. The clinical course and severe adverse events should be reported to the coordinator or one of the authors immediately and may result in modifications and amendments!**
5 Chronic Granulomatous disease

Gungor et al. have successful experience of HSCT in more than 20 CGD patients with ongoing infection and/or inflammation using a submyeloablative protocol B including in-vivo T cell depletion and CSA/MMF resulting in full myeloid donor chimaerism.

For adolescents/school children - aim for cumulative AUC for Busulfan between 50-65 mg/L x h to achieve full donor myeloid chimaerism (equivalent to 55-75% of the fully myeloablative dose of Busulfan (cumulative AUC of 90 mg/L x h)).

For preschool children/infants (below 6 yrs of age) – aim for cumulative AUC at the upper limit of the submyeloablative range (65-70 mg/L x h).

Calculation of the AUC for Busulfan:

\[
\text{a Bu AUC in ng/ml x h divided by 4.105} = \text{BU AUC in micromol x min} \\
\text{Example 10 000 ng/ml x h divided by 4.105} = 2436 \text{ micromol x min} \\
(10 000 \text{ ng/ml x h corresponds to 10 mg/L x h})
\]

If AUC for Busulfan cannot be measured, protocol A with full-dose Busulfan (dosage according to weight based recommendations) is recommended. Protocol D is an alternative conditioning in any type of HSCT for CGD, however, there is - as yet - no experience with this protocol for MSD, MMUD or MUD donors. Hence, the use of protocol D is regarded to be experimental. Any conditioning regimen is experimental for UCB and haploidentical transplantation since only anecdotal reports available. Therefore protocol A or D can be equally used if a UCB or haplo PBSCT is indicated. For MSD, MMUD and MUD transplants Protocol B with targeted Busulfan or fully myeloablative Protocol A is favoured.

I Genotypically identical donor

- Protocol B (use targeted Busulfan with a cumulative AUC of 65-70 mg/Lxh) or D
- Use ATG-Genzyme 7.5 mg/kg (d-5 to -3) because of the increased incidence of GvHD and for rejection prophylaxis
- BM is the preferred stem cell source
- CyA for 6 mo and MMF (until day +120)

II MUD or phenotypically matched family donor

- Protocol B (use targeted Busulfan with a cumulative AUC of 65-70 mg/Lxh) or D
- Use Campath 0.5 mg/kg (d-8 to -6) for BM (preferred stem cell source) and 1 mg/kg (d-8 to -4) in case of PBSC
- CyA for 6 mo and MMF (until day +120)

III UCB

- Protocol A (use targeted Busulfan with a cumulative AUC of 85-90 mg/Lxh) or D
- Serotherapy with ATG Genzyme (7.5 mg/kg)
- CyA for 6 mo and MMF (until day +120)

IV Haploidentical donor (use maternal donor in autosomal recessive disease; use paternal or non-X-CGD carrier donors in X-linked disease)

- Protocol A (use targeted Busulfan aiming at a cumulative AUC of 85-90 mg/Lxh) or D
- Use T depleted graft (CD34+ selection) (CD3 < 3 x 10^4/kg; CD34 5-10 x 10^6/kg)
- Use Campath 0.5 mg/kg (d-8 to -6) to prevent rejection
- CyA (only if CD3 cells are > 3 x 10^4/kg)
6 Haemophagocytic disorders inc: HLH, CHS, Griscelli, XLP with HLH

I Genotypically identical donor (inc 1 Ag mismatch)
- Protocol A or B or D
- Use serotherapy if 1 Ag mismatch
- CyA/MMF or CyA/MTX

II MUD or phenotypical matched donor
- Protocol A, B, C or D
- PBSCs are preferred stem cell source for matched (10/10) MUD and MFD with RIC protocols C and D
- Serotherapy
- CyA
- MTX or MMF as second agent (CyA/MMF if using PBSCs as stem cell source)

III UCB
- Protocol A, B or D
- Serotherapy
- Consider omitting serotherapy if well matched (6/6 or 5/6) donor and/or concern of viral infection
- CyA (+ MMF or steroids if increased concern of GvHD or if omitting serotherapy)

IV Haploidentical donor
- Protocol A plus Thiotepa as specified in the SUMMARY-Table: Myelo-Ablative Conditioning in Inborn Errors, page 30
- Use T depleted graft (CD34+ selection)
- Serotherapy (ATG)
- CyA or none
APPENDIX 1

In previous versions of these guidelines, recommendations regarding management and supportive care for specific disorders have been made which have been very useful to treating physicians. These are made available here for those conditions.
SCID X-1

SCID-X1

MSD/MFD/MUD search

MSD/MFD available

MUD/UCB available

MUD/UCB HSCT with conditioning

No MSD/MFD/MUD/UCB available

Haplo HSCT

<3mths
Haplo HSCT No conditioning

>3mths
Haplo HSCT with conditioning

Enrol into Gene therapy trial

Gene therapy No conditioning
ADA deficiency

PEG-ADA or Gene Therapy are options when a genotypically matched donor is unavailable.

The following algorithm for treatment of ADA-SCID has been agreed by members of the IEWP in conjunction with other experts (Gaspar et al., Blood 2009):
Wiskott Aldrich Syndrome

clinical diagnosis of WAS

mutation analysis

WAS mutation?

yes

no

WAS protein analysis

protein expressed?

yes

no

initiate donor search

HSCT from best available donor or gene therapy (clinical trial)

WAS score 5?

yes

no

MSD 10/10** MUD

HSCT

conservative therapy

HSCT from best available donor

gene therapy (clinical trial)

* don't search should be initiated immediately for severely affected children with life-threatening complications
** high resolution DNA based typing for all alleles
*** no sufficient evidence which therapy is most appropriate
Conservative therapy:

**IVIG:** Indicated for any WAS patient with a score ≥3. May be indicated in selected milder cases.

**Cotrimoxazole:** Indicated for any WAS patient with a score ≥3. May be indicated in selected milder cases.

**Splenectomy:** May reduce bleeding risk but increases risk for severe (fatal) post-splenectomy infections and increases risk for transplant related complications. The risk for post splenectomy infections may be lower with appropriate antibiotic prophylaxis and vaccination, but strict compliance to life-long antibiotic prophylaxis (also during adolescence and post transplant) must be ensured. If HSCT is contemplated, then splenectomy should not be pursued.

### WAS score:

<table>
<thead>
<tr>
<th>Score</th>
<th>XLN 0</th>
<th>iXLT &lt;1</th>
<th>XLT 1</th>
<th>XLT 2</th>
<th>Classic WAS</th>
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<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>-</td>
<td>-/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Small platelets</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Eczema</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>+</td>
<td>++ -/+/++</td>
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<td>Myelodysplasia</td>
<td>-/+</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

-/+, absent or mild;
-/+, intermittent thrombocytopenia, possible myelodysplasia;
(+), mild, transient eczema or mild, infrequent infections not resulting in sequelae;
+, thrombocytopenia, persistent but therapy-responsive eczema, and recurrent infections requiring antibiotics and often IVIG prophylaxis;
++, eczema that is difficult to control and severe, life-threatening infections.

Michael Albert, 11/17/2010
Chronic Granulomatous Disease

Revised and modified by Güngör T (according to Seger R, Flood T)

A1. Indications

X-CGD or a/r-CGD with MSD, MUD, MMUD donors plus one of the following (UCB and haploidentical stem cell sources are still experimental):

- Non-availability of specialist medical care
- Non-compliance with long-term antibiotic/antimycotic prophylaxis
- ≥ 1 life-threatening infection in the past
- Severe granulomatous disease with progressive organ dysfunction (e.g. lung restriction)
- Steroid-dependent granulomatous disease (e.g. colitis)
- Ongoing therapy-refractory infection (e.g. Aspergillosis)
- After emergence of premalignant clones or MDS (e.g. after gene therapy)

A2. Pre- and post-transplant work-up

2.1. Immunologic

- Quantitative measurement of respiratory burst
- Cytochemical NBT-test of maternal cells and of donor cells (mosaicism in X-CGD)
- Surface gp91phox expression
- Immunoblot (gp91, p22, p47, p67phox)
- Mutation analysis (if gp91 or p22phox def. suspected)
- Look for McLeod blood group in X-linked CGD

2.2. Pulmonary

- Chest x-ray and CT, O₂-saturation, lung-function,
- If pulmonary infection/inflammation:
  CT, PET or PET/CT-scan, bronchoscopy + lavage ± lungbiopsy and cultures

2.3. Gastrointestinal

- Weight/length curves, malabsorption parameters
- If colitis:
  Abdominal contrast-CT, colonoscopy, colonic biopsy and cultures. Calprotectin in stools.

2.4. Renal

Rule out CGD glomerulonephritis and protein losses

A3. Supportive therapy

3.1 Pre-transplant

Optimal reduction of infectious/inflammatory foci by antibiotics/antimycotics. Try to avoid transfusions of leukocytes from unrelated donors (sensitization towards HLA or HNA or McLeod). Use surgery or steroids if needed prior to HSCT. Stop Itraconazole at least 7 days prior the administration of alkylating agents, e.g. Busulfan. Stop gamma-Interferon 4 weeks prior HSCT.

3.2 Post-transplant
3.2.1. **Antimycotics**

If proven Aspergillosis in the past and elevated CRP prior HSCT:

administer iv Voriconazole alone (adjusted to blood levels > 1 and < 5 mg/L). (adjust CsA-levels!)

If ongoing florid Aspergillosis, treat with

Voriconazole* 14 mg/kg/day in 2x  
+Caspofungin ± 1 mg/kg/day in 1x  
(adjust CsA-levels!)  
*adjust Voriconazole to blood levels > 1 and < 5 mg/L).  
(adjust CsA-levels!)

3.2.3. **GvHD treatment**

If acute GvHD emerges: aggressive and early treatment is mandatory.  
At first signs: Methylprednisolone 2 mg/kg/day iv in 2 doses.  
If no response within 5-7 days: Consider early administration of other immunosuppressive drugs including further Campath 1H.

3.2.5. **Antiinfectious prophylaxis**

Continue Itraconazole or Voriconazole prophylaxis at least until day +120. Cotrimoxazole or Ciprofloxacin daily until day +120 or according preexisting infections.

3.2.6. With normal neutrophil counts (>1000/microliter) and the absence of GvHD fungus infections will clear within 2-3 months after HSCT. Granulomatous colitis will also clear within 2-3 months after HSCT.

A4. **Reporting**

Please report your transplant to Tayfun Güngör (Zürich) (tayfun.guengoer@kispi.uzh.ch) (Tel. 0041-44-2667492 and fax 0041-44-2667914) and to the Immunodeficiency registry in Paris (P. Landais, landais@necker.fr).

A6. **Literature:**

BMT for CD40 Ligand Deficiency Guidelines

Revision and studied by Graham Davies and Andy Gennery

B1. **Optimal management of newly diagnosed cases**
   - PCP prophylaxis
   - Adequate IVIG replacement
   - Cryptosporidium avoidance
   - Boiled / filtered water
   - +/- Antimicrobial Prophylaxis
   - Monitoring for organ disease
   - Tissue Typing

B2. **Monitoring for liver disease**
   - Regular measurements of transaminases + Gamma GT
   - Ultrasound scan at least 1/year
   - Stool for Cryptosporidium
   - If abnormal biochemistry or ultrasound need:
     - Liver biopsy
     - ERCP

B3. **When to do BMT?**
   Depends on type of donor available
   - Matched Sibling Donor
     - At diagnosis (and without complications)
   - Matched Unrelated Donor
     - Possibly at diagnosis
     - Definitely if early complications detected
   - Mis-matched Unrelated Donor
     - Only at stage of established early complications
   - Mismatched Related (Haplo)
     - There is no experience of these
     - Consider if progressive organ damage

B4. **Cryptosporidial Prophylaxis during BMT**
   - No evidence for efficacy in BMT
   - Three possible drugs
     - Azithromycin, Nitazoxa
     - Paromomycin
     - Potentially ototoxic
     - May be absorbed from GI tract if mucositis occurs
   - Azithromycin & Nitazoxantide have low toxicity – may get some disturbance of transaminases with the latter
   - Propose:
     - CP negative cases (PCR neg) use Azithromycin alone
     - CP positive (or + history) cases use Azithromycin + 1 other drug
     - Add third drug if overt Cryptosporidial disease occurs

B5. **Complications meriting consideration of transplantation**
   - Histological or radiological abnormalities of liver
. Lung damage – early brochiectasis
. Enteropathy
. Neutropenia not responsive to increased dosing with IVIG
. Persistent Cryptosporidium excretion
. Infection with Toxoplasma

If you should face adverse events, please inform as soon as possible the coordinator of this study:

**Graham Davies** (Hospital for Children NHS Trust – United Kingdom) Tel: 44.207.8138403 – Fax: 44.207.813.8552 – Email: davieg1@gosh.nhs.uk

**Andrew R Gennery** (Newcastle General Hospital – United Kingdom) Tel: 44.191.2336161 – Fax: 44.191.2730861 – Email: A.R.Gennery@ncl.ac.uk
Osteopetrosis

These guidelines are part of a prospective study « Osteopetrosis, Consensus guidelines for diagnosis, therapy and follow-up » which will be available on the ESID website.

For further questions you can contact
Ansgar SCHULZ    ansgar.schulz@uniklinik-ulm.de
Despina Moshous   despina.moshous@nck.aphp.fr

1 General Considerations

The clinical presentation of patients with OP is very heterogeneous; it is thus not possible to define a distinct strategy for each situation. However, following the pathophysiological and genetic defect and the experience of the retrospective data analysis (as described in chapter 1 of the prospective study), some general suggestions can be made. Type of disease, risk factors and donor availability are main determinants for the therapeutic procedure ranging from “urgent transplantation” to “wait and see”.

1.1 Indication for HSCT

Haematological failure and imminent loss of vision (e.g. nystagmus and/or narrowed foramina of optical nerves in MRI/CT scans) represent absolute (and urgent) indications for HSCT. Since the spectrum of haematological problems ranges from mild anaemia (with preserved haematopoiesis and no extramedullary haematopoiesis) to transfusion dependent anaemia and thrombocytopenia (with no relevant bone marrow space and important hepatosplenomegaly), this indication must be carefully evaluated and considered in the context of other symptoms and donor availability. Bone marrow biopsy, the count of reticulocytes and CD34 positive stem cells in the peripheral blood, as well as the LDH level and ultrasonography of spleen and liver, may help to evaluate the bone marrow function in less severe cases.

Severe problems from disease other than haematological failure or imminent visual loss may be considered as relative indications for HSCT, for instance: multiple fractures after inadequate trauma; severe bone malformations, particularly of the head bones, repeated bacterial infections and/or CNS problems such as hydrocephalus and/or Arnold Chiari-like lesion or central nerve compressions. Beside the medical history, MRI and CT scans (possibly with serial scans) should help to evaluate the clinical relevance of these symptoms.

Up to now, there are two absolute contraindications for standard HSCT:
1) the extrinsic osteoclast defect characterised by mutations of the RANKL gene
2) the neuronopathic form of OP characterised by encephalopathy and neurodegeneration with irritability, hypertonicity, seizures not due to hypocalcaemia (primary neurological defect) and progressive developmental delay, associated with mutations of the OSTM1 gene (and sometimes of the CLCN7 gene).

Relative contraindications for HSCT may arise from severe problems in the individual patient, such as bad clinical condition (infection, pulmonary hypertension, elevated intracranial pressure) or severe handicaps (e.g. blindness and deafness).

In some patients – for instance in a patient with a known genetic defect (TCIRG1, CLCN7 or RANK) but without haematological insufficiency - the decision for or against HSCT may be rather difficult. It seems to be essential to respect not only the patient’s individual clinical situation but also the individual feelings and choices of the patient and their family, particularly in atypical cases.

1.2 Donor

We suggest the following ranking of donors:
• HLA-genoidentical family donors (matched sibling donor/MSD)
• matched family donor/MFD in consanguineous families
• HLA-matched donors (matched family donors in non-consanguineous families > matched unrelated donors: BM > PBSC > cord blood)
• HLA-haplodentical family or HLA-mismatched cord blood donors

HLA-matching (e.g. to the 4 digit level for HLA-A, -B, -C, -DRB1 and -DQB1) and sub-ranking (e.g. according to CMV status, gender, age) of donors can be evaluated following the internal guidelines of the transplant centre and/or other transplant studies (e.g. the ALL-SCT-study of the EBMT).

If no matched donor will be available within a reasonable time period, HSCT from alternative donors (HLA-haploidentical parents or HLA-mismatched cord blood) should be initiated without delay. The use of cord blood has been explored in some small series with mixed success rates \(^{26,35-38}\) and the Eurocord experience will be summarised in an upcoming paper. On the other hand, also HLA-haploidentical transplants have been explored with mixed success in some European centres \(^{27,39-44}\), summarised in an upcoming retrospective registry of the ESID and the EBMT. The choice of the alternative donor may be dependent mainly on the local experience of the transplant centre.

1.3 Conditioning regimens
Conditioning in OP has to strike a difficult balance between the need for myeloablation and immunosuppression and the risk of regimen-related toxicity. Different regimens have been used in the past, but the “ideal” regimen remains subject of discussion \(^{21,27,43,45-47}\).

Following considerations seem to be reasonable:

a) the i.v. use of Busulfan with adequate dose adjustments
b) the substitution of Cyclophosphamide by Fludarabine because of the more favourable toxicity profile of Fludarabine;
c) the use of Thiotepa in non-genoidentical transplants because of its highly immunosuppressive and relatively myeloablative potential;
d) furthermore, in high risk situations, the substitution of Busulfan by Treosulfan may be explored in experienced centres.

The following risk factors are associated with a poor outcome in the retrospective analysis: significant extramedullary haematopoiesis (marked enlargement of spleen +/- liver), respiratory problems (choanal stenosis or pulmonary hypertension), CNS symptoms, age more than 1 year and an HLA-haploidentical transplant setting. It must be stressed that, particularly in these high risk situations, HSCT should be performed in experienced centres only. Up to now we are not able to give a general recommendation for conditioning in cord blood transplantation.

1.4 Transplant and Boost

• In the case of (geno- or pheno-) identical transplants, bone marrow is the stem cell source of first choice and no graft manipulation is necessary. If only peripheral blood stem cells are available, T-cell number in the graft may be reduced by ex vivo procedures to adjust the T-cell content of the graft to a maximum of 10-50 x 10^6 per kg body weight of the recipient.
• In the case of HLA-nonidentical (> 1/10 HLA-mismatch or HLA-haploidentical) transplants, peripheral blood stem cells should be used and the amount of T-cells in the graft must be roughly reduced by ex vivo procedures (CD34-positive selection and/or CD3/CD19 negative selection) to yield a T-cell content of the graft below 2.5 x 10^4 per kg body weight of the recipient.

To ensure engraftment, it is important to obtain an excellent graft with more than 5 x 10^8 nucleated cells per kg body weight of the recipient in the case of bone marrow and more than 10 x 10^7 per kg body weight of the recipient in the case of peripheral blood stem cells, respectively. Furthermore, since many patients will have a delayed reconstitution due to the narrowed bone marrow space, the preservation and storage of additional stem cells for a stem cell boost should be considered. In
particular, in the case of a T-depleted stem cell source, the preparation and cryoconservation of an additional graft with a cell content equal to the primary graft is highly recommended and should be “pre-emptively” done during the first stem cell preparation procedure. The “ideal” time point for a boost is considered around one month after transplantation, when the risk of acute GvHD can be evaluated and osteoclasts maturing from the primary transplant may have opened the bone marrow space in the recipient. A pre-emptive boost around day +28 may have been mainly attributed to the relative good results of the HLA-haploidentical transplants at the Ulm transplant centre27.

1.5 Risk prophylaxis
An adapted risk prophylaxis regimen is highly recommended taking into account the “special risk factors” of infants with OP:

- **GvHD and rejection prophylaxis:**
  In the case of an unmanipulated bone marrow graft, the standard CSA/MTX regimen has been substituted by a CSA/MMF prophylaxis regimen, since MMF is less toxic to the liver (VOD) and the graft (graft failure). In the case of a sibling donor below the age of about 14 years, CSA mono prophylaxis may be considered. Serotherapy should be used in any cases other than HLA-genoidentical transplants. We recommend ATG (10 mg/kg Thymoglobulin) in standard situations. If the T-cell content of an HLA-nonidentical graft accidentally exceeds 3 to 5 x 10⁴/kg body weight, a GvHD prophylaxis using CSA and/or MMF should be introduced.

- **VOD prophylaxis:**
  Whereas the new recommendations with regard to chemotherapeutic regimen and GvHD prophylaxis have been designed to be less toxic than the standard Busulfan-Cyclophosphamid-CSA-MTX regimen, patients with OP are at very high risk of developing liver (and pulmonary) VOD ³⁷,⁴⁸. In addition to a careful monitoring of VOD symptoms (untreatable thrombocytopenia, weight gain, liver enlargement, ascites, bilirubin elevation), prophylaxis or early therapy with Defibrotide is highly recommended.

- **Respiratory problems:**
  Respiratory problems are common during transplantation for several reasons. Upper airway obstructions (e.g. choanal stenosis) and secondary ventilation problems due to fluid overload and hepatosplenomegaly (VOD, CLS) or CNS diseases (hydrocephalus, hypocalcaemic convulsions) must be distinguished from primary pulmonary diseases due to infections and primary pulmonary hypertension. Secondary respiratory problems should be prevented and treated according to the individual situation (e.g. local steroids, assisted ventilation, tracheostomy, Defibrotide, anticonvulsant drugs). In the case of primary pulmonary problems, it is important to consider, monitor and treat pulmonary hypertension ³¹,³²,⁴⁹, (see treatment section below). Furthermore, patients with OP seem to harbour an elevated risk for pneumocystic jirovecii pneumonia (PCP), possibly because of the lack of prophylaxis before HSCT and the prolonged haematological and immunological recovery. Since at least three patients acquired PCP even in the laminar airflow environment (Moshous and Schulz, unpublished observation) we recommend the pre-treatment of patients with Cotrimoxazol (5mg/kg day of Trimethoprim / 25mg/kg day of Sulfamethoxazol) at least 2 weeks before HSCT and the re-introduction of Cotrimoxazol either on neutrophil engraftment or even earlier in the case of prolonged cytopenia.

- **CNS problems:**
  One of the most difficult and puzzling complications in OP is attributed to the CNS. Malformations of head bones and primary malformations of the brain should be distinguished. Primary malformations of the brain substance are characteristic for the “neuronopathic forms” of OP, which are genetically associated to mutations in OSTM1 and sometimes in CLCN7, and are considered as contraindications for HSCT in OP ²⁸. Malformations of the bones may lead to macro- or microcephalus, prominent large
fontanelle, hydrocephalus and/or Arnold-Chiari-like malformations. Whereas these malformations are in principle reversible after successful HSCT, they may lead to severe clinical complications. Careful and interdisciplinary diagnostic, monitoring and treatment regimens are mandatory in the individual affected patient.

- **Serum calcium disturbance:**
  Hypocalcaemia eventually associated with convulsions before engraftment contrasts hypercalcaemic complications thereafter. In the case of hypocalcaemia, supplementation with calcium gluconate and vitamin D (1000 IU per day) is recommended before HSCT. (Please read the paper by Schinke et al. \(^{33}\) for best administration regimen in patients with gastric pH disturbance as those affected by mutations in the TCIRG1 gene.) This supplementation should be reduced or even stopped with engraftment after HSCT to avoid hypercalcaemic crisis. Serum levels of calcium and phosphate should be carefully monitored before and for several months after transplantation and in the case of hypercalcaemia an individual treatment procedure is recommended.

**FOR SPECIFIC HSCT REGIMEN GUIDELINES (SEE SECTION 4 OF MAIN DOCUMENT)**

*Treatment of Complications*

Severe complications are common after HSCT in OP. Nevertheless, most “disease specific” complications are treatable and reversible. Therefore diagnostic and therapeutic intervention should be performed in a relatively short time period and in an “aggressive” manner. Using this strategy, even patients transferred to the ICU because of respiratory insufficiency (attributed to VOD, pulmonary hypertension, CNS complications) in the majority of cases survived in a larger series in Ulm.

Of course, “standard” complications of HSCT as infections (CMV, EBV, fungal infections) and GvHD should be monitored and treated according to established protocols.

**2.1 Non-engraftment and rejection**

Most patients with OP show a slow haematological recovery after HSCT, possibly because of due to narrowed marrow space and/or hepatosplenomegaly. A delayed haematological reconstitution must be carefully distinguished from an immunological rejection by chimerism analysis. If rejection can be excluded (see below), a stem cell boost (about at least one month after transplantation) should be considered (see chapter 1.4). In the case of mixed chimerism, chimerism analysis of different cell populations should be performed. Most importantly, using the conditioning regimens recommended in this protocol, we observed full donor chimerism or stable mixed chimerism (with persistent recipient T-cells) up to several months after transplantation resulting finally in full donor chimerism or stable mixed chimerism without signs of disease.

In the case of an active acute rejection (rising recipient T-cells with CD8-phenotype, disappearing donor granulocytes and stem cells), a secondary conditioning regimen should be considered before a stem cell boost can be given. Depending on the individual patient condition and the donor setting, chemotherapy (Cyclophosphamide 120 mg/kg) and/or anti-T-cell serotherapy (Alemtuzumab [Campath-1H] and/or Okt-3) may be administered. Particularly in the case of rejection of a non-identical graft, an alternative donor should be considered assuming sensitisation of the patient against the first donor.

**2.2 Venous Occlusive Disease (VOD)**

Preliminary data suggest that administration of prophylactic Defibrotide may efficiently prevent VOD in OP-patients. However in the absence of Defibrotide being freely available for this indication, early diagnosis and start of specific therapy is a prerequisite of a successful treatment of this common complication. Immediate start of Defibrotide infusions is highly recommended when VOD is
suspected by the typical, but sometimes unspecific clinical signs. A carefully balanced fluid and diuretic therapy (central venous pressure +1 to +5 cm H$_2$O) may prevent cardiovascular, pulmonary and renal insufficiency. In the acute phase of VOD and clinical presentations overlapping with capillary leakage syndrome (CLS), a short course of steroid therapy may be helpful.

2.3 Pulmonary hypertension
In the case of oxygen requirement and/or tachypnoea in the absence of microbiological documentation, repeated echocardiographic and ECG investigations (enlarged right ventricle) is helpful to detect this severe complication, documentation by cardiac catheterisation is recommended. Treatment is difficult and should reflect the individual situation. Oxygen administration (oxygen saturation > 95%), magnesium substitution and moderate diuretic therapy represent the first step of treatment. In progressive cases, Sildenafil (Viagra) or inhaled NO administration may be considered. In severe cases, complicated by cardiovascular and pulmonary insufficiency, Epoprostenol (Flolan) has been successfully used. Bosentan, an endothelin-receptor antagonist, may be considered. A tight interdisciplinary collaboration of haematologists, cardiologists, respiratory physicians and the intensive care team is mandatory, when this life threatening complication is suspected or evident.

2.4 Hypercalcaemia
Engraftment of donor cells may be accompanied by elevation of serum calcium, potentially to life threatening levels, especially in older patients with high bone mass. Low calcium (and phosphate) nutrition is recommended during this phase. Hypercalcaemia can arise despite this at any time during the first few months after transplantation. In severe cases, in particular when relevant nephrocalcinosis is detected in ultrasound, inhibition of osteoclast function by bisphosphonate therapy may be considered.

2.5 Secondary graft failure and mixed chimerism
As stated above, delayed normalisation of peripheral blood cell counts and mixed chimerism are common problems after HSCT. Nevertheless, normalisation of haematopoietic function and absence of OP may be achieved spontaneously. Therefore, therapeutic interventions must be carefully balanced against possible side effects. Since the therapeutic options are highly dependent on the individual situation, no general recommendation can be drawn. However, in the cases of progressive loss of donor chimerism in an HLA-identical setting, DLI may be considered.

2.6 “Late adverse effects”
Even after successful transplantation patients with OP have a high risk of specific sequelae. Therefore, a careful and long-lasting follow-up is mandatory (see time schedule in chapter 4 of the prospective study). Some examples are listed below:

- **Dwarfism:** After HSCT, the height of the majority of patients is between 3rd and 10th percentile and about 20% of patients show dwarfism (< 3rd percentile) after transplantation (A. Schulz, unpublished results). There seems to be no primary growth hormone deficiency in OP patients and treatment by growth hormone is not established but may be considered in severe cases after careful evaluation of pros and cons.

- **Craniosynostosis and intracranial hypertension:** In very few patients increased cranial pressure secondary to craniosynostosis has been described, which was treated surgically (A. Schulz, unpublished observation).

- **Autism:** In a small subset of patients, symptoms of autism have been described. These may be due to visual or auditory compromise or to the particular genetic defect or both (Schulz and Moshous, unpublished observation).
• **Osteoporosis:** Very recent mouse data indicate the risk of impaired calcium homeostasis due to impaired gastric acidification leading to late hypocalcaemic osteoporosis (particularly in patients with TCIRG1 mutations), which may be treated by calcium gluconate. Therefore, BMD measurements are recommended in long-term follow up after HSCT.

**Bibliography**
Available in the prospective study via the ESID website or by contacting the investigators (email address see above).
APPENDIX 2

In this appendix are detailed the specific protocols A-D. These should be followed as closely as possible with respect to dosing and drug administration schedule.

We recognise there are different options for GvHD prophylaxis depending on donor, such as CyA alone, CyA/MMF, CyA/MTX BUT we have shown not all the different permutations for every protocol. Similarly in some protocols, centres may want to use ATG instead of Alemtuzumab. In such cases, centres should use the ATG protocol they normally use.

We have also not shown the different antibiotic/anti-fungal/anti-viral/anti-VOD prophylactic regimens in these protocols. These will vary between different centres and also between different patients and so these should be individual decisions made by the individual transplant team.
## PROTOCOL A

### SUMMARY: Myelo-Ablative Conditioning in Inborn Errors

<table>
<thead>
<tr>
<th>Day</th>
<th>Bu Flu core protocol</th>
<th>GvHD/rejection prophylaxis</th>
<th>Additional Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSD</td>
<td>Cord Blood (4-6/6)</td>
<td></td>
</tr>
<tr>
<td>-9</td>
<td></td>
<td>Thymoglobuline</td>
<td>Thymoglobuline</td>
</tr>
<tr>
<td>-8</td>
<td></td>
<td>Thymoglobuline</td>
<td>Thymoglobuline</td>
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<tr>
<td>-7</td>
<td></td>
<td>Thymoglobuline</td>
<td>Thymoglobuline</td>
</tr>
<tr>
<td>-6</td>
<td></td>
<td>Thymoglobuline</td>
<td>Thymoglobuline</td>
</tr>
<tr>
<td>-5</td>
<td>Thymoglobuline</td>
<td>GvHD/rejection prophylaxis</td>
<td>For HR disease + haplo: + TT 10mg/kg</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Thymoglobulin (or Campath)</td>
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<td></td>
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<tr>
<td>0</td>
<td></td>
<td>Thymoglobulin (or Campath)</td>
<td></td>
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</tbody>
</table>

- **High risk (HR) disease:** Osteopetrosis (EBMT-guideline)
- **Standard risk:** PID (including HLH), Inborn Errors of Metabolism (IEM)
- Fludarabine to be given immediately before the busulfan.
- The PK adjusted doses of Bu are given at the same rate (mg/min) as the initial dose.
- Where PK results are not immediately available, other options are:
  1) Give two half doses on day -6 and -5, over 90 minutes
  2) Bring the day -5 treatment forward a day
- The total target AUC = 90 +/- 5 mg*h/L.

(For more detailed information, see the cell source/serotherapy specific sheets)
PROTOCOL A: Ablative with MSD

**CONDITIONING**  Busulfan/Fludarabine160
**BMT DONOR**  Matched sibling
**GVHD PROPHYLAXIS**  Cyclosporin/MTX

<table>
<thead>
<tr>
<th>DAY</th>
<th>DATE</th>
<th>D-DAY</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-6</td>
<td></td>
<td></td>
<td>Option for therapy – see below</td>
</tr>
<tr>
<td>D-5</td>
<td></td>
<td></td>
<td>Fludarabine 40mg/m² once a day (IV infusion 30-60 mins) prior to IV Busulfan over 3h (take and run PK)</td>
</tr>
<tr>
<td>D-4</td>
<td></td>
<td></td>
<td>Fludarabine 40mg/m² once a day IV Busulfan over 3h*</td>
</tr>
<tr>
<td>D-3</td>
<td></td>
<td></td>
<td>Fludarabine 40mg/m² once a day IV Busulfan over 3h*</td>
</tr>
<tr>
<td>D-2</td>
<td></td>
<td></td>
<td>Fludarabine 40mg/m² once day IV Busulfan over 3h* Start Cyclosporine</td>
</tr>
<tr>
<td>D-1</td>
<td></td>
<td>Rest Day</td>
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</tr>
<tr>
<td>D-0</td>
<td></td>
<td>Infusion of BM, MTX day 1,3,6</td>
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</table>

Initial busulfan dose is based on weight:

<table>
<thead>
<tr>
<th>Body-weight</th>
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<tbody>
<tr>
<td>3 to 15kg</td>
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<tr>
<td>15 to 25kg</td>
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<td>3.3</td>
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<tr>
<td>75 to 100kg</td>
<td>2.7</td>
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</tbody>
</table>

*Ref: PK-study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)

Subsequent doses are based on TDM.

*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h

For full myeloablative dose, aim for cumulative Busulfan dose:

\[ \text{AUC 85-95 mg/L} \times \text{h (target 90) } = 85000 – 95000 \text{ ng/ml} \times \text{h} = 20706 -23180 \text{ mmol.min} \]

If there is a delay in obtaining PK results to allow adjustment of day 2/3/4 Bu, then the first day of Fludarabine and busulfan could be given one day earlier (day -6)

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with immediate PK analysis is also recommended to be done in for patients who are young (<2y), sick (drugs that may modify Bu clearance) or where a large change in dose (>25%) is recommended.

**Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK monitoring should still be undertaken after the first dose and the required AUC will remain the same**
PROTOCOL A: Ablative with Alemtuzumab and unrelated donor

<table>
<thead>
<tr>
<th>CONDITIONING</th>
<th>Busulfan/Fludarabine160/Alemtuzumab 0.6</th>
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</thead>
<tbody>
<tr>
<td>BMT DONOR</td>
<td>Unrelated BM/PBSC (9-10/10 allele match)</td>
</tr>
<tr>
<td>GVHD PROPHYLAXIS</td>
<td>Cyclosporine/Alemtuzumab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DAY</th>
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<tr>
<td>D-6</td>
<td>Option for therapy – see below</td>
<td></td>
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</tr>
<tr>
<td>D-5</td>
<td>Fludarabine 40mg/m² once a day (IV infusion 30-60 mins) prior to IV Busulfan over 3h (take and run PK)</td>
<td>Alemtuzumab 0.2mg/kg with pre-med</td>
<td></td>
</tr>
<tr>
<td>D-4</td>
<td>Fludarabine 40mg/m² once a day IV Busulfan over 3h* Alemtuzumab 0.2mg/kg with pre-med</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-3</td>
<td>Fludarabine 40mg/m² once a day IV Busulfan over 3h* Alemtuzumab 0.2mg/kg with pre-med</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-2</td>
<td>Fludarabine 40mg/m² once day IV Busulfan over 3h Start Cyclosporine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-1</td>
<td>Rest Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-0</td>
<td>Infusion of BM/PBSC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Initial busulfan dose is based on weight:

<table>
<thead>
<tr>
<th>Body-weight</th>
<th>mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 to 15kg</td>
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</tr>
<tr>
<td>50 to 75kg</td>
<td>3.3</td>
</tr>
<tr>
<td>75 to 100kg</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*Ref: PK study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)

Subsequent doses are based on TDM.

*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h
For full myeloablative dose, aim for cumulative Busulfan dose:

\[
\text{AUC } 85-95 \, \text{mg/L x h (Target 90)} = 85000 - 95000 \, \text{ng/ml x h} = 20706 - 23180 \, \text{mmol.min}
\]

If there is a delay in obtaining PK results to allow adjustment of day 2/3/4 Bu, then the first day of Fludarabine and busulfan could be given one day earlier (day -6)

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with immediate PK analysis is also recommended to be done in for patients who are young (<2y), sick (drugs that may modify Bu clearance) or where a large change in dose (>25%) is recommended.

Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK monitoring should still be undertaken after the first dose and the required AUC will remain the same.
PROTOCOL A: Ablative with ATG and unrelated donor

CONDITIONING  Busulfan/Fludarabine 160/ATG 10 (thymoglobuline)
BMT DONOR    Unrelated BM/PBSC (9-10/10 allele match)
GVHD PROPHYLAXIS  Cyclosporin/MTX

<table>
<thead>
<tr>
<th>DAY</th>
<th>DATE</th>
<th>D-DAY</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-6</td>
<td>Option for therapy – see below</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-5</td>
<td>Fludarabine 40mg/m² once a day (IV infusion 30-60 mins) prior to IV Busulfan over 3h (take and run PK)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-4</td>
<td>Fludarabine 40mg/m² once a day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-3</td>
<td>Fludarabine 40mg/m² once a day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-2</td>
<td>Fludarabine 40mg/m² once day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-1</td>
<td>Rest Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-0</td>
<td>Infusion of BM/PBSC, MTX day 1,3,6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Initial busulfan dose is based on weight:

<table>
<thead>
<tr>
<th>Body-weight</th>
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</tr>
</thead>
<tbody>
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</tr>
</tbody>
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*Ref: PK-study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)
Subsequent doses are based on TDM.
*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h
For full myeloablative dose, aim for cumulative Busulfan dose:

\[ \text{AUC} \text{ 85-95 mg/L x h (Target 90)} = 85000 - 95000 \text{ ng/ml x h} = 20706 - 23180 \text{ mmol.min} \]

If there is a delay in obtaining PK results to allow adjustment of the day 2/3/4 Bu, then the first day of Fludarabine and busulfan could be given one day earlier (day -6)

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with immediate PK analysis is also recommended to be done in for patients who are young (<2y), sick (drugs that may modify Bu clearance) or where a large change in dose (>25%) is recommended.

Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK monitoring should still be undertaken after the first dose and the required AUC will remain the same
PROTOCOL A: Ablative with ATG and Cord Blood Donor

CONDITIONING
Busulfan/Fludarabine 160/ ATG10

BMT DONOR
Cord Blood (4-6 / 6 match)

GVHD PROPHYLAXIS
Cyclosporin / Pred (or MMF)

<table>
<thead>
<tr>
<th>DAY</th>
<th>DATE</th>
<th>D-DAY</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-9</td>
<td></td>
<td>Thymoglobulin, 2.5mg/kg</td>
<td></td>
</tr>
<tr>
<td>D-8</td>
<td></td>
<td>Thymoglobulin, 2.5mg/kg</td>
<td></td>
</tr>
<tr>
<td>D-7</td>
<td></td>
<td>Thymoglobulin, 2.5mg/kg</td>
<td></td>
</tr>
<tr>
<td>D-6</td>
<td></td>
<td>Thymoglobulin, 2.5mg/kg (optional for therapy: see below)</td>
<td></td>
</tr>
<tr>
<td>D-5</td>
<td></td>
<td>Fludarabine 40mg/m² once a day (IV infusion 30-60 mins) prior to IV Busulfan over 3h (take and run PK)</td>
<td></td>
</tr>
<tr>
<td>D-4</td>
<td></td>
<td>Fludarabine 40mg/m² once a day IV Busulfan over 3h*</td>
<td></td>
</tr>
<tr>
<td>D-3</td>
<td></td>
<td>Fludarabine 40mg/m² once a day IV Busulfan over 3h*</td>
<td></td>
</tr>
<tr>
<td>D-2</td>
<td></td>
<td>Fludarabine 40mg/m² once day IV Busulfan over 3h* Start Cyclosporine</td>
<td></td>
</tr>
<tr>
<td>D-1</td>
<td></td>
<td>Rest Day</td>
<td></td>
</tr>
<tr>
<td>D-0</td>
<td></td>
<td>Infusion of CB Prednison 1mg/kg/d (in 2) till +28, taper in 2 weeks Or MMF 3 x 15mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

Initial busulfan dose is based on weight:

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*Ref: PK-study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)

Subsequent doses are based on TDM.
*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h
For full myeloablative dose, aim for cumulative Busulfan dose:

**AUC 85-95 mg/L x h Target 90 = 85000 - 95000 ng/ml x h = 20706 -23180 mmol.min**

If there is a delay in obtaining PK results to allow adjustment of the day 2/3/4 Bu, then the first day of Fludarabine and busulfan could be given one day earlier (day -6)

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with immediate PK analysis is also recommended to be done in for patients who are young (2y), sick (drugs that may modify Bu clearance) or where a large change (>25%) in dose is recommended.

Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK monitoring should still be undertaken after the first dose and the required AUC will remain the same
PROTOCOL A: Ablative with ATG and Haplo-identical Donor (for SCID)

CONDITIONING
Busulfan/Fludarabine 160/ ATG10
BMT DONOR
Haplo-identical donor (BM or PBSC) – T depleted graft

GVHD PROPHYLAXIS
None or Cyclosporin

<table>
<thead>
<tr>
<th>DAY</th>
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</tr>
<tr>
<td>D-7</td>
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<td>Thymoglobulin, 2.5mg/kg</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>Thymoglobulin, 2.5mg/kg</td>
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<tr>
<td>D-5</td>
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<td>Fludarabine 40mg/m² once a day (IV infusion 30-60 mins) prior to IV Busulfan over 3h (take and run PK)</td>
<td></td>
</tr>
<tr>
<td>D-4</td>
<td></td>
<td>Fludarabine 40mg/m² once a day IV Busulfan over 3h*</td>
<td></td>
</tr>
<tr>
<td>D-3</td>
<td></td>
<td>Fludarabine 40mg/m² once a day IV Busulfan over 3h*</td>
<td></td>
</tr>
<tr>
<td>D-2</td>
<td></td>
<td>Fludarabine 40mg/m² once day IV Busulfan over 3h* Start Cyclosporine</td>
<td></td>
</tr>
<tr>
<td>D-1</td>
<td></td>
<td>Rest Day</td>
<td></td>
</tr>
<tr>
<td>D-0</td>
<td></td>
<td>Infusion of Haplo graft</td>
<td></td>
</tr>
</tbody>
</table>

Initial busulfan dose is based on weight:

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*Ref: PK-study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)

Subsequent doses are based on TDM.
*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h
For full myeloablative dose, aim for cumulative Busulfan dose:

**AUC 85-95 mg/L x h Target 90 = 85000 – 95000 ng/ml x h = 20706 -23180 mmol.min**

If there is a delay in obtaining PK results to allow adjustment of the day 2/3/4 Bu, then the first day of Fludarabine and busulfan could be given one day earlier (day -6)

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with immediate PK analysis is also recommended to be done in for patients who are young (2y), sick (drugs that may modify Bu clearance) or where a large change (>25%) in dose is recommended.

**Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK monitoring should still be undertaken after the first dose and the required AUC will remain the same**
PROTOCOL A: Ablative with ATG and Haplo-identical Donor (for NON-SCID)

CONDITIONING
Busulfan/Fludarabine 160/ ATG10/Thiotepa

BMT DONOR
Haplo-identical donor (BM or PBSC) – T depleted graft

GVHD PROPHYLAXIS
None or Cyclosporin

GVHD PROPHYLAXIS
None or Cyclosporin

DAY DATE D-DAY TREATMENT
D-9 Thymoglobulin, 2.5mg/kg
D-8 Thymoglobulin, 2.5mg/kg
D-7 Thymoglobulin, 2.5mg/kg
D-6 Thymoglobulin, 2.5mg/kg; Thiotepa 5mg/kg twice per day
D-5 Fludarabine 40mg/m^2 once a day (IV infusion 30-60 mins) prior to
IV Busulfan over 3h (take and run PK)
D-4 Fludarabine 40mg/m^2 once a day
IV Busulfan over 3h*
D-3 Fludarabine 40mg/m^2 once a day
IV Busulfan over 3h*
D-2 Fludarabine 40mg/m^2 once day
IV Busulfan over 3h*
Start Cyclosporine
D-1 Rest Day
D-0 Infusion of Haplo graft

Initial busulfan dose is based on weight:

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<tbody>
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*Ref: PK-study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)

Subsequent doses are based on TDM.
*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h
For full myeloablative dose, aim for cumulative Busulfan dose:

**AUC 85-95 mg/L x h Target 90 = 85000 – 95000 ng/ml x h = 20706 -23180 mmol.min**

If there is a delay in obtaining PK results to allow adjustment of the day 2/3/4 Bu, then the first day of
Fludarabine and busulfan could be given one day earlier (day -6)

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with
immediate PK analysis is also recommended to be done in for patients who are young (2y), sick (drugs that
may modify Bu clearance) or where a large change (>25%) in dose is recommended.

Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK
monitoring should still be undertaken after the first dose and the required AUC will remain the same
### PROTOCOL B

**SUMMARY: Non-Myelo-Ablative Conditioning in Inborn Errors**

<table>
<thead>
<tr>
<th>Day</th>
<th>Bu Flu core protocol: Non-myeloablative</th>
<th>GvHD/rejection prophylaxis</th>
<th>Additional treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSD</td>
<td>Cord Blood (4-6 /6)</td>
<td>9-10/10 URD</td>
</tr>
<tr>
<td>-12</td>
<td></td>
<td>Thymoglobuline</td>
<td></td>
</tr>
<tr>
<td>-11</td>
<td></td>
<td>Thymoglobuline</td>
<td></td>
</tr>
<tr>
<td>-10</td>
<td></td>
<td>Thymoglobuline</td>
<td></td>
</tr>
<tr>
<td>-9</td>
<td></td>
<td>Thymoglobuline</td>
<td></td>
</tr>
<tr>
<td>-8</td>
<td></td>
<td>Thymoglobuline</td>
<td></td>
</tr>
<tr>
<td>-7</td>
<td><strong>Fludarabine</strong> 45mg/m² in 30-60min</td>
<td>Thymoglobuline</td>
<td>Campath 0.2mg/kg</td>
</tr>
<tr>
<td>-6</td>
<td><strong>Fludarabine</strong> 45mg/m²</td>
<td>Thymoglobuline</td>
<td>Campath 0.1-0.2mg/kg</td>
</tr>
<tr>
<td>-5</td>
<td><strong>Fludarabine</strong> 45mg/m² 1h followed by Busulfan with PK</td>
<td>Thymoglobuline</td>
<td></td>
</tr>
<tr>
<td>-4</td>
<td><strong>Fludarabine</strong> 45mg/m² + Busulfan (based on PK first day)</td>
<td>Thymoglobuline</td>
<td></td>
</tr>
<tr>
<td>-3</td>
<td>Busulfan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td><strong>Busulfan</strong> (PK check)</td>
<td>CyA</td>
<td>CyA</td>
</tr>
<tr>
<td>-1</td>
<td></td>
<td>CyA</td>
<td>CyA</td>
</tr>
<tr>
<td>0</td>
<td>CyA</td>
<td>CyA MMF</td>
<td>CyA</td>
</tr>
<tr>
<td></td>
<td>CyA 150-250, MTX 1,3,6</td>
<td>CyA 200-300 Pred 1mg/kg (or MMF 3 x 15mg/kg)</td>
<td>CyA 200-300 + MMF (3 x 15mg/kg)</td>
</tr>
</tbody>
</table>

- Indications for the NMA regimen: CGD > 6 years of age, PID (including HLH) with organ toxicity / reduced performance scale
- (Preferably) Not in patients with IEM
- Preferably no CB using NMA
- Based on the day 1 PK, the subsequent 2 doses, a total AUC of 60+/- 5 mg*h/L

**See for more detailed information the cell source / serotherapy specific sheets**
PROTOCOL B: Reduced Intensity (NMA) with MSD

CONDITIONING          Busulfan/Fludarabine 180 /ATG 7.5
BMT DONOR             Matched sibling
GVHD PROPHYLAXIS      Cyclosporine/MMF

DAILY TREATMENT

D-7  Thymoglobuline 2.5 mg/kg with pre-med
      Fludarabine 45 mg/m² once a day (IV infusion 30-60 mins)
D-6  Thymoglobuline 2.5 mg/kg with pre-med
      Fludarabine 45 mg/m² once a day
D-5  Thymoglobuline 2.5 mg/kg with pre-med
      Fludarabine 45 mg/m² once a day
      IV Busulfan over 3h (take and run PK)
D-4  Fludarabine 45 mg/m² once a day
      IV Busulfan over 3h*
D-3  IV Busulfan over 3h*
      Start Cyclosporine
D-2  IV Busulfan over 3h*
D-1  Rest Day
D-0  Infusion of BM/PBSC
      Start MMF

Initial busulfan dose is based on weight (dosed to reach target in 4 days):

<table>
<thead>
<tr>
<th>Body-weight</th>
<th>mg/kg/day</th>
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<tbody>
<tr>
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<td>75 to 100kg</td>
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*Ref: PK-study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)

Subsequent doses are based on TDM.
*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h

If there is a delay in obtaining PK results to allow adjustment of the day 2/3/4 Bu, then the first day of Fludarabine and busulfan could be given one day earlier (day -6)

For non-myeloablative dose, aim for cumulative Busulfan dose:

**Cumulative AUC for Busulfan: 55000 – 65000 ng/ml x h = 13398 -15834 mmol.min**

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with immediate PK analysis is recommended to be done for patients who are young (2y), sick (drugs that may modify Bu clearance) or where a large change in dose is recommended.

Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK monitoring should still be undertaken after the first dose and the required AUC will remain the same.
**PROTOCOL B**: Reduced Intensity (NMA) with unrelated donor

**CONDITIONING**  
**BMT DONOR**  
**GVHD PROPHYLAXIS**  
**Day** | **Date** | **Treatment**
---|---|---
D-7 | | Alemtuzumab 0.2 mg/kg with pre-med  
Fludarabine 45 mg/m² once a day (IV infusion 60 mins)
D-6 | | Alemtuzumab 0.2 mg/kg with pre-med  
Fludarabine 45 mg/m² once a day (IV infusion 60 mins)
D-5 | | Alemtuzumab 0.1-0.2 mg/kg with pre-med  
Fludarabine 45 mg/m² once a day (IV infusion 60 mins)  
IV Busulfan over 3h (take and run PK)
D-4 | | Fludarabine 45 mg/m² once a day (IV infusion 60 mins)  
IV Busulfan over 3h*
D-3 | | IV Busulfan over 3h*  
Start Cyclosporine
D-2 | | IV Busulfan over 3h*
D-1 | | Rest Day
D-0 | | Infusion of BM/PBSC  
Start MMF

Initial busulfan dose is based on weight (dosed to reach target in 4 days):

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*Ref: PK-study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)

Subsequent doses are based on TDM.  
*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h

If there is a delay in obtaining PK results to allow adjustment of the third and fourth dose of Bu, then the first day of Fludarabine and busulfan could be given one day earlier (day -6)

For non-myeloablative dose, aim for cumulative Busulfan dose:  
**Cumulative AUC for Busulfan**  
55000 – 65000 ng/ml x h = 13398 -15834 mmol.min

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with immediate PK analysis is recommended to be done in patients who are young (2y), sick (drugs that may modify Bu clearance) or where a large change in dose is recommended.

Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK monitoring should still be undertaken after the first dose and the required AUC will remain the same.
PROTOCOL B: Reduced intensity (NMA) with Cord Blood

CONDITIONING

Busulfan/Fludarabine 180/ATG 10

BMT DONOR

CB 4/6 – 6/6 (A and B on low-res / DR on high-res)

Cell dose:

6/6: >2.5*10^7 NC/kg for 6/6 or > 1*10^5 CD34+/kg
5/6: >3*10^7 NC/kg for 6/6 or > 2*10^5 CD34+/kg
4/6: >5*10^7 NC/kg for 6/6 or > 3*10^5 CD34+/kg

GVHD PROPHYLAXIS

Note: High-Resolution typing + selection optional

Cyclosporin / Pred (or MMF)

GVHD PROPHYLAXIS

<table>
<thead>
<tr>
<th>BODY-WEIGHT</th>
<th>mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 to 15kg</td>
<td>3.5</td>
</tr>
<tr>
<td>15 to 25kg</td>
<td>3.2</td>
</tr>
<tr>
<td>25 to 50kg</td>
<td>2.8</td>
</tr>
<tr>
<td>50 to 75kg</td>
<td>2.2</td>
</tr>
<tr>
<td>75 to 100kg</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Ref: PK-study busulfan I.H.Bartelink, JJ.Boelens et al. 2011 (submitted)*

Subsequent doses are based on TDM.
*TDM doses of Bu are given at same rate (in mg/h) as initial dose, so may not be over 3h

If there is a delay in obtaining PK results to allow adjustment of the third and fourth dose of Bu, then the first day of Fludarabine and busulfan could be given one day earlier (day -6)

For non-myeloablative dose, aim for cumulative Busulfan dose:

AUC 55-65 mg/L x h Target 60 mg/L x h: 55000 – 65000 ng/ml x h = 13398 -15834 mmol.min

A repeat PK set on at least one of the subsequent days is desirable, but can be run later. A repeat set with immediate PK analysis is recommended to be done in patients who are young (2y), sick (drugs that may modify Bu clearance) or where a large change in dose (>25%) is recommended.

Busulfan can be given as a once daily dose or in divided doses (often 4 times per day). PK monitoring should still be undertaken after the first dose and the required AUC will remain the same
Busulfan new dosing regimen (Honolulu 2011)

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Myeloablative 4days 1ddd, total target 90mg*h/L</th>
<th>Non-myeloablative 4days 1ddd, total target 60mg*h/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 -15</td>
<td>5.1</td>
<td>3.5</td>
</tr>
<tr>
<td>15 – 25</td>
<td>4.9</td>
<td>3.2</td>
</tr>
<tr>
<td>25 – 50</td>
<td>4.1</td>
<td>2.8</td>
</tr>
<tr>
<td>50 – 75</td>
<td>3.3</td>
<td>2.2</td>
</tr>
<tr>
<td>75 – 100</td>
<td>2.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

This dosing regimen will lead to a more predictable exposure as compared to the licensed dose. However, dose targeting using therapeutic drug monitoring is advised to reach the target within 10% range:

Busulfan blood samples should be taken after the first dose

☐ Blood sample 1: Approx. 5 minutes after end of infusion
☐ Blood sample 2: Approx. 1 hours after end of infusion
☐ Blood sample 3: Approx. 2 hours after end of infusion
☐ Blood sample 4: Approx. 3 hours after end of infusion

Calculation of the following doses should be performed based on these blood samples.

References:
1. Bartelink et al. BMT Tandem Meetings 2011 abstract 73, oral presentation
2. Bartelink et al. BBMT 15; 231-241 (2009)
3. Bartelink et al BMT Tandem Meetings 2011 abstract 289

Contact: J.J.Boelens@umcutrecht.nl or I.Bartelink@umcutrecht.nl
# Protocol C

## Conditioning

### BMT Donor

### GVHD Prophylaxis

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-9</td>
<td>Start Allopurinol 4 mg/kg po tds for 6 days</td>
</tr>
<tr>
<td>D-8</td>
<td>Alemtuzumab 0.2mg/kg with pre-med</td>
</tr>
<tr>
<td>D-7</td>
<td>Fludarabine 30 mg/m² (iv infusion over 30 minutes) Alemtuzumab 0.2mg/kg with pre-med</td>
</tr>
<tr>
<td>D-6</td>
<td>Fludarabine 30 mg/m² Alemtuzumab 0.2mg/kg with pre-med</td>
</tr>
<tr>
<td>D-5</td>
<td>Fludarabine 30 mg/m² Alemtuzumab 0.2mg/kg with pre-med</td>
</tr>
<tr>
<td>D-4</td>
<td>Fludarabine 30 mg/m² Alemtuzumab 0.2mg/kg with pre-med</td>
</tr>
<tr>
<td>D-3</td>
<td>Fludarabine 30 mg/m²</td>
</tr>
<tr>
<td>D-2</td>
<td>Pre-Melphalan hydration: 4% dextrose/ 0.18% saline + 20 mmol/l of KCl at 200 ml/m²/hr for 3 hrs Melphalan 140 mg/m² (iv bolus push) Post-Melphalan hydration: 0.45% saline / 2.5% dextrose + 40 mmol/m²/24 hrs of KCl at 3 l/m² for 24 hrs</td>
</tr>
<tr>
<td>D-1</td>
<td>Cyclosporin 1.5 mg/kg iv twice daily</td>
</tr>
<tr>
<td>D 0</td>
<td>Infusion of BM/PBSCs MMF 15mg/kg tds orally from D0 to D27 – continue or tail depending on presence or absence of GVHD</td>
</tr>
</tbody>
</table>
## PROTOCOL D

**SUMMARY:** Myelo-Ablative, reduced toxicity conditioning in Inborn Errors

<table>
<thead>
<tr>
<th>Day</th>
<th>Treo Flu core protocol</th>
<th>GvHD/rejection prophylaxis</th>
<th>Additional Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MSD</td>
<td>Cord Blood (4-6/6)</td>
</tr>
<tr>
<td>-9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-7</td>
<td><strong>Fludarabine</strong> 30mg/m2</td>
<td></td>
<td><strong>Campath</strong> 0.2mg/kg</td>
</tr>
<tr>
<td></td>
<td><strong>Treosulfan</strong> (&gt; 1 y: 14 g/m², &lt; 1 y 12 g/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-6</td>
<td><strong>Fludarabine</strong> 30mg/m2</td>
<td></td>
<td><strong>Campath</strong> 0.2mg/kg</td>
</tr>
<tr>
<td></td>
<td><strong>Treosulfan</strong> (&gt; 1 y: 14 g/m², &lt; 1 y 12 g/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5</td>
<td><strong>Fludarabine</strong> 30mg/m2</td>
<td></td>
<td><strong>Campath</strong> 0.2mg/kg</td>
</tr>
<tr>
<td></td>
<td><strong>Treosulfan</strong> (&gt; 1 y: 14 g/m², &lt; 1 y 12 g/m²)</td>
<td><strong>PBSC or 9/10 BM</strong></td>
<td></td>
</tr>
<tr>
<td>-4</td>
<td><strong>Fludarabine</strong> 30mg/m2</td>
<td></td>
<td><strong>Campath</strong> 0.2mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>PBSC or 9/10 BM</strong></td>
</tr>
<tr>
<td>-3</td>
<td><strong>Fludarabine</strong> 30mg/m2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1</td>
<td></td>
<td><strong>CyA</strong></td>
<td><strong>CyA</strong></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td><strong>CyA</strong></td>
<td><strong>CyA</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>CyA 100-150</strong></td>
<td><strong>CyA100-150</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>MMF 3 x 15mg/kg</strong></td>
<td><strong>MMF till +28, taper and stop by D+50</strong></td>
</tr>
</tbody>
</table>

- **Thymoglobuline** may be used instead of Campath at centre’s discretion
- **Serotherapy** for cord blood at centre’s discretion