# 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.

# Myeloablative Conditioning

| PROTOCOL | CHEMOTHERAPY | SEROTHERAPY | GVHD PROPHYLAXIS |
|---|---|---|---|
| **A** | **Busulfan (iv)**<br>**(wt or AUC dosing)[1]**<br><br>**Fludarabine**<br>**160 mg/m2** | **†Campath 1H (TD 0.6-1mg/kg)**<br>**OR**<br>**††ATG (TD 10mg/kg)** | **CyA**<br>**or**<br>**CyA + MMF or MTX (as 2nd agent)** |

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

***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)***

# Reduced Intensity Conditioning

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

- [2]AUC dosing for iv Bu - = 60+/- 5 mg*h/L. (see appendix for specific protocols for different donor sources and dosing)
- Avoid Melphalan 140mg/$m^2$ < 1 year of age unless HLH
- Treosulphan 36g/$m^2$ < 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 2$^{nd}$ 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/m$^2$<br>Alemtuzumab* 0.2 mg/kg |
| Day -8 | Fludarabine 30 mg/m$^2$<br>Alemtuzumab* 0.2 mg/kg |
| Day -7 | Fludarabine 30 mg/m$^2$<br>Alemtuzumab* 0.2 mg/kg |
| Day -6 | Fludarabine 30 mg/m$^2$<br>Alemtuzumab* 0.2 mg/kg |
| Day -6 | Cyclophosphamide 5 mg/kg<br>Fludarabine 30 mg/m$^2$<br>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<br>Cyclosporin from day – 1<br>GCSF 5 micrograms/kg from day +8 |

***Suggest removal of Alemtuzumab in MSD transplants**

**References**

Buck D, Moshous D, de Chasseval R, Ma Y, le Deist F, Cavazzana-Calvo M, Fischer A, Casanova JL, Lieber MR, de Villartay JP. Severe combined immunodeficiency and microcephaly in siblings with hypomorphic mutations in DNA ligase IV. *Eur J Immunol* . 2006; 36: 224-35

Enders A, Fisch P, Schwarz K, Duffner U, Pannicke U, Nikolopoulos E, Peters A, Orlowska-Volk M, Schindler D, Friedrich W, Selle B, Niemeyer C, Ehl S. A severe form of human combined immunodeficiency due to mutations in DNA ligase IV. *J Immunol* 2006; 176: 5060-8

Gruhn B, Seidel J, Zintl F, Varon R, Tönnies H, Neitzel H, Bechtold A, Hoehn H, Schindler D. Successful bone marrow transplantation in a patient with DNA ligase IV deficiency and bone marrow failure. *Orphanet J Rare Dis* . 2007; 2:5

Riballo E, Critchlow SE, Teo SH, Doherty AJ, Priestley A, Broughton B, Kysela B, Beamish H, Plowman N, Arlett CF, Lehmann AR, Jackson SP, Jeggo PA. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr Biol* 1999;9: 699-702

van der Burg M, van Veelen LR, Verkaik NS, Wiegant WW, Hartwig NG, Barendregt BH, Brugmans L, Raams A, Jaspers NG, Zdzienicka MZ, van Dongen JJ, van Gent DC. A new type of radiosensitive T-B-NK+ severe combined immunodeficiency caused by a LIG4 mutation. *J Clin Invest* 2006; 116: 137-45

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

**IGenotypically identical donor (inc 1 Ag mismatch)**

- Protocol A or B or D
- Use serotherapy if 1 Ag mismatch
- CyA/MMF or CyA/MTX

**IIMUD 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)

**IIIUCB**

- 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, < 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*

### Transplant

- BM (1st choice): > 5 x $10^8$ NC / kg BW
- PBSC (2nd choice): >10 x $10^6$ 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
- 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+: neuropatic forms should be excluded contact one of the Authors
- HLA-matched 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 (**15 mg/kg**): 2 x 5 mg/kg at day -4, 1 x 5 mg/kg at day -3
  - 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):*
  - *Treosulfan (> 5 kg: 42 g/m²): 14 g/m², day -7 to day -5*
  - *Fludarabine (150 mg/m²): 30 mg/m²/day, day -7 to day -3*
  - *Thiotepa (**15 mg/kg**): 2 x 5 mg/kg at day -4, 1 x 5 mg/kg at day -3*
  - *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):
  - stem cells: >10 x $10^6$ CD34+ / kg BW;
  - T-cells: < 2 x $10^4$ 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 $10^4$ 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**:

| | | |
|---|---|---|
| **a Bu AUC in ng/ml x h** | **divided by 4.105** | **= BU AUC in micromol x min** |
| **Example 10 000 ng/ml x h** | **divided by 4.105** | **= 2436 micromol x min** |

**(10 000 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)

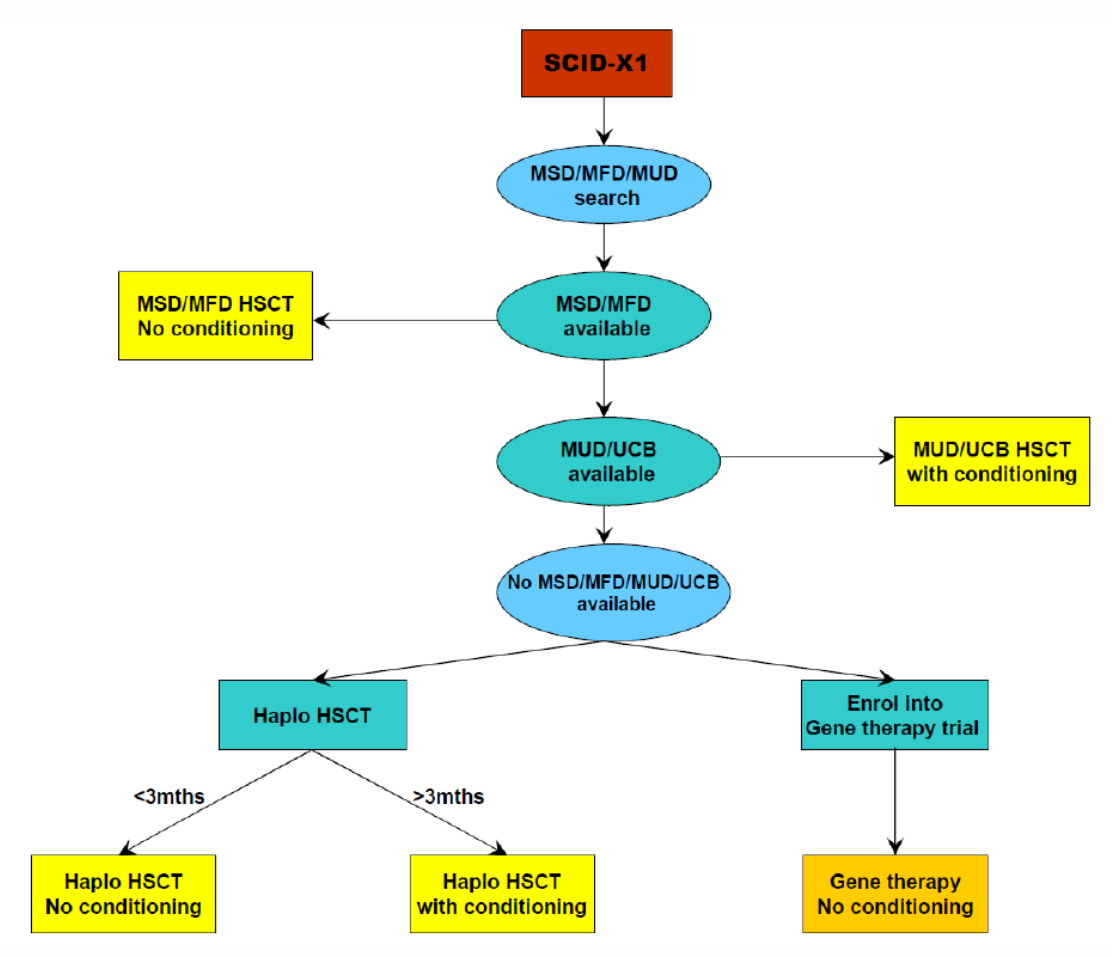
## IV Haploidentical donor

- 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

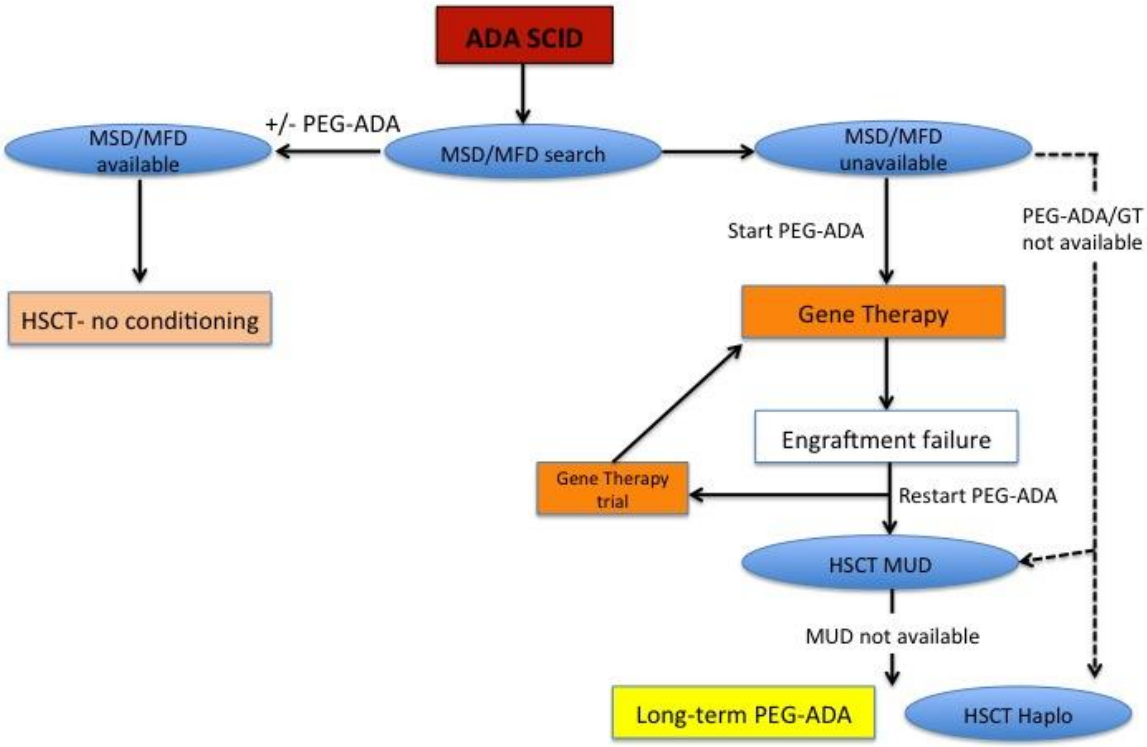
**SCID X-1**


SCID-X1
MSD/MFD/MUD search
MSD/MFD available
MSD/MFD HSCT No conditioning
MUD/UCB available
MUD/UCB HSCT with conditioning
No MSD/MFD/MUD/UCB available
Haplo HSCT
Enrol into Gene therapy trial
<3mths
>3mths
Haplo HSCT No conditioning
Haplo HSCT with conditioning
Gene therapy No conditioning

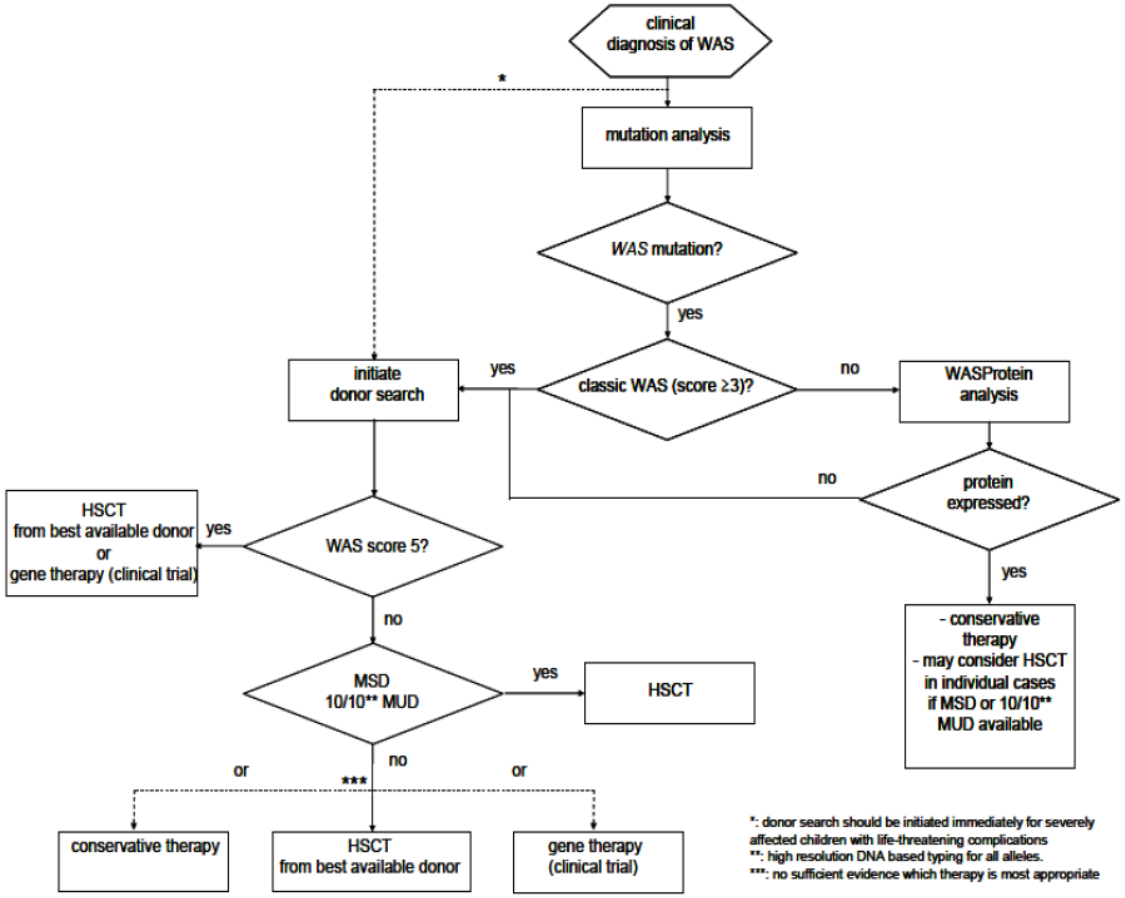
## ADA deficiency

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

The following algorithm, updated from the 2011 guidelines, for treatment of ADA-SCID has been agreed by members of the IEWP in conjunction with other experts.



# Wiskott Aldrich Syndrome



**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:**

| | XLN | iXLT | XLT | | classic WAS | | |
|---|---|---|---|---|---|---|---|
| **Score** | **0** | **<1** | **1** | **2** | **3** | **4** | **5** |
| Thrombocytopenia | - | -/+ | + | + | + | + | + |
| Small platelets | - | + | + | + | + | + | + |
| Eczema | - | - | - | (+) | + | ++ | -/(+)/+/++ |
| Immunodeficiency | -/(+) | - | -/(+) | (+) | + | + | (+)/+ |
| Infections | -/(+) | - | - | (+) | + | +/++ | -/(+)/+/++ |
| Autoimmunity and/or malignancy | - | - | - | - | - | - | + |
| Congenital neutropenia | + | - | - | - | - | - | - |
| Myelodysplasia | -/+ | - | - | - | - | - | - |

-/(+), 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.
(modified from Stiehm ER, Ochs HD, Winkelstein IA, eds. Immunologic Disorders in Infants and Children. Fifth edition. Philadelphia: Saunders; 2004. Used with permission.)

**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, $O_2$-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 tranfusions 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.

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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. Co-trimoxazole 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:


*1. R. Seger et al: Treatment of CGD with myeloablative conditioning and an unmodified allograft. Blood, 2002, 100, 4344-4350*

*2. S. Yang et al: Exuberant inflammation in nicotinamide adenine dinucleotide phosphate-oxidase-deficient mice after allogeneic marrow transplantation. J. Immunol. 2002, 168, 5840 - 5847*

*3. T. Güngör et al.: Successful Low Toxicity Hematopoietic Stem Cell Transplantation for High Risk Adult Chronic Granulomatous Disease Patients. Transplantation; 2005, 79: 1596-1606.*

*4. E. Soncini et al. Unrelated donor and HLA-identical sibling haematopoietic stem cell transplantation cure chronic granulomatous disease with good long-term outcome and growth. Br J Haematol. 2009,145, 73-83*

*5. R.Seger Hematopoietic stem cell transplantatation for chronic granulomatous disease. Immunol Allergy Clin North Am. 2010;30, 195-208.*

# 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
. Cryptosporidum 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
- Potientially ototoxic
- May be absorbed from GI tract if mucositis occurs
. Azithromycin & Nitazoxanide 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 (v3) (2015)

These guidelines are part of a prospective study **« Osteopetrosis, Consensus guidelines for diagnosis, therapy and follow-up ».**

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

## 3.2 Conditioning Protocols

### 3.2.1 Matched Sibling Donor (MSD)

**Inclusion Criteria**

- Infantile Osteopetrosis
- Intermediate Osteopetrosis → discuss with Authors

**Exclusion Criteria**

- Neurodegenerative forms due to mutations in *OSTM1* mutations or in *CLCN7* (in approximately 50% of the patients; for clinical signs see above) → contact the authors
- Osteoclast extrinsic form (*RANKL* mutations and/or wild type in other known genes) → contact the authors

**Conditioning**

- Standard Protocol, Busulfan-based:
    - Busulfex (weight adapted, kinetics strongly recommended, myeloablative AUC): day -8 to day -5
    - Fludarabine (160 mg/m²): 40 mg/m²/day, day -6 to day -3

- *Pilot Protocol, Treosulfan.based*:*
    - *Treosulfan*
      *Less than 1 year of age: 12 g/m²/day days -7 to day -5, i.e total dose 36 g/m²*
      *More than 1 year of age: 14 g/m²/day days -7 to day -5, i.e total dose 42 g/m²*
    - *Fludarabine (160 mg/m²): 40 mg/m²/day, day -6 to day -3*
    - *Thiotepa (10 mg/kg): 2 x 5 mg/kg at day -4*

**Transplant**

- BM (1st choice): > 5 x $10^8$ NC / kg BW
- PBSC (2nd choice): >10 x $10^6$ CD34+ / kg BW

**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 60

** The pilot protocol may be used in high risk situations (significant extramedullary haematopoiesis, significant hepatosplenomegaly, hydrocephalus, pulmonary hypertension, infection, patients > 1 year of age, 2nd transplant).*

### 3.2.2 Matched Family Donor (MFD) or Matched Unrelated Donor (MUD)

**Inclusion Criteria**

- Infantile Osteopetrosis
- Intermediate Osteopetrosis → discuss with authors

**Exclusion Criteria**

- Neurodegenerative forms due to mutations in *OSTM1* mutations or in *CLCN7* (in approximately 50% of the patients; for clinical signs see above) → contact the authors
- Osteoclast extrinsic form *(RANKL* mutations and/or wt in other known genes) → contact the authors
- MSD available

**Conditioning**

- Standard Protocol, Busulfan-based:
    - Busulfex (weight adapted, kinetics strongly recommended, myeloablative AUC): day -8 to day -5
    - Fludarabine (160 mg/m²): 40 mg/m²/day, day -6 to day -3
    - Thiotepa (10 mg/kg): 2 x 5 mg/kg at day -4
    - Serotherapy (day.10 to -7)**, alternatively:
        - Alemtuzumab (Campath1-H) (0.7mg/kg**):** 0.1 mg/kg, 1st day, then 0.2 mg/kg 2nd-4th day
        - Thymoglobulin (10 mg/kg): 1 mg/kg 1st day;3 mg/kg 2nd-4th day or
        - ATG Fresenius (60 mg/kg): 3 mg/kg 1st day;19 mg/kg 2nd-4th day

- *Pilot Protocol, Treosulfan.based*:*
    - *Treosulfan*

      *Less than 1 year of age: 12 g/m²/day days -7 to day -5, i.e total dose 36 g/m²*

      *More than 1 year of age: 14 g/m²/day days -7 to day -5, i.e total dose 42 g/m²*
    - *Fludarabine,Thiotepa and Serotherapy as in standard protocol (see above)*

**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 5 to 10 x $10^6$ CD3+ / kg BW

**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 60

** 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)*

*** "Late" serotherapy around transplantation particularly with Alemtuzumab (Campath-1H) in treosulfan based conditioning seems to be associated with an increased risk of (secondary) graft failure (M. Sirin, M. Albert and A. Schulz EBMT 2015, abstract).*

### 3.2.3 HLA-Haploidentical Donor

**Inclusion Criteria**

- Infantile Osteopetrosis

**Exclusion Criteria**

- Neurodegenerative forms due to mutations in *OSTM1* mutations or in *CLCN7* (in approximately 50% of the patients; for clinical signs see above) → contact the authors
- *OSTM1, CLCN7* Osteoclast extrinsic form (*RANKL* mutations) and/or wild type in all known genes) → contact the authors
- HLA-matched donor available in an adequate delay

**Conditioning**

- Standard Protocol, Busulfan-based:
    - Busulfex (weight adapted, kinetics strongly recommended, myeloablative AUC): day -8 to day -5
    - Fludarabine (160 mg/m²): 40 mg/m²/day, day -6 to day -3
    - Thiotepa (**15 mg/kg**): 2 x 5 mg/kg at day -4, 1 x 5 mg/kg at day -3
    - Serotherapy: Thymoglobulin (10 mg/kg); ATG Fresenius (60 mg/kg), or Campath1-H (0.7mg/kg): **start 2-4 days prior to transplantation**

**Transplant**

- T cell depleted PBSC (method according to local protocols):
    - stem cells: >10 x $10^6$ CD34+ / kg BW
    - T-cells: < 1 to 2 x $10^4$ 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 < 2 to 4 x $10^4$ CD3+ / kg BW

**GvHD prophylaxis**

- T-cell depletion
- If CD3+ T cells in the graft exceed 2 x $10^4$ CD3+ / kg BW: MMF 1200 mg/m² start i.v. at day 0, stop at day 60

**Remarks:**

- ***HLA-haploidentical transplantation in OP is associated with high risks such as graft rejection, graft failure, toxic and infectious complications particularly in patients with advanced disease (> 10 months of age). This procedure should be performed in experienced centres only!***
- ***Treosulfan should NOT be used in T-cell depleted HSCT because of the high risk of non-engraftment in this setting. 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.***
- ***T replete haploidentical HSCT with cyclophosphamide post-HSCT (Fuchs 2012) is currently under evaluation in patients with advanced disease; please contact the authors for updated***

## 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

| Day | Bu Flu core protocol | GvHD/rejection prophylaxis | | | | Additional Treatment |
|---|---|---|---|---|---|---|
| | | **MSD** | **Cord Blood (4-6/6)** | **9-10/10 URD** | **Haplo** | For HR disease + haplo: **+ TT 10mg/kg** |
| -9 | | | **Thymoglobuline** | | **Thymoglobuline** | |
| -8 | | | **Thymoglobuline** | | **Thymoglobuline** | |
| -7 | | | **Thymoglobuline** | | **Thymoglobuline** | |
| -6 | | | **Thymoglobuline** | | **Thymoglobuline** | **Thiotepa (TT)- 2 x 5mg/kg**<br>**Only indicated for:**<br>• Haplo for NON-SCID<br>• high risk disease e.g OP |
| -5 | **Fludarabine** 40mg/$m^2$ over 30-60 minutes<br>**Busulfan** mg/kg **(new dosing regimen)** over 180 minutes with PK | | | **Thymogl. (or Campath)** | | |
| -4 | **Fludarabine** 40mg/$m^2$ +<br>**Busulfan** (based on first day) | | | **Thymogl. (or Campath)** | | **Thymogl. 3 x 3.3mg/kg: if more practical** |
| -3 | **Fludarabine** 40mg/$m^2$ + **Busulfan** | | | **Thymogl. (or Campath)** | | |
| -2 | **Fludarabine** 40mg/$m^2$ + **Busulfan** (PK check) | CyA | CyA | CyA<br>**Thymoglobuline** | CyA | |
| -1 | | CyA | CyA | CyA | CyA | |
| 0 | | CyA | CyA Pred | CyA | CyA | |
| | | CyA 200-300, MTX 1,3,6 | CyA 200-300<br>Pred 1mg/kg (or MMF 3 x 15mg/kg) | CyA 200-300, MTX 1,3,6 | CyA 200-300 | Pred till +28, taper in 2 weeks |

- **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**

| **DAY** | **DATE** | **D-DAY** | **TREATMENT** |
|---|---|---|---|
| | | D-6 | Option for therapy – see below |
| | | 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 BM, MTX day 1,3,6 |

Initial busulfan dose is based on weight:

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 5.1 |
| 15 to 25kg | 4.9 |
| 25 to 50kg | 4.1 |
| 50 to 75kg | 3.3 |
| 75 to 100kg | 2.7 |

*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 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

**CONDITIONING** **Busulfan/Fludarabine160/Alemtuzumab 0.6**
**BMT DONOR** **Unrelated BM/PBSC (9-10/10 allele match)**
**GVHD PROPHYLAXIS** **Cyclosporine/Alemtuzumab**

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

Initial busulfan dose is based on weight:

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 5.1 |
| 15 to 25kg | 4.9 |
| 25 to 50kg | 4.1 |
| 50 to 75kg | 3.3 |
| 75 to 100kg | 2.7 |

*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 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**

| **DAY** | **DATE** | **D-DAY** | **TREATMENT** |
|---|---|---|---|
| | | D-6 | Option for therapy – see below |
| | | D-5 | Fludarabine 40mg/m$^2$ once a day (IV infusion 30-60 mins) prior to IV Busulfan over 3h (take and run PK)<br>Thymoglobulin, 2.5mg/kg |
| | | D-4 | Fludarabine 40mg/m$^2$ once a day<br>IV Busulfan over 3h*<br>Thymoglobulin, 2.5mg/kg |
| | | D-3 | Fludarabine 40mg/m$^2$ once a day<br>IV Busulfan over 3h*<br>Thymoglobulin, 2.5mg/kg |
| | | D-2 | Fludarabine 40mg/m$^2$ once day<br>IV Busulfan over 3h*<br>Thymoglobulin, 2.5mg/kg<br>Start Cyclosporine |
| | | D-1 | Rest Day |
| | | D-0 | Infusion of BM/PBSC, MTX day 1,3,6 |

Initial busulfan dose is based on weight:

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 5.1 |
| 15 to 25kg | 4.9 |
| 25 to 50kg | 4.1 |
| 50 to 75kg | 3.3 |
| 75 to 100kg | 2.7 |

*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 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)**

| **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 (optional for therapy: see below) |
| | | 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 CB Prednison 1mg/kg/d (in 2) till +28, taper in 2 weeks Or MMF 3 x 15mg/kg |

Initial busulfan dose is based on weight:

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 5.1 |
| 15 to 25kg | 4.9 |
| 25 to 50kg | 4.1 |
| 50 to 75kg | 3.3 |
| 75 to 100kg | 2.7 |

*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**

| **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 |
| | | 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:

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 5.1 |
| 15 to 25kg | 4.9 |
| 25 to 50kg | 4.1 |
| 50 to 75kg | 3.3 |
| 75 to 100kg | 2.7 |

*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**

| **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:

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 5.1 |
| 15 to 25kg | 4.9 |
| 25 to 50kg | 4.1 |
| 50 to 75kg | 3.3 |
| 75 to 100kg | 2.7 |

*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

| Day | Bu Flu core protocol: Non-myeloablative | GvHD/rejection prophylaxis | | | Additional treatments |
|---|---|---|---|---|---|
| | | **MSD** | **Cord Blood (4-6 /6)** | **9-10/10 URD** | |
| -12 | | | **Thymoglobuline** | | |
| -11 | | | **Thymoglobuline** | | |
| -10 | | | **Thymoglobuline** | | |
| -9 | | | **Thymoglobuline** | | |
| -8 | | | | Campath 0.2mg/kg | |
| -7 | **Fludarabine** 45mg/m$^2$ in 30-60min | **Thymoglobuline** | | Campath 0.2mg/kg | |
| -6 | **Fludarabine** 45mg/m$^2$ | **Thymoglobuline** | | Campath 0.1-0.2mg/kg | Dose Campath third day depending on cell source |
| -5 | **Fludarabine** 45mg/m$^2$ 1h followed by **Busulfan** with **PK** | **Thymoglobuline** | | | |
| -4 | **Fludarabine** 45mg/m$^2$ + **Busulfan** (based on PK first day) | | | | |
| -3 | **Busulfan** | | | | |
| -2 | **Busulfan** (PK check) | CyA | CyA | CyA | |
| -1 | | CyA | CyA | CyA | |
| 0 | | CyA | CyA MMF | CyA | |
| | | CyA 150-250<br>MTX 1,3,6 | CyA 200-300<br>Pred 1mg/kg (or MMF 3 x 15mg/kg) | CyA 200-300 + MMF (3 x 15mg/kg) | Pred till +28, taper in 2 weeks |

- 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**

| **DAY** | **DATE** | **D-DAY** | **TREATMENT** |
|---|---|---|---|
| | | D-7 | Thymoglobuline 2.5 mg/kg with pre-med |
| | | | Fludarabine 45 mg/m$^2$ once a day (IV infusion 30-60 mins) |
| | | D-6 | Thymoglobuline 2.5 mg/kg with pre-med |
| | | | Fludarabine 45 mg/m$^2$ once a day |
| | | D-5 | Thymoglobuline 2.5 mg/kg with pre-med |
| | | | Fludarabine 45 mg/m$^2$ once a day |
| | | | IV Busulfan over 3h (take and run PK) |
| | | D-4 | Fludarabine 45 mg/m$^2$ 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):**

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 3.5 |
| 15 to 25kg | 3.2 |
| 25 to 50kg | 2.8 |
| 50 to 75kg | 2.2 |
| 75 to 100kg | 1.8 |

*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** **Busulfan/Fludarabine 180 /Alemtuzumab 0.5 (BM), 0.6 mg (PBSC)**
**BMT DONOR** **Unrelated BM/PBSC (9-10/10 allele match)**
**GVHD PROPHYLAXIS** **Cyclosporine/MMF**

| **DAY** | **DATE** | **D-DAY** | **TREATMENT** |
|---|---|---|---|
| | | D-7 | Alemtuzumab 0.2 mg/kg with pre-med<br>Fludarabine 45 mg/m$^2$ once a day (IV infusion 60 mins) |
| | | D-6 | Alemtuzumab 0.2 mg/kg with pre-med<br>Fludarabine 45 mg/m$^2$ once a day (IV infusion 60 mins) |
| | | D-5 | Alemtuzumab 0.1-0.2 mg/kg with pre-med<br>Fludarabine 45 mg/m$^2$ once a day (IV infusion 60 mins)<br>IV Busulfan over 3h (take and run PK) |
| | | D-4 | Fludarabine 45 mg/m$^2$ once a day (IV infusion 60 mins)<br>IV Busulfan over 3h* |
| | | D-3 | IV Busulfan over 3h*<br>Start Cyclosporine |
| | | D-2 | IV Busulfan over 3h* |
| | | D-1 | Rest Day |
| | | D-0 | Infusion of BM/PBSC<br>Start MMF |

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

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 3.5 |
| 15 to 25kg | 3.2 |
| 25 to 50kg | 2.8 |
| 50 to 75kg | 2.2 |
| 75 to 100kg | 1.8 |

*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*10e7 NC/kg for 6/6 or > 1*10e5 CD34+/kg**
**5/6: >3*10e7 NC/kg for 6/6 or > 2*10e5 CD34+/kg**
**4/6: >5*10e7 NC/kg for 6/6 or > 3*10e5 CD34+/kg**
**Note: High-Resolution typing + selection optional**
**GVHD PROPHYLAXIS** **Cyclosporin / Pred (or MMF)**

| **DAY** | **DATE** | **D-DAY** | **TREATMENT** |
|---|---|---|---|
| | | D-12 | Thymoglobulin, 2.5mg/kg D-11 Thymoglobulin, 2.5mg/kg D-10 Thymoglobulin, 2.5mg/kg D-9 Thymoglobulin, 2.5mg/kg D-8 |
| | | D-7 | Fludarabine 45g/m2 |
| | | D-6 | Fludarabine 45g/m2 |
| | | D-5 | Fludarabine 45g/$m^2$ prior to IV Busulfan over 3h (take and run PK) |
| | | D-4 | Fludarabine 45g/$m^2$ IV Busulfan over 3h* |
| | | D-3 | IV Busulfan over 3h* |
| | | D-2 | IV Busulfan over 3h Start Cyclosporine |
| | | D-1 | Rest Day |
| | | D-0 | Infusion of CB Prednison 1mg/kg/d (in 2) till +28, taper in 2 weeks Or MMF 3 x 15mg/kg |

Initial busulfan dose is based on weight (dosed to reach target in 3 days):

| Body-weight | mg/kg/day |
|---|---|
| 3 to 15kg | 3.5 |
| 15 to 25kg | 3.2 |
| 25 to 50kg | 2.8 |
| 50 to 75kg | 2.2 |
| 75 to 100kg | 1.8 |

*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/Lx 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)

| **Body weight (kg)** | **Myeloablative** 4days 1dd, total target 90mg*h/L | **Non-myeloablative** 4days 1dd, total target 60mg*h/L |
|---|---|---|
| **3 -15** | 5.1 | 3.5 |
| **15 – 25** | 4.9 | 3.2 |
| **25 – 50** | 4.1 | 2.8 |
| **50 – 75** | 3.3 | 2.2 |
| **75 – 100** | 2.7 | 1.8 |

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

D Blood sample 1: Approx. 5 minutes **after end of infusion**

D Blood sample 2: Approx. 1 hours after end of infusion

D Blood sample 3: Approx. 2 hours after end of infusion

D 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

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# PROTOCOL C

**CONDITIONING** **Fludarabine/Melphalan/Alemtuzumab**
**BMT DONOR**
**GVHD PROPHYLAXIS** **CYCLOSPORIN/MMF**

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

# PROTOCOL D

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

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

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