



on behalf of the ESID and the EBMT WP Inborn Errors



Osteopetrosis

Consensus guidelines for diagnosis, therapy and follow-up

Version 3

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TABLE OF CONTENTS

1 SUMMARY	3
1.1 Background	4
1.2 Classification	4
1.3 Treatment	9
1.3.1 Contraindications to HSCT	10
1.3.2 Donor selection	11
1.3.3 Prevention of specific complications	11
2 DIAGNOSTIC GUIDELINES	12
2.1 General Considerations	12
2.2 Specific examinations	12
2.2.1 Molecular genetics	12
2.2.2 Peripheral blood and bone marrow	13
2.2.3 Radiology	14
2.2.4 Neurological examination	14
2.1.5 Scientific laboratory tests	14
3 THERAPEUTIC GUIDELINES	15
3.1 General Considerations	15
3.1.1 Indication	15
3.1.2 Donor	16
3.1.3 Conditioning regimens	16
3.1.4 Transplant and Boost	17
3.1.5 Risk prophylaxis	17
3.2 Conditioning Protocols	19
3.2.1 Matched Sibling Donor (MSD)	19
3.2.2 Matched Family Donor (MFD) or Matched Unrelated Donor (MUD)	20
3.2.3 HLA-Haploidentical Donor	21
3.3 Treatment of Complications	22
3.3.1 Non-engraftment and rejection	22
3.3.2 Venous-Occlusive Disease (VOD)	22
3.3.3 Pulmonary hypertension	22
3.3.4 Hypercalcaemia	23
3.3.5 Secondary graft failure and mixed chimerism	23
3.3.6 "Late adverse effects"	24
4 DOCUMENTATION	25
4.1 General Considerations	25
4.2. Registration and follow up	25
4.2.1 Registration and genetics	25
4.2.2 Transplantation	25
4.2.3 Status and Follow up	25
5 REFERENCES	29

1 Summary

Title: Osteopetrosis: Consensus guidelines for diagnosis, therapy and follow-up

Design: Prospective multi-centre survey

Objective: To provide a consensus protocol for diagnosis, treatment and follow-up of patients suffering from infantile (“malignant”) osteopetrosis and to build up a central registry for this disease

Inclusion Criteria: Paediatric patients suffering from osteopetrosis

General Remarks:

- These Guidelines represent the consensus recommendations of experts in this field, collected and reviewed by the authors on behalf of the ESID and the EBMT, and supported by Grants of the EU and E-RARE. They are NOT part of a formal treatment study according to GCP requirements in accordance with National and EU regulations.
- Collection and storage of patient data and material as well as specific laboratory tests (particularly genetic analysis and investigations related to accompanying research projects, as marked in the text), are important parts of this project but require written informed consent of parents and patients according to National and EU regulations.

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1.1 Background

Osteopetrosis (OP) is the generic name of a number of rare single gene diseases characterised by sclerosis of the skeleton (for a review see Tolar et al. 2004, Balemans et al. 2005, Villa et al. 2009, Steward 2010, Sobacchi et al. 2013). At least eleven forms are known with different modes of inheritance and severity, which cumulatively have an incidence of >1:100,000.

The disease originates from reduced or complete lack of osteoclast function and, as a consequence, impairment of bone resorption. In two thirds of the patients, osteoclasts are formed normally, but are unable to resorb bone effectively due to mutations affecting either H⁺ or Cl⁻ transport (Frattini et al. 2000, Kornak et al. 2000, Pangrazio et al. 2012). Recently, mutations in the sorting nexin 10 (*SNX10*) gene, whose product is suggested to interact with the proton pump, have been identified (Aker et al. 2012, Pangrazio et al. 2013). More rarely, osteoclasts are absent and the genetic defects in some of these “osteoclast-poor” forms have been found to reside in either the *RANKL* or the *RANK* gene, which encode key factors involved in preosteoclast fusion (Sobacchi et al. 2007, Guerrini et al. 2008, Pangrazio et al. 2012). Other very rare forms have been described, caused by mutations in *CAII*, *OSTM1*, *NEMO*, *PLEKHM1*, *KINDLIN3*, and *SLC29A3*, which impair osteoclast activity by different mechanisms (Sly et al. 1983, Sly et al. 1985, Dupuis-Girod et al. 2002, Chalhoub et al. 2003, Henriksen et al. 2005, Lange et al. 2006, Van Wesenbeeck et al. 2007, , Pangrazio et al. 2011, Schmidt et al. 2011, Campeau et al. 2012, Ott et al. 2013, Crazzolara et al. 2015). This genetic variability results in extreme phenotypic heterogeneity, with forms ranging from asymptomatic to rapidly fatal (Villa et al. 2009, Sobacchi et al. 2013). Diagnosis may be difficult and delayed, since some symptoms lead to suspicion of other diseases, as for instance JMML (Strauss et al. 2015).

1.2 Classification

For practical clinical use the different OP forms may be classified according to their clinical severity, bone marrow histology and genetic basis:

1. Clinical presentation
 - a. **Severe** - autosomal recessive inheritance pattern (ARO – Autosomal Recessive Osteopetrosis): Presenting at birth or in the first few months. Dense sclerotic bones, fractures, neurological symptoms with predominantly optic nerve impairment due to compression, bone marrow failure, infections and early death are the hallmarks of ARO, and the infants rarely survive >2 years in the absence of treatment. Long-term survivors have a poor quality of life and require frequent blood and platelet transfusions, surgery for dental diseases, nerve and cranial decompression and osteomyelitis. ARO is caused by mutations in *TCIRG1*, *CLCN7*, and less frequently *SNX10*, *OSTM1*, *RANK* and *RANKL*. *OSTM1* and *RANKL* must be distinguished early, since transplantation is contraindicated. Mental retardation and progressive neurodegeneration may be observed, and can be particularly severe in some *CLCN7* and in *OSTM1* gene mutation-dependent forms (de Vernejoul et al. 1993, Kasper et al. 2005, Lange et al. 2006, Pangrazio et al. 2010). In some patients with Leukocyte Adhesion Deficiency Type III (LAD-III), characterized by mutations in Kindlin-3, a high

bone density can be found, since Kindlin-3 signalling is required for osteoclast-mediated bone resorption (Schmidt et al. 2011). Clinically, LAD-III patients present with recurrent infections and a bleeding diathesis regardless of platelet or leukocyte count (Crazzolara et al. 2015, Stepensky et al. 2015).

- b. **Intermediate** - dominant or recessive inheritance: This group of OP is characterised by an intermediate, but still severe, course. The spectrum, severity and timepoint of clinical presentations are heterogeneous, but usually blood transfusions are not necessary. The genetic basis of intermediate OP is heterogeneous. An intermediate recessive form associated with brain calcifications and renal tubular acidosis is due to mutations in the carbonic anhydrase enzyme (*CAII*) gene. Mental retardation is frequently observed in these patients (Sly et al. 1985, McMahon et al. 2001, Cotter et al. 2005). Notably, clinical intermediate forms with no or only mild sensory or haematological impairments have been described in some patients with biallelic *CLCN7* mutations and more recently in patients with *RANK*, *SNX10* and *TCIRG1* mutations as well (Frattini et al. 2003, Waguespack et al. 2007, Pangrazio et al. 2010, Pangrazio et al. 2013, Sobacchi et al. 2014, Palagano et al, 2015). Other intermediate forms, characterised by mild sclerosis, short stature and fractures, remain genetically unrecognised except for one patient harbouring a loss-of-function mutation of the *PLEKHM1* gene (Van Wesenbeeck et al. 2007, Del Fattore et al. 2008). Single allelic *CLCN7* mutations may also cause an intermediate (or even severe) phenotype (ADO, see below) (Benichou et al. 2000).
- c. **Mild/late onset** - dominant inheritance pattern (ADO – Autosomal Dominant Osteopetrosis) is defined as a "benign" adult form. ADO (previously designated ADO type II), caused by single allelic mutations in *CLCN7*, has a heterogeneous course ranging from an asymptomatic to a severe phenotype (Benichou et al. 1998, Del Fattore et al. 2006). The latter is characterised by thickness of the vertebral end plates (sandwich vertebrae or Rugged-Jersey spine), pelvis and skull base associated with diffuse pain, secondary haematological and neurological impairment, osteomyelitis and frequent pathological fractures. Early death in these patients is rare, but some patients experience a very poor quality of life (Benichou et al. 1998, Tolar et al. 2004). What was previously designated ADO1 turned out to be a high bone mass phenotype caused by a missense mutation in the first beta propeller of *LRP5* (Van Wesenbeeck et al. 2003, Bollerslev et al. 2013).

Hematopoietic stem cell transplantation is the treatment of choice in patients with severe forms denominated “malignant infantile osteopetrosis” (MIOP), except in patients with neurodegeneration and with RANKL mutations. However, HSCT may be also considered in patients with intermediate phenotypes after careful evaluations of pros and cons (P. Stepensky and A. Schulz, manuscript in preparation).

2. Bone trephine or open biopsy characterisation

- a. **Osteoclast rich:** The osteoclast count in bone marrow is normal or even increased. The function of osteoclasts is impaired due to an intrinsic defect. Mutations in *TCIRG1*, *CLCN7*, *SNX10* and *OSTM1* lead to an osteoclast rich phenotype.
- b. **Osteoclast poor:** Osteoclasts are decreased or even absent in the bone marrow. This subtype is rare and linked to defects in osteoclast differentiation (*RANK*, *RANKL*). Note that osteoclast-poor OP caused by *RANKL* defects does not respond to HSCT since *RANKL* is expressed in osteoblasts (extrinsic osteoclast defect) which are not derived from the hematopoietic stem cell.

3. Genetic basis

A growing number of genetic defects have been described in OP, the most frequent genetic causes identified to date are summarised in Table 1. The following genes may be affected in malignant infantile OP:

INTRINSIC OSTEOCLAST DEFECTS

- a. ***TCIRG1*** (OMIM #259700; OPTB1; vacuolar H(+)-ATPase; ARO, causing 50% of osteopetrosis cases): The affected patients show “classical” MIOP (Pangrazio et al. 2012). Milder forms have been described but only very rarely (Sobacchi et al. 2014, Palagano et al. 2015).
- b. ***CLCN7*** (OMIM #611490; OPTB4; ClC-7 chloride channel; ARO, 10%): Most patients show “classical” infantile MIOP, but there is concern that some (but not all) children may develop progressive neurodegeneration. Developmental delay, failure to thrive and a pathological EEG are early signs of the neurodegenerative MIOP. MRI changes may occur at a later stage (A. Schulz and B. Winter, manuscript in preparation). Importantly, infants with OP caused by *CLCN7* mutations may develop progressive neurodegeneration also after successful transplantation performed at an asymptomatic stage (A. Schulz and B. Winter, manuscript in preparation; de Vernejoul et al. 1993, Mazzolari et al. 2009, Pangrazio et al. 2010, Ott et al. 2013).
- c. ***OSTM1*** (OMIM #259720; OPTB5; Chloride channel 7 beta subunit; grey lethal, rare): All described children have a very severe phenotype and severe neurological problems resembling progressive neurodegeneration. Common in Arab populations (Maranda et al. 2008, Mazzolari et al. 2009). HSCT is contraindicated, as the neurological impairment cannot be prevented by HSCT.
- d. ***SNX10*** (OMIM #615085; OPTB8; sorting nexin molecule 10, ARO, rare): Including 3 cases of “Västerbottenian osteopetrosis” (Swedish Province with a higher incidence of the disease) (Pangrazio et al. 2013). Patients present with “classical” MIOP, HSCT is curative. Vision impairment is frequent. Very rarely patients may present with deafness (so far one patient described) or neurodevelopmental impairment (so far also only one patient described).
- e. ***RANK*** (OMIM #612301; OPTB7; TNFRSF11A; ARO, rare): This subtype of OP is characterized by absence of osteoclasts and heterogeneity of clinical presentation (Pangrazio et al. 2012). However, since the defect is located within the osteoclasts, this form can be treated by HSCT as well.

EXTRINSIC OSTEOCLAST DEFECTS

- f. **RANKL** (#259710; OPTB2; TNFSF11; RANK ligand, rare) – patients with OP caused by RANKL mutation commonly lack osteoclasts on bone biopsy examination. They do not respond to HSCT since RANKL is produced by cells of mesenchymal origin (Lo Iacono et al. 2013).

Although these genotypes are correlated with distinct phenotypic features, “atypical” manifestations are possible and should be carefully considered prior to HSCT. Particular care must be taken in patients with osteoclast-poor (RANKL) and neurodegenerative forms (OSTM1 and some CLCN7 mutations) of OP in whom HSCT is contra-indicated.

In a minority of patients no mutation can be identified by classical Sanger sequencing in any of the genes so far associated with osteopetrosis. In these patients, large genomic deletions or splice site mutations of known genes may be present, which cannot be detected by classical molecular protocols analyzing exons and exon-intron boundaries, as described for TCIRG1 (Pangrazio et al. 2009, Pangrazio et al. 2012, Palagano et al. 2015). In addition, other yet undefined genes may be involved (Pangrazio et al. 2012). In these cases, further laboratory tests such as whole exome sequencing and osteoclast function assays may be necessary to determine the best treatment strategy (Sobacchi et al. 2013).

Table 1: Classification, genetics and clinical manifestations of osteopetrosis

OP	Age at onset	Inheritance	Gene (OMIM#)	Growth retardation	Hypo-Calcaemia	Haematol. impairment	Visual impairment	CNS Symptoms	Bone / Bone Marrow Morphology	HSCT indication
Infantile "malignant" Autosomal Recessive Osteopetrosis (ARO)	< 1 years	Autosomal Recessive	<i>TCIRG1</i> (#259700; OPTB1)	+ to +++	+++	+++	+ to +++	0 to ++ (Hydrocephalus)	Normal or high osteoclast counts	Yes
			<i>CLCN7</i> (#611490; OPTB4)	+ to +++	+++	+ to +++	+ to +++	0 to +++ (Hydrocephalus, neurodegeneration)		Yes in absence of neurodegen.
			<i>OSTM1</i> (#259720; OPTB5)	+ to +++	++	+ to +++	+ to +++	+++ (Neurodegeneration)		No
			<i>SNX10</i> (#615085; OPTB8)	?	++	+ to +++	+ to +++	0 to + (Hydrocephalus)	Smaller osteoclasts, high counts	Yes
			<i>KINDLIN-3</i> (#612840; LAD3)	+	0	+	0	0	Larger osteoclasts. high counts	Yes
			<i>RANK</i> (#612301; OPTB7)	++	+	+	+ to +++	0	Reduced osteoclast counts	Yes
			<i>RANKL</i> (#259710; OPTB2)	++	+	+	+ to +++	0		No
Intermediate Osteopetrosis	1-10 years	Autosomal Recessive or Dominant	<i>Some mutations in CLCN7 (dominant or recessive), TCIRG1, SNX10, RANK and RANKL may cause intermediate OP as well</i>					0 to +++	See above	Experimental in absence of neurodegen.
			<i>CAII</i> (#259730; OPTB3)	+	+	0 to +	0 to +++	Cerebral Calcifications, Mental Retardation	Renal tubular acidosis	
Benign Osteopetrosis (ADO)	10-40 years	Autosomal Dominant	<i>CLCN7</i> (#166600; OPTA2)	0	0	0 to +	rare	0	Scoliosis, arthritis, osteomyelitis	No

1.3 Treatment

Due to the haematological origin of osteoclasts, most forms of OP can be treated with Haematopoietic Stem Cell Transplantation (HSCT). In most cases HSCT greatly improves but does not fully rescue the phenotype. This approach has mostly been used to treat ARO, with >50% of patients being engrafted successfully but several undesired effects, including the progression of visual loss in the early post-transplant period (Driessen et al. 2003, Mazzolari et al. 2009, Steward 2010). Some attempts have been made to cure *CAII* deficiency (defined as IAO) with HSCT with similar outcome (Tolar et al. 2004). The results of pharmacological treatments with corticosteroids, vitamin D/calcium supplementation, PTH or gamma-interferon are inconsistent and generally cannot substitute for HSCT, with very few exceptions (Key et al. 1995, Shankar et al. 1997, Iacobini et al. 2001).

Treatment of **ADO** is generally based on empiric approaches. No guidelines for therapy are currently available and usually patients are treated symptomatically. Notably, an extremely heterogeneous phenotype is observed, not only in patients with different mutations of *CLCN7* for instance, but also in family members harbouring the same mutation (Frattini et al. 2003, Chu et al. 2005, Del Fattore et al. 2006). This begs the question whether other determinants may affect the gene penetrance (Chu et al. 2005). The **conservative treatment of patients with OP** who are not eligible for HSCT is not the primary subject of this protocol. Patients should be managed in a multi-disciplinary setting according to their clinical problems (as reviewed for instance in <http://www.geneclinics.org> (de Vernejoul et al. 1993)).

HSCT in MIOP: indication, conditioning and follow-up.

Until the last large ESID/EBMT survey in 2003, only HSCT using HLA-identical donors had an acceptable outcome, with a 73% five-year disease-free survival (Gerritsen et al. 1994, Driessen et al. 2003). However, the success rate of HSCT in experienced centres using alternative sources such as T-cell depleted haematopoietic stem cells from HLA-haploidentical family donors has improved significantly (Schulz et al. 2002, Driessen et al. 2003). In a retrospective analysis of the ESID and the EBMT, 125 transplanted patients with osteopetrosis were recorded (Sobacchi et al. 2013). The five-year DFS was estimated as 88% for genoidentical transplants, 80% for matched unrelated transplants and 66% for haploidentical transplants (Sobacchi et al. 2013). In a very recently published report of 193 patients transplanted in various centres by a cyclophosphamide based regimen, the five-year probabilities of survival were 62% after HLA-matched sibling and 42% after alternative donor transplantation (Orchard et al. 2015). Notably, the most recent data of the Ulm and Paris transplant units revealed an improved outcome with survival rates of 93% and 80% for T cell replete matched donor and T cell depleted haploidentical donor transplants, respectively, using a fludarabine-based conditioning regimen as proposed in these guidelines (A. Schulz et al., EBMT 2015, Abstract).

Cautions in Diagnosis and Management. The heterogeneity and the biology of the group of diseases involving OPs determine particular aspects in the management of affected patients. In particular **before HSCT** is initiated, the following questions must be addressed in each individual patient:

- a) is there a clear indication based on the severity of existing or imminent clinical manifestations (such as haematological failure or visual impairment) or based on the identified genetic defect?
- b) are there no contraindications based on the pathophysiology of the disorder (such as neurodegenerative or osteoclast extrinsic forms)?

1.3.1 Contraindications to HSCT

- **NEURODEGENERATIVE OP:** Children with any form of infantile OP may be extremely irritable due to fractures (common), hydrocephalus (rarer) or hypocalcemia (especially in the neonatal period) (Srinivasan et al. 2000). The commonest cause of severe neurological manifestations, however, is neurodegenerative OP, a metabolic disease causing early CNS deterioration and characterised by CNS inclusions as seen in ceroid lipofuscinosis (Lange et al. 2006). Children may exhibit spasticity, retinal changes, cerebral atrophy and agenesis of the corpus callosum (Steward 2003). All known patients with *OSTM1* mutations and about half of those with biallelic *CLCN7* mutations show neurodegenerative features (Pangrazio et al. 2006, Pangrazio et al. 2010). Some mutations seem to be clearly associated to a neurodegenerative course that cannot be prevented by HSCT even if performed in neurologically asymptomatic patients (B. Winter and A. Schulz, manuscript in preparation; A. Schulz, D. Moshous and C. Steward, unpublished observation). A careful neurological evaluation including EEG and cerebral MRI is therefore mandatory and should be repeated prior to the conditioning for HSCT, particularly for patients presenting *CICN7* mutations that have not been described previously. The indication for HSCT should be evaluated carefully on an individual basis and discussed with an expert.
- **TRANSIENT OP:** Transient cases of osteosclerosis have been described (Monaghan et al. 1991), and some children following this pattern are probably carriers of OP genes. In less severely affected infants repeated X-ray and comparison with original imaging is therefore recommended just before commencing conditioning therapy.
- **MILDER FORMS:** Some patients harboring mutations in *CLCN7* and *RANK* (but also in other) genes may present with milder (or no) haematological impairment. However, prognosis and quality of life are often poor and the risk of transplantation failure rises with age (see below). Therefore, HSCT is also indicated in most patients with a milder phenotype and should be performed as soon as possible after an individual risk assessment. Very few patients with milder forms of ARO older than 5 years, with ADO, or *CAII* deficiency have been transplanted so far (McMahon et al. 2001, Frattini et al. 2003, Othman et al. 2009, Shroff et al. 2012; P. Stepensky and A. Schulz, manuscript in preparation).
- **EXTRINSIC OSTEOCLAST DEFECTS:** HSCT is contraindicated in OP caused by mutations in the *RANKL* gene. Systemic treatment of *RANKL* has recently been shown to revert osteopetrosis in *RANKL* gene knockout mice (Lo lacono et al. 2012). So far there are no clinical trials for this treatment in humans.
- **OLDER CHILDREN:** Transplant problems regarding toxicity seem to accumulate in older patients, especially those over the age of 3 years. A reduced intensity regimen may be suitable for this group (e.g. treosulfan-based in the T replete setting). In addition, severe post-transplant hypercalcaemia is more likely in this group, although this may be treatable using hydration, steroids, calcitonin and bisphosphonates (Rawlinson et al. 1991, Kulpiya et al. 2012). In addition, Denosumab (PROLIA, Amgen), a monoclonal *RANK-L* antibody,

has been successfully used in two patients with *RANK* mutations suffering from severe hypercalcemia at Great Ormond Street Hospital, London and at the University Medical Center Ulm (Shroff et al. 2012). There is a correlation of transplant failure (rejection / graft failure and major toxic complications) with age at HSCT above 10 months, particularly after haploidentical transplantation (A. Schulz, unpublished observation). New transplant strategies such as the Baltimore protocol of T replete haploidentical transplantation with cyclophosphamide post-transplant (Fuchs 2012) may be suitable for these patients, and has been successfully used in several patients (retransplantation after rejection or non-engraftment, but also as first line approach in patients >10 months, D. Moshous, personal communication).

1.3.2 Donor selection

If a genotypically identical donor is not available and the patient has a severe phenotype (with existing or incipient visual loss) a mismatched family donor allows rapid transplantation. In these cases, HLA-haploidentical transplantation should be initiated without delay. The use of unrelated cord blood is no longer recommended, independently of the match, as the UCB is frequently associated with primary engraftment failure (Chiesa et al. 2013). The use of a genoidentical cord blood should be discussed with an expert.

1.3.3 Prevention of specific complications

- **STEM CELL BACK-UP:** A stem cell back-up should be considered before HSCT in patients with high risk of rejection (non-identical HSCT). There may be high numbers of circulating CD34 positive cells spontaneously, allowing collection of cells merely by limited exchange transfusion (Steward et al. 2005).
- **VENO-OCCLUSIVE DISEASE (VOD):** OP patients seem to be prone to VOD, although this risk may be reduced with the use of conditioning therapy based on intravenous busulfan and fludarabine instead of oral busulfan and cyclophosphamide. There is promising experience with the use of defibrotide as prophylaxis (Corbacioglu et al. 2004, Corbacioglu et al. 2006, Corbacioglu et al. 2012).
- **PULMONARY ARTERIAL HYPERTENSION (PAH):** Acute PAH has been frequently reported in MIOP patients, who have been conditioned with a busulfan and cyclophosphamide based regimen (Steward et al. 2004). PAH usually develops in the first 90 days after HSCT. Typical presentations are acute dyspnoea, hypoxia and brady/tachycardia. As these signs can also be observed in other types of post-HSCT pneumonitis, careful evaluation by an experienced cardiologist is warranted. However, EEG and echocardiographic changes may take weeks to become apparent and if the diagnosis is strongly suspected this may need to be investigated by cardiac catheter study, as timely treatment is required. Combined therapy with bosentan (endothelin receptor antagonist) and sildenafil should be initiated as soon as possible. Epoprostenol (prostacyclin) should be introduced if the situation is not controlled by these agents (Steward et al. 2004).

PAH must therefore be considered in any child who becomes acutely breathless after HSCT for OP.

2 Diagnostic Guidelines

2.1 General Considerations

In view of the complexity and heterogeneity of this disease, coupled with the possibility of rapid neurological deterioration, expert investigation/consultation/referral should be expedited. In co-operation with expert centres (or with one of the authors of these guidelines), a diagnostic workup should be initiated and co-ordinated immediately. Transfer of the patient to a centre experienced in HSCT in OP should be considered and planned.

The diagnostic workup of patients must respect the genetic defects, biology, atypical manifestations and possible complications mentioned in Chapter 1 and summarised in Table 1. In addition to a complete medical history, a physical examination should be performed by an experienced (neuro-) paediatrician. Laboratory testing of complete blood count, electrolytes, calcium profile, liver, renal and immunological parameters, and HLA-typing should be performed together with additional tests described below as appropriate to the case.

2.2 Specific examinations

2.2.1 Molecular genetics

Although there is no clear genotype-phenotype correlation in all forms of this disease, genetic analysis should be initiated immediately to recognise “classical” and “atypical” forms, which is maximally important for treatment strategies. Next Generation Sequencing (whole exome sequencing or panel approaches) may be performed in particular, if no mutation could be identified in the most abundant genes *TCIRG1* and *CLCN7* by Sanger sequencing:

- In severe infantile forms, the positive finding of ***TCIRG1*** mutations precludes neurodegenerative OP (associated usually with *OSTM1* mutations) and extrinsic untransplantable forms (such as *RANKL* mutations). Classical *TCIRG1* positive forms of OP are usually STRONG CANDIDATES FOR IMMEDIATE HSCT.
- This also holds true for patients with mutations in ***SNX10***.
- A positive finding of ***OSTM1*** (associated with severe neurological defect) or ***RANKL*** mutations (not rescued by HSCT) is helpful to preclude patients from ineffective and inappropriate HSCT. If DNA analysis revealed no mutations in other genes (as *TCIRG1* and *CLCN7*), ***OSTM1* and *RANKL* defects must be excluded prior to HSCT.**
- ***CLCN7*** mutations are associated with a VARIABLE PHENOTYPE ranging from “classical” *TCIRG1*-like infant forms, through intermediate or even mild forms to NEURODEGENERATIVE *OSTM1*-like forms. Contact the investigators of this study as there are some known genotype-phenotype correlations that may predict the neurological course of a given patient. In case of mutations that have not been described so far, an extensive clinical workup is necessary in affected children, including repeated and careful neurological evaluations by experienced neuropaediatricians, in order to detect patients at risk for neurodegenerative forms. Be aware, that early HSCT even prior to the onset of neurodegeneration cannot prevent the poor outcome. *OSTM1* patients (and probably also *CLCN7* patients with neurodegeneration) can have normal brain volume on CT/MRI very early in life but rapidly develop cerebral atrophy later. Failure to thrive, developmental delay and pathological EEG seem to be early signs of neurodegeneration prior to morphological brain pathologies.

- In OP with **low or absent osteoclasts** on bone marrow trephine examination, a positive finding of **RANK** mutation helps to differentiate patients from **extrinsic defects of osteoclast formation** (such as **RANKL** deficiency). In these patients, both **RANK** and **RANKL** genes should be sequenced to choose the appropriate intervention. Patients with **RANK** mutations respond well to HSCT, whereas **RANKL** defects cannot be rescued by HSCT.
- **Other genes:** If no mutations can be found in the known genes, therapy depends on clinical presentation. An extensive clinical workup is necessary. **In such cases blood cells should be sent to reference centres to analyse further candidate genes and osteoclast activity.**

2.2.2 Peripheral blood and bone marrow

Analysis of **blood cell count** including **reticulocyte** count and blood smear as well as **LDH** in serum are mandatory to evaluate the extent of haematological impairment. Decreased haemoglobin, reticulocyte and platelet parameters correlate with the extent of BONE MARROW FAILURE. In contrast, an increased leukocyte count and immature granulocytes in the PB (sometimes termed a leukoerythroblastic picture), as well as an increased LDH level, usually indicate EXTRAMEDULLARY HAEMATOPOIESIS. In some cases acute leukaemia may be suspected from these findings but can be excluded easily in most instances by other typical signs of OP.

pH of blood and urine should be analysed to detect RENAL TUBULAR ACIDOSIS. Metabolic acidosis with a persistently normal anion gap, together with a mild degree of hypokalaemia and a failure to achieve maximally low urine pH are detected in patients with renal tubular acidosis due to *CAII* deficiency. Basic parameters of **bone metabolism** including calcium (total and ionised) phosphate, and alkaline phosphatase in serum must be analysed to detect HYPOCALCAEMIA, which may cause convulsions in severe cases (Srinivasan et al. 2000).

Analysis of the **bone marrow** can be indicated under special circumstances. It can help to detect OSTEOCLAST POOR FORMS. In atypical and milder forms, the extent of REDUCED HAEMATOPOIESIS in the marrow should help to determine indication for and timing of HSCT. Since marrow aspirates usually fail in OP, **trephine biopsy** may be considered in patients with unclear aetiology. Bone biopsy should be performed only by an experienced operator as it is associated with the risk of bone fracture (ideally to be performed under the same general anaesthesia as MRI scanning or central line insertion). In rare cases, particularly if the findings are not conclusive regarding number of osteoclasts, open bone biopsy may even be required. Part of the biopsy (which must **not be decalcified** but should be suspended in formalin), may be sent to the coordinator in Ulm for reference analysis.

Some patients, especially those with *TCIRG1* and *RANK* mutations demonstrate immunological impairments such as hypogammaglobulinaemia (Guerrini et al. 2008, Mazzolari et al. 2009, Pangrazio et al. 2012) and numerical and functional disturbances of peripheral lymphocytes (A. Schulz, unpublished observation). Furthermore, the amount of CD34 positive stem cells in peripheral blood is usually elevated (more than 1%) in patients with haematological impairment possibly reflecting extramedullary haematopoiesis (Steward et al. 2005). Therefore, we recommend analysis of **IgG, IgA, IgM and IgE** and analysis of specific

Ab response upon vaccination. Furthermore, we suggest evaluating **lymphocyte subpopulations** by FACS (at least CD3, CD4, CD8, CD19, CD56/63 and CD34) and analysing **T-cell function** *in vitro*.

2.2.3 Radiology

Osteopetrosis is primarily a radiological diagnosis including features such as increased bone density, bone modelling defects, bone within bone appearance, fractures of the long bones, ribs and acromial processes and a dense skull all seen in conventional **X-ray**. Complete skeletal survey should be avoided and spared for atypical cases for radiation protection. However, radiological evaluation of at least one extremity, the head and the thorax should be performed to describe the morphology and extent of **OSTEOSCLEROSIS**, bone marrow narrowing and head deformities in the individual patient. Also check for growth plate widening as a sign of osteopetrorickets. In addition, imaging of the brain and skull is highly recommended to detect **HYDROCEPHALUS**, **NARROWING OF CENTRAL NERVE CHANNELS AND NEURODEGENERATIVE CHANGES** (such as cerebral atrophy and agenesis of corpus callosum) (Cure et al. 2000, Steward 2003). We recommend **MRI** for radiation protection and better quality. A CT scan (sometime claimed by radiologists to be better for assessment of bone morphology) is expendable in most instances. Keep in mind that the radiological narrowing of the optic nerve does not correspond to the damage and visual impairment in the individual patient, possibly because atrophic nerves tend to be shrunk away from the bone as their blood supply has already died.

Ultrasound evaluations of the brain, the abdomen and the hips should be done, particularly before HSCT, as objective and easy follow-up investigation methods. Doppler sonographic investigation of liver vessels may help to detect changes according to VOD after transplant. In the same way **Echocardiography and ECG** before and after HSCT may help to detect changes due to pulmonary hypertension.

2.2.4 Neurological examination

Since the spectrum of disease manifestations gives a variety of sensory and neurological impairments, careful work-up is mandatory in each individual patient. This work-up includes:

- detailed **neurological examination** by an experienced paediatric neurologist including examination of the developmental status. Repeated **EEGs** are necessary in selected cases.
- detailed visual assessment of the retina, optic nerve, vision and VEP (if possible) by an experienced paediatric **ophthalmologist**
- examination of ears, nose and throat by an experienced **ENT** specialist, including a hearing test +/- AEP

2.1.5 Scientific laboratory tests

It is highly recommended to preserve blood cells and bone marrow (if possible) for further analysis. In atypical cases, material should be sent to the coordinator in Ulm and/or other specialised laboratories after consultation.

Obligatory and scientific / optional investigations are listed in Table 2 in Chapter 4.

3 Therapeutic Guidelines

3.1 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. Type of disease, age at presentation, risk factors and donor availability are the main determinants for the therapeutic procedure ranging from “urgent transplantation” to “wait and see”.

3.1.1 Indication

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 major hepatosplenomegaly), this indication must be carefully evaluated and considered in the context of other symptoms and donor availability. Bone marrow biopsy and assay of peripheral blood reticulocytes and CD34 positive stem cells, 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 OP related problems other than haematological failure may be considered as **relative indications** for HSCT. These include: 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 compression). 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) **extrinsic osteoclast defects** characterised by mutations of the *RANKL* gene.
- 2) **neurodegenerative forms of OP**, characterised by encephalopathy and neurodegeneration with irritability, hypertonicity, seizures not due to hypocalcaemia (i.e a primary neurometabolic defect) and progressive developmental delay. This pattern of disease is associated with all known mutations of the *OSTM1* gene and about half of the mutations of the *CLCN7* gene.

Relative contraindications for HSCT may arise from severe problems in the individual patient, such as poor clinical condition (infection, pulmonary hypertension, elevated intracranial pressure).

In some patients – for instance those with a known genetic defect (TCIRG1, SNX10, CLCN7 or RANK) but without haematological insufficiency - the decision for or against HSCT may be difficult. Since clinical symptoms as well as HSCT risks seem generally accumulate over time, HSCT may still be considered appropriate in such patients.

3.1.2 Donor

We suggest the following ranking of donors:

- HLA-genoidentical family donors (matched sibling donor/MSD)
- HLA-matched family donor/MFD (especially in consanguineous families)
- HLA-matched unrelated donors/MUD (BM > PBSC)
- HLA-haploidentical family 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.

If no matched donor is available within a reasonable time period, HSCT from alternative donors (HLA-haploidentical parents) should be initiated without delay. Recent results of HLA-haploidentical transplant using a busulfan-fludarabine based conditioning regimen show acceptable toxicity and efficacy resulting in a disease free survival of 80 % (A. Schulz and D. Moshous unpublished observation; EBMT 2015, abstract). The use of cord blood has been explored in some small series with mixed success rates, but frequent primary graft failures have been noted resulting in an overall survival at 3 years of about 45% (Tsuji et al. 2005, Jaing et al. 2006, Jaing et al. 2008, Chiesa et al. 2013, Behfar et al. 2015). **Therefore, unrelated cord blood donors are no longer recommended independently of the HLA match.**

3.1.3 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 the subject of discussion (Eapen et al. 1998, Schulz et al. 2002, Driessen et al. 2003, Behfar et al. 2015). A fludarabine based protocol seems to be superior to a conventional cyclophosphamide based protocol (A. Schulz et al., EBMT 2015, abstract; P. Stepensky, personal communication). The following considerations seem to be reasonable:

- a) Use of intravenous busulfan with measurement of blood levels and dose targeting
- b) Substitution of cyclophosphamide by fludarabine because of the more favourable toxicity profile of fludarabine
- c) Use of thiotepa in non-genoidentical transplants because of its highly immunosuppressive and relatively myeloablative potential
- d) Furthermore, in high risk situations and in the T-replete setting, the substitution of busulfan by treosulfan has been explored with some success (A. Schulz, unpublished observation; P. Stepinsky, personal communication).

The following risk factors were associated with a poor outcome in retrospective analysis: significant extramedullary haematopoiesis (marked enlargement of spleen +/- liver), respiratory problems (choanal stenosis or pulmonary hypertension), CNS symptoms, age more than 10 months and an HLA-haploidentical transplant setting. It must be stressed that in these high risk situations HSCT should be performed in experienced centres only.

An alternative approach is the use of a T-replete haploidentical graft with the administration of cyclophosphamide after HSCT as GVHD prophylaxis (Fuchs 2012). This procedure is currently under evaluation in patients > 10 months at HSCT who are at high risk for primary engraftment failure and has been used successfully in several MIOF patients (D. Moshous, personal communication).

3.1.4 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.

- In the case of **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, CD3/CD19 negative selection, or CD3 alpha beta / CD19 negative selection) to yield a (alpha-beta TCR positive) T-cell content of the graft below 2.5×10^4 per kg body weight of the recipient.
- **T replete haploidentical HSCT** with cyclophosphamide post-HSCT (Fuchs 2012) is currently under evaluation in patients with advanced disease (and after rejection of the first graft); please contact the authors for updated information. In this setting bone marrow is the stem cell source of first choice and no graft manipulation is necessary.
- To ensure engraftment, it is important to obtain an **excellent graft** with more than 5×10^8 nucleated cells per kg body weight of the recipient in the case of bone marrow and more than 10×10^7 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 cryopreservation 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 donor cell engraftment has been documented, 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.

3.1.5 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 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) than MTX. Serotherapy should be used in any cases other than HLA-genoidentical transplants. In T replete transplantation, particularly if treosulfan is used instead of busulfan, serotherapy should be initiated 10 days prior to transplantation to ensure engraftment. If the T-cell content of an HLA-nonidentical graft accidentally exceeds 2×10^4 /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 former busulfan-cyclophosphamide-CSA-MTX regimen, patients with OP may still be at very high

risk of developing liver (and pulmonary) VOD (Steward et al. 2004, Corbacioglu et al. 2006). In addition to a careful monitoring of VOD symptoms (refractory thrombocytopenia, weight gain, liver enlargement, ascites, bilirubin elevation), prophylaxis or early therapy with Defibrotide is recommended.

- **Respiratory problems (choanal stenosis/CLS/PAH):** Respiratory problems are common during transplantation for several reasons. **Upper airway obstructions** (e.g. pre-existing choanal stenosis) and secondary ventilation problems due to mucositis, fluid overload and hepatosplenomegaly (**VOD, capillary leak syndrome, CLS**) or **CNS diseases** (hydrocephalus, hypocalcaemic convulsions) must be distinguished from primary pulmonary problems 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 (Kasow et al. 2004, Steward et al. 2004, Kasow et al. 2008)(see treatment section below).
- **Pneumocystic jirovecii pneumonia (PCP):** Patients with OP seem to harbour an elevated risk for **pneumocystic jirovecii pneumonia (PCP)**, possibly because of the lack of prophylaxis before HSCT, the young age at HSCT and the prolonged haematological and immunological recovery, since at least three patients developed PCP even in the laminar airflow environment (Moshous and Schulz, unpublished observation). Alternative or combined strategies to prevent PCP may be a) pre-treatment of patients with daily cotrimoxazole at least 2 weeks prior to HSCT until HSCT, b) twice weekly cotrimoxazole throughout transplantation and c) the use of Caspofungin in the post HSCT period could be considered as antifungal and PCP prophylaxis during cytopenia (Annaloro et al. 2006, Utili et al. 2007, Tu et al. 2013).
- **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 from the “neurodegenerative” form of MIOP, associated with all *OSTM1* and about half of the *CLCN7* cases (see above). Malformations of the bones may lead to hydrocephalus and/or Arnold-Chiari-like malformations. Whereas these malformations are in principle reversible after successful HSCT, they may lead to severe clinical complications particularly during the period of fluid retention early after transplantation/during an episode of VOD. Careful and interdisciplinary diagnostic, monitoring and treatment regimens are mandatory in the individual affected patient.
- **Serum calcium disturbance: Hypocalcaemia and the attendant risk of** convulsions before engraftment contrasts with **hypercalcaemic complications** thereafter. In the case of hypocalcemia, supplementation with calcium gluconate and one alpha vitamin D (1000 IU per day) is recommended before HSCT. [See paper by Schinke et al. for best administration regimen in patients with gastric pH disturbance as those affected by mutations in the *TCIRG1* gene (Schinke et al. 2009)]. This supplementation should be reduced or even stopped upon 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 (see treatment section below).

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 10⁸ NC / kg BW
- PBSC (2nd choice): >10 x 10⁶ 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 10⁸ NC / kg BW
- PBSC (2nd choice): >10 x 10⁶ CD34+ / kg BW; T-cells may be reduced in vitro to 5 to 10 x 10⁶ 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 10⁶ CD34+ / kg BW
 - T-cells: < 1 to 2 x 10⁴ 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 10⁴ CD3+ / kg BW

GvHD prophylaxis

- T-cell depletion
- If CD3+ T cells in the graft exceed 2 x 10⁴ 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 information***

3.3 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) survived in the majority of cases in a large series performed in Ulm.

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

3.3.1 Non-engraftment and rejection

Most patients with OP show a slow haematological recovery after HSCT, possibly 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 should be considered at around one month after transplantation (see chapter 3.1.4). 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 a 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, disappearance of donor granulocytes and stem cells), a secondary conditioning regimen and another, alternative donor is recommended. Whereas rejection is a major and not uncommon complication - in particular in the haploidentical setting - it can be managed successfully in many cases (Stepensky et al. 2011) (A. Schulz and D. Moshous, unpublished observation). T replete haploidentical HSCT with cyclophosphamide post-HSCT may be appropriate in this situation and is currently under investigation (Fuchs 2012). The retransplant strategy should be discussed with the authors.

3.3.2 Venous Occlusive Disease (VOD)

Preliminary data suggest that administration of prophylactic defibrotide may efficiently prevent VOD in OP patients. Early diagnosis and start of specific therapy is a prerequisite for successful treatment of this common complication. Immediate commencement of **Defibrotide** is highly recommended when VOD is suspected by the typical, but sometimes non-specific clinical signs. A carefully balanced fluid and diuretic therapy (to keep central venous pressure at +1 to +5 cm H₂O) may prevent cardiovascular, pulmonary and renal insufficiency.

3.3.3 Pulmonary hypertension

In the case of oxygen requirement and/or tachypnoea in the absence of identification of relevant pathogens, repeated echocardiographic and ECG investigations (to detect an enlarged right ventricle and tricuspid regurgitation) is helpful to detect this severe complication. Documentation by cardiac catheterisation is recommended where possible. Treatment is difficult and should reflect the individual situation. Continuous oxygen administration,

magnesium supplementation and moderate diuretic therapy represent the first steps of treatment.

In case of proven (or highly suspected) pulmonary hypertension simultaneous use of 1 and 2 below should be initiated immediately:

1 – BOSENTAN (Endothelin Receptor Antagonist): 2 x 2 mg/kg/day p.o.

2 – SILDENAFIL: 4 x 1.5 mg/kg/day p.o.

If the situation is not controlled by this therapy, a prostaglandin antagonist may be added (Steward et al. 2004):

3- EPOPROSTENOL (FLOLAN, prostacyclin): continuously sc or iv, dosage depending on the efficacy (up to 20-30 ng/kg/min). **Beware cardiovascular side effects including tachycardia, bradycardia fall in diastolic blood pressure.**

Careful interdisciplinary collaboration of haematologists, cardiologists, respiratory physicians and the intensive care team is mandatory when this life threatening complication is suspected or evident. Even severe cases of pulmonary hypertension can reverse dramatically to complete resolution allowing withdrawal of all therapy after only a few weeks and it is important that intensive care physicians are aware of this potential.

Since the introduction of busulfan/fludarabine based conditioning regimens, the incidence of PAH seems to be regressing significantly.

3.3.4 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 at any time during the first few months after transplantation. First measures include hyperhydration and use of diuretics. Steroids may be useful. In severe cases, in particular when relevant nephrocalcinosis is detected in ultrasound, Denosumab (PROLIA, Amgen), a monoclonal *RANK-L* antibody, has been successfully used in two patients with *RANK* mutations suffering from severe hypercalcemia at Great Ormond Street Hospital, London and at the University Medical Center, Ulm (Shroff et al. 2012). An initial dosage of about 0.1 mg/kg body weight seems to be appropriate. Repeated low dose Denosumab applications may be superior to other agents such as calcitonin and bisphosphonates because of its high efficacy and low toxicity (A. Schulz, unpublished observation). Consult the study investigators for details.

3.3.5 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 regression of OP may be achieved spontaneously within 6 to 12 months after HSCT, even if only 20% donor cells are present (A. Schulz, personal observation; Behfar et al. 2015, Hashemi Taheri et al. 2015), but osteosclerosis typically redevelops at around 10% donor haematopoiesis (Steward, personal observation). 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 given.

3.3.6 “Late adverse effects”

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

- **Impact on stature:** Patients tend to show no catch-up growth after HSCT. But the height of the majority of patients remains in the lower range. There seems to be no primary growth hormone deficiency in OP patients (al Herbish et al. 1994).
- **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; Krimmel et al. 2004, Dowlati et al. 2007). The head circumference should be monitored at regular intervals after transplantation.
- **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 (Mazzolari et al. 2009; A. Schulz and D. 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 in patients with *TCIRG1* mutations, which may require treatment by calcium gluconate (Schinke et al. 2009, Koehne et al. 2013). Therefore, bone density should be measured in long-term follow up after HSCT (by DEXA scan in older patients), in particular in patients with *TCIRG1* mutations and/or mixed chimerism.

4 Documentation

4.1 General Considerations

The aim of the registry is a detailed analysis of the outcome of patients with OP, depending on the clinical presentation, the genetic defect and the chosen therapy. Outcome analysis should comprise not only overall and disease free survival but also short term and long term sequelae of disease and of therapy. In addition to known general side effects of stem cell transplantation OP-specific problems will be monitored, particularly growth delay, sensory impairment and neurological problems. Furthermore, the quality of life (QoL) as judged by patients and their parents and the age dependent integration of patients within the society will be evaluated.

For this purpose, specific questionnaires have been designed to cover these issues. Following the concept of other registries in the context of HSCT (e.g. the EBMT MED -A/-B forms), patient data will be stored together with initials, date of birth and the (registering or transplanting) centre. Data from the questionnaires will be integrated into a database covering data protection regulations of the EU. Transfer of data out of the questionnaire into the electronic database will be done at the registry centre in Ulm only to optimise quality, efficacy and privacy.

To cover legal regulations of the EU, patients and parents must give their informed consent to provide data including genetic tests and to allow the electronic storage of these data.

4.2. Registration and follow up

4.2.1 Registration and genetics

Each patient will be registered **once** using the registration questionnaire. Basic ethnic and family information, as well as results of molecular analysis, specifying the disease are covered in this form. Updated results of DNA analysis may be recorded by this questionnaire as well.

4.2.2 Transplantation

The transplantation questionnaire records transplant related issues as well as acute side effects of the transplant procedure. This questionnaire should be completed **for each transplant** at about 3 to 6 months after transplantation.

4.2.3 Status and Follow up

The physical and the mental status of the patient (including QoL and school attendance), paraclinical findings (including chimerism data in transplanted patients) as well as the survival status are recorded by this questionnaire. The questionnaire can be completed independently of the treatment procedure chosen also in non-transplanted patients. This form should be completed **for each patient repeatedly at different time points** (at registration and in regular yearly intervals).

Usable forms are listed below.

Time Schedule for Diagnostics and Documentation				
all patients at diagnosis	HSCT patients		non-HSCT patients	Procedure
	at day +100	every 1-2 years	every 1-2 years	
				Laboratory Tests
●	●	●	●	Peripheral Blood: Cell count and clinical chemistry including LDH, CK, pH Urine: pH, Ca
●	●	●	●	Bone metabolism - Serum: Ca/Ph, Alkaline Phosphatase, Parathormone, VitD3
●	●	⊘	⊘	Immunological Parameters - Serum: IgG, IgA, IgM, specific Ab; Heparinised-peripheral Blood: Lymphocyte subsets and function tests*
●				Molecular Genetic Analysis (see text for priorities; include specific consent if sent to Authors)*
●				Donor Search - high resolution HLA-typing of patient (and family)
⊘		○	○	In selected patients only Bone Marrow - Trepine biopsy or open biopsy (non-calcified, in Formalin)*
	●	●		Chimerism Analysis - PB cells; additionally in case of mixed chimerism cellular subtypes
⊘		○	○	In selected patients with unusual presentation and/or no known genetic alterations, specific biomarkers and the osteoclast function may be analyzed (contact the authors)
				Radiology
●		○	○	X-ray - One extremity; in older patients, bone density should be measured by DEXA scan particularly in cases with mixed chimerism
⊘	○	○	○	Ultrasound (abomen, kidneys, brain and hips)
⊘		○	○	MRI - brain (CT may be considered)*
				Consultants
●	⊘	⊘	⊘	Paediatric Neurologist - status, development, QoL; prior to HSCT, EEG is mandatory to detect neurodegeneration (particularly in CLCN7 patients)
●	○	●	●	Ophthalmologist - visual testing, optic nerve assessment, VEP
●	○	○	⊘	ENT - morphology, hearing tests
				Documentation
●				Consent
●				Registration questionnaire
	●			Transplantation questionnaire
●		●	●	Status and Follow-up questionnaire
●	Obligatory			
⊘	Recommended (until normal, if pathological in prior analysis)			
○	Optional			
*	send to Coordinator in Ulm or to one of the Authors			

Checklist for investigations at diagnosis

Patient ID

Clinic ID

Obligatory / Recommended

Optional (may be performed / initiated by the coordinator in Ulm)

Laboratory

Cell count (EDTA blood, including blood smear and reticulocyte count)

Clinical chemistry (serum: Ca, Ph, LDH, kidney and liver parameters)

pH (serum and urine)

Bone metabolism (serum: PTH, ALP, 1,25-dihydroxyvitamin D3, Osteocalcin, TRAcP, CTX or NTX, *RANKL*, OPG)

Immunoglobulins (serum: IgG, IgA, IgM, IgE); Ab response to vaccination (DT, Hib)

Lymphocyte subsets (FACS analysis: CD3, CD4, CD8, CD19, CD14, CD56/63, CD34)

Lymphocyte function (in vitro stimulation: mitogens and antigens)

Molecular genetics (EDTA blood: *TCIRG1*, *CLCN7*), if normal:

Panel diagnostics / whole exome sequencing (Lab.: _____)

HLA-typing of patients and family (EDTA blood: 10 loci, 4 digits)

in selected patients only: BM histology (NOT decalcified in Formalin; send part to coordinator in Ulm)

Technical investigations

Date

X-ray (1 extremity)

_____._____._____

Sonography (head, abdomen, kidney, hip)

_____._____._____

EEG

_____._____._____

MRI of head

_____._____._____

CT of head

_____._____._____

Consultants

Paediatric neurologist

_____._____._____

Ophthalmologist

_____._____._____

ENT specialist

_____._____._____

Forms

Consent form of the Registry

Consent form for genetic analysis

Registration questionnaire

Status questionnaire

Transplant questionnaire

Separate forms

File

Questionnaires

Registration and genetics:	01_Registration
Transplantation:	02_Transplantation
Status and Follow up:	03_Follow-Up
Short follow up	03a_Follow-Up v1.9 short

Consent and request forms:

English, also available in French (please contact the authors)

Information for Parents	04_OP_registry_Information Parents
Informed Consent	06_OP_registry_Consent

German

Aufklärungsbogen Eltern	07_OP_registry_Aufklärung Eltern
Aufklärungsbogen Patient	08_OP_registry_Aufklärung Kinder
Einverständniserklärung	09_OP_registry_EVE

Genetic Diagnostic Forms	10_OP_registry_Genetic Test_Ulm
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Votum Ethic Committee Ulm	11_OP_registry_Ethikvotum_Ulm
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