



on behalf of the ESID and the EBMT WP Inborn Errors



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# Osteopetrosis

## Consensus guidelines for diagnosis, therapy and follow-up

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## 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 a generic name of a number of rare single gene diseases characterised by sclerosis of the skeleton. At least nine forms are known with different modes of inheritance and severity, which cumulatively have an incidence >1:100,000. The disease originates from reduced or complete lack of osteoclast function and, as a consequence, impairment of bone resorption<sup>1</sup>. In two thirds of children, osteoclasts are formed normally, but are unable to resorb bone effectively due to mutations affecting either H<sup>+</sup> or Cl<sup>-</sup> transport<sup>2-4</sup>. Rarely, osteoclasts are totally absent (so called “Osteoclast-poor” forms) and the genetic defect in some of these forms has recently been found to reside in the RANKL gene and RANK molecules, which are key factors involved in preosteoclast fusion<sup>5,6</sup>. Other forms are due to environmental defects reducing osteoclast activity<sup>7,8</sup>, or to enzymatic deficits leading to insufficient proton production<sup>9</sup>. In addition, in very few patients with OP, mutations of the NEMO factor (involved in the NF-κB signal), of the OSTM1 gene (whose product is a transmembrane protein functionally associated to the Cl<sup>-</sup> transport) and of the PLEKHM1 gene (which is linked to the activity of small-GTPase proteins of the Rab family), have been described<sup>10,11,12,13</sup>. This genetic variability results in extreme heterogeneity, with forms ranging from asymptomatic to fatal (recently summarised by Villa *et al.*<sup>14</sup>).

On behalf of the ESID and the EBMT, data of patients with OP have been collected retrospectively. Between 1983 and 2008, data from 173 patients were recorded from 28 European referring centres. Most patients were defined as suffering from Autosomal Recessive OP (ARO), but about 10% with Autosomal Dominant OP (ADO) have been collected in this registry as well. In about 30% of cases no genetic aberration could be defined. In most of these cases not all known candidate genes have been analysed. The genetic distribution of the disease is shown in Figure 1.

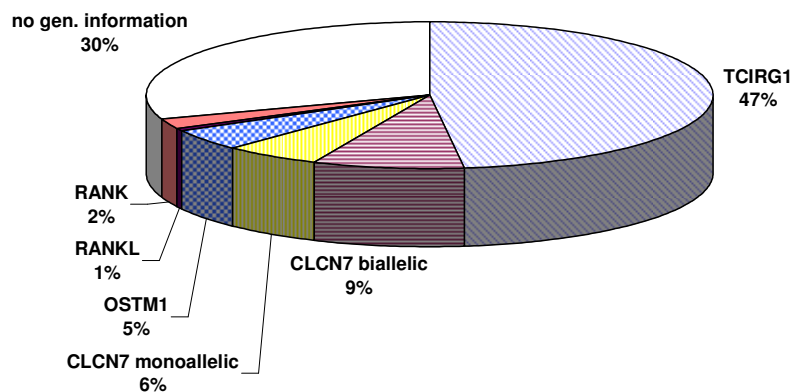


Figure 1: Genetic distribution of osteopetrosis

## 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, bone marrow failure, infections and early death are the hallmarks of ARO, and the infants rarely survive >2 years. Longer survivors have a poor quality of life and require frequent blood transfusions, surgery for dental diseases, nerve and cranial decompression and osteomyelitis. ARO is caused by mutations in TCIRG1, CLCN7 and (rarely) OSTM1, RANK and RANKL. OSTM1 and RANKL must be distinguished early, since transplantation is contraindicated. Mental retardation may be observed, and can be particularly severe in some CLCN7 and in OSTM1 gene mutation-dependent forms<sup>13,15</sup>. Children with TCIRG1 mutations are usually urgent candidates for transplantation. Those with CLCN7 mutations should be discussed with an expert.
- b. **Intermediate** - may be of dominant or recessive inheritance: This group of OP is characterised by an intermediate but still severe course. The spectrum, severity and time point of clinical presentations are heterogeneous, but usually blood transfusions are not necessary. The genetic basis of intermediate OP is heterogeneous. An IRO form is associated with brain calcifications and renal tubular acidosis and is due to mutations of the carbonic anhydrase enzyme (CAII) gene<sup>16</sup>. Mental retardation is frequently observed in these patients<sup>16-18</sup>. Other IRO 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<sup>1,19</sup>. Single allelic CLCN7 mutations may also cause an intermediate (or even severe) phenotype (ADO II, see below). Patients with intermediate OP may be candidates for Hematopoietic Stem Cell Transplantation (HSCT), but pros and cons should be evaluated carefully on an individual basis and discussed with an expert.
- c. **Mild/late onset** - dominant inheritance pattern: **ADO** is defined as a benign adult form ADO type I is generally very mild, with a diffuse sclerosis and no alterations in bone turnover biochemical markers and blood cell counts, ADO type II has an extremely heterogeneous course ranging from an asymptomatic to a severe phenotype. The latter is characterised by thickness of the vertebral end plates (sandwich vertebrae or Rugger-Jersey spine), pelvis and skull base associated with diffuse pain, secondary haematological and neural failure, osteomyelitis and frequent pathological fractures. Early death in these patients is rare, but some patients can experience a very poor quality of life<sup>1,20</sup>. Patients with mild OP are usually no candidates for HSCT.

## 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, 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 do not respond to HSCT since RANKL is expressed on osteoblasts (extrinsic osteoclast defect).

## 3. Genetic basis

A growing number of genetic defects have been described in OP summarised (incompletely) in Table 1. The following genes may be affected in malignant **infantile OP**:

### INTRINSIC OSTECLAST DEFECTS

- a. **TCIRG1** (ATP6i, ~50%): The affected patients show “classical” malignant infantile OP; very rarely milder forms have been described.
- b. **CLCN7** (about 10%) - there is concern that some (but not all) children with OP caused by CLCN7 mutations may develop cerebellar problems some time after transplant and/or may belong to the neuropathic form of OP.
- c. **OSTM1** (grey lethal, rare, less than 2%) - all described children have a very severe phenotype and obviously severe neurological problems resembling progressive neurodegeneration. Common in Arab races. HSCT is contraindicated.
- d. **RANK** (rare, about 1%) - heterogeneity of presentation and absence of osteoclasts are common to patients with RANK mutations. However, since the defect is located within the osteoclasts, this form can be treated by HSCT as well.

### EXTRINSIC OSTECLAST DEFECTS

- e. **RANKL** (RANK ligand, rare, about 1%) – 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 osteoblasts.

***Although these genotypes are correlated with distinct phenotypic features, “atypical” manifestations are possible and should be carefully considered before HSCT. Particular care must be taken in patients with osteoclast-poor forms of OP.***

Table 1: Classification, genetics and clinical manifestations of osteopetrosis

OP	Age at presentation	Inheritance	Gene	Growth retardation	Hypo-Calcemia	Haematol. impairment	Visual impairment	CNS Symptoms	Bone / Bone Marrow Morphology
Infantile "malignant" Autosomal Recessive Osteopetrosis (ARO)	< 1 years	Autosomal Recessive	<i>TCIRG1</i>	+ to +++	+++	+++	+ to +++	0 to ++ (Hydrocephalus)	Normal or high osteoclast counts
			<i>CLCN7</i>	+ to +++	+++	+ to +++	+ to +++	0 to +++ (Hydrocephalus, neurodegeneration)	
			<i>OSTM1</i>	+ to +++	++	+ to +++	+ to +++	+++ (Neurodegeneration)	
			<i>RANK</i>	++	+	+	+ to +++	0	No or reduced osteoclast counts
			<i>RANKL</i>	++	+	+	+ to +++	0	
Intermediate Autosomal Osteopetrosis (IAO)	1-10 years	Autosomal Recessive or Dominant (see <i>CLCN7</i> )	<i>CLCN7</i> may be involved in IAO as well				0		
			<i>CAII</i>	+	+	0 to +	- to +++	Cerebral Calcifications, Mental Retardation	Renal tubular acidosis
			<i>PLEKHM</i>	++	0	0	0	0	Bone deformities, pain, chondrolysis
Benign Osteopetrosis (ADOII / Morbus Albers-Schönberg)	10-40 years	Autosomal Dominant	<i>CLCN7</i>	0	0	0 to +	very rare	0	Skoliosis, arthritis, osteomyelitis
			<i>PLEKHM</i>	0	0	0	0	0	Focal osteopetrosis osteopenia

### Outcome of stem cell transplantation for osteopetrosis

~ mutation ~

(n = 106, TCIRG1 = 56, CLCN7 = 8, unknown = 42)

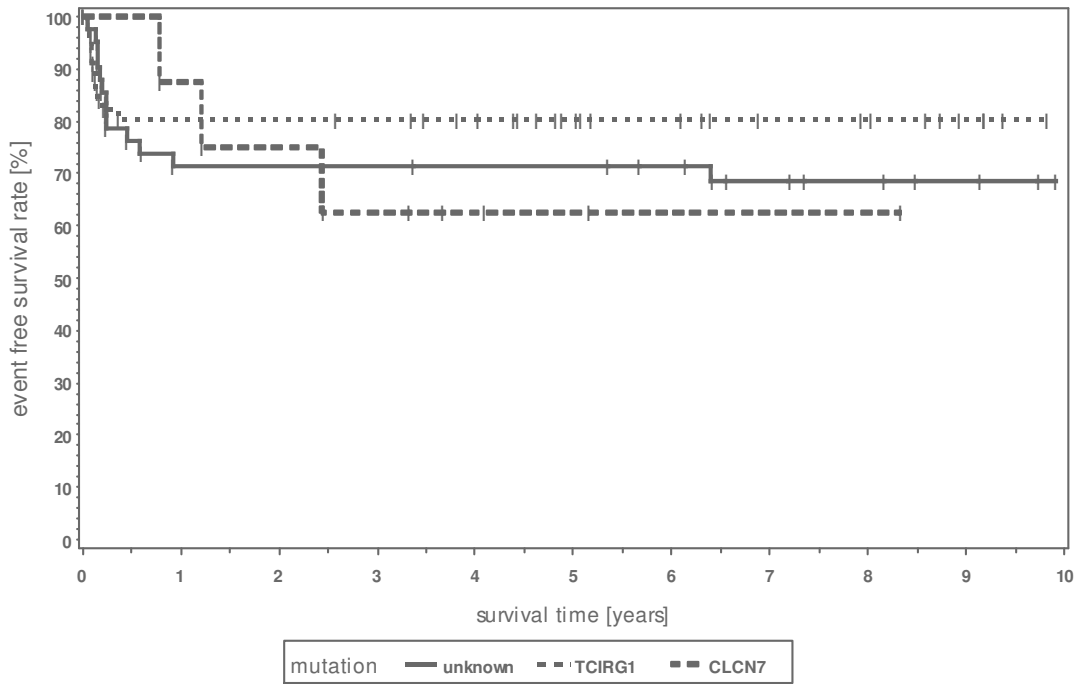


Figure 2: Survival following HSCT according to genetic defects

### Outcome of stem cell transplantation for osteopetrosis

~ donor ~

(n = 106, MSD = 35, haplo = 51, MUD = 15, MMUD = 5)

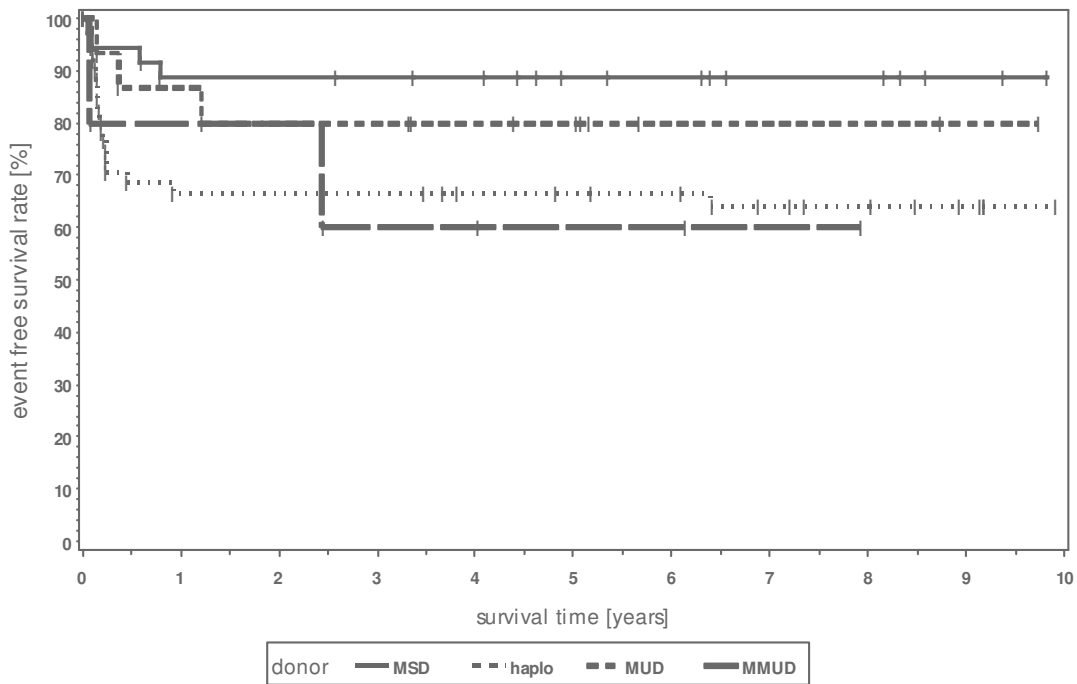


Figure 3: Survival following HSCT according to donor



### 1.3 Treatment

Since the haematological origin of osteoclasts was discovered, the disease has been treated with Haematopoietic Stem Cell Transplantation (HSCT), which in most cases improves but does not fully rescue the phenotype<sup>21</sup>. This approach has mostly been used to treat ARO, with >50% of successful engraftment and several undesired effects, including the progression of neural failure with vision deterioration<sup>21</sup>. Some attempts have been made to cure CAII deficiency (defined as IAO) with HSCT with similar outcome<sup>17</sup>. The results of pharmacological treatments with corticosteroids, vitamin D/calcium supplementation, PTH or gamma-interferon are inconsistent and generally cannot substitute for HSCT, with a very few exceptions<sup>22</sup>.

Treatment of ADO is generally based on empiric approaches. No guidelines are available so far for therapy and usually patients are treated symptomatically. Notably 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<sup>23-25</sup>. This begs the question of what other determinants may affect the gene penetrance in this form<sup>24</sup>.

The **conservative treatment of patients with OP** is not the primary subject of this protocol and patients should be managed in a multi-disciplinary setting according to their clinical problems (as reviewed for instance in <http://www.geneclinics.org>). These guidelines will focus on HSCT with particular respect to its indication, conditioning and follow up. HSCT using HLA-identical donors has an acceptable outcome (73% five-year disease-free survival)<sup>21</sup>. The success rate of HSCT from experienced centres using alternative sources as T-cell depleted hematopoietic stem cells from HLA-haploidentical family donors or cord blood from unrelated donors has improved markedly in recent years<sup>26,27</sup>. In the retrospective analysis of the ESID and the EBMT, 134 transplanted patients were recorded, and for 106 patients detailed follow-up data are available. The results of HSCT according to this survey are depicted in Figure 2 with respect to the genetic background defect and in Figure 3 with respect to the donor.

**Cautions in Diagnosis and Management.** The heterogeneity and the biology of the group of diseases involving OPs implicate 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 failure and b) are there strict contraindications based on the pathophysiology of the disorder (such as neuropathic or osteoclast extrinsic forms)

#### 1.3.1 Contraindications to HSCT

- **IRRITABILITY / CNS ABNORMALITY:** Children with any form of infantile OP may be irritable due to fractures (common) or hydrocephalus (rarer). The commonest cause of severe irritability, however, is neuropathic OP, a metabolic disease causing early CNS deterioration and characterised by CNS inclusions as seen in ceroid lipofuscinosis; most cases are due to OSTM1 mutations. Children may exhibit spasticity, retinal changes, cerebral atrophy and agenesis of the corpus callosum<sup>28</sup>.

- **TRANSIENT OP:** Transient cases of osteosclerosis are well described, and some children following this pattern are probably carriers of OP genes. In less severely affected infants repeat X-ray and comparison with original films is recommended just before commencing conditioning therapy.
- **MILDER FORMS:** In patients harbouring mutations in CLCN7 and RANK (but also the other) genes may present with milder (or no) haematological impairments. However, prognosis and quality of life are often poor and the risk of transplantation failure rise with age (see below). Therefore, HSCT is also indicated in most cases and should be performed as soon as possible after an individual risk assessment. HSCT has not been tested in patients with known ADO nor in PLEKHM1 ARO.
- **EXTRINSIC OSCEOCLAST DEFECTS:** HSCT is not indicated in OP cause by mutations in the RANKL gene.
- **OLDER CHILDREN:** Beware older children, especially those over the age of 3 years. Severe post-transplant hypercalcaemia is much more likely in this group, although this may be treatable using bisphosphonates <sup>29</sup>. 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, London and at the Ulm university clinics. Consult the study investigators for details.

There is a strong correlation of transplant failure (rejection / graft failure and major toxic complications) with age above 10 months (A. Schulz, unpublished observation). In patients with a suitable donor therefore the transplantation should be performed as soon as possible after diagnosis.

### 1.3.2 Donor selections

There have been promising results in recent years with the use of mismatched family donors, i.e. mismatched for 1-5 of 10 antigens tested. 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 (or partially matched cord blood) transplantation should be initiated without delay.

### 1.3.3 Prevention of specific complications

- **STEM CELL BACK-UP:** A stem cell back-up should be considered before HSCT in patient with high risk of rejection (nonidentical HSCT). There may be high numbers of circulating CD34 positive cells spontaneously, allowing collection of cells merely by limited exchange transfusion <sup>30</sup>.
- **VENO-OCCLUSIVE Disease (VOD):** OP patients seem prone to VOD, although this risk may be reducing with the use of i.v. Busulfan and Fludarabine instead of p.o. Busulfan and Cyclophosphamide. There is promising experience with the use of defibrotide as prophylaxis.
- **POST-TRANSPLANT RESPIRATORY PROBLEMS:** As many as one child in three will develop acute severe Pulmonary Arterial Hypertension (PAH) in the first 90 days after HSCT for ARO, although this is extremely easy to mistake for other types of

pneumonitis<sup>31,32</sup>. It seems to occur regardless of donor type, stem cell manipulation or conditioning therapy. Typical presentations are with acute dyspnoea, hypoxia and brady/tachycardia. Successful treatment with Epoprostenol (Prostacyclin) in conjunction with nitric oxide and/or Defibrotide have been reported<sup>31</sup>. **PAH MUST THEREFORE BE EXCLUDED 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 as mentioned in Chapter 1 and as summarised in Table 2. In addition to a complete medical history, a physical examination should be performed by an experienced paediatrician. Laboratory testing of electrolytes, calcium homeostasis, 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 some forms of this disease, genetic analysis should be initiated immediately to recognise “classical” and “atypical” forms, which is maximally important for treatment strategies. Gene analysis may be done in a hierarchical sequence with respect to clinical manifestation and age as well as consequence for therapy:

- In severe infant forms, the positive finding of **TCIRG1** mutations precludes neuropathic 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**.
- 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**), **THESE PARTICULAR DEFECTS MUST BE EXCLUDED BEFORE HSCT**.
- **CLCN7** mutations are associated with a **VARIABLE PHENOTYPE** ranging from “classical” **TCIRG1**-like infant forms, through intermediate or even mild forms to **NEUROPATHIC OSTM1**-like forms. An extensive clinical workup is necessary in affected children and seems to be more important for therapy than the immediate and exact genetic description.
- In OP with **LOW OR ABSENT OSTEOCLASTS** in bone marrow, a positive finding of **RANK** mutation helps to differentiate patients from **EXTRINSIC DEFECTS OF OSTEOCLAST FORMATION** (such as **RANKL** deficiency). In

these patients, both genes should be analysed to choose the appropriate intervention. Patients with RANK mutations well respond to HSCT.

- **Other genes:** If no gene mutations can be found, therapy depends on clinical presentation. An extensive clinical workup is necessary. IN SUCH CASES BLOOD CELLS SHOULD BE SEND TO REFERENCE CENTRES TO ANALYSE FURTHER CANDIDATE GENES.

### 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 as well as an increased LDH level are usually found possibly resembling 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. A persistent normal anion gap type of metabolic acidosis, a mild degree of hypokalemia and a failure to achieve maximally low urine pH were detected in patients with renal tubular acidosis due to CAII deficiency. Basic parameters of **bone metabolism** as calcium (total and ionised) and phosphate in serum must be analysed to detect disturbances as HYPOCALCAEMIA, which may cause convulsions in severe cases. Other parameters as PTH, ALP, 1,25-dihydroxyvitamin D3, Osteocalcin, TRAcP, bone resorption markers (CTX or NTX), RANKL and OPG are subjects of accompanying research projects on OP. Serum may be sent to the coordinator for (free) analysis of these markers.

Analysis of the **bone marrow** is required to detect OSTEOCLAST POOR FORMS and speed genetic analysis. In atypical and milder forms, the extent of REDUCED HAEMATOPOIESIS in the marrow should help to determine indication and time point for HSCT. Since marrow aspirates usually fail in OP, **trepine biopsy** should be performed by an experienced operator (ideally under the same general anaesthesia as MRI or central line). In rare cases, particularly if the findings are not conclusive regarding number of osteoclasts, open bone biopsy may be even be required. A research project of members of the E-RARE constortium should help to distinguish osteopetrosis from osteopetrorickets, which has recently be described in oc/oc mice (TCIRG1 negative mice) by Schinke *et al.*<sup>33</sup>. Therefore and also for reference analysis, part of the biopsy, which must **not be decalcified** but suspended in Formalin, should be sent to the coordinator in Ulm.

In some patients, **immunological impairments** as HYPOGAMMAGLOBULINAEMIA (in patients with TCIRG1 and RANKL mutations<sup>6,34</sup> as well as numeral and functional disturbances of peripheral lymphocytes (A. Schulz, unpublished results) have been observed. 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 hematopoiesis<sup>30</sup>. Therefore, we recommend analysis of **IgG, IgA, IgM and IgE** and analysis of specific **Ab response** upon vaccination. Furthermore, we suggest to evaluate **lymphocyte subpopulations** by FACS (at

least CD3, CD4, CD8, CD19, CD56/63 and CD34) and analysis of **T-cell function** in vitro. Five to 10 ml heparinised blood may be sent to the coordinator in Ulm for the latter tests.

### 2.2.3 Radiology

The diagnosis of OP is primarily defined by an elevated radiodensity and particular findings in **X-ray** analysis – and usually leads to diagnosis. X-rays of all bones should be avoided and spared for atypical cases for radiation protection. However, pictures 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, radiological analysis of the brain and skull by **MRI or CT** is highly recommended to detect HYDROCEPHALUS, NARROWING OF CENTRAL NERVE CHANNELS AND NEUROPATHIC CHANGES (such as cerebral atrophy and agenesis of corpus callosum) <sup>28, 53</sup>. 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 optical nerve does obviously not correspond to damage of the nerves and vision 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 EEC** before and after HSCT may help do detect changes due to pulmonary hypertension.

### 2.2.4 Neurological examination

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

- detailed **neurological examination** by an experienced paediatric neurologists including examination of the developmental status, an intelligence test (if applicable) and an **EEG**
- 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, and a hearing test

### 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. However, following the pathophysiological and genetic defect and the experience of the retrospective data analysis (as described in chapter 1), some general suggestions can be made. Type of disease, risk factors and donor availability are main determinants for the therapeutic procedure ranging from “urgent transplantation” to “wait and see”.

#### 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 important hepatosplenomegaly), this indication must be carefully evaluated and considered in the context of other symptoms and donor availability. Bone marrow biopsy, the count of reticulocytes and CD34 positive stem cells in the peripheral blood, as well as the LDH level and ultrasonography of spleen and liver, may help to evaluate the bone marrow function in less severe cases.

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

Up to now, there are two **absolute contraindications** for standard HSCT:

- 1) the extrinsic osteoclast defect characterised by mutations of the RANKL gene
- 2) the neuropathic form of OP, characterised by encephalopathy and neurodegeneration with irritability, hypertonicity, seizures not due to hypocalcaemia (primary neurological defect) and progressive developmental delay, associated with mutations of the OSTM1 gene (and sometimes of the CLCN7 gene).

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

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

### 3.1.2 Donor

We suggest the following ranking of donors:

- HLA-genotypical family donors (matched sibling donor/MSD)
- matched family donor/MFD in consanguineous families)
- HLA-matched donors (matched family donors in non-consanguineous families > matched unrelated donors: BM > PBSC > cord blood)
- HLA-haploidentical family or HLA-mismatched cord blood donors

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

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

### 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 subject of discussion<sup>21,27,43,45-47</sup>.

Following considerations seem to be reasonable:

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

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

### 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. If only peripheral blood



stem cells are available, T-cell number in the graft may be reduced by *ex vivo* procedures to adjust the T-cell content of the graft to a maximum of  $10\text{-}50 \times 10^6$  per kg body weight of the recipient. In the case of HLA-nonidentical ( $> 1/10$  HLA-mismatch or HLA-haploidentical) transplants, peripheral blood stem cells should be used and the amount of T-cells in the graft must be roughly reduced by *ex vivo* procedures (CD34-positive selection and/or CD3/CD19 negative selection) to yield a T-cell content of the graft below  $2.5 \times 10^4$  per kg body weight of the recipient.

To ensure engraftment, it is important to obtain an excellent graft with more than  $5 \times 10^8$  nucleated cells per kg body weight of the recipient in the case of bone marrow and more than  $10 \times 10^7$  per kg body weight of the recipient in the case of peripheral blood stem cells, respectively. Furthermore, since many patients will have a delayed reconstitution due to the narrowed bone marrow space, the preservation and storage of additional stem cells for a stem cell boost should be considered. In particular, in the case of a T-depleted stem cell source, the preparation and cryoconservation of an additional graft with a cell content equal to the primary graft is highly recommended and should be “pre-emptively” done during the first stem cell preparation procedure. The “ideal” time point for a boost is considered around one month after transplantation, when the risk of acute GvHD can be evaluated and osteoclasts maturing from the primary transplant may have opened the bone marrow space in the recipient. A pre-emptive boost around day +28 may have been mainly attributed to the relative good results of the HLA-haploidentical transplants at the Ulm transplant centre<sup>27</sup>.

### 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 an unmanipulated bone marrow graft, the standard CSA/MTX regimen has been substituted by a CSA/MMF prophylaxis regimen, since MMF is less toxic to the liver (VOD) and the graft (graft failure). In the case of a sibling donor below the age of about 14 years, CSA mono prophylaxis may be considered. Serotherapy should be used in any cases other than HLA-genoidentical transplants. We recommend ATG (10 mg/kg Thymoglobulin) in standard situations. If the T-cell content of an HLA-nonidentical graft accidentally exceeds  $3$  to  $5 \times 10^4$  /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 standard Busulfan-Cyclophosphamid-CSA-MTX regimen, patients with OP are at very high risk of developing liver (and pulmonary) VOD<sup>31,48</sup>. In addition to a careful monitoring of VOD symptoms (untreatable thrombocytopenia, weight gain, liver enlargement, ascites, bilirubin elevation), prophylaxis or early therapy with Defibrotide is highly recommended.

- **Respiratory problems:**

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

- **CNS problems:**

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

- **Serum calcium disturbance:**

**Hypocalcaemia** eventually associated with convulsions before engraftment contrasts **hypercalcaemic complications** thereafter. In the case of hypocalcaemia, supplementation with calcium gluconate and vitamin D (1000 IU per day) is recommended before HSCT. (Please read the paper by Schinke et al.<sup>33</sup> for best administration regimen in patients with gastric pH disturbance as those affected by mutations in the *TCIRG1* gene.) This supplementation should be reduced or even stopped with engraftment after HSCT to avoid hypercalcaemic crisis. Serum levels of calcium and phosphate should be carefully monitored before and for several months after transplantation and in the case of hypercalcaemia an individual treatment procedure is recommended (see treatment section below). In addition, Denosumab (PROLIA, Amgen), a monoclonal RANK-L antibody, has been successfully used in 2 patients with RANK mutations suffering from severe hypercalcemia.

## 3.2 Conditioning Protocols

### 3.2.1 Matched Sibling Donor

#### Inclusion Criteria

- HLA-genoidentical Donor

#### Exclusion Criteria

- Neuropathic form (MRI, genetics: OSTM1+) → contact one of the Authors
- Osteoclast poor form (bone biopsy evaluation or genetics: TCIRG1-, C1CN7-, RANK-, RANKL+) → contact one of the Authors
- CLCN7+: neuropathic 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 (160 mg/m<sup>2</sup>): 40 mg/m<sup>2</sup>/day, day -6 to day -3
- Pilot Protocol, Treosulfan-based\*:
  - Treosulfan (> 1 y: 42 g/m<sup>2</sup>, < 1y 36 g/m<sup>2</sup>): 14 g/m<sup>2</sup>/day or. 12 g/m<sup>2</sup>/day, day -7 to day -5
  - Fludarabine (160 mg/m<sup>2</sup>): 40 mg/m<sup>2</sup>/day, day -6 to day -3
  - Thiotepa (10 mg/kg): 2 x 5 mg/kg at day -4

#### Transplant

- BM (1<sup>st</sup> choice): > 5 x 10<sup>8</sup> NC / kg BW
- PBSC (2<sup>nd</sup> choice): >10 x 10<sup>6</sup> 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/m<sup>2</sup> 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, 2<sup>nd</sup> transplant).

### 3.2.2 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

- Neuropathic form (MRI, genetics: OSTM1+) → contact one of the Authors
- Osteoclast poor form (bone biopsy evaluation or genetics: TCIRG1-, C1CN7-, RANK-, RANKL+) → contact one of the Authors
- CLCN7+: neuropathic forms should be excluded → contact one of the Authors
- HLA-genotypical donor available

#### Conditioning

- Standard Protocol, Busulfan-based:
  - Busulfex (weight adapted, kinetics recommended): day -8 to day -5
  - Fludarabine (160 mg/m<sup>2</sup>): 40 mg/m<sup>2</sup>/day, day -6 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 *or* Alemtuzumab (Campath-1H): (0.9 mg/kg): 0.1 mg/kg day -3, 0.2 mg/kg/Day day -2 to day +1
- *Pilot Protocol, Treosulfan-based\**:
  - Treosulfan (> 12 kg BW: 42 g/m<sup>2</sup>, < 12 kg BW 36 g/m<sup>2</sup>): 14 g/m<sup>2</sup>/day *or* 12 g/m<sup>2</sup>/day, day -7 to day -5
  - Fludarabine (160 mg/m<sup>2</sup>): 40 mg/m<sup>2</sup>/day, day -6 to day -3
  - Thiotepa (10 mg/kg): 2 x 5 mg/kg at day -4
  - Serotherapy: ATG-Thymoglobulin (Genzyme) (10 mg/kg): 1 mg/kg day -3, 3 mg/kg/day day -2 to day 0 *or* Alemtuzumab (Campath-1H): (0.9 mg/kg): 0.1 mg/kg day -3, 0.2 mg/kg/Day day -2 to day +1

#### Transplant

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

### 3.2.3 HLA-Haploidentical Donor

#### Inclusion Criteria

Haematopoietic insufficiency (transfusion dependent)

#### Exclusion Criteria

- Neuropathic form (MRI, genetics: OSTM1+) → contact one of the Authors
- Osteoclast poor form (bone biopsy evaluation or genetics: TCIRG1-, C1CN7-, RANK-, RANKL+) → contact one of the Authors
- CLCN7+: neuropathic 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 (160 mg/m<sup>2</sup>): 40 mg/m<sup>2</sup>/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, ATG-Thymoglobulin (Genzyme) suggested (**13 mg/kg**): 1 mg/kg day -3, 3 mg/kg/day day -2 to day +1 *or*  
Alemtuzumab (Campath-1H): (0.9 mg/kg): 0.1 mg/kg day -3, 0.2 mg/kg/Day day -2 to day +1
- *Pilot Protocol, Treosulfan-based (cave: this protocol has not been explored so far – contact the coordinator, if considered):*
  - Treosulfan (> 5 kg: 42 g/m<sup>2</sup>): 14 g/m<sup>2</sup>, day -7 to day -5
  - Fludarabine (160 mg/m<sup>2</sup>): 40 mg/m<sup>2</sup>/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, ATG-Thymoglobulin (Genzyme) suggested (**13 mg/kg**): 1 mg/kg day -3, 3 mg/kg/day day -2 to day +1 *or*  
Alemtuzumab (Campath-1H): (0.9 mg/kg): 0.1 mg/kg day -3, 0.2 mg/kg/Day day -2 to day +1

#### Transplant

- T cell depleted PBSC (method according to local protocols):
  - stem cells: >10 x 10<sup>6</sup> CD34+ / kg BW
  - T-cells: < 2 x 10<sup>4</sup> 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<sup>4</sup> 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 particularly in patients with advanced disease (> 10 months of age). This procedure should be performed in experienced centres only!**
- **A standard conditioning regimen for HLA-haploidentical HSCT in OP has not been established. In particular, the treosulfan based protocol has not been explored 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.**

### 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) in the majority of cases survived in a larger series in Ulm.

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

#### 3.3.1 Non-engraftment and rejection

Most patients with OP show a slow haematological recovery after HSCT, possibly because of due to narrowed marrow space and/or hepatosplenomegaly. A delayed haematological reconstitution must be carefully distinguished from an immunological rejection by chimerism analysis. If rejection can be excluded (see below), a stem cell boost (about at least one month after transplantation) should be considered (see chapter 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, disappearing donor granulocytes and stem cells), a secondary conditioning regimen should be considered before a stem cell boost can be given. Depending on the individual patient condition and the donor setting, chemotherapy (Cyclophosphamide 120 mg/kg) and/or anti-T-cell serotherapy (Alemtuzumab [Campath-1H] and/or Okt-3) may be administered fludarabine has also been used to precondition patients refractory to previous DLI or for stem cell top-ups. Might this be less toxic than cyclophosphamide?. Particularly in the case of rejection of a non-identical graft, an alternative donor should be considered assuming sensitisation of the patient against the first donor.

#### 3.3.2 Venous Occlusive Disease (VOD)

Preliminary data suggest that administration of prophylactic Defibrotide may efficiently prevent VOD in OP-patients. However in the absence of Defibrotide being freely available for this indication, early diagnosis and start of specific therapy is a prerequisite of a successful treatment of this common complication. Immediate start of **Defibrotide** infusions is highly recommended when VOD is suspected by the typical, but sometimes unspecific clinical signs. A carefully balanced fluid and diuretic therapy (central venous pressure +1 to +5 cm H<sub>2</sub>O) may prevent cardiovascular, pulmonary and renal insufficiency. In the acute phase of VOD and clinical presentations overlapping with capillary leakage syndrome (CLS), a short course of steroid therapy may be helpful.

### 3.3.3 Pulmonary hypertension

In the case of oxygen requirement and/or tachypnoea in the absence of microbiological documentation, repeated echocardiographic and ECG investigations (enlarged right ventricle) is helpful to detect this severe complication, documentation by cardiac catheterisation is recommended. Treatment is difficult and should reflect the individual situation. Oxygen administration (oxygen saturation > 95%), magnesium substitution and moderate diuretic therapy represent the first step of treatment. In progressive cases, **Sildenafil** (Viagra) or inhaled **NO** administration may be considered. In severe cases, complicated by cardiovascular and pulmonary insufficiency, **Epoprostenol** (Flolan) has been successfully used<sup>31</sup>, **Bosentan**, an endothelin-receptor antagonist, may be considered. A tight interdisciplinary collaboration of haematologists, cardiologists, respiratory physicians and the intensive care team is mandatory, when this life threatening complication is suspected or evident.

### 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 despite this at any time during the first few months after transplantation. In severe cases, in particular when relevant nephrocalcinosis is detected in ultrasound, inhibition of osteoclast function by bisphosphonate therapy may be considered<sup>29</sup>.

### 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 absence of OP may be achieved spontaneously. Therefore, therapeutic interventions must be carefully balanced against possible side effects. Since the therapeutic options are highly dependent on the individual situation, no general recommendation can be drawn. However, in the cases of progressive loss of donor chimerism in an HLA-identical setting, **DLIs** may be considered.

### 3.3.6 “Late adverse effects”

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

- **Dwarfism:** After HSCT, the height of the majority of patients is between 3<sup>rd</sup> and 10<sup>th</sup> percentile and about 20% of patients show dwarfism (< 3<sup>rd</sup> percentile) after transplantation (A. Schulz, unpublished results). There seems to be no primary growth hormone deficiency in OP patients<sup>50</sup> and treatment by growth hormone is not established but may be considered in severe cases after careful evaluation of pros and cons.
- **Craniosynostosis and intracranial hypertension:** In very few patients increased cranial pressure secondary to craniosynostosis has been described, which was treated surgically<sup>51,52</sup> (A. Schulz, unpublished observation).

- **Autism:** In a small subset of patients, symptoms of autism have been described. These may be due to visual or auditory compromise or to the particular genetic defect or both <sup>34</sup> (Schulz, unpublished observation).
- **Osteoporosis:** Very recent mouse data indicate the risk of impaired calcium homeostasis due to impaired gastric acidification leading to late hypocalcaemic osteoporosis (particularly in patients with TCIRG1 mutations), which may be treated by calcium gluconate <sup>33</sup>. Therefore, BMD measurements are recommended in long-term follow up after HSCT.



## 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 therapy chosen. 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 of the questionnaires will be integrated into a web based database covering data protection regulations of the EU (funded by the ERARE project). 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	yearly	yearly	
<b>Laboratory Tests</b>				
●	○	●	●	Peripherale Blood: Cell count and clinical chemistry, pH, CK Urine: pH, Ca, pH
●	●	●	●	Bone metabolism - Serum: Ca/Ph, ALP; bone density measurements
●	●	◐	◐	Immunological Parameters - Serum: IgG, IgA, IgM, specific Ab; Heparin-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)
◐		○	○	Bone Marrow - Trephine biopsy or open biopsy ( <b>non-calcified, in Formaline</b> )*
	●	●		Chimerism Analysis - PB cells; additionally in case of mixed chimerism cellular subtypes
◐		○	○	Scientific Program - Serum (PTH, 1,25-dihydroxi-/25-hydroxy-Vitamin D3, Gastrin, Osteocalcin, TRcAP, CTX/NTX, OPG, RANKL)
<b>Radiology</b>				
●		◐	○	X-ray - 1 extremity; additionally at diagnosis head, thorax*
●	○	○	○	Ultrasound (abomen, kidneys, brain and hips)
●		○	○	MRI - brain (CT may be considered)*
<b>Consultants</b>				
●	○	◐	◐	Paediatric Neurologist - status, development, IQ, EEG
●	○	●	●	Ophthalmologist - visus, optical nerve, VEP
●	○	◐	◐	ENT - morphology, hearing tests
<b>Documentation</b>				
●				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 upon 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:
  - Rare genes (EDTA blood: RANK, RANKL, OSTM1, CAII, PLEKHM, others)
- HLA-typing of patients and family (EDTA blood: 10 loci, 4 digits)
- BM histology (NOT decalcified in Formaline; send part to coordinator in Ulm)

### Technical investigations

Date

- X-ray (1 extremity, head, thorax) \_\_\_\_\_
- 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

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D-89075 Ulm  
Germany

### OSTEOPETROSIS REGISTRY – Request for investigations

Dear Dr. Schulz,  
the diagnosis of osteopetrosis has been established or is assumed in the patient:

\_\_\_\_\_, born \_\_\_\_\_.

- We wish to include this patient in the Osteopetrosis Registry and include
  - Consent form of the Registry
  - Registration questionnaire
  - Transplant questionnaire
  - Status and follow-up questionnaire
  - Medical report(s)
- We ask you to perform genetic analysis for genes not analysed and include
  - Consent form for genetic analysis
  - Peripheral Blood (5 ml EDTA)
  - Genes found to be normal: \_\_\_\_\_
- We ask you to analyse immunologic parameters and bone metabolism
  - Peripheral blood (5-10 ml heparinised, sent at RT by express mail)
  - Serum (5 ml, sent by express mail)
- We ask you to review the bone marrow histology and include
  - part of a bone biopsy/trephine suspended in formalin
- We ask you to review the radiological data of the patient and include
  - a CD containing X-rays
  - a CD containing MRI/CT scans
- We request to perform further scientific analysis (contact coordinator):

Remarks: \_\_\_\_\_

\_\_\_\_\_  
Completing Physician / Stamp

\_\_\_\_\_,  
Date

\_\_\_\_\_  
Signature

## Separate forms

### File

#### Questionnaires

Registration and genetics:	<a href="#">01_Registration v1.7</a>
Transplantation:	<a href="#">02_Transplantation v1.7</a>
Status and Follow up:	<a href="#">03_Follow-Up v1.7</a>

#### Consent and request forms:

##### English

Information Parents	<a href="#">04_OP_registry_Information Parents_E02122011</a>
Informed Consent	<a href="#">06_OP_registry_Consent_E02122011</a>

##### German

Aufklärungsbogen Eltern	<a href="#">07_OP_registry_Aufklärung Eltern_D01112011</a>
Aufklärungsbogen Patient	<a href="#">08_OP_registry_Aufklärung Kinder_D01112011</a>
Einverständniserklärung	<a href="#">09_OP_registry_EVE_D05082010</a>

<b>Genetic Diagnostic Forms</b>	<a href="#">10_OP_registry_Genetic Test_Ulm</a>
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<b>Votum Ethic Committee Ulm</b>	<a href="#">11_OP_registry_Ethikvotum_Ulm</a>
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<b>Accompanying Letter</b>	<a href="#">12_OP_registry_Letter</a>
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## 5. LITERATURE

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