Clinical Trial Protocol

EWOG-MDS 2006

Prospective non–randomized multi-center study for epidemiology and characterization of Myelodysplastic Syndromes (MDS) and Juvenile Myelomonocytic Leukemia (JMML) in childhood

Final Version

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List of Abbreviations

AA	*Aplastic Anemia*
AML	*Acute Myeloid Leukemia*
ANC	*Absolute Neutrophil Count*
AP	*Alkaline Phosphatase*
Ara-C	*Cytosine Arabinosid*
ATG	*Anti Lymphocyte Globulin*
BM	*Bone Marrow*
BMT	*Bone Marrow Transplantation*
BUN	*Blood Urea Nitrogen*
CBC	*Complete Blood Count*
CBMF	*Congenital Bone Marrow Failure*
CGH	*Comparative Genomic Hybridization*
CI	*Cumulative Incidence*
CMML	*Chronic Myelomonocytic Leukemia*
CNS	*Central Nervous System*
COG	*Childrens Oncology Group*
CRF	*Case Report Form*
CSA	*Cyclosporine A*
CSC	*Coordinating Study Center*
CT	*Computerized Tomography*
CTC	*Common Toxicity Criteria*
DBA	*Diamond Blackfan Anemia*
DC	*Dyskeratosis Congenita*
DLI	*Donor Lymphocyte Infusion*
DMC	*Data Monitoring Committee*
DS	*Down Syndrome*
EC	*European Community*
EFS	*Event Free Survival*
EORTC	*European Organization for Research and Treatment of Cancer*
ET	*Essential Thrombocythemia*
FA	*Fanconi Anemia*
FAB	*French American British Group*
GCP	*Good Clinical Practice*
GGT	*Gamma Glutamic Transferase*
GLP	*Good Laboratory Practice*
GM-CSF	*Granulocyte-Macrophage Colony Stimulating Factor*
GMP	*Good Manufacturing Practice*
GvHD	*Graft versus Host Disease*
GvL	*Graft versus Leukemia*
HbF	*Hemoglobin F*
HSCT	*Hematopoietic Stem Cell Transplantation*
IEC	*Independent Ethics Committee*
ICH	*International Conference on Harmonization*
IP	*Investigational Product*
IPSS	*International Prognostic Scoring System*
IST	*Immuno Suppressive Therapy*
jCML	*Juvenile Chronic Myeloid Leukemia*
JMML	*Juvenile Myelomonocytic Leukemia*

LDH	*Lactate Dehydrogenase*
MCV	*Mean Corpuscular Volume*
MDR-AML	*Myelodysplasia Related Acute Myeloid Leukemia*
MDS	*Myelodysplastic Syndrome*
MFD	*Matched Family Donor*
MMFD	*Mismatched Family Donor*
MNC	*Mononuclear Cells*
NF1	*Neurofibromatosis 1*
NIH	*National Institute of Health (US)*
NMDP	*National Marrow Donor Program (US)*
n.s.	*Non significant*
PB	*Peripheral Blood*
PBSCT	*Peripheral Blood Stem Cell Transplantation*
PI	*Principal Investigator*
PNH	*Paroxysmal Nocturnal Hemoglobinuria*
PV	*Polycythemia Vera*
QOL	*Quality Of Life*
RA	*Refractory Anemia*
RAEB	*Refractory Anemia with Excess Blasts*
RAEB-T	*Refractory Anemia with Excess Blasts in Transformation*
RARS	*Refractory Anemia with Ringsideroblasts*
RC	*Refractory Cytopenia*
RBC	*Red Blood Cell Count*
SAA	*Severe Aplastic Anemia*
SAE	*Serious Adverse Event*
SAP	*Statistical Analysis Plan*
SCN	*Severe Congenital Neutropenia*
SDS	*Shwachman Diamond Syndrome*
SDV	*Source Data Verification*
SGOT	*Serum Glutamic Oxaloacetic Transaminase*
SGPT	*Serum Glutamic Pyruvic Transaminase*
TBI	*Total Body Irradiation*
TRM	*Transplant Related Mortality*
UD	*Unrelated Donor*
WBC	*White Blood Cell Count*
WHO	*World Health Organization*

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Synopsis

Title of the study:
PROSPECTIVE NON–RANDOMIZED MULTI-CENTER STUDY FOR EPIDEMIOLOGY AND CHARACTERIZATION OF MDS AND JMML IN CHILDHOOD

Protocol No.: *EWOG-MDS 2006*

Objectives: To assess the epidemiology and to characterize subtypes of MDS and JMML in childhood.

Design: Prospective, non-randomized, multi-center study

Planned Study Duration:
The total study duration is of 5 years. The study ends 12 months after enrollment of the last patient (total study end). Study duration for each patient is a minimum of 12 months (from inclusion) to a maximum of 5 years.

Study Population:
A total of 260 patients are expected to be enrolled.

Inclusion Criteria:

- Written informed consent by the caretakers and whenever possible the patient's assent.
- Confirmed diagnosis of MDS or JMML (morphology, cytogenetics)
- Myeloid leukemia of Down syndrome (patients aged > 6 years).
- Age less than 18 years

Exclusion Criteria:

- Denied informed consent and/or assent by caretakers/patient.
- Myeloid leukemia of Down syndrome (patients < 6 years).
- Participation in another study within the last 4 weeks (except for therapy optimizing studies in cancer or bone marrow failure disorders and studies in diagnostics).

Endpoints
Primary:

- To evaluate the frequency of the different subtypes of MDS in childhood and adolescence by a standardized diagnostic approach
- To evaluate the frequency of cytogenetic and molecular abnormalities, using array-CGH to evaluate the frequency of subtle chromosomal imbalances, using mFISH to identify unknown chromosomal aberrations

Secondary:

- To assess survival for children and adolescents with MDS and JMML
- To evaluate relapse rate, morbidity and mortality in children with MDS and JMML treated by HSCT

Methodology:
All patients undergo a standardized initial diagnostic approach. The follow-up covers 12 month periods for the whole study duration.

Statistical Methods:
The final analysis will be performed six months after the end of the study. One interim analysis two years after start is planned. Survival times will be calculated according to the Kaplan-Meier method and comparisons between probabilities in different patient groups will be performed using the log-rank test.

Timetable: Start of Study: Begin 2006 Enrollment: 48 months End of Study: Fourth quarter 2010 Data available: Begin 2011 Study report: Second quarter 2011	
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Flow Chart JMMLDiagnostic Approach and Follow-Up

	Diagnostic Procedures	Follow-up Every 12 months	HSCT Data	After HSCT Day 100	After HSCT Yearly
Inclusion/ Exclusion Criteria	X				
Physical examination + vital signs Including signs of NF1	X		X		
Labs (Hematology, Serum Chemistry)	X	X	X		
Viral Serology	X				
Transfusion history	X	X	X		
Concomitant therapy	X	X	X		
Buccal swab or skin biopsy	X				
Cytogenetics	X	X*	X		
Mutational study (*PTPN11*, *RAS)*	X				
GM-CSF hypersensitivity in vitro (optional)	X				
Bone marrow aspirate/ (biopsy optional)	X	X*	X		
Survival		X	X	X	X
Lansky score		X	X		
Secondary malignancy		X			X
Allograft data (graft, conditioning, transplantation, engraftment)			X		
GvHD				X	X
Chimerism				X	X*
Relapse/ DLI				X	X
Complications (infections, non-infectious)				X	X

*** if available**

Flow Chart **Refractory Cytopenia Diagnostic Approach and Follow-Up**

	Diagnostic Procedures	Follow-up Every 12 months	HSCT Data	After HSCT Day 100	After HSCT Yearly
Inclusion/ Exclusion Criteria	X				
Physical examination + vital signs Including signs of associated abnormalities	X		X		
Labs (Hematology, Serum Chemistry)	X	X	X		
Viral serology	X				
Exclusion of Fanconi anemia	X				
Stool elastase or serum typsinogen, serum isoamylase	X				
PNH clone	X				
Transfusion history	X	X	X		
Concomitant Therapy	X	X	X		
Cytogenetics	X	X*	X		
Bone marrow aspirate/biopsy	X	X*	X		
Second bone marrow aspirate/biopsy #	X				
Survival		X	X	X	X
Lansky score		X	X		
Secondary malignancy		X			X
Allograft data (graft, conditioning, transplantation, engraftment)			X		
GvHD				X	X
Chimerism				X	X*
Relapse/ DLI				X	X
Complications (infections, non-infectious)				X	X

*** if available**

 # recommended within 3 months

Flow Chart High grade MDS Diagnostic Approach and Follow-Up

	Diagnostic Procedures	Follow-up Every 12 months	HSCT Data	After HSCT Day 100	After HSCT Yearly
Inclusion/ Exclusion Criteria	X				
Physical examination + vital signs Including signs of associated abnormalities	X		X		
Labs (Hematology, Serum Chemistry)	X	X	X		
Viral Serology	X				
Exclusion of Fanconi anemia	X				
Transfusion history	X	X	X		
Concomitant Therapy	X	X	X		
Cytogenetics	X	X*	X		
Bone marrow aspirate/ biopsy	X	X*	X		
Second bone marrow aspirate/biopsy	X				
Diagnostic lumbar puncture	X				
Survival		X	X	X	X
Lansky score		X	X		
Secondary malignancy		X			X
Allograft data (graft, conditioning, transplantation, engraftment)			X		
GvHD				X	X
Chimerism				X	X*
Relapse/ DLI				X	X
Complications (infections, non-infectious)				X	X

*** if available**

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2 Introduction

2.1 Significance and Background

Myelodysplastic and myelodysplastic/myeloproliferative disorders in childhood are separated into three groups: juvenile myelomonocytic leukemia (JMML), myelodysplastic syndrome (MDS), and Down syndrome leukemia (2). Myeloid leukemia in Down syndrome has unique features and patients should be included in the appropriate protocols for treatment of *de novo* AML with special consideration of drug doses. The protocol EWOG-MDS 2006 includes only patients with JMML and MDS.

2.2 Classification

The first classification of MDS was introduced in 1982 by the French-American-British (FAB) group (3). It divided MDS according to morphology in 5 subgroups (table 1). The FAB classification is based on the number of blasts in the PB and BM, the presence of ring sideroblasts in the BM and the absolute PB monocyte count. Subsequently, it was recognized that the presence of Auer rods is not always an indicator of fast progression of the disease; many investigators like EWOG-MDS omitted Auer rods as a criterion for classification.

Type		Blood	Bone Marrow
Refractory anemia	RA	<1% blasts	< 5% blasts
Refractory anemia with ring sideroblasts	RARS	<1% blasts	< 5% blasts and ring sideroblasts $\geq$ 15%
Refractory anemia with excess of blasts	RAEB	< 5% blast	5 - 19% blasts
Refractory anemia with excess of blasts in transformation	RAEB-T	$\geq$ 5% blasts, or Auer rods	20 - 29% blasts or Auer rods
Chronic myelomonocytic leukemia	CMML	Monocytes > $1x10^9$/L.	< 20% blasts

Table 1 FAB classification as initially described in 1982

In 2000, the World Health Organization (WHO) classification of neoplastic diseases of the hematopoietic and lymphoid tissues incorporating both morphology and genetic changes was introduced (4). It recognizes JMML as distinct entity and places the disorder in a group of myelodysplastic/myeloproliferative disorders. For the definition of MDS, the WHO classification eliminated RAEB-T by reducing the threshold of blasts required to make the diagnosis of AML to 20% (5). At the same the subtype of RAEB was redefined, now accommodating all cases with up to 20% blasts in PB. Six MDS subtypes are described by WHO (table 2).

- Refractory anemia
- Refractory anemia with ringed sideroblasts
- Refractory cytopenia with multi-lineage dysplasia
- Refractory anemia with excess blasts
- Myelodysplastic syndrome, unclassifiable
- Myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality

Table 2 WHO classification of MDS

In children, there are no data to indicate whether a blast threshold of 20% is better than the traditional 30% to distinguish MDS from *de novo* AML. In addition, the subdivision of MDS does not reflect the hematological and clinical picture of MDS in childhood: Ringed sideroblasts are very infrequently seen in children, the importance of multi-lineage dysplasia is unknown and the unique 5q- syndrome has not been described. The last category, "MDS not otherwise categorized" may not be very useful. Therefore, a pediatric modification of the WHO classification MDS and myelodysplastic/myeloproliferative diseases (table 3) was developed (2).

Myelodysplastic / Myeloproliferative Disease
- Juvenile myelomonocytic leukemia (JMML)
- Chronic myelomonocytic leukemia (CMML) (secondary only)
- *BCR-ABL* negative chronic myeloid leukemia (Ph^- CML)

Myelodysplastic Syndrome (MDS)
- Refractory cytopenia (RC) (PB blasts <2% and BM blasts <5%)
- Refractory anemia with excess blasts (RAEB) (PB blasts 2-19% or BM blasts 5-19%)
- RAEB in transformation (RAEB-T) (PB or BM blasts 20-29%)

Table 3 Classification of MDS and myelodysplastic/myeloproliferative disorders of childhood

JMML incorporates those disorders previously referred to as jCML (6) or chronic myelomonocytic leukemia (CMML) (7) of infancy, as well as some cases of the infantile monosomy 7 syndrome (8). Because there is no evidence that monosomy 7 represents a discrete entity, it should no longer be referred to as the monosomy 7 syndrome (9;10). In MDS secondary to chemo- or radiation therapy a hematological picture best described as CMML is occasionally noted. *BCR-ABL* negative chronic myeloid leukemia (Ph^- CML) is exceedingly rare in children.

In adult patients with refractory anemia (RA) anemia is generally the main presenting symptom. Children differ in their hematological presentation because neutropenia and thrombocytopenia are more frequently observed. Therefore, the term "refractory cytopenia" (RC) was felt to be more appropriate (2;11). The category of RAEB-T was kept. To have consistency with the definition of RAEB in the WHO classification, cases with up to 20% blasts in PB were incorporated in this subgroup. Disease evolving from MDS with a blast count of > 30% will be referred to as myelodysplasia-related AML (MDR-AML). Table 4 gives the composition of study patients in EWOG-MDS 98 according to the pediatric classification (interim analysis 2005).

	JMML and MDS		MDS only
	N	%	%
JMML	119	23	
RC	207	40	52
RAEB	137	27	35
RAEB-T/MDR-AML	51	10	13

Table 4 Study patients of EWOG-MDS 98 according to the pediatric MDS classification

The genetic changes predisposing children to MDS at a young age are largely unknown. The presumed underlying mechanism may also give rise to subtle phenotypic abnormalities noted in many children with MDS. Therefore, there was a need to clearly define "secondary" MDS which will only refer to MDS following neoplasia, congenital or acquired bone marrow failure disorders or MDS in familial disease (table 5) (2).

Secondary MDS	- prior malignancy and/or prior chemo- or radiation therapy
	- known congenital bone marrow failure disorder
	- prior acquired aplastic anemia
	- MDS in a first degree relative (familial MDS)
Primary MDS	all others

Table 5 Definition of primary and secondary MDS

2.3 Epidemiology

Combined population-based data from Denmark and British Columbia in Canada identified 38 cases of MDS, representing 4% of all hematological malignancies in children (Table 6), which corresponds to an annual incidence of MDS of 1.8 per million children aged 0-14 years (12;13). MDS and JMML combined constituted 7.7% of the Japanese cases of childhood leukemia[8] with a high proportion of therapy-related cases (23%). Recent data from the United Kingdom suggest a considerably lower annual incidence of MDS, 0.8 per million (Table 6) (14). The UK study excluded secondary MDS, partly explaining the lower incidence.

	N	%	Annual incidence per million	UK incidence per million
ALL	815	79	38.5	nd
AML[1]	115	11	5.4	5.8
MDS[1]	38	4	1.8	0.8
Myeloid leukemia of DS	19	2	0.9	0.6
JMML	25	2	1.2	0.6
CML	13	1	0.6	0.5
PV/ET[2]	3	0	0.1	nd
Unclassified	3	0	0.1	
Total	1030	100	48.7	

[1]Excluding Down syndrome (DS); [2] PV: polycythemia vera; ET: essential thrombocythemia

Table 6 Annual incidences of hematological malignancies in children 0 - 14 years. Combined data from Denmark 1980 - 1991 and British Columbia 1982 - 1996 (12;13) and, for comparison, UK data from 1990-1999 (14)

2.4 Juvenile Myelomonocytic Leukemia (JMML)

Clinical presentation

Juvenile myelomonocytic leukemia (JMML) is a rare clonal myeloproliferative disorder afflicting young children (2;7;15). JMML predominates in infants (median age at diagnosis, 2 years) (7;16). There is a male predominance with a male: female ratio of 2:1. Pallor, fever, infection, skin bleeding and cough are the most commonly presenting symptoms. There is generally marked splenomegaly and hepatomegaly. Unlike acute monoblastic leukemia, JMML rarely involves the central nervous system (CNS).

Hematological picture and karyotype

Leukocytosis, anemia and thrombocytopenia are common findings in JMML patients. The median white count (WBC) is 33 x 10^9/L (7). An absolute monocyte count exceeding 1 x 10^9/L is required for the diagnosis of JMML (17). The median blast cell percentage in PB smears is less than 2% (16) (7) and rarely exceeds 20%. The morphological evaluation of the peripheral blood smear is often the most important step in establishing the diagnosis.

JMML lacks the Philadelphia chromosome and the *BCR/ABL* fusion gene. Chromosomal studies of leukemic cells show monosomy 7 in about 25% of patients, other abnormalities in 10%, and a normal karyotype in 65% (7;8;18). Clinical characteristics of patients with monosomy 7 do not differ from those of patients with a normal karyotype (7). However, patients with monosomy 7 present with a lower median WBC but similar absolute monocyte count, as they have a higher percentage of monocytes on the differential count. Red blood cells are often macrocytic, and erythropoiesis in bone marrow is more pronounced than in cases with a normal karyotype. In addition, patients with monosomy 7 present with a normal or only moderately elevated HbF, which is often elevated in patients with normal karyotype (7) (Figure 1).

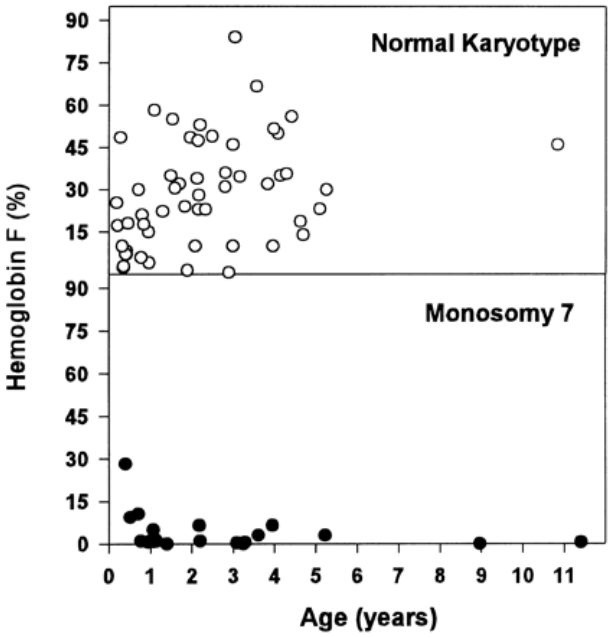


Figure 1 Hemoglobin F concentration for patients with JMML and either normal karyotype or monosomy 7 (7)

Molecular pathophysiology

JMML myeloid progenitors show a characteristic hypersensitivity for granulocyte-macrophage colony stimulating factor (GM-CSF) (19). This hypersensitivity is mediated by the RAS-RAF-MAP (mitogen-activated protein) kinase signaling pathway, which is pathologically activated by mutations in *RAS*, *NF1* (the gene for Neurofibromatosis 1 [NF1]), and *PTPN11* (20-24) (Figure 2). Mutations in *PTPN11*, *RAS* and *NF1* are mutually exclusive in JMML, suggesting the importance of the pathological activation of RAS dependent pathways in the pathophysiology of the disease.

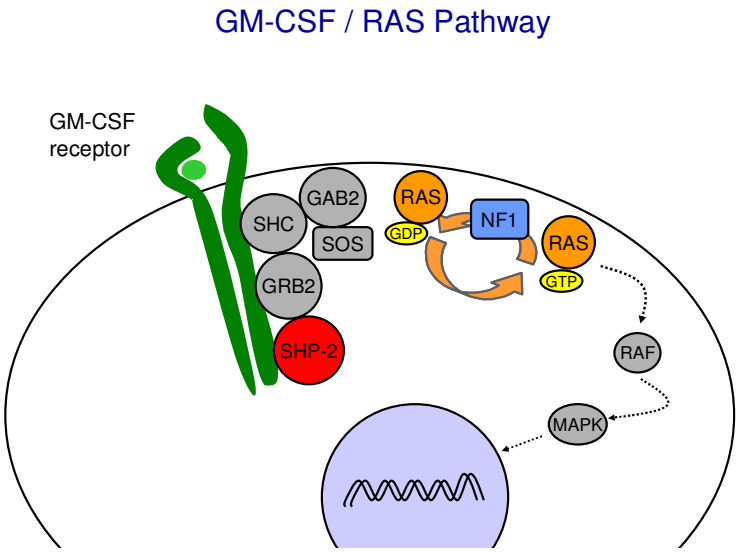


Figure 2. Model outlining the roles of SHP-2, RAS, and NF1 in the GM-CSF signal transduction pathway. Oncogenic point mutations in codons 12, 13 and 61 of *NRAS* and *KRAS* are observed in leukemic cells of about 15% to 20% of children with JMML (21;24-27).

Neurofibromin, the protein encoded by the gene for NF1, functions as GTPase activating protein and negatively regulates RAS. A clinical diagnosis of NF1 can be made in up to 11% of patients with JMML (8;16). Moreover, about 15 % of JMML patients without the clinical diagnosis of NF1 have mutations in the gene for NF1 (22;28) suggesting that approximately 25% to 30% of JMML cases are associated with NF1. Analysis of JMML cells from patients with NF1 revealed homozygous NF1 inactivation due to somatic loss of the normal allele, which results in hyperactivation of RAS (29-32).

The somatic mutation in *PTPN11*, a gene that encodes the non-receptor protein tyrosine phosphatase SHP-2, is found in about 35% of the JMML patients (33). SHP-2 contains 2 src homology 2 (SH2) domains and a catalytic PTPase domain. The SHP-2 PTPase is activated by binding to phosphotyrosyl peptides through its N-SH2 domain. In JMML the mutations are located in exon 3 and 13 which encode segments of the N-SH2 and PTPase domains, respectively. All mutations cause a gain of function in SHP-2 through preferential occupation of the activated state.

JMML-like disorder in Noonan Syndrome

Heterozygous germline missense mutations in *PTPN11* are known to cause Noonan syndrome, a developmental disorder characterized by dysmorphic facial features, growth retardation and heart disease (33). The mutations are found in exon 3 (most common), 7, 8, and 13, which encode segments of the N-SH2 and PTPase domains. Although many of the germ line *PTPN11* mutations identified in Noonan syndrome and somatic mutations in JMML alter the same codons, the spectrum is distinct with respect to the pattern of amino acid substitutions (34). A small number of patients with Noonan syndrome develop a JMML-like disorder (21;35-39). In these patients with Noonan syndrome and JMML-like disorder, the spontaneous remission of the disorder is well documented. The distribution of mutations in *PTPN11* in Noonan syndrome with JMML-like disorder also showed specificity, as most of the cases harbored the C218T mutation, which was observed in only 2% of isolated Noonan syndrome and none of isolated JMML patients. It has been demonstrated that the gain-of-function mutations in *PTPN11* identified in JMML and Noonan syndrome have distinct effects. The specific mutations in isolated JMML which occur

as somatic changes but are not observed as germ line defects are stronger and most likely associated with embryonic lethality. Conversely, most of the mutations in isolated Noonan syndrome are milder and sufficient to perturb development processes are not fully leukemogenic. In this scheme, mutants in Noonan syndrome with JMML-like disorder are predicted to have intermediate effects, potentially explaining the milder course of the disorder. Although the JMML-like disorder in Noonan syndrome may not be a clonal, and the majority of cases are not fatal, children with this disorder will be included in this study.

Neurofibromatosis 1 (NF1)

A clinical diagnosis of NF1 can be established in about 11% of patients with JMML (7;8). Up to 15 % of JMML patients without the clinical diagnosis of NF1 have mutations in the *NF1* gene (22;28), suggesting that approximately 25% of JMML cases are associated with NF1. NF1 is an autosomal dominant disorder affecting between 1/2000 and 1/4500 individuals (40). About half of all NF1 cases are familial cases, while the other half is caused by *de novo* mutations. Similarly, about 50% of JMML patients with the clinical diagnosis of NF1 are known to have an affected parent (own unpublished data). Because the *NF1* gene is large and mutations do not cluster in certain "hot spots", mutational studies are still challenging. Therefore, the diagnosis of NF is generally based on the clinical diagnostic criteria proposed by the National Institute of Health (NIH) (Table 7).

Cardinal clinical features (any two or more are required for diagnosis)
• 6 or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in post pubertal individuals
• 2 or more neurofibromas of any type or 1 plexiform neurofibroma
• Freckling in the axillary or inguinal regions
• Optic glioma
• 2 or more Lisch nodules (irishamartomas)
• A distinctive osseous lesion such as sphenoid dysplasia or thinning of the long bone cortex with or without pseudarthrosis
• A first degree relative (parent, sibling, or offspring) with NF1 by the above criteria

Table 7 NIH Diagnostic Criteria for NF1

Applying these criteria, some individuals, who are later shown to have NF1, cannot be diagnosed in early childhood (41). Thirty percent of NF1 patients <1 year of age have only one of the cardinal clinical features (41) and must have an affected first degree relative to be diagnosed with NF1 by these criteria. Café-au-lait macules are usually the first appearing clinical feature; 99% of patients show $\geq$ 6 café-au-lait macules > 5 mm in diameter by 1 year of age (Table 8).

• Café-au-lait macules:	99% of patients ≥ 6 café-au-lait macules (> 5 mm) by 1 year of age
• Inguinal or axillary freckling:	90% of patients by 7 years old.
• Lisch nodules:	>70% of patients by 10 years old.
• Neurofibromas:	48% of patients by 10 years old
	84% of patients by 20 years old
• Symptomatic optic glioma:	1% of NF1 patients by 1 year old
	It reaches maximum frequency (~4%) by 3 years old.
• Characteristic osseous lesions:	Usually apparent within the first year of life.
	They occur in ~14% of patients.

Table 8 The age of appearance of each cardinal clinical feature of NF1

In addition to the features listed among the NIH criteria we suggest to consider JMML as one of the cardinal clinical features of NF1. With this adjustment a diagnosis of NF1 can be made in infants with the following criteria shown in table 9.

In patients with a confirmed diagnosis of JMML a clinical diagnosis of NF1 can be made in the presence of

- ≥ 6 café-au-lait macules greater than 5 mm in diameter

or

- a first degree relative (parent or sibling) with NF1.

Table 9 Diagnostic criteria for NF1 modified for JMML patients

Diagnostic Criteria of JMML

The diagnostic criteria proposed by the International JMML Working Group in 1998 have been widely applied (17). They are based on clinical and laboratory findings. Recently, molecular studies have greatly facilitated the diagnostic approach. As described above, somatic mutations in *PTPN11* and oncogenic point mutations in *RAS* are found in 34% and 25% of JMML patients (21;25). In addition, approximately 25% of JMML cases are associated with NF1. In summary, one of these abnormalities can be detected in about 80% of JMML patients. Therefore, molecular studies to detect mutations in *PTPN11*, *RAS*, and if available *NF1,* have to be introduced in the diagnostic process of JMML.

Splenomegaly, monocytosis (> $1x10^9$/L) in PB, and less than 20% of blasts count in the BM are the essential clinical and hematological criteria for JMML. In patients fulfilling these criteria, JMML can be diagnosed in the presence of one of the following criteria: *PTPN11* / *RAS* / *NF1* mutation, clinical diagnosis of NF1, or monosomy 7. For patients without *PTPN11* / *RAS* / *NF1* mutation, clinical diagnosis of NF1, or monosomy 7, the diagnosis of JMML should be made by the classical criteria (17). In these patients, the colony assay for spontaneous growth and hypersensitivity to GM-CSF and the exclusion of *BCR/ABL* rearrangement (Philadelphia chromosome) are mandatory.

I. Clinical and hematological features (all three features mandatory)
- Peripheral blood monocyte count > $1x10^9$/L
- Blast percentage in PB and BM < 20%
- Splenomegaly

II. Oncogenetic studies (1 parameter sufficient)
- Somatic mutation in *PTPN11** or *RAS*
- *NF1* mutation or clinical diagnosis of NF1
- Monosomy 7

III. In the absence of one parameter listed under II, the following criteria have to be fulfilled:
- Absence of Philadelphia chromosome (*BCR/ABL* rearrangement) (mandatory)
  And at least two of the following criteria
- Spontaneous growth or GM-CSF hypersensitivity in colony assay
- Hemoglobin F increased for age
- Myeloid precursors on peripheral blood smear
- White blood count > $10x10^9$/L
- Clonal abnormality besides monosomy 7

Table 10 Revised diagnostic criteria of JMML

* In cases with somatic *PTPN11* mutation a germline mutation has to be excluded (Noonan syndrome). JMML-like disorder seen in Noonan syndrome is considered separately.

Differential diagnosis in JMML

The clinical and morphologic pictures of JMML can be mimicked by a variety of infectious agents such as cytomegalovirus (42), Epstein-Barr virus (43), human herpes virus 6 (44) and parvovirus B19 (45). However, positive results of these viruses do not exclude the diagnosis of JMML.

Clinical course of JMML

JMML is a rapidly fatal disorder for most children if left untreated. Some young children with JMML (i.e., those diagnosed before 1 year of age) may experience a longer course characterized by temporary clinical improvement in the absence of therapy. The median survival time without hematopoietic stem cell transplantation (HSCT) is about 1 year (Figure 3).

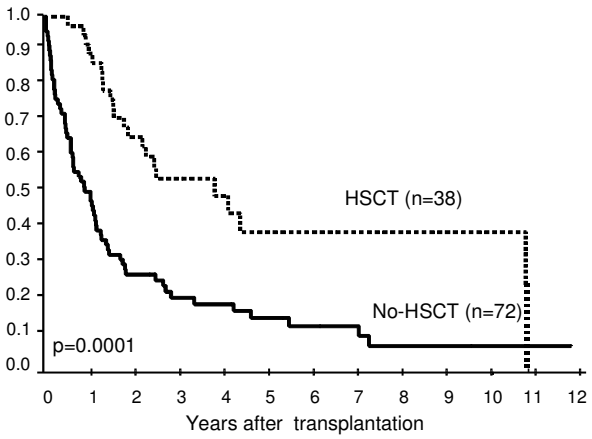


Figure 3 Survival with and without HSCT in patients with JMML (7)

Low platelet count, age above 2 years at diagnosis and high HbF at diagnosis are the main predictors of short survival (7;8;16). In a retrospective series of 110 cases, all children presenting with a platelet count of 33 x 10^9/L or less had died within a year from diagnosis, while those with higher counts and age less than 2 years of age at diagnosis had a median survival of 3 years (7) (Figure 4). Blastic transformation is infrequent in JMML, and most untreated patients die from respiratory failure due to pulmonary infiltration with mature leukemic cells.

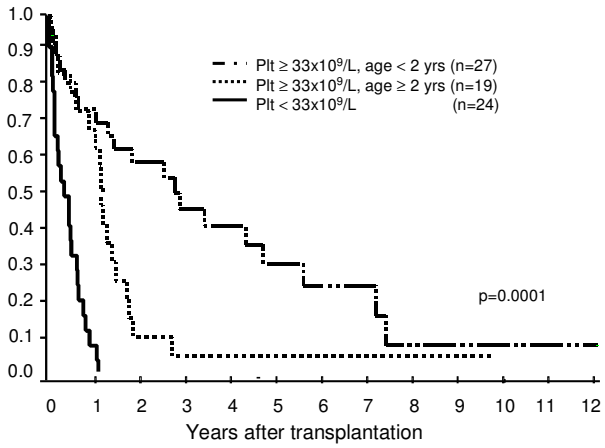


Figure 4 Survival without HSCT in patients with JMML according to platelet count and age at diagnosis (7).

Therapy prior to HSCT for JMML

Long-term survival has only been achieved with HSCT, and there are no confirmed drugs which are curative for JMML in the absence of HSCT. The role of anti-leukemic therapy prior to transplantation is currently uncertain. 6-mercaptopurine (6-MP) is probably the drug most commonly applied in JMML prior to HSCT to control the tumor burden, administered either as single-agent (50 mg/m^2) (16;46) in combination with low-dose cytarabine (46;47). However, the response is transient and there are no data indicating that it influences the duration of survival. Clinical remissions and long-term survival after AML-type combination therapy have been reported in small series of children with JMML (48);(49);(10). However, other investigators pointed out that intensive chemotherapy is notably unsuccessful, especially in patients with aggressive disease (16;46;50), and durable remission may not be achievable. In a recent prospective analysis of EWOG-MDS/EBMT trial, neither EFS was improved, nor relapse incidence was reduced in patients who had received intensive chemotherapy before the allograft (51). Thus, in view of these results, intensive chemotherapy prior to HSCT cannot be recommended outside clinical trials.

Castleberry et al. reported that treatment with isoretinoin (100 mg/m^2/day) resulted in complete or partial response in 6 of 10 children with JMML (52). However, other investigators did not observe significant clinical responses with retinoic acid (46;53;54). The treatment with interferon-α (IFN-α) showed some clinical improvements in a limited number of patients (46;55-59). A prospective study with IFN-α, 30.000 units/m^2 subcutaneously daily for 14 days followed by the same dose 3 times weekly was stopped for excessive toxicity (60). By contrast, in a JMML patient relapsing after HSCT, IFN-α induced a sustained and complete remission (61).

E21R is a modified GM-CSF protein which results in antagonism of GM-CSF function via selective binding to the GM-CSF receptor complex, exerting an anti-leukemic action (62;63). There is a report on a patient with JMML who was treated with 3 courses of E21R (64). A clear efficacy was observed after 2 courses of E21R but the disease appeared completely refractory during the third course. Unpublished experience indicates transient responses to E21R as well. Farnesyl transferase inhibitors (FTIs) are a novel class of compounds that inhibit an enzymatic step (farnesylation) critical to the activation of several cellular proteins, including the RAS proteins. The Phase II window evaluation of R115777 (Zarnestra®) of the Children's Oncology Group (COG) showed some responses.

Splenectomy in JMML

The benefit of splenectomy for prevention of post-transplant relapse is unknown (46;54). In the current HSCT study of the EWOG-MDS, splenectomy did not improve the survival of the patient after HSCT (51) (Figure 5).

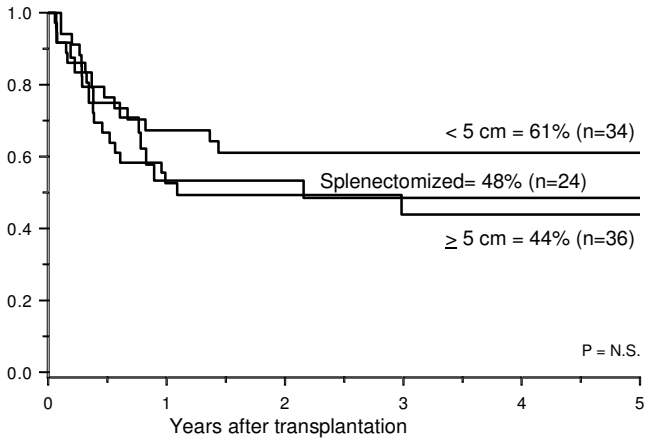


Figure 5 Spleen size and the event free survival after HSCT

Allogeneic HSCT in JMML

Allogeneic HSCT is currently the only curative treatment for JMML. The results of the most recent trials on HSCT in JMML are summarized in table 11. The analysis of the EWOG-MDS/EBMT trial of 100 patients with JMML transplanted with preparative regiment of busulfan (BU), cyclophosphamide (CY) and melphalan shows a 5-year probability of EFS was 52% (51). The EFS of patients transplanted from a matched family donor (MFD) and unrelated donor (UD) are not significantly different (Figure 6).

Study group	Study design	Nr. pts	Study period	EFS (%)	Donor (MFD/ MMFD/ UD)	Regimen Non-TBI/ TBI	Relapse (%)	TRM (%)	Acute GvHD ≥II/ ≥III (%)	Chronic GvHD (%)
EWOG	prospect.	100	1993-02	52 (5 yrs)	48/ 0/52	100/ 0	35*	13*	40/17*	17*
Japan	retrospect.	27	1990-97	54 (4 yrs)	12/ 4/11	9/ 18	26	11	58/ 31	45
NDMP	retrospect.	46	1990-97	24 (2 yrs)	0 / 0/46	11/ 35	58*	18*	54/ 33*	34*

Table 11 Recent large studies of HSCT in JMML

EFS: event-free-survival, MFD: matched familial donor, MMFD: mismatched familial donor, UD: unrelated donor. TBI: total body irradiation, TRM: transplant related mortality, GVHD: graft versus host disease, * Cumulative incidence

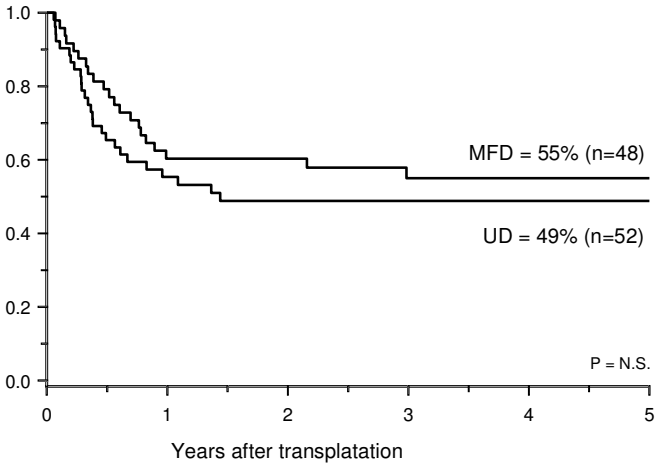


Figure 6 Event free survival in transplant with matched family donor (MFD) and unrelated donor (UD)

Relapse is a major treatment failure which is observed in up to 50% patients (65-67). In the current EWOG-MDS/EBMT trial, the 5-year cumulative incidence of relapse was 35% (51). Relapse occurs early, at a median of 2 to 6 months from transplantation (67;68) and generally within the first year.

Generally, HSCT shortly after diagnosis is recommended, and younger age at HSCT predicts improved survival (68;69). In the prospective HSCT study from EWOG-MDS, multivariate analysis shows age greater than 4 years and female sex predicted poorer outcome (51). Cytogenetic abnormalities do not confer a worse prognosis (51). A recent study from the United Kingdom reported that monosomy 7 was associated with an outcome comparable to or even better than that of JMML patients with normal karyotype (70).

The impact of acute and chronic graft-versus-host disease (GVHD) on relapse and survival rate has been reported controversially among studies (51;67;69). The result from the National Marrow Donor Program (NMDP) in the US on unrelated HSCT in JMML showed that chronic GVHD was associated with lower risk of relapse and better survival, while acute GvHD (≥ grade III) was associated with poor survival (67). This result suggests a role for a graft versus leukemia (GvL) effect in JMML. Similarly, the Japanese retrospective study showed a trend for a more favorable outcome for patients with grade 0-I acute GVHD or chronic GvHD (69), and the interim analysis of the prospective Japanese study indicated a beneficial impact of chronic GvHD (71). The prospective EWOG-MDS study, however, did not show an impact of neither acute nor chronic GvHD on outcome after HSCT. This result may be explained by the low incidence of chronic GvHD in the EWOG-MDS study cohort (17%) (51).

Approach for the patients relapsing after HSCT

Treatment options for patients relapsing with leukemia after HSCT are limited. Withdrawal of immunosuppressive drugs is usually the first measure, which by itself can control leukemia in a limited number of patients (72;73). In case of non-response and for all patients suffering disease recurrence after cessation of immunosuppressant agents, donor leukocyte infusion (DLI) or second HSCT may be considered. There are some case reports on the successful treatment with relapsed patients with DLI (65;67;74-76). The recent analysis from EWOG-MDS showed that 6 of the 21 JMML patients, who received DLI for mixed chimerism or hematological relapse, responded to DLI and achieved complete chimerism (77). Response rate was significantly higher in patients receiving a higher number of T cells ($\geq$ $1x10^7$/kg) and in patients with abnormal karyotype. Notably, none of the 6 patients given DLI from a matched sibling responded. The outcome of even the responders was unfavorable. Only one of the responders is alive in remission, 2 relapsed and 3 died of complications of DLI or HSCT. Infusion of a high enough number of T cells, strategies to reduce toxicity, and cytoreduction prior to DLI may possibly improve the results.

Despite the generally aggressive re-emergence of the malignant clone and the short interval between first and second HSCT, a substantial number of children have been cured by a second HSCT (46;78;79). In the most recent analysis of 23 patients with JMML who relapsed after first HSCT and underwent second HSCT (BMT/PBSCT n=15/8), 10 patients are alive in remission, 8 relapsed and 5 died of transplant related mortality (unpublished data of the EWOG-MDS study). The same donor was used for both first and second HSCT in 19 of 23 patients. In the majority of patient, a Bu based regimen was used for first HSCT and a TBI based regimen for second HSCT**.** It is reasonable to hypothesize that less intensive GVHD prophylaxis was applied during the second allograft in these patients, thereby preserving a GVL effect. In contrast to this encouraging result, the report from the Seattle group shows only one of 6 patients after the second HSCT alive in remission. At present, second HSCT seems to offer the best chance of cure following relapse after HSCT, although the role of DLI under optimal conditions has not yet been established.

Current guidelines for therapy in JMML

All children should receive an allogeneic HSCT as soon as the diagnosis is established. A busulfan-based myeloablative preparative regimen is recommended. The current EWOG-MDS HSCT trial in JMML applies busulfan, cyclophosphamide and melphalan, for details see separate protocol.

There is currently no evidence that therapy prior to HSCT improves survival following HSCT, and therapy other than 6-MP or isoretinoin is generally not recommended. Conventionally, oral 6-MP (50 mg/m^2) has been administered in patients with very high WBC count, pulmonary problems and/or prominent organomegaly. Some investigators administer oral isoretinoin (100 mg/m^2, single dose) alone or with 6-MP.

More intensive chemotherapy should only be considered in severely ill children. Low-dose intravenous cytarabine (Ara-C) (i.e. 40mg/m^2 x 5 days, schedule as needed) can be administered first line. In case this fails, high dose Ara-C can be considered [e.g. fludarabine 30 mg/m^2/day (day 1-5) and Ara-C 2g/m^2/day (day 1-5), COG protocol]. After intensive therapy, patients should be carefully observed, because a rapid rebound of disease may cause fatal pulmonary infiltration.

Patients with Noonan Syndrome and a JMML-like picture are followed closely generally without therapy.

2.5 Myelodysplastic Syndrome (MDS)

The following section first describes morphology and cytogenetics for all myelodysplastic syndromes. Subsequently a division into refractory cytopenia, high grade MDS and secondary MDS is made.

2.5.1 Morphology

The evaluation of the morphology on BM aspirate, BM biopsy and PB smear is the *sine qua non* for the diagnosis of MDS.

Dysplasia

Cytologically, MDS is characterized by dysplasia of at least 2 cell lines. Figure 7 indicates dysplastic features noted in MDS in the 3 cell lineages.

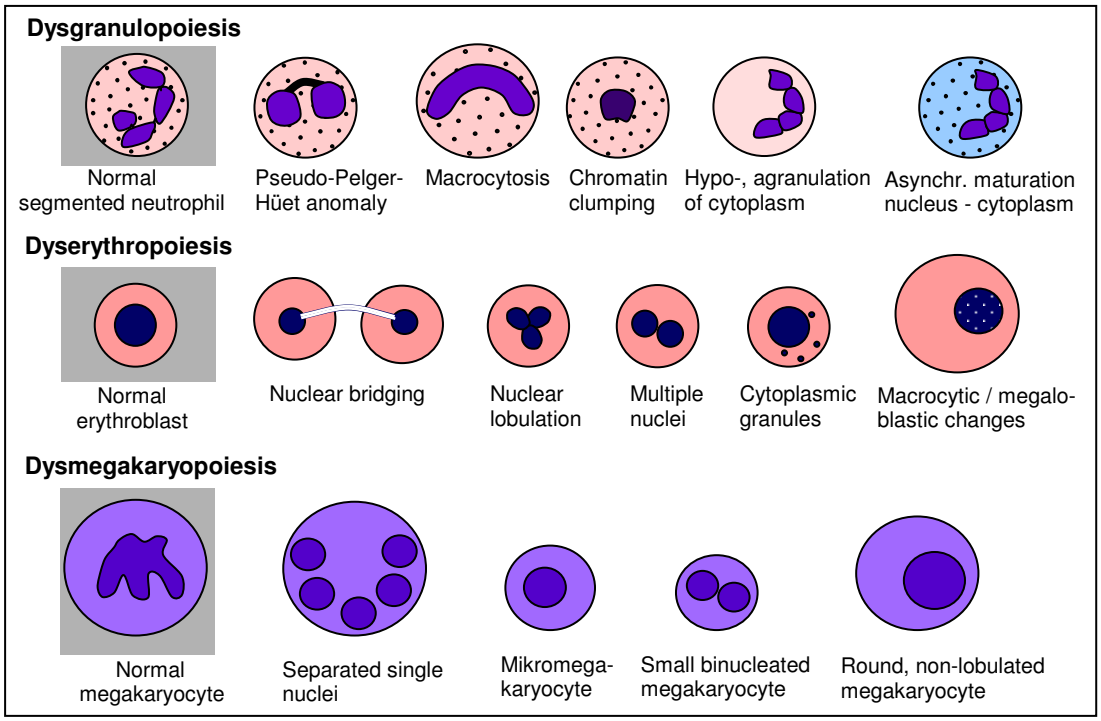
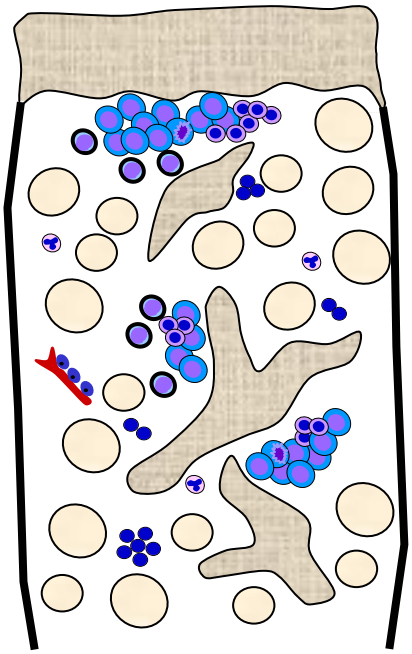


Figure 7 Dysplastic features in MDS, modified from (1)

Differential diagnosis of refractory cyotopenia and aplastic anemia

The morphological differential diagnosis, in particular the histological pattern of hypoplastic RC and aplastic anemia (AA), may be challenging. Since hypoplastic RC shows patchy distribution of hematopoiesis in an otherwise fatty marrow (Figure 8), the biopsy including only fat cells and missing hematopoiesis, may mimic AA. The bone marrow biopsy is mandatory to obtain the essential information for the diagnosis of RC, such as disruption of the regular hematopoietic topography, typical clustering of left-shifted erythropoiesis, detection of dysplastic megakaryocytes and marrow fibrosis (Figure 8).



Erythropoieses: Uni- or multifocal clustering of predominantly immature forms with increased mitoses
Occasionally with atypia

Granulopoieses: Sparsely dispersed or lacking

Megakaryopoiesis: Usually decreased
Micromegakaryocytes or other dysplastic changes

Figure 8 Histological pattern of hypoplastic refractory cytopenia (1)

Cellularity

BM cellularity can only be evaluated on BM biopsies. Therefore, a BM biopsy is suggested for all cases of MDS. It is mandatory if a hypocellular BM is suspected. In RC, many cases are hypocellular (table 12), therefore a BM biopsy is to be recommended. Similarly, in high grade MDS, progression of disease is often associated with myelofibrosis which can only be diagnosed on biopsy.

	Retrospective study *		Prospective study **	
Cellularity	n=48	%	n=139	%
increased	6	13	11	8
normal	15	31	16	12
low	27	56	112	81

Table 12 Cellularity in refractory cytopenia

** Kardos et al. Blood 2003;102:1977 **Interim Analysis of EWOG-MDS 98 in August 2004*

Ring sideroblasts

Ring sideroblasts are qualified by erythroid precursors with more than 5 iron granules which comprise more than 30% of the nuclear rim. Sideroblastic anemia refers to cases with more than 15% ring sideroblasts in BM. Refractory anemia with ring sideroblasts (RARS) is extremely rare in children and there are no data to document whether patients with RARS share distinctive clinical features. Therefore, we suggest that RARS should be included in the category of “refractory cytopenia”. The finding of sideroblasts in the bone marrow in children should prompt investigations for rare disorders like mitochondrial cytopathies (Pearson syndrome) or inherited sideroblastic anemia (80;81). In Pearson syndrome, vacuolated erythroid and myeloid precursors are observed besides ring sideroblasts. Ring sideroblasts are also occasionally seen in MDS with increase in blasts; these cases, like RC, are classified according to blast count.

Description of blast cells and separation from promyelocytes

To clearly define blast cells and to distinguish them from promyelocytes, the FAB group in 1982 provided the definition of blasts type I and II (table13) (3;82). In 1991 a type III blast was defined on the basis of a higher number of cytoplasmatic granules (83). Promyelocytes, promonocytes, proerythroblasts and megakaryoblasts are not included in the blast count but scored separately.

Blast type I	Large central nucleus with finely dispersed uncondensed (reticular) chromatin, at least one and usually 2 - 3 prominent nuclei, slight to moderate basophilic cytoplasm, no paranuclear hof (Golgi zone), no granules.
Blast type II	As type I but a few (< 20) granules in the cytoplasm
Blast type III	As type II but $\geq$ 20 granules in the cytoplasm
Promyelocyte	Large eccentric nucleus, slightly condensed chromatin, one prominent nucleus, paranuclear golgi zone, less basophilic cytoplasm with evenly dispersed granules. In MDS promyelocytes are often hypogranular. A cell can therefore be recognized as a promyelocyte when the following features are present: a paranuclear golgi zone and an eccentrically placed nucleus in combination with a low nuclear to cytoplasmic ratio compared to a blast cell (EWOG-MDS Morphology Board).

Table 13 Description of blasts type I - III and promyelocytes

Myelofibrosis

BM fibrosis (myelofibrosis) refers to the abnormal deposition of the reticulin network by BM fibroblasts.
The prominent myelofibrosis leads extramedually hematopoiesis (EHM), which contributes to hepatosplenomegaly. Typically tear drop shaped red cells and nucleated erythroid and myeloid precursors are observed on PB smears.

Myelofibrosis can be secondary to various hematological disorders; MDS, AML (most frequently M7), myeloproliferative disorders like CML, polycythemia vera. It also occurs associated with other underlying diseases such as autoimmune diseases, Down syndrome, and metabolic disorders.

In adult MDS, bone marrow biopsy frequently (17-55%) reveals focal or patchy reticulin fibrosis. It is often associated with BM hyperplasia and disturbed differentiation of megakaryopoiesis. In adults, the distribution of myelofibrosis is similar in RA, RARS, RAEB and RAEB-T, but considerably higher in CMML (84). Some reports suggests that myelofibrosis in adult MDS is associated with poor prognosis (84). Most patients show no or only mild hepatosplenomegaly. However, the minorities of cases develop striking myelofibrosis with prominent EMH and may be best described as MDS with myelofibrosis.

In childhood MDS, myelofibrosis is, however, less frequent than in adult MDS. It is generally observed in advanced cases and extremely rare in low grade MDS *(I. Baumann, unpublished observation).* Myelofibrosis in the absence of an increased blast percentage and without disturbed megakaryopoiesis is very rarely seen (85-87). Some of these patients show a stable clinical course for years, and spontaneous regression is also reported. Therefore, the initial management should be conservative (85;86). Vitamin D deficiency should be excluded (87). Some cases may respond to steroid therapy (88). The nature of these disorders remains unknown, but differs significantly from adult chronic idiopathic myelofibrosis (CIMF) which usually runs an aggressive course (85;86;89).

2.5.2 Cytogenetics and Molecular Genetics

Conventional cytogenetics reveals an abnormal karyotype in about half of MDS patients (table 14) at diagnosis. However, the frequency varies dependent on whether the disease is primary refractory cytopenia, primary advanced MDS or secondary MDS. In contrast to AML, numerical abnormalities dominate; structural abnormalities are frequently part of a complex karyotype with numeric abnormalities. Monosomy 7 is the most common cytogenetic abnormality being identified in approximately 25% of cases. Constitutional trisomy 8 mosaicism may remain unrecognized (90) and should be tested for when trisomy 8 is found in the bone marrow.

Karyotype	Primary MDS (%)			Secondary MDS (%)
	All n = 199	Refractory cytopenia n = 105	Advanced MDS n = 94	Prior chemotherapy/ radiation therapy n = 44
Normal	57	72	40	18
Monosomy 7 (+/- 1 additional aberration)	24	17	32	23
Trisomy 8 (+/- 1 additional aberration)	4	3	5	0
Complex (>2 aberrations)	6	3	9	36
Other aberrations	9	5	14	23

Table 14 Karyotype of patients with primary and secondary MDS (EWOG-MDS unpublished)

Monosomy 7 is associated with a shorter time to progression in RC of childhood (91). In advanced MDS, monosomy 7 as the sole cytogenetic aberration has not been an unfavorable feature in most studies (7;9;10). Cytogenetic aberrations, which generally implicate a favourable prognosis like -Y, del (5q), have been often reported in adults, but these aberrations are so infrequent in children that they are of no practical importance (92).

AML-specific translocations, including t(8;21)(q22;q22), t(15;17)(q22;q12), or inv(16)(p13q22), may occur in cases of *de novo* AML with a low blast cell count. Their response to therapy is generally favorable and they should not be considered MDS.

In situ hybridization (FISH) for identification in monosomy 7 or trisomy 8 can be helpful in the absence of conventional banding cytogenetics. However, the importance of small clones (< 30%) of monosomy 7 cells remains unknown.

Multicolor FISH (mFISH) can be helpful to identify unknown genetic material in cases with an abnormal karyotype. In this analysis, whole chromosome painting DNA probes are labelled with different fluorochromes or fluorochrome combinations. Special software analyses the colour information and identifies the chromosomal origin of each individual pixel within the image. Using this method, even complex rearrangements are readily detectable. Figure 9 gives an example how mFISH can provide additional information even if conventional banding cytogenetics are satisfactory. Thus, mFISH can provide new information that may help to identify special target genes which play a role in leukaemogenesis.

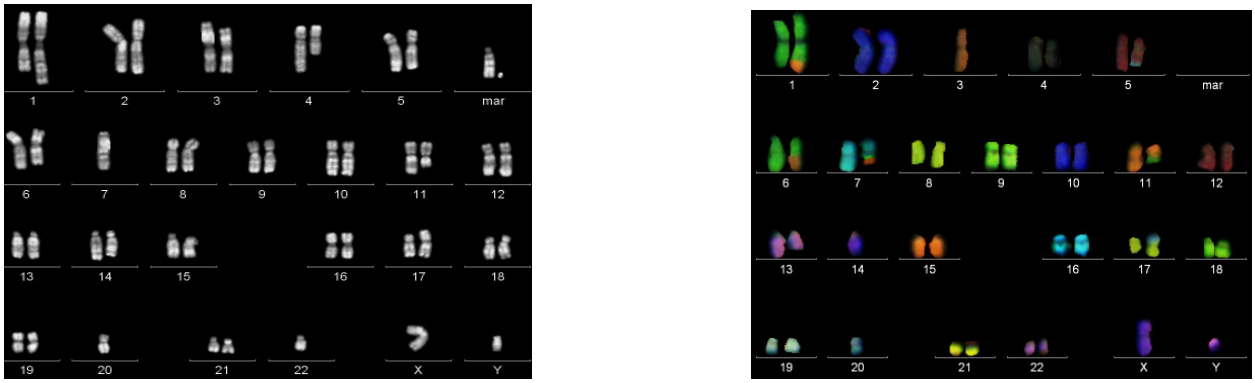


Figure 9 Conventional banding cytogenetics (left panel) and mFISH (right panel) of a child with primary MDS and complex karyotype

The karytoype was determined by conventional cytogenetics as: 42~49,XY,t(1;11)(q43;q21),del(4)(q25), -5,del(5)(q23q34),der(6)t(3;6)(q13;q13),-7,del(13)(q13q21),-14,add(14)(p13),del(14)(q32),add(17)(p12), -20,-22,+1~3mar[cp16]. The 5q-marker recognized by conventional cytogenetics had initially been interpreted as chromosome 5 with an interstitial deletion at 5q23~q34. With mFISH it was found to contain chromosomal material translocated from chromosome 7. Moreover, the derivative chromosome 17 was recognized to present a whole-arm-translocation with a chromosome 20. Summarized we could further indentify a der(5)t(5;7) and a der(17;20).

Matrix-/array-based comparative genomic hybridization (CGH) is a method that utilizes DNA chips consisting of arrayed genomic DNA fragments to screen for microdeletions and duplications. Differentially labelled patient and reference DNA is co-hybridized on DNA microarrays to identify fluorescent signal differences due to loss or excess of genomic sequences. In a pilot study, we analyzed DNA of 10 children with primary MDS and a cytogenetically detected monosomy 7 by array-CGH to clarify if additional subtle chromosomal gains or losses were present (D. Steinemann, B. Schlegelberger, unpublished). Three patient groups could be identified: one group with sole monosomy 7, a second group with additional aberrations also detectable by conventional cytogenetics, and a third group with high chromosomal imbalances that had not been detected by karyotyping. In one example additional aberrations of chromosomes 3, 12 and 20 were detected in addition to the events involving chromosome 5, 7 and 17 that had been identified by conventional cytogenetics (see fig 10).
Comparing the genomic profile in DNA from granulocytes to that obtained in mononuclear cells, subclones could be detected in the mononuclear cell fraction.

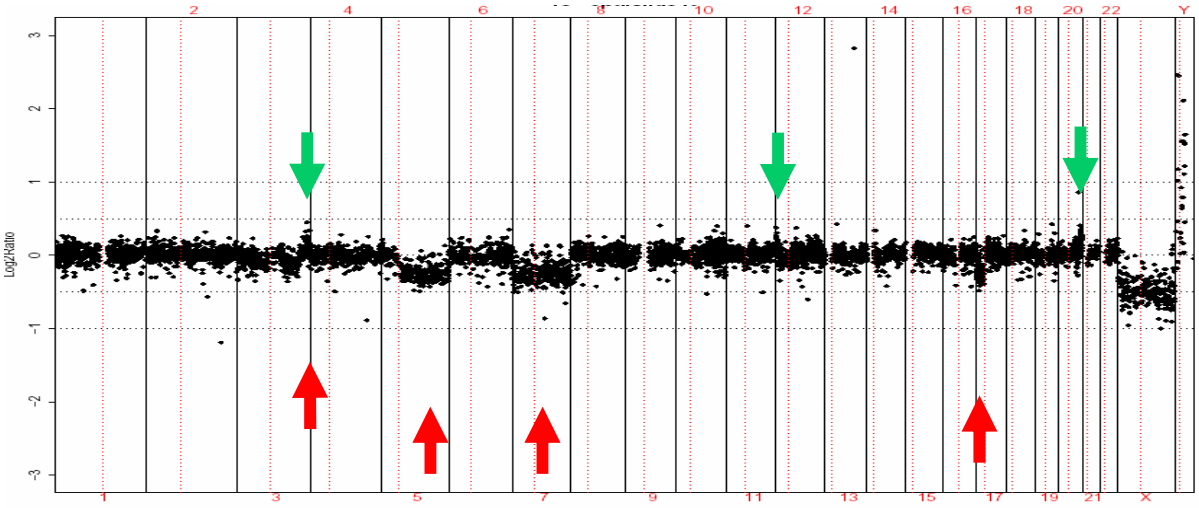


Figure 10. The genomic profile of a patient showing multiple aberrations by means of array-CGH. Gains in 3q, 12p and 20q are indicated by green arrows, losses in 3q, 5q, 7 and 17p by red arrows

2.5.3 Refractory Cytopenia

Clinical presentation and hematological picture in RC

Refractory cytopenia (RC) is the most common subtype of childhood MDS accounting for about half of all MDS cases (93). Patients usually present with symptoms related to pancytopenia, such as anemia, infection, and bleeding tendency. Organomegaly is generally absent. The term “refractory cytopenia” indicates, that in children, in contrast to adults, thrombocytopenia and neutropenia are more frequently observed than anemia (93). The MCV is usually elevated in MDS, but it is also high in majority of patients with congenital bone marrow failure syndromes, in some patients with severe aplastic anemia (SAA) at diagnosis, and in most patients with SAA during their clinical course. In contrast to adult MDS, bone marrow cellularity is often reduced in RC. In an interim analysis of study EWOG-MDS 98, approximately 80% of patients with RC have a hypocellular biopsy specimen (unpublished data, see Table 12).

Cytogenetics in RC

Karyotype is the most important factor for progression to high grade MDS and survival (Figure 11). The median time to progression for children with RC and monosomy 7 is less than 2 years. Spontaneous disappearance of monosomy 7 and cytopenia has been noted in some infants, but remains a rare event. In contrast to monosomy 7, patients with trisomy 8 and other karyotypes may experience a long stable course of their disease.

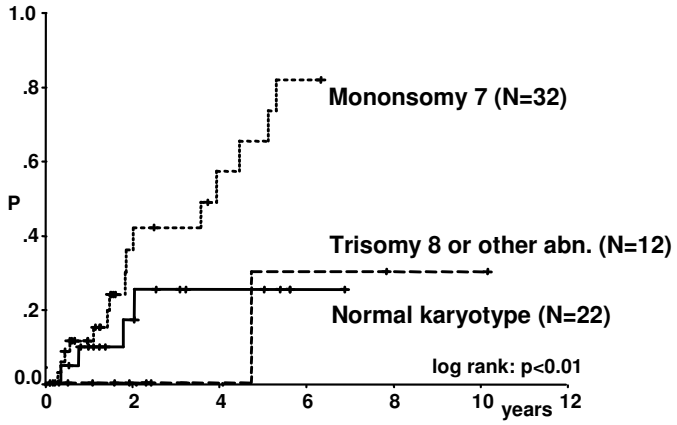


Figure 11 Cumulative incidence of progression to high grade MDS for patients with refractory anemia and either normal karyotype, monosomy 7, or trisomy 8 or other abnormalities at the time of diagnosis. Patients who had received a stem cell transplantation were censored at time of transplantation (93)

Differential diagnosis of RC

MDS with less than 5% blasts in BM is particularly difficult to diagnose, because dysplasia of hematopoietic cells is frequently observed in association with infections, metabolic disorders, nutritional deficiencies, and a variety of other diseases. The congenital bone marrow failure disorders such as Fanconi anemia, dyskeratosis congenita, Shwachman Diamond syndrome, amegakaryocytic thrombocytopenia, and pancytopenia with radioulnar synostosis should be excluded by careful physical examination for skeletal and other organ abnormalities and taking past and family history. Currently many of these diseases can be diagnosed molecularly. The examination for Fanconi anemia by chromosomal breakage, G_2 cell cycle arrest, Western blot or mutational analysis, is mandatory for all the patients with primary MDS. Differentiating hypoplastic RC from SAA remains an intriguing challenge. Moreover, MDS develops in 10-15% of those children with SAA not treated with HSCT (94;95). In the absence of a cytogenetic marker, the clinical course will have to be carefully evaluated; 2 BM examinations with biopsies at least 2 weeks (but not more than 3 months) apart are recommended before a diagnosis of RC can be established.

Paroxysmal nocturnal hemoglobinuria (PNH) is a unique acquired clonal stem cell disorder caused by somatic mutation in the *PIGA* gene on the X-chromosome (Xp22.1) encoding a protein in the synthesis of the glycosylphosphatidylinositol (GPI) anchor by which many proteins are attached to the cell membrane. About 15 proteins have been found to be deficient on the abnormal blood cells in PNH. These defects can result in clinical symptoms including intravascular hemolysis, thrombotic events and bone marrow failure. The hemolysis is caused by increased susceptibility of red cells to complement-mediated lysis, as demonstrated *in vitro* by the acidified serum lysis test or Ham´s test, which is the classical diagnostic test for PNH. GPI-anchor deficient clones (PNH clones) can be detected more sensitively by flowcytometric screening.

PNH develops as a late complication in patients with SAA and MDS. In the absence of clinical signs of PNH, PNH clones can be noted by flowcytometry in 10% - 28% of adults with MDS at diagnosis and during follow-up (96;97). These PNH clones are more frequently observed in refractory anemia than in high grade MDS. While it is unknown, how many children with RC have a PNH clone, the presence of a PNH clone does not speak against the diagnosis of MDS. PNH, the clinical disorder with hemolysis and thrombosis is very rare disease in childhood, most often seen in adolescents.

Clinical course of RC and treatment approach

The therapeutic aim in children with MDS is cure and not palliation. Therefore, therapeutic efforts concentrate on HSCT rather than on novel therapeutics like anti-angiogenic therapy, farnesyl transferase inhibitors or DNA methylation inhibitors. HSCT from an HLA compatible related or unrelated donor early in the course of the disease is the treatment of choice for patients with RC and monosomy 7, 7q- or complex karyotype. For children with a normal karyotype or chromosomal abnormalities other than monosomy 7, 7q- or a complex karyotype, and absence of transfusion dependency or neutropenia a watch and wait strategy can be appropriate. If cytopenia necessitates treatment, current therapy options include HSCT with either myeloablative or reduced intensity preparative therapies. Some patients will respond to immunosuppressive therapy (IST) with cyclosporine A (CSA) and anti-lymphocyte globulin (ATG).

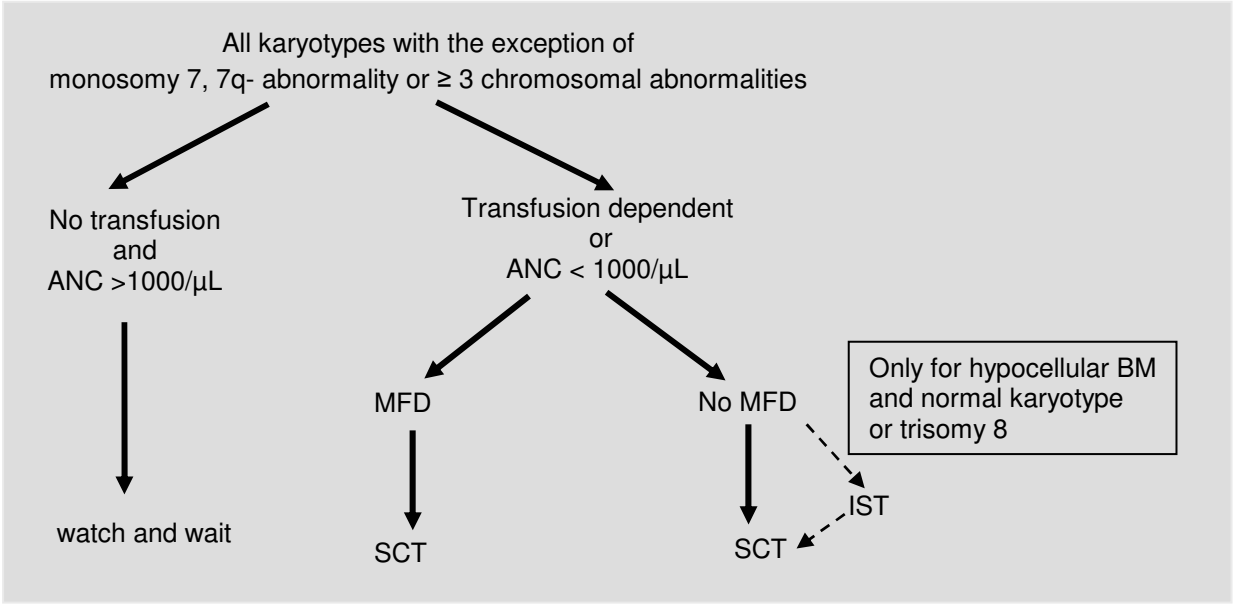


Figure 12 Algorithm for management of refractory cytopenia in the absence of monosomy 7, 7q- abnormality or complex karyotypes

Allogeneic HSCT in RC

***Myeloablative Preparative Regimen*:** Currently allogeneic HSCT remains the only confirmed curative treatment. In study EWOG-MDS 97 a preparative regimen consisting of busulfan, cyclophosphamide and melphalan was applied. In the interim analysis of October 2005 the probability of survival at 5 years for children was 76% for MFD and 83% for UD transplants (figure 13) (p=n.s.). Chromosomal analysis had

revealed a normal karyotype, monosomy 7 or other abnormalities in 20, 12 or 5 patients, respectively; in 5 patients the karyotype was unknown. There were no relapses, all failures were treatment related.

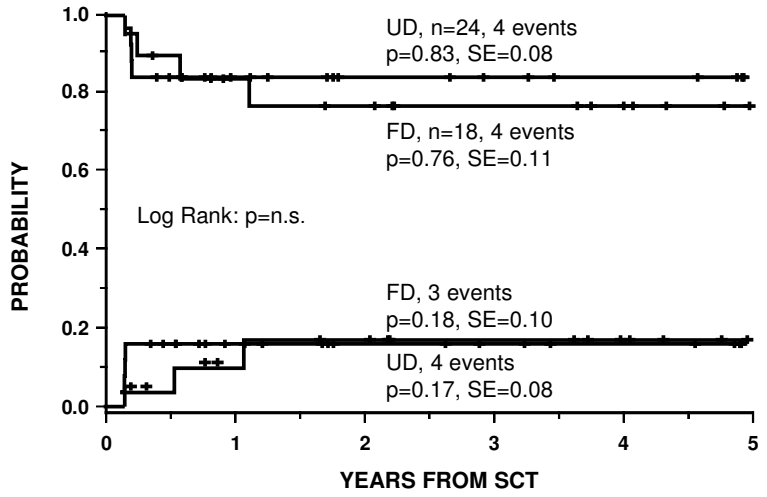


Figure 13: Event free survival and transplant related mortality in patients with refractory cytopenia transplanted with the preparative regimen of busulfan, cyclophosphamide, and melphalan

Reduced intensitiy conditioning: The favorable result with absence of relapse draws attention to late clinical complications of HSCT, especially infertility caused by BU. In a pilot study for patients with normal karyotype and UD, a reduced intensive regimen consisting of thiotepa (5 mg/kg/day x 3days), fludarabine (40 mg/m² x4 days) and ATG resulted in an EFS similarly to what has been observed with the ablative regimen (EWOG-MDS unpublished). In 19 children with a hypocellular bone marrow and normal karyotype the probablility of survival 3 years after HSCT from an UD was 83% (Figure 14).

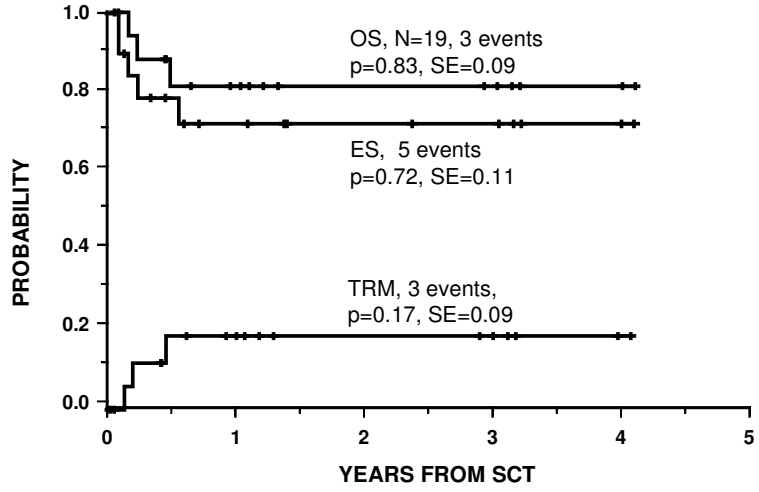


Figure 14: Overall Survival, Event free survival and transplant related mortality in patients with a hypocellular bone marrow and normal karyotype

Immunosuppressive therapy (IST) in RC

Initiating events of MDS are thought to occur at a genetic level of the hematopoietic stem cell. However, early bone marrow failure can result at least in part from T-cell mediated suppression of hematopoiesis. Studies in adults demonstrated that 34% - 50% of MDS patients have clinically relevant responses to IST (98;99). In a pilot study of EWOG-MDS, IST according to the German protocol for SAA was applied [ATG (0.75ml/kg/day for 8 days), prednisolone (1mg/kg/day, day 1-14, then taper till day 28), CSA (5 mg/kg/day for > 6 months), and G-CSF (5µg/kg/day depending on neutrophil count)]. In an interim analysis (October 2005), 18 of 29 patients (69%) responded to IST (currently complete response: n=9, partial response: n=10, toxic death n=1). Response was generally observed within 3-6 months. There was no obvious correlation between the degree of neutropenia (ANC < 200/µl [n=6], 200 - 500/µl [n=9] or > 500/µl [n=14]) and response to IST. Data on long term follow-up are not yet available, and it is currently unknown whether IST can result in sustained responses in a substantial number of children with RC. It is however, reasonable to assume that survival after 5 years will not be superior to what has been reported for AA and IST. In a recent report from the German Pediatric SAA study patients with SAA and an ANC > 200µl had an 5 year survival rate of 81% (100).

Current guidelines for therapy of RC

Therapy options in RC are dependend on karyotype, peripheral blood counts and bone marrow cellularity.

1. Patients with **monosomy 7, 7q-, or complex karyotypes** should be transplanted soon after the diagnosis is established (generally within 3 months). The recommended preparative regimen is myeloablative consisting of busulfan, cyclophosphamid and melphalan (details see EWOG-MDS RC-SCT 2006)
2. Patients with all **other karyotypes** can be followed according to a watch and wait strategy if their ANC is > 1000/µl and there is no need for transfusions.
3. If these patients have an ANC < 1000 /µl or are transfusion dependent they will require therapy.

3.a. In case of hypocellularity of the BM

- HSCT with reduced intensity from a sibling or unrelated HLA matched donor (8/8 or 7/8 antigens) is recommended (details see EWOG-MDS RC-SCT 2006).
- Therapy with IST can be a treatment option (details see EWOG-MDS RC IST 2006) for patients with normal karyotype or trisomy 8. For patients on IST who are non responders on day 120, an unrelated donor search is to be initiated. In the presence of non-response it is advised to transplant the patient as soon as a suitable donor is identified.

3b. Patients with normocellular or hypercellular BM are not candidates for IST, therapy consists of HSCT following a myeloablative preparative regimen.

Therapy options are outlined in the algorism depicted in Figure 12.

2.5.4 High Grade MDS

MDS with increased blast count comprises the MDS-subtypes RAEB and RAEB-T (table 1). In contrast to the original FAB classification (3), RAEB includes cases with up to 19% blasts in PB, and Auer rods are no longer used for classification. The international prognostic scoring system (IPSS) for MDS in adults has recommended subdivision of RAEB according to the BM blasts into BM blasts 5-10% (RAEB I) and 11-20% (RAEB II) (101). This scheme warrants further investigation in pediatrics.

High grade MDS and de novo AML

The separation of MDS with increased blast count from *de novo* AML remains challenging and thresholds of blast counts, whether set at 20% or 30%, are arbitrary. Assuming that the underlying genetic changes between MDS and *de novo* AML are different and therapy approaches will differ, the distinction between these entities becomes important. *De novo* AML is a chemo-sensitive disease characterized by balanced translocations, while the typical genetic changes in MDS, typically resistant to chemotherapy, are numerical aberrations. Patients with recurrent cytogenetic abnormalities typically associated with AML, e.g. t(15;17) (PML/RARα), t(8;21) (AML1/ETO), inv(16)(CBFβ/MYH11), t(9;11) (MLL/AF9), should be diagnosed and treated as *de novo* AML regardless of the blast count (102). The only chromosomal abnormality which may be regarded as marker of MDS-like biology is monosomy 7 (Table 14).

MDS progressing to disease with BM blast counts > 30% is referred to as myelodysplasia-related AML (MDR-AML). For monosomy 7, it is unknown, whether cases evolving from MDS to MDR-AML have the same biology than cases diagnosed as AML with monosomy 7. In AML studies, patients diagnosed as AML with monosomy 7 have a lower response rate to chemotherapy (9;10) and a higher relapse rate (103) compared with AML without -7.

It should be emphasized, however, that most MDS patients have a blast percentage < 20% at diagnosis, while the vast majority of children with *de novo* AML present with a frank leukemic BM. Therefore, the blast count can be considered a surrogate marker for the underlying biology of the disease in most cases (Figure 15).

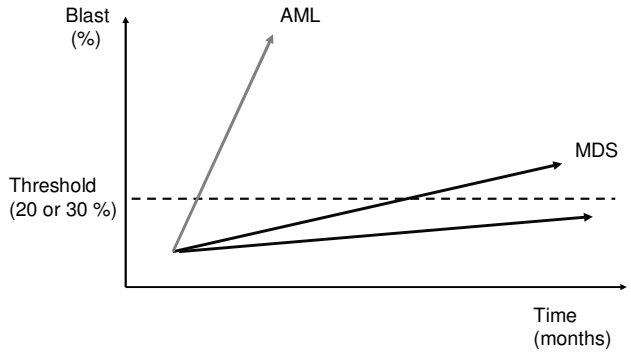


Figure 15 Disease progression in *de novo* AML and MDS
Modified from (104)

For patients with an ambiguous blast count, organomegaly, CNS infiltration or chloroma are indicative of de *novo* AML (figure 16). In patients presenting with a BM blast percentage > 20% and no clinical or cytogenetic changes characteristic of MDS or *de novo* AML, it is recommended to repeat the BM examination after 2 weeks. If the blast count has increased to ≥ 30% the patient most likely has *de novo*-AML. If the blast count is stable over an arbitrary period of 4 weeks the diagnosis of RAEB-T can be made.

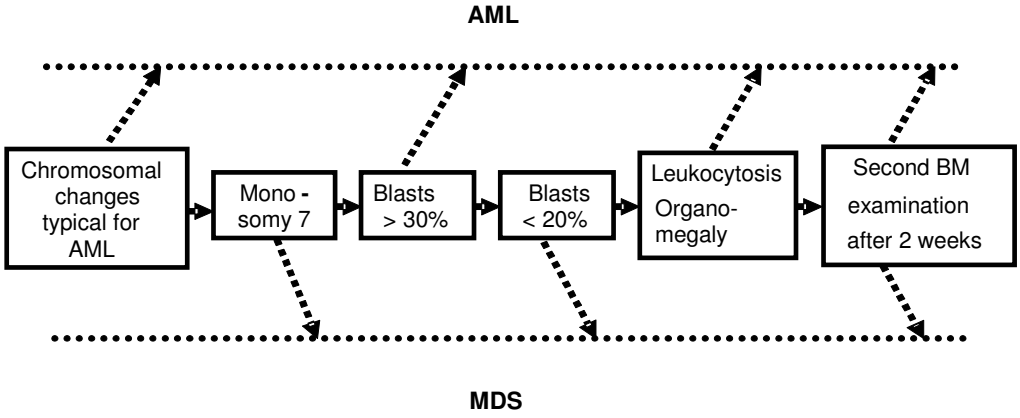


Figure 16 Algorithm for discrimination of *de novo* AML and MDS by blast count, monosomy 7 and clinical characteristics

In contrast to *de novo* AML, leukemic cells in MDS infiltrate the central nervous system (CNS) in rare cases only. A mere 1% of the patients in EWOG-MDS 98 exhibit blasts in the CNS. Cranial nerve palsy was not observed. However, at the time of diagnosis, a diagnostic lumbar puncture is recommended. In the absence of blasts, CNS therapy is not warranted.

Therapy in high grade MDS

Allogeneic HSCT is the treatment of choice in high grade MDS and can rescue a large proportion of children. Whether intensive chemotherapy prior to stem cell transplantation should routinely be employed is highly controversial. Data from EWOG-MDS (EWOG-MDS trial 97, 88 patients, preparative regimen with busulfan 16 mg/kg, cyclophosphamid 120 mg/kg and melphalan 140 mg/m²) indicate that AML-type therapy prior to the grafting procedure does not prolong survival. With a median observation time after SCT of 35 mo. the EFS at 5-yrs. was 68% and 46% for SCT from MFD and UD, respectively (EWOG-MDS, Interim analysis, 2005). Overall, in the patients transplanted for RAEB/ RAEB-T/ AML the EFS at 5 years were 0.50 ± 0.08. Toxicity of the procedure and relapse rate contributed equally to the number of events. There were no significant differences in relapse incidence among the patient groups with RAEB and RAEB-T (highest FAB-type prior to SCT), but patients with MDR-AML experienced a higher relapse rate. Therefore, intensive AML-type therapy prior to HSCT is not recommended for patients with high grade MDS.

Current guidelines for therapy in MDS with increased blast count

Allogeneic HSCT should be performed as soon as possible. The current HSCT trial of EWOG-MDS utilizes a preparative regimen consisting of busulfan, cyclophosphamide and melphalan. Intensive chemotherapy prior to HSCT is not recommended.

2.5.5 MDS after Chemo- or Radiation Therapy

Hematological, therapy-related secondary MDS (tMDS) after chemo- or radiotherapy can manifest as RC, high grade MDS, or CMML (105). Between 7% to 18% of MDS cases in children are tMDS (38;70). The pathophysiology of the development of tMDS is supposed to be similar in children, adolescents and adults. It likely depends on complex interactions between three kinds of factors: (1) The effect of the primary leukemogens, i.e., topoisomerase II inhibitors and alkylators; enhanced by (2) additional facilitating treatment-related risk factors like irradiation, asparaginase, and thiopurines; modified by (3) host factors, for example CYP3A4 polymorphisms (106). In children and adolescents, the classical distinction between alkylator-type (107) and topoisomerase II inhibitor-type (108) tMDS does neither exist clinically (EWOG database, unpublished data) nor cytogenetically (109).

There is little information on clinical and cytogenetic characteristics of tMDS in children and adolescents. The largest series published, a retrospective study from Japan reporting on 24 patients, found a higher proportion of abnormal karyotypes (often complex aberrations) and a worse prognosis compared to primary MDS (110). HSCT improved survival (111).

The following data result from an interim analysis of study EWOG-MDS 98 (April 2005, unpublished): tMDS occurred most frequently after ALL (34%), followed by tMDS after solid tumors outside the CNS (27%) and after CNS tumors (20%). In some ALL patients signs of tMDS can already be noted during maintenance therapy. They include low platelet counts, prominent monocytosis, and unusually high hematological toxicity of maintenance therapy. These clinical features can persist for months before blasts emerge, and the CMML-like picture converts to frank high grade MDS. This observation corresponds to the short interval between diagnosis of the primary malignancy and tMDS (median 3.3 years; range, 0.5 to 11.1). Autoimmune phenomena like skin rashes or effusions are sometimes noted at presentation or during clinical course of tMDS, and may require steroid treatment.

Suspected tMDS after primary AML is a diagnostic dilemma. Even if there is strong myelodysplasia and the course of the hematological disease is relatively stable over prolonged time, the disorders should be considered relapse of *de novo* AML with a “smouldering” course. Like in AML relapse, patients should receive intensive therapy for remission induction prior to HSCT.

In the absence of an increased blast percentage, the morphological diagnosis of tMDS may be difficult. Conventional cytogenetics may be helpful in demonstrating an abnormal clone (see Table 14). Early diagnosis of tMDS is vital for cure. Because many patients suddenly deteriorate after a relatively stable course, HSCT should be performed as soon as possible, preferably within 3 months from diagnosis. One of the unresolved problems is relapse of the primary malignancy after HSCT for tMDS.

Current guidelines for therapy in tMDS

Diagnostic procedures should be performed as soon as a suspicion of tMDS arises. If a curative therapy approach is chosen the patient should receive an allogeneic HSCT as soon as possible. Intensive therapy prior to HSCT is not recommended. The preparative regimen prior to HSCT is the same than for primary high grade MDS, currently consisting of busulfan, cyclophosphamide and melphalan.

2.5.6 MDS after Bone Marrow Failure Disorders

2.5.6.1 MDS in congenital bone marrow failure disorders

A number of inherited disorders are characterized by BM failure associated with or without somatic abnormalities. They may present in infancy or thereafter, some cases as late as adulthood. The BM failure may involve all 3 lineages [e.g. Fanconi anemia (FA), dyskeratosis congenita (DC)] or a single lineage [e.g. severe congenital neutropenia (SCN), Diamond Blackfan anemia (DBA)]. Most congenital BM failure disorders (CBMF) are associated with a high preposition for secondary MDS and secondary acute myeloid leukemia. The definition of secondary MDS in CBMF without increase in blast count is difficult, because myelodysplastic features are often observed in the absence of clonal evolution. We recommend diagnosing secondary MDS after CBMF only when the criteria outlined in Table 15 are fulfilled.

Secondary MDS in CBMF is defined by a consistent acquired BM abnormality such as

- increase in blasts
- acquired chromosomal abnormality
- increasing bone marrow cellularity in the presence of blood pancytopenia

Table 15 Definition of secondary MDS in congenital CBMF

The incidence of secondary MDS in the different CBMF is summarized in Table 16. FA is the disorder most frequently followed by hematological malignancy. Among congenial neutropenias, MDS/AML develops in SCN and Shwachman-Diamond syndrome (SDS), but neither in cyclic neutropenia nor glycogen storage disease Type Ib. In patients with congenital amegakaryocytic thrombocytopenia, cancer preposition is not documented.

CBMF differ in their natural history and display different disease-specific problems. Consequently, the primary disorder has an impact on treatment strategies of secondary MDS (e.g. the indication of HSCT

and the preparative regimen), and a uniform therapy approach to all CBMF patients is not advisable. In general, patients with CBMF should be registered in national or international registries/studies, and MDS/AML should be treated according to guidelines of the respective registries/studies.

	Incidence (% of total cohort)		Age at the time of MDS/AML	Ref.
	hematological malignancy	non-hematol. malignancy		
FA	7-15%	5-9%	median 11-14 yrs	(112-115)
DC	2.7%	8.8%	10, 22, 27 and 29 yrs	(116)
SCN	6%	-	median 8 -13 yrs	(117;118)
Cyclic neutropenia	0%	-	-	(117;118)
SDS	5-33%	-	median 12-27 yrs	(118-120)
DBA	0%-5.2%	1.5-1.6 %	median 22 yrs	(121-123)

Table 16 Incidence of hematological and non-hematological malignancy in patients with congenital bone marrow failure disorder

Fanconi Anemia

Fanconi Anemia (FA) is an autosomal recessive disorder characterized by BM failure, short stature, hyperpigmentation, developmental abnormalities such as radial and thumb defects, microcephaly, and renal anomaly (124;125). FA often manifests during early childhood. Progressive BM failure affects platelets first, followed by pancytopenia (115;126). Increased HbF and high MCV are commonly observed (115). Because up to 30 % of patients show no apparent congenital defects and hematological and clinical features are highly variable, clinical diagnosis can be difficult. Therefore, FA should be considered and excluded in all children with hypoplastic cytopenias. The standard screening test for FA is based on the characteristic hypersensitivity of FA cells to cross-linking agents such as mitomycin C (MMC) and diepoxybutane (DEB). At least 12 complementation groups are known to date with FANCA being the most common group; genes for 8 groups have been cloned (FANCA, C, D2, E, F, G, L, and BRCA (127;128). The FA proteins cooperate in a common FA/BRCA pathway responding to DNA damage (124;129).

The median survival of patients with FA is approximately 30 years (112). Treatment with androgens can improve pancytopenia in many patients. HSCT is the only treatment for establishing normal hematopoiesis. Because FA patients are extraordinarily sensitive to the chemo-therapeutic agents and radiation, the dosage of conditioning agents have be reduced to avoid fatal toxicities. HSCT with MFD can result in a cure rate for BM failure of 60% - 90% (130-135), results with UD are less favorable with 30% - 60% survival (132;136-138) Currently, regimens with reduced intensity using fludarabine are being investigated (139). Post HSCT, there is a high incidence of malignancy, particularly cancers of the head and neck.

FA patients are at increased risk for hematological and non-hematological malignancies (112). The cumulative incidence for myeloid neoplasia at the age of 40 years is 33% (113). In about one third of patients, the initial diagnosis of FA is established at the time of myeloid neoplasia, without an apparent preceding AA (115). Consequently, FA has to be excluded in all patients with MDS (excluding some cases of therapy-related MDS). As outlined above, the morphological diagnosis of MDS in FA is difficult in the absence of an increased blast percentage and should be restricted to cases with increasing cellularity and ineffective hematopoiesis. Despite these morphological limitations it is believed that most cases of myeloid leukemia in FA are preceded by an MDS phase (115). With the underlying chromosomal instability cytogenetic abnormalities are frequently observed. The relationship between fluctuating cytogenetic clones and progression to myeloid neoplasia is not always clear. The most common aberrations are monosomy 7 and trisomy 1 (140). Gains of the chromosomal segment 3q26q29 have been shown to be an adverse risk factor (141). Although only HSCT offers the potential for cure in patients with FA and myeloid neoplasia, the survival is generally poor.

Dyskeratosis Congenita

DC is an inherited disease characterized by the triad of abnormal skin pigmentation, nail dystrophy and mucosal leukoplakia (116). The median age for the onset of mucocutaneous abnormalities is 6-8 years and nail change occurs first. Dental, gastrointestinal, genitourinary, neurological, ophthalmic, pulmonary and skeletal abnormalities have also been reported. DC is caused by inherited defects in the telomerase complex. Autosomal dominant DC (10% of patients) is associated with mutations in the RNA component of telomerase, hTERC, while X-linked DC is due to mutations in the gene encoding dyskerin, a protein implicated in both telomerase function and ribosomal RNA processing (142).

Immunological abnormalities can occur in a subgroup of patients, and fatal infection can be attributable to immunodeficiency rather than AA (143). BM failure is the major cause of early mortality; the median age for the onset of pancytopenia is 10 years. By age 40 years, 90% of DC patients have at least a single cytopenia, 50% have pancytopenia (116). In a number of cases AA preceded the onset of abnormal skin, dystrophic nails, or leukoplakia. HSCT for pancytopenia is generally less successful than in FA because of fatal pulmonary and vascular complications. In a report from the DC registry, malignancies developed in 13 (8.8%) out of the 148 patients, including 4 cases of MDS, which developed at age 10, 22, 27 and 29 years (116).

Severe Congenital Neutropenia

SCN (Kostmann´s Syndrome) is a CBMF disorder characterized by severe neutropenia. It is associated with a maturation arrest at the promyelocyte/myelocyte level in most cases (117). The majority of SCN patients respond to G-CSF with increasing numbers of neutrophils and absence of infections. Most SCN patients have inherited a heterozygous mutation in the gene encoding neutrophil elastase (144).

SCN patients are at risk for developing MDS/AML regardless of presence or absence of G-CSF therapy. According to data from the SCN International Registry (SCHIR) the cumulative incidence of myeloid

neoplasia is 12 % - 13% without a statistically significant relationship between age at onset of MDS/AML and dose or duration of G-CSF therapy (117). In contrast, a French study group showed that high time-averaged and cumulative G-CSF doses are associated with high risk of progression to MDS/AML (118).

Acquisition of a G-CSF receptor point mutation precedes MDS/AML in > 80% of cases. These nonsense mutation result in the truncated C-terminal cytoplasmic region that is crucial for G-CSF induced maturation. SCN patients with an acquired G-CSF receptor mutation have a high risk of developing MDS/AML, time to MDS/AML progression varies from months to years (117;118). Almost all patients with transformed disease show a cytogenetic abnormality such as -7, 7p-, and +21(118), and half have activating *RAS* mutations.

HSCT is indicated for SCN patients without response to G-CSF. In patients with MDS/AML HSCT is the only curative therapy option and should be performed as soon as possible. Like in other forms of high grade MDS, intensive therapy prior to HSCT is not recommended. The outcome of HSCT in patients with SCN and myeloid neoplasia has been significantly inferior to those without an excess blast count (145). Therefore, some investigators advocate HSCT as soon as a clonal cytogenetic abnormality or a G-CSF receptor mutation is detectable. Annual BM examination with cytogenetic and molecular analysis is recommended in all SCN patients.

Shwachman-Diamond Syndrome

SDS is an autosomal recessive disorder characterized by BM failure, exocrine pancreatic insufficiency and short stature (119). It is the second most common cause of inherited pancreatic insufficiency after cystic fibrosis and ordinarily presents in early childhood as failure to thrive. SDS can be associated with skeletal abnormality (metaphyseal chrondro dysplasia mainly affecting the hip), psychomotor retardation, liver and renal dysfunction.

SDS is caused by inactivating mutations of the SBDS gene located on chromosome 7 (146). The SBDS gene encodes a 250 amino acid protein of unknown function; indirect genetic evidence suggests that SBDS may be involved in RNA processing. The diagnosis SDS is usually made clinically by exocrine pancreatic insufficiency (low serum trypsinogen and isoamylase, abnormal 72 hour fecal fat content) and characteristic hematological abnormalities.

Intermitted (2/3 of patients) or persistent (1/3) neutropenia is the common hematological abnormality in SDS (119). In addition, neutrophils show impaired chemotaxis. About 20 % of patients present with pancytopenia (119;120). Increased HbF is noted in most of patients. While pancreatic insufficiency will improve later in life in about half of the patients, BM failure persists and clonal disease may develop. MDS is documented in 5% - 33% of SDS patients, but the lifetime risk of MDS/AML is still unknown (120;147). Chromosome abnormalities can be detected in 7% - 29% of SDS patients. Isochromosome 7q is a fairly specific change in SDS that may be related to the mutant SBDS gene on 7q11. The clinical course of SDS patients with abnormal cytogenetics is highly variable, and stable disease or disappearance of the abnormal clone have been observed in some patients (147). Therefore, some investigators recommend a

watch and wait strategy with careful monitoring for SDS patients with chromosome abnormality and stable disease. Signs of disease progression like increase in blasts, recurrent infections, transfusion dependency etc should prompt HSCT (147).

Supportive care, pancreatic enzyme replacement and G-CSF for severe neutropenia are the standard of care for SDS. HSCT is indicated in patients with severe pancytopenia or MDS/AML. Recently, the EBMT reported on 21 patients, who underwent HSCT for BM failure (n=13), MDS/AML (n=4), or unknown reason (n=4). Overall survival was 65% (76% in patients with BM failure, 25% in MDS/AML) (148).

Diamond-Blackfan Anemia

DBA is a congenital hypoplastic pure red cell aplasia generally diagnosed within the first year of life (121;149). In about 40% of patients with DBA diverse physical abnormalities are noted. Patients with DBA show features characteristic of fetal hematopoiesis, including persistent macrocytosis, elevated fetal hemoglobin and erythrocyte deaminase (eADA) level. DBA patients can develop neutropenia or thrombocytopenia, although infants often display thrombocytosis at diagnosis (149). The majority of patients with DBA respond to steroid therapy, non-responders will have to undergo transfusion therapy.

Recent molecular studies have identified mutations in the gene encoding the ribosomal protein RPS19 on chromosome 19 in 25% of patients with DBA. In another subset of patients, linkage analysis has identified another locus on chromosome 8p in association with DBA (150). There are, however, other cases of DBA that are linked neither to the RPS19 gene nor to the locus on 8p, implying the involvement of undefined genetic defects in the cause of DBA (150). There are no genotype-phenotype correlations.

DBA carries a preposition for malignancy. A review of the literature reveals 10 cases of hematological malignancy and 19 case of other malignancy (5 of them are osteogenic sarcoma) (122). Data from the DBA registries of North America and Europe indicate a incidence of frequency of malignancy of 1.3% - 1.7% (121) (149). In contrast, in a single center study from Boston with the cumulative risk for the development of MDS/AML at age 30-40 years was 23% (123).

Current guidelines for therapy of MDS in congenital bone marrow failure disorders

Only HSCT offers cure for patients with CBMF disorders and MDS. Indications for HSCT differ between disorders. In FA, DC and possibly SDS, the underlying genetic defect will exclude treatment with standard myeloablative preparative regimens like those applied for primary high grade MDS. Treatment should be in accordance with the recommendations of the respective National/International Studies on that particular CBMF disorder. Patients with CBMF and MDS should also be registered in EWOG-MDS 2006.

2.5.6.2 MDS in Acquired Bone Marrow Failure Disorders

MDS after acquired aplastic anemia

The prognosis of severe AA (SAA) has dramatically been improved by HSCT and IST. Although IST with ATG and CSA allows recovery of autologous hematopoiesis in 70-80% of children (151-153), patients remain at risk for clonal disease. Previous reports show that in the absence of HSCT the cumulative incidence of myeloid neoplasia is 10% - 16% (94;151;154-161). A similar incidence has been reported for hepatitis associated SAA(162). In contrast, MDS/AML after HSCT is not observed.

Because the distinction between hypoplastic RC and aplastic anemia has been controversial (see 2.1.4.2 Morphology), one may assume that incorrect diagnoses may increase the incidence of subsequent clonal disorders in SAA. The initial diagnosis of SAA has to be questioned when secondary MDS/AML is diagnosed within a few months after presentation. The median interval from the diagnosis of SAA to the onset of MDS/AML is 37-56 months. Risk factors for clonal disease in SAA are not well clarified, but some reports showed that older age, splenectomy, multiple courses of IST, duration of G-CSF therapy, and unresponsiveness to IST are related with high risk of clonal evolution (94;154;163).

In some series of AA patients, cytogenetic studies at the time of diagnosis have demonstrated clonal abnormalities in 4% -12% patients (164;165). Numeric aberrations (trisomy 8, trisomy 6 and monosomy 7) were the most frequent abnormalities observed. When conventional cytogenetics is unsuccessful, FISH analysis for monosomy 7 and trisomy 8 may be helpful. Patients with trisomy 8 often respond to IST, and the prognosis is favorable. In contrast, monosomy 7 generally implies a poor prognosis with a high likelihood of transformation to leukemia. Currently there is no consensus as to whether certain chromosome abnormalities such as trisomy 8 or monosomy 7 exclude the diagnosis SAA. In cases of clearly secondary MDS/AML, abnormalities of chromosome 7 are most commonly detected (94;151).

Current guidelines for therapy of MDS after acquired bone marrow failure disorders

HSCT without prior intensive chemotherapy is recommended as soon as the diagnosis is established. Current preparative regimens of EWOG-MDS utilize a preparative regimen consisting of busulfan, cyclophosphamid, and melphalan.

3 Study Objectives

3.1 Rationale of the Study

The aim of the study is to improve the accuracy of diagnosis for children and adolescents with MDS by a standardized review of morphology and standardized cytogenetic and molecular analyses.

3.2 Primary Objectives

The primary objectives of the study are:

- To evaluate the frequency of the different subtypes of MDS in childhood and adolescence by a standardized diagnostic approach
- To evaluate the frequency of cytogenetic and molecular abnormalities:
  Specifically using array-CGH to evaluate the frequency of subtle chromosomal imbalances, i.e. gains and losses of defined chromosomal regions, and amplifications.
  Specifically using mFISH to identify unknown chromosomal aberrations, particularly subtle translocations involving new candidate genes, and to better define chromosomal breakpoints.

3.3 Secondary Objectives

The secondary objectives of the study are:

- To assess survival for children and adolescents with MDS and JMML
- To evaluate relapse rate, morbidity and mortality in children with MDS and JMML treated by HSCT

4 Investigational Plan

4.1 Study Design

This is a prospective, non-randomized, multicenter study. Around 260 patients will be enrolled. Patients who are identified as being eligible according to the Inclusion and Exclusion Criteria will enter the study. The study will take place at centers in 12 European countries.

The final analysis of the trial will be performed at the beginning of 2011.

Based on the assumption of a recruitment of 65-70 patients per year, the total study duration will be approximately 5 years.

Timetable:
Start of Study: 01.01.2006
Enrollment: 48 months
End of Study: Fourth quarter 2010
Data available: First quarter 2011
Study report: Second quarter 2011

4.2 Participating Centers

The center of Freiburg, Germany, is the Coordinating Study Center (CSC). Patients for this study are recruited in European centers which are located in the following countries: Austria, Czech Republic, Denmark, Finland, Germany, Iceland, Italy, the Netherlands, Norway, Poland, Sweden and Switzerland. Centers and the locally responsible investigators are listed in Appendix 1. All study centers are tertiary care centers with sufficient experience in clinical trials in pediatric oncology. In every country a Regional Coordinator is responsible for forwarding the national data to the Coordinating Study Center (CSC). For the Nordic Countries (Denmark, Finland, Iceland, Norway, Sweden) there is only one EWOG-MDS Regional Coordinator.

The EWOG-MDS Regional Coordinators are solely responsible for conducting the study in their country/study group. They assure that diagnostic BM and PB samples of all study patients are evaluated

by a member of the EWOG-MDS Morphology Board. Cytogenetic and molecular studies as well as immunophenotyping are generally performed in national reference laboratories and are evaluated by members of the EWOG-MDS Committee on Molecular Genetics and the Regional Coordinators. Data are collected regionally by the Regional Coordinator and transferred on a 3-monthly schedule to the Coordinating Study Center (CSC) in Freiburg, Germany.

4.3 Number of Patients

Approximately 260 patients are expected to enter the study during the study period.

4.4 Central and Reference Laboratories

Every region has its own national reference laboratories for pathology and oncogenetic studies. They are listed in the section 1.

5 Study Population

5.1 Study Population

Patients will only be allowed to enter the trial if they or their caretakers provide written informed consent about their participation (following full explanation of the trial) and if the physician has verified that the patient meets all of the Inclusion Criteria and none of the Exclusion Criteria.

5.2 Inclusion Criteria

Patients enrolled in this study are to meet the following Inclusion Criteria:

- Confirmed diagnosis of MDS or JMML (morphology, cytogenetics)
- Myeloid leukemia of Down syndrome (patients aged > 6 years).
- Age: age less than 18 years

The caretakers will have given their written informed consent to participate in the study. Consent will be documented by the caretaker's dated signature which will be also signed and dated by the investigator in the participating center. If the patient is able to understand the meaning and consequences of the study and its procedures his/her written informed assent is also needed. Written informed consent has to be obtained prior to enrollment into the study.

5.3 Exclusion Criteria

Patients who do not fulfill the Inclusion Criteria may not be included into study.

Specific Exclusion Criteria are:

- Denied informed consent and/or assent by caretakers/patient.

- Fanconi anemia (diagnosed by chromosomal breakage, G_2 cell cycle arrest, Western blot or mutational analysis) or other congenital bone marrow failure disorders (diagnosed clinically or by disease specific germ line mutations) without secondary MDS. Secondary MDS in congenital bone marrow failure is defined by a consistent acquired bone marrow abnormality as
  a) increase in blasts
  b) acquired consistent chromosomal abnormality
  c) increasing bone marrow cellularity in the presence of blood pancytopenia
- Shwachman syndrome or Fanconi anemia with a single aberration not typical of MDS.
- Translocation characteristic for *de novo* AML like
  t(8;21)(q22;q22) [AML1/ETO fusion gene]
  t(15,17)(q22;q12) [PML/RARα rearrangement]
  inv(16)(p13q22) [CBFβ/MYH11rearrangement]
- Myeloid leukemia of Down syndrome (patients aged < 6 years).
- Participation in another interventional study within the last 4 weeks (except for therapy optimizing studies in cancer or bone marrow failure, diagnostic protocols).

6 Enrollment and Patient Registration

6.1 Time of Enrollment

It is planned to start enrollment 01.01.2006. Enrollment is planned to be finished 31.12.2009.

6.2 Minimal Requirements for Enrollment

The following minimal requirements have to be fulfilled before a patient can be registered in the study:

- Patient identification by name and sex
- Date of birth
- Date of diagnosis
- White blood cell count
- Bone marrow with differential count
- Peripheral blood with differential count

6.3 Mode of Enrollment

The caring physician has to receive the written informed consent of the patient before any study specific examination. The Regional Coordinator investigator will register the patient on a **patient identification list** located at the regional coordinating center.

The Regional investigator has to record the following information about the patient on the patient identification list:

- full name
- date of birth

- gender
- Inclusion and Exclusion Criteria of the study fulfilled yes/no

The patient receives a consecutive **patient identification number** which is given by the Coordinating Study Center (CSC) in Freiburg. This patient identification number consists of 2 letters, 3 digits and an extra code number:

1. The first two letters are the country code.
2. The next three digits stand for successively included patients. As the patients have been registered to the MDS-study for many years, the patient number will continue in that region as a successive number.
3. All patients recruited from 1st May 2006 receive as extra coding the digit "_06" to mark them as belonging to the EWOG-MDS study 2006.

7 Methodology

7.1 Study Schedule

A patient with suspected MDS undergoes the diagnostic procedures as outlined in section 7.3.1. Once entered the study and assigned to a defined subgroup a defined data set is recorded for each patient every 12 months for minimum of 1 and a maximum of 5 years.

7.2 Written Informed Consent

A written Informed Consent will be obtained from caretakers and possibly also patients prior to participation to the study.

7.3 Patient Evaluation

Every patient undergoes a specific diagnostic evaluation. In regular time intervals (at least 12 months) follow-up information is requested. The diagnostic procedures are part of the routine examinations. No additional punctures are performed for the study. For study purposes 5 ml of peripheral blood and 5 ml of bone marrow are collected at the same timepoints of routine examinations.

7.3.1 Initial Diagnostic Procedures

The investigator will record the following information and will perform the following diagnostic evaluations:

7.3.1.1 Demographic Assessments:

- Inclusion/Exclusion Criteria: Compliance with Inclusion and Exclusion Criteria must be verified and recorded on the source documents and in the CRF prior to enrollment of the patient into the study.
- Demography: date of birth, sex.
- Medical History: complete past and current medical conditions (including neurofibromatosis, hepatitis, clinical PNH)

- Family history
- Transfusion history (yes/no)
- Disease Details
- Concomitant Medication/ Therapy: steroids, immunoglobulins, growth factors, immunosuppression, chemo- and radiotherapy

7.3.1.2 Physical Examination:

- Height (cm), weight (Kg), head circumference (cm) with percentiles respectively
- Birth weight
- Congenital abnormalities, xanthomas, café au lait spots, lymphadenopathy, hepatosplenomegaly, cranial nerve palsy, respiratory tract symptoms; signs of neurofibromatosis type I

7.3.1.3 Screening Laboratory Tests:

- Hematology (hemoglobin, hematocrit, MCV, reticulocyte count, CBC with differential). Hemoglobin electrophoresis (Hb A_2, Hb F)
- Serum Chemistry: LDH
- Direct and indirect Coombs-test
- Serology (IgG, IgM): EBV, CMV, HHV-6, HSV, Parvovirus B19, HBV, HCV, HIV

7.3.1.4 Special Examinations:

All MDS and JMML:

- Evaluation of morphology on PB smear, BM aspirate and BM biopsy (BM biopsy optional for JMML)
- Standard Cytogenetics
- FISH analysis for complex karyotypes
- Comparative hybridization
- HLA-Typing

Refractory Cytopenia: Diagnostic Procedures

- Second bone marrow biopsy mandatory (reference pathology) within 3 months prior to any therapy; for transfusion-dependent or neutropenic patients consider second biopsy within 2 weeks
- Exclusion of Fanconi anemia by G_2 cell cycle arrest, chromosomal breakage test, Western blot or mutational analysis
- Exclusion of pancreatic insufficiency: Stool elastase or serum trypsinogene and serum isoamylase
- PNH clone (for details see EWOG-MDS RC IST 2005 protocol)

Differential diagnosis in refractory cytopenia: diagnostic procedures to consider if reasonable

- Viral screening: PCR for CMV, EBV, parvovirus, HHV 6 in bone marrow and peripheral blood
- Screening for vitamin deficiencies: folinic acid, vitamin B 12, copper, iron
- Exclusion of Shwachman syndrome: stool elastase, transaminases
- Exclusion of Pearson syndrome
- Screening for metabolic diseases: venous blood gases, screening for urinary organic acids, lactate,

glucose

- Exclusion of immunological disorders: complement factors, subpopulations of lymphocytes, direct and indirect antibodies against surface of neutrophils, organ-specific and non-organ-specific auto-antibodies

JMML: Diagnostic Procedures

- Buccal swab or skin biopsy for germline DNA
- *PTPN11* / *RAS* mutation (somatic and germline)

  For patients without any of the above mutations or monosomy 7 but with the typical clinical and morphological picture of JMML the following examinations have to be performed:

- *BCR/ABL* rearrangement (Philadelphia chromosome)
- Colony assay: spontaneous growth or GM-CSF hypersensitivity

High Grade MDS: Diagnostic Procedures

- Second bone marrow biopsy.
- Exclusion of Fanconi anemia by G_2 cell cycle arrest, chromosomal breakage test, Western blot or mutational analysis
- Diagnostic lumbar puncture

According to the obtained results patients will be assigned to one of the following groups:

1.1) JMML

1.2) JMML-like picture in Noonan-Syndrome

2) MDS

2.1) Refractory cytopenia

2.2) MDS with increased blast count

2.3) Secondary MDS

7.3.3 Laboratory Tests/Special Examinations During Study Period

For each patient the follow-up data is recorded at 12 month intervals for the whole duration of the study (minimum one year, maximum 5 years).

7.3.3.1 Patients Receiving Chemotherapy or No Therapy

The following data is recorded for this subgroup of patients:

- Treatment other than AML-therapy: weight, length; begin/end/ongoing therapy, drug, dosage
- Splenectomy (date), splenic irradiation
- AML-therapy, remission
- Hematological data:

  Every bone marrow examination along with peripheral blood counts.

  In the absence of a bone marrow examination the first peripheral blood count indicating progress.

  In the absence of progress the last peripheral blood count.

  - complete blood count with differential (including reticulocytes), transfusion history

- bone marrow with differential

– Cytogenetics: Every analysis before HSCT (metaphase and FISH)

– Date of last examination
– Karnofsky score
– Survival: stable disease, complete remission (CR), relapse (date, site, kind of relapse)
– Secondary malignancy (date of diagnosis, diagnosis)
– Death (date, cause)

7.3.3.2 Patients Undergoing HSCT

For patients with HSCT the recorded data corresponds to the EBMT-follow-up but adding MDS-specific topics.

– Initial data at transplantation until day 100:
  - Pre-Transplant Treatment: subclassification at primary treatment, therapy, complete remission
  - At Transplantation: clinical features, subclassification at transplantation, status of disease, bone marrow investigation, cytogenetics, hematological values before start of conditioning regimen
  - Allograft data: patient, donor, graft manipulation, conditioning, transplantation, engraftment, acute GvHD
  - Status at day 100: best response of disease; complications within the first 100 days (infection/ non infection related); additional treatment post-transplant; donor leukocyte infusion (DLI)
– Follow-up year one:
  - Complications after day 100: infection/ non infection related, chimerism studies, immunosuppressive therapy, chronic GvHD (onset, treatment, resolution), relapse, treatment of relapse, follow-up (disease status, death)
– Follow-up yearly from year two:
  - complications (infection/ non infection related); events sine last follow-up (chronic GvHD, chimerism, first relapse or progression after transplant, secondary malignancy, death); disease status

7.4 Duration of Study Participation

All patients who have entered the study are followed for a maximum of 5 years according to this protocol. They will most probably be followed for a longer period according to a study following in 2010. There are no criteria for planned drop-outs.

For patients lost to follow-up the last known data should be retrieved and recorded.

7.5 Use of Patients' Material

Peripheral blood, bone marrow aspirate, bone marrow biopsies and fibroblasts are retrieved from the patient for diagnostic procedures. This material may be partially stored for study purpose. A separate consent has to be obtained from the patient/ caretakers if this material is stored for later research work. Every patient/ caretaker may state if he wants to be informed about the research results. No patient is

undergoing an additional invasive procedure just to gain material for research. It has to be stated that the material will only be used for doing research on the disease and that genetic analysis will only concern the biology of the disease. The research is not commercial. The material is stored nationally. Material will be sent to other laboratories anonymously. The right to perform analysis lies within the national coordinator's research group.

7.6 Asservation of Patients' Material

At diagnosis, prior to HSCT and at relapse material form peripheral blood and bone marrow will be retrieved and stored for research purposes. If there are other diagnostic bone marrow examinations at other time points (prior to HSCT), the material will be handled the same way.

The following material will be retrieved:

- 8 smears from PB
- 8 smears from BM
- at least 5 ml of heparinized PB
- at least 5 ml of heparinized BM

The material will be stored as follows:

- Smears from PB and BM will be frozen at -80 °C.
- Cells in PB and BM will be separated by a Ficoll procedure, mononuclear cells (MNC) will be frozen according to standard procedure
- DNA and RNA from MNC will be extracted according to standard procedure and stored adequately
- DNA from granulocytes in the Ficoll pellet will be extracted after red cell lysis and stored adequately

To obtain germ-line DNA a buccal swab is to be obtained from all patients with JMML at diagnosis. Prior to HSCT a skin biopsy is recommended to allow for culturing of fibroblasts. Optimally, the biopsy is taken when general anesthesia is required for therapeutic purposes (e.g. with central line placement).
Cytogenetic material is stored at the reference laboratory.
Material should be stored at the attention of the regional coordinator.
Details concerning laboratory procedures can be found in the study manual.

8 Data Handling and Reporting

8.1 Reporting and Recording of Data

Follow-up information is asked for on a 12 month basis. Data is requested every four months. The follow-up list with the due patients will be sent automatically from the Coordinating Study Center (CSC) in Freiburg to the Regional Coordinator. The Regional Coordinator will stay in contact with the local center and will return the completed form within 3 months. HSCT patients will be documented the same way as non-HSCT patients.

Once a year, the Coordinating Study Center (CSC) in Freiburg will provide the Regional Coordinators with the data base of their respective national data.

All protocol-required information collected during the study must be entered and signed by the investigator, or designated representative in the CRF. The data has to be complete, clear, accurate, legible, and plausible. Missing examinations or data have to be marked along with a justification/explanation.

Documentation of data on any study paper forms should be made with a black pen and well readable note.

Potential corrections are to be made by the investigator or an authorized person (according to the centers' signature form).

Corrections are to be made according to the GCP-guidelines, i.e.:

- the version that has to be corrected will be crossed in a way that it is still readable
- the correct version will be written above or beside the first version
- the correction (or any remark) will be marked with date, initials, and a justification by the investigator or an authorized person.

8.2 Data Management and Handling

Once the diagnosis of MDS and JMML is confirmed, the registration form is completed by the local center and sent to the regional Coordinator. The Regional Coordinator is obliged to register the patient within 3 months of diagnosis to the Coordinating Study Center (CSC) in Freiburg. The data will be transferred into the target data base at the CSC. Data will only be entered in the data base by advice of the regional coordinator.

Advices are:

1. completed MDS forms
2. information about events, status of the patient sent by mail or email

The CSC will not enter any information received by local centers.

For yearly follow ups the Coordinating Study Center (CSC) will contact the regional coordinator in January.

For state of the art the data manager will send a list of all patients diagnosed since 01.07.98 (start of EWOG-MDS) by email. On this list the patients who need an actual FUP form (clinical course, event) will be marked. HSCT patients will be documented the same way as non-HSCT patients.

For security reasons the email will be sent with a password-based data protection.

Although the CSC would appreciate to receive the completed forms until the end of March, forms can be sent at any time.

To inform the national coordinators about changes in the national data the CSC will send an email every 4 months (January, May, September). This email will include:

1. Patient's name, birth date, presumptive diagnosis for material that has been sent to Freiburg for in vitro or molecular studies
2. Events the CSC heard of by email from local centers
3. Forms the CSC received within the last 4 months

9 Quality assurance

In the framework of the clinical trial quality-control and quality-assurance will be guaranteed by a data supervision board a steering committee and an authorized supervision.

9.1 Data Supervision Board

The Data Supervision Board consists of all Regional Coordinators and two independent members. Their duty is to supervise the Coordinating Study Center (CSC) at least every two years. Correct data handling from the CRF to the database is their main area of monitoring. They will have access to all study documents including the trial master file and the standard operating procedures.

9.2 Steering Committee

All Regional Coordinators are part of the Steering Committee in their role as principal investigators for their countries/ regions. The Committee meets once a year in one of the member countries. Study problems and interim data and analysis are discussed. The Committee guarantees the scientific value and actuality of the study.

9.3 Authorized Supervision

During the course of the study, the study assistant located at the Coordinating Study Center (CSC) will have the duty of an authorized supervisor together with the study coordinator and the principal investigator.

The authorized supervisor will stay in regular contact with the study centers to get information about the compliance with the study protocol requirements, consensus of the data in the CRF and the originals, the updated patient identification lists, and the archiving system. The contacts will be mostly done by e-mail and telephone and are supposed to control the progress of the trial, realize problems early and potentially solve them.

The authorized supervisor will review the case report forms of the patients in the study to make certain that the items have been completed and that the data provided are plausible and obtained in the manner specified in the protocol.

The authorized supervisor signs to handle all data that are under professional secrecy or show the patient's identity confidentially and will use the data only for the purpose the patient gave informed consent for. No data disclosing the identity of patients should leave the study center as a result of the monitoring procedure.

9.4 Data Verification

There will not be a source data verification in the sense of controlling the recorded data of the CRFs in regard to correctness and completeness compared to the original data.

There will be an inherent plausibility check contained in the principal data set. If primary data is suspected to be incorrect a query form is going to be generated. The local investigator has to respond to the query and provide written information as soon as possible. The authorized supervisor will also generate written queries if data entered into the data base does not seem plausible. A query trail will document all changes to the data set.

9.5 Auditing Procedures

In addition to the quality assurance procedures outlined above, audits can be done in the framework of the auditing system according to the ICH-GCP-guidelines. It can be an inspection initialized by authorities (even after the study has been completed).

In the context of an audit it will be checked if planning, conduction and analysis of a clinical trial are in agreement with the law and the requirements of the ICH-GCP-guidelines.

This includes controlling of the data keeping and organization of the study center as well as controlling of laboratories and the original documents. The aim of auditing is to assure that all results and conclusions written in the final report can be drawn from the raw data.

All persons who are auditing are obliged to sign to handle data that are professional secrecy or show the patient's identity confidentially and use the data only for the purpose the patient gave informed consent for.

The investigator will be informed in time about planned audits. The investigator is required to inform the Coordinating Study Center (CSC) immediately of an inspection requested by a regulatory authority.

10 Statistics

10.1 Trial Design

This is a prospective, non-randomized, multicenter study. The aim of the study is to improve the accuracy of diagnosis for children and adolescents with MDS by a standardized review of morphology and standardized cytogenetic and molecular analyses. The primary and secondary objectives are defined in chapters 3.2/ 3.3.

10.2 Patients Included in the Analyses

Based on the assumption of a recruitment of 65-70 patients per year and a recruitment time of 5 years around 260 patients will be enrolled. Patients who are identified as being eligible according to the Inclusion and Exclusion Criteria will enter the study (see 5.2). The study will take place at centers in 12 European countries.

The final analysis of the trial will be performed in autumn 2010.

10.3 Statistical Analysis

The final analysis of the EWOG-MDS 2005 study will be performed within six months after the end of the study. One Interim analysis two years after study start is planned.

Overall survival (OS) is defined as the probability of survival from date of diagnosis or the beginning of treatment with only death as event; children alive were censored at their last follow-up. Event-free survival (EFS) is defined as the probability of survival following treatment without any events including disease progression, relapse or death of any cause. Graft rejection or failure were considered events. Survival times will be calculated according to the Kaplan-Meier method and comparisons between probabilities in different patient groups will be performed using the log-rank test. Relapse incidence (RI) is defined as the probability of having a relapse before time t; death without experiencing a relapse is considered a competing event. On the contrary, transplant-related mortality (TRM) is defined as the probability of dying without previous occurrence of a relapse, which was the competing event. Both these probabilities will be estimated as cumulative incidence curves. All results will be expressed as 5-year probability or 5-year cumulative incidence (%) and 95% confidence interval (95% CI).

Univariate analyses of EFS, RI and TRM will be performed. For multivariate analyses, the Cox proportional hazard regression model will be used, including in the models all the variables with $P < 0.1$ in univariate analysis.

Statistical analyses will be done using the statistical software SPSS (Statistical Package for Social Sciences) and SAS (Statistical Analysis System). All analyses will be documented and saved. The transfer of the data from the database hold in the Coordinating Study Center (CSC) will be performed after checking the data for plausibility. The procedures of later transformation of data during the statistical analysis will be validated by check of random samples. The statistical analysis will be done under the supervision of the responsible statistician.

11 Conditions for protocol amendments

11.1 Changes in Protocol

Any change or addition to this protocol requires a written protocol amendment. No change to the protocol may be made without the joint agreement of the Principal Investigator and the Regional Coordinators. Any amendment has to be signed by all parties before the change of or addition to the final protocol is effective.

If an amendment significantly affects the safety of the patients, the scope of the investigation or the scientific quality of the study, it should be formally approved by the Ethics Committee, and communicated to the regulatory authority, as required by local law.

After approval, an amendment becomes an integral part of the protocol. All Regional Coordinators will be informed immediately after approval by the Coordinating Study Center (CSC) in Freiburg.

The Principal Investigator is authorized to decide the discontinuation of the study due to relevant medical or administrative reasons.

The above-mentioned requirements do not preclude any immediate action taken by the investigator in the interests of the patient's safety. In the case where such an immediate change to the protocol is implemented and the principal investigator should be notified immediately.

12 Ethical and Legal Considerations

The study will be conducted in accordance with the Declaration of Helsinki (Appendix 2), the current revision of ICH Topic E6 (Appendix 3), Guideline for GCP: "Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95), and the legal requirements of each participating country in its valid version. It is mandatory that all considerations regarding the protection of the patients be carried out in accordance with the Declaration of Helsinki. The data protection will be granted according to the local law.

To ensure compliance the investigator agrees, by written consent to this protocol, to fully cooperate with compliance checks by allowing access to all documentation by authorized individuals.

12.1 Patient Information and Informed Consent

All patients must sign and personally date an approved Informed Consent Form after receiving detailed written and verbal information about the reason, the nature and the methods of the study. The information comprises also information about the patient insurance and the conditions subsequent to this policy. The Informed Consent complies with regulatory requirements.

The written informed consent must be obtained before the entry of the patient into the study!

Furthermore, the patient must be notified that participation is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her right to the most appropriate medical treatment or affect the doctor/patient relationship. A written patient information leaflet will be handed to the patient, whose contents have to be discussed with the patient by the investigator. The investigator will provide the patient ample time and opportunity to inquire about details of the study and to decide whether or not to participate in the study. All questions about the trial will be answered to the satisfaction of the patient. The patient should be given sufficient time to read and understand the statement him/herself before signing his/her consent and dating the document. Neither the investigator nor the trial staff will coerce or unduly influence a patient to participate or to continue to participate in the trial.

Personal information will be treated as strictly confidential and will not be publicly available.

The patient will receive a copy of the written informed consent once signed, and the original version of the informed consent has to be kept in the investigator file.

12.1.1 Patient Withdrawal

A patient may withdraw from the study at any time, at his or her own request, for any reason, specified or unspecified, and without penalty or loss of benefits to which the patient is otherwise entitled. Patients who are withdrawn from the study will not be allowed to re-enter later.

Date of discontinuance, all recorded results at this time and, if known the reasons for discontinuance are to be documented in the CRF. If possible a final examination has to be done.

12.1.2 Premature discontinuation of the study by the investigator

The responsible investigator has the right to exclude patients from the study if the diagnosis of MDS/JMML turns out to be wrong. Centers may be excluded from the study if there is no appropriate data documentation or data transmission.

12.2 Disclosure and Confidentiality

Throughout this study all data will be treated confidentially.

Throughout the whole data-recording and -analysis patients will be identified only by a patient identification number and medication number - never by their full name, initials and date of birth. The legal provisions by the respective Laws will be heeded.

The **investigator is responsible** for keeping sufficient information for every patient (name, date of birth, internal clinic number, patient identification number, gender, informed consent), in order to identify the patient. According to the ICH-GCP-guidelines these documents **(Patient Identification List)** have to be archived for at **least 15** years.

By conducting this study, the investigator agrees that he and his staff will maintain all information in strict confidence. The investigator is requested to insist on similar confidentiality for this information from other bodies such as the Hospital Scientific Committees and Ethic Committees/Institutional Review Boards that have been consulted by the investigator. Study documents provided by (protocols, CRFs and other material) will be stored appropriately to further ensure their confidentiality. It is understood that the confidential information provided to the investigator will not be disclosed to others without direct written authorization from the patient. Such information will not be communicated by telephone to potential or enrolled patients or to any other individual.

The evaluation of the study will be done by the Coordinating Study Center (CSC) in Freiburg.

12.3 Independent Ethics Committee (IEC) / Institutional Review Board

Prior to implementation of this study, the protocol, Patient Information Sheet and the proposed Informed Consent must be reviewed and approved by the Ethics Committee. Signed and dated approval by the Ethics Committee must be obtained by prior to study initiation and patient enrollment.

The investigator is committed in accordance with local requirements to inform the IEC of any emergent problem and/or protocol amendments.

12.4 General Disclosure Duty

Before starting the clinical trial authorization from the national authorities has to be obtained. All regional authorities will be informed.

According to the law of participating centers in other countries the corresponding authorities will be informed correctly as well.

12.5 Insurance

The aim of this study is the collection of epidemiological data based on a standardized diagnostic approach and not the investigation of clinical or pharmacological properties of drugs. The study is

therefore exempt from clinical trials insurance coverage according to law. Patients are covered by the public liability insurance of their hospitals.

13 Study Documents and Archiving of Records

13.1 Investigator's File

The investigator's file contains all essential and relevant documents (e.g. regulatory and study documents, correspondence with ethics committee and general information). The investigator's file has to be accessible during audits and authorized inspections. After finishing the trial the investigator's file has to be kept within the study center according to the ICH-GCP-guidelines and legal regulations at least for 15 years.

13.2 Documentation of Patient Data

13.2.1 Case Report Form (CRF)

The investigator or his representatives (according to the signature form) document the data currently and continuously on the trial relevant CRF. If possible, documentation should be made immediately. No section of the CRF is to be left blank without an appropriate explanation by the Investigator.

All data have to be entered in an accurate, plausible and complete way by the investigator or a person authorized by him.

13.2.2 Documentation of data in the patient's file

The investigator documents in the patient's file the participation to the study, the frequency of the trial visits, all relevant data of disease, all examinations and diagnostic evaluations and concomitant treatment.

13.2.3 Patient Identification List

According to the ICH-GCP-guidelines the investigator has to keep a patient identification list which allows an accurate relation of the patient's identity to his/her enrollment into the trial.

The following information will be recorded on the patient identification list:

- full name
- date of birth
- gender
- Inclusion and Exclusion Criteria of the study fulfilled yes/no

13.3 Archiving of Records

According to the German law, all investigational records must be retained at the investigational site for a minimum of 15 years. Patient files and other source documents must be kept for the maximum period of time permitted by the hospital/institution, but for not less than 15 years.

Originals of all documentation and copies of outgoing correspondence concerning the study will be stored and retained in a safe area in the Master File at the Coordinating Study Center (CSC) in Freiburg.

14 Administrative Considerations

14.1 Financing

The study is supported by public grants. There is no support from the industry. The main supporter in Germany is the Deutsche Krebshilfe.
In every country the study is supported by national public grants.

14.2 Final Report

The Coordinating Study Center (CSC) in Freiburg will perform the statistical analysis and present a written statistical and medical report. The final report of the trial will be written by the Coordinating Study Center (CSC) in Freiburg in cooperation with the Regional Coordinator.

Except for cogent reasons nobody will pass on data to third persons unless all parties agreed upon the analysis and interpretation of the results.

14.3 Publication of Study Results

Any formal presentation or publication of data collected as a direct or indirect result of this trial will be considered as a joint publication by the investigators. It requires the agreement of the Principal Investigator and all Regional Coordinators. Authorship will be determined by mutual agreement.

The results of the study may be presented during scientific symposia or published in a scientific journal only after review and written approval by the Principal Investigator and all regional coordinators. Investigators participating in multicenter studies must agree not to engage in presentations based on data gathered individually or by a subgroup of centers before publication of the first main publication, unless this has been agreed otherwise by all other investigators.

Every clinical study should be published to avoid the problem of 'Publication Bias'.

At least within 1 year of termination of the study, a manuscript for publication has to be jointly finalized.

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16 Protocol Approval

Coordinating Investigator (LKP)		15.03.2006
	Signature	Date
Study-Coordinator (Germany)		15.03.2006
	Signature	Date
Statistician		15.03.2006
	Signature	Date

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17 Investigator Statement

Regional Coordinator:

Trial center and country:

COMMITTMENTS

By signing this document, I agree to conduct the trial as outlined in the protocol and in accordance with the Declaration of Helsinki (Appendix 2) as well as all applicable government regulations and GCP. I declare:

1. I am well qualified by scientific training and experience to conduct investigational studies in the clinical area of the proposed trial and I am affiliated with a recognized medical school or with an independent institution recognized for its excellence.
2. I shall provide information to all staff members involved in the trial about their obligations as described in this document.
3. I shall submit the protocol, Informed Consent form/Information Sheet and other required documentation to the EC for review and approval.
4. I shall make no changes to the protocol without formal amendment (signed by the principal investigator and submitted to the EC for notification/approval), except when necessary to protect the safety, the rights or welfare of patients. In this last case I will inform the principal investigator of the change.
5. I shall require Informed Consent from each patient prior to enrollment into the study. The Informed Consent shall be documented by use of a written consent form approved by the national authority and the EC.
6. I shall complete the Study's Case Report Form (CRF) in a timely and legible manner.
7. I shall maintain accurate source records (hospital or other institutional records), which will support the data entered into Case Report Forms and I shall maintain these as specified by the protocol.
8. I shall allow monitoring visits by representatives of the supervising boards as needed.
9. I shall allow any Regulatory Authorities to inspect the facilities and pertinent records at reasonable times and in a manner which ensure patients confidentiality.

Following completion of the study, the data may be considered for reporting at a scientific meeting and/or for publication in a scientific journal. A copy of the manuscript or abstract will be provided to all main regional co-investigators for review before submission to a scientific journal for publication and/or a scientific meeting selection committee for oral or poster presentation.

_____ _____

Investigator Signature Date

The original of this page is to be filed in the Central Trial Master File. A copy is kept by the Investigator.

Appendix 1 List of participating study centers

Appendix 2 Declaration of Helsinki

http://www.wma.net/e/policy/b3.htm

Appendix 3 ICH-GCP-Guidelines

http://www.ich.org

E6 Good Clinical Practice: Consolidated Guideline

E11 Clinical Investigations of Medicinal Products in the Pediatric Population

Appendix 4 CRFs

Appendix 5 Invoice Forms

Appendix 6 Patient Information/ Informed Consent

Appendix 7 Approval by Ethics Committee