STANDARD CLINICAL PRACTICE RECOMMENDATIONS FOR ACUTE MYELOID LEUKEMIA (AML) IN CHILDREN AND ADOLESCENTS

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INTRODUCTORY PAGES

- Acute Myeloid Leukemia (AML) in Children and Adolescents
- Recommendations for diagnostics, therapy and follow-up care of children and adolescents with Acute Myeloid Leukemia (AML) 2020 V1

This document has been developed by:

Prof. Dr. med. Dirk Reinhardt
Chair: AML-BFM Study Group
*Director EuPAL foundation (**Eu**ropean **P**ediatric **A**cute **L**eukemia), Utrecht, NL*
University Hospital Essen, Pediatrics III
Hufelandstr. 55, 45147 Essen, Germany
Dirk.reinhardt@uk-essen.de
reinhardt.dirk@aml-bfm.de

Asocciate Professor Mareike Rasche
University Hospital Essen, Pediatrics III
Hufelandstr. 55, 45147 Essen, Germany
Mareike.Rasche@uk-essen.de

Dr.med. Evangelia Antoniou
University Hospital Essen, Pediatrics III
Hufelandstr. 55, 45147 Essen, Germany
Evangelia.antoniou@uk-essen.de

Prof. Dr. med. Jan-Henning Klusmann
University Hospital of Frankfurt
Theodor-Stern-Kai 7, 60590, Frankfurt, Germany
jan-henning.klusmann@kgu.de

Prof. Dr. med. Michael Dworzak
Chair: AML-BFM Study Group Austria
St. Anna Children's Hospital
Kinderspitalgasse 6, 1090 Vienna, Austria
michael.dworzak@ccri.at

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PREAMBLE

This recommendations/guideline include diagnostics, treatment and supportive care.

The following recommendations summarize the experiences of treatment in pediatric / adolescent AML within the last 4 decades. The describe treatment is very intensive and induces a high rate of toxicities.

These guidelines are based in the environment of a fully developed interdisciplinary management including the availability of every treatment option concerning

- Access to imaging including MRI, CT, ultrasound at any time
- Access to PICU
- Access to surgery (Pediatrics, Neurosurgery)
- Access to all drugs
    - antibiotics
    - anti-viral drugs
    - antimycotic drugs
- Access to all kind of blood products (erythrocytes/ platelets/ PBS / Immunoglobulin etc.)

Treatment centers must be experienced in the therapy of AML in children and at least three pediatric hematologists/oncologists should be available.

Therefore, if the experience of treating AML and conditions are not given, consider the transferal of patients to a more experienced center.

This even more important in case of allogeneic stem cell transplantation!

If this is not possible, consider the reduction of intensities (reduction of dosages by 1/3) and/or contact the authors

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Disclosure:

In any case, the treating physician remains responsible for the application of any procedures and treatment to children and adolescents.

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1. BACKGROUND AND RATIONALE

1.1 Background

Pediatric acute myeloid leukemia (AML) is a rare type of childhood cancer, encompassing a heterogeneous group of diseases, whose classification is based on lineage-commitment and genetics[1]. The incidence in childhood is approximately seven new cases per 1 million children/ adolescents (<15 years) annually[2]. Although the prognosis of this disease remains poor if compared to acute lymphoblastic leukemia, overall survival significantly improved over the past 30 years. This improvement was achieved by the use of intensive chemotherapy and post-remission treatment with additional anthracyclines and high-dose cytarabine or hematopoietic stem cell transplantation (HSCT) for those subgroups with high risk of recurrence. A significant contribution was also given by advances in supportive care allowing the administration of intensive therapy with low morbidity and mortality rates. Along with the progress of therapeutic strategies, a significant evolution in genomic research has resulted in important steps towards better stratification of patients, with a consequent risk-directed therapy. Given that pediatric AML is relatively rare, multicenter clinical trials have been required for continued progress and multiple international cooperative groups have contributed to an evolving treatment strategy[2] (Table 1). As a result, several groups now achieve complete remission (CR) rates of 80–90%, relapse rates of 30–40%, event-free survival (EFS) rates of 50%, and overall survival (OS) rates of nearly 70%[3].

Study	Years of Enrolment	Eligible Age (Years)	Number of Patients	CR (%)	EFS (%) and median follow-up	OS (%) and median follow-up
AML-BFM 2012 registry	2012-2017	<18	324	90	60 (5-year)	81 (5-year)
AML-BFM 2004	2004–2010	<18	611	89	All: 55 (5-year); L-DNR: 59; Ida: 53	All: 74 (5-year); L-DNR: 76; Ida: 75
DB AML01	2010-2014	<17	112	94	53 (3-year)	74 (3-year)
AIEOP AML 2002/01	2002–2011	≤18	482	87	55 (8-year)	68 (8-year)
ELAM02	2005-2011	<18	438	89	57 (4-years)	73 (4-years)
JPLSG AML05	2006-2010	≤18	443	na	54 (3-year)	73 (3-year)
SJCRH AML02	2002–2008	≤21	216	94	63 (3-years)	71 (3-years)
NOPHO AML 2004	2004–2009	≤18	151	92	57 (3-years)	69 (3-years)
NOPHO AML 2012	2012–202	≤18	151	92	57 (3-years)	78 (3-years)
COG AAML0531	2006–2010	≤29	1022	87	GO: 53; No GO: 47 (3-year)	GO: 69; No GO: 65 (3 year)
MRC AML12	1995–2002	<16	529	92	54 (10-year)	63 (10-year)

Table 1: Results of clinical trials by cooperative Study Groups [2]

AIEOP, Associazione Italiana di Ematologia e Oncologia Pediatrica; **BFM,** Berlin-Frankfurt-Münster Study Group; **COG,** Children's Oncology Group; **CR**, complete remission; **DB**, Dutch Belgian; **EFS**, event-free survival; **GO**, gemtuzumab ozogamicin arm; **Ida**, idarubicin arm; **JCACSG**, Japanese Childhood AML Cooperative Study Group; **L-DNR**, liposomal daunorubicin arm; **MRC**, Medical Research Council; **NOPHO,** Nordic Society of Pediatric Haematology and Oncology; **OS**, overall survival; **SJCRH**, St Jude Children's Research Hospital.

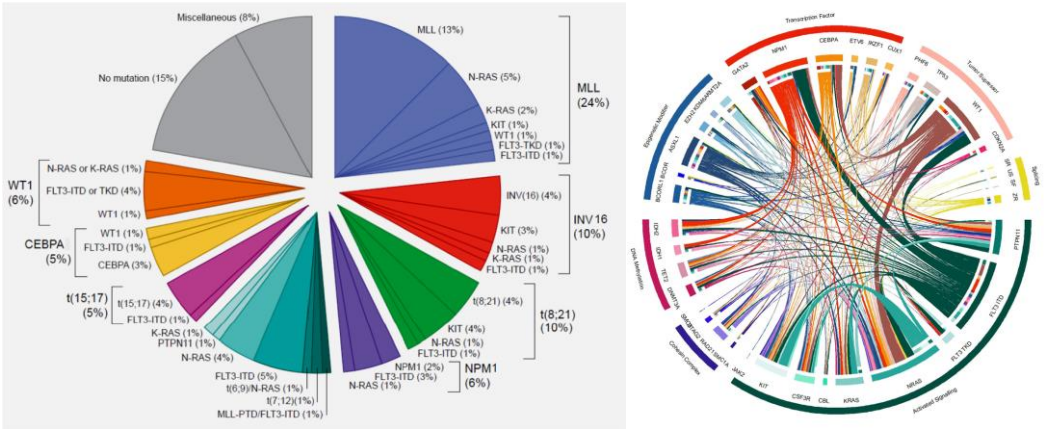
AML is caused by the uncontrolled clonal proliferation of immature myeloid cells, which can display a progenitor-like (i.e., $CD34^+/CD38^+$) or stem cell-like (i.e., $CD34^+/CD38^-$) immunophenotype. This uncontrolled clonal growth leads to the depletion of healthy hematopoiesis in the bone marrow with the

clinical consequences of granulocytopenia (infections, sepsis), thrombocytopenia (bleeding) and anemia (dyspnea, decreased performance). In addition to gene translocations such as t(8; 21), t(15; 17) or inv(16), numerical changes such as trisomy 8, monosomy 7 or complex changes involving more than three recurrent chromosomal aberrations in one clone can be found. It was shown that these changes have a very important role in disease pathogenesis and also prognosis. With the advent of new molecular techniques, particularly Next Generation Sequencing (NGS), it has become apparent that even within one patient the disease may consists of genetically distinct subclones and the proportion of different clones can change over the course of the disease. In the NGS analysis, an average of 5 recurrent changes were detected per patient. Nearly all patients had at least one mutation in one of nine functionally defined groups of genes, critical for transformation:

- Activating mutations of signal transduction (*FLT3*, *KIT*, *KRAS*, *NRAS* etc.)
- Mutations of myeloid transcription factors (*RUNX1*, *CEBPA* etc.)
- Fusion genes involving transcription factors (*PML-RARA*, *MYH11-CBFB* etc.)
- Mutations of chromatin modifiers (*MLL1*, *ASXL1* etc.)
- Mutations in the cohesin complex (*SMC1S* etc.)
- Mutations in splicing factors (*SF3B1*, *U2AF1* etc.)
- Mutations in tumor suppressor genes (*TP53*, *WT1* etc.)
- NPM1 mutations
- Mutations in factors regulating DNA methylation (TET1, TET2, IDH1, IDH2, DNMT3B, DNMT1, DNMT3A).

Further studies showed that in about 50% of the patients at least one subclone was detectable besides the major clone; individual patients had up to three additional leukemia clones. This clonal heterogeneity could have a significant impact on the response to therapy or the development of recurrence.

In a comprehensive European collaboration on AML in children and adolescents, the frequency and prognostic relevance of many mutations have been presented (figure 1). These molecular changes may provide direction towards targeted individualized therapies using novel drugs with specific molecular targets.[1]



A **B**

Figure 1 A: Type I/II mutations in AML amongst those results relating to children in international, cooperative studies[4]. B: Cooperation of AML-associated mutations

External factors, known to have a carcinogenic effect, play a limited role in children. Only a small proportion of AML in children and adolescents develop AML as part of hereditary syndrome, most frequently transient leukemia (TL) and myeloid leukemia in children with trisomy 21 (ML-DS). [2, 5, 6]

1.2 Relapsed/ refractory AML

Nevertheless, newly diagnosed AML is a rare disease in children. The prognosis has improved considerably using intensive chemotherapy with or without stem cell transplantation. However, 5-10% of patients do not achieve first CR due to resistant disease (primary refractory AML), and 30-50% of CR patients relapse. Primary refractory AML and relapsed AML have a poor prognosis with a long-term OS of 36%[7].

For AML in first relapse, the prognosis mainly depends on the time to relapse, cytogenetic/molecular lesions and the early response to reinduction therapy. For early relapses, defined as within 1 year from initial diagnosis, the second CR rate is about 50%, and OS about 20%. For late relapses, defined as after 1 year from diagnosis, the second CR rate is 70-75% and the OS up to 50%. Multiple relapsed AML has an even worse prognosis. Several treatment schedules have been studied recently, and improvement seems feasible using new drugs and new drug combinations. Therefore, there is an unmet medical need for further development of new treatment options to rescue these children. Gemtuzumab ozogamicin (GO) holds potential in this respect. This observation is very important, since the use of anthracyclines is limited by long-term cardiotoxicity, which is a dose-dependent late effect of therapy. Clinical cardiotoxicity has been reported to occur in more than 10% of patients that were treated with higher cumulative doses of anthracyclines, and patients may die from this complication, or may need heart transplantation. The cytotoxic compound of GO is calicheamicin and is related to anthracyclines. However, because calicheamicin is linked to anti-CD33, it does not affect the cardiac muscle to the same degree as free anthracyclines.

1.3 The role of stem cell transplantation in AML

Allogeneic stem cell transplantation (HSCT) has proven to be potential curative therapy option in many indications. However, in AML, the role of HSCT in first complete remission is still a matter of investigation.

Depending on the underlying disease, fractionated total body irradiation (TBI) or Busulfan-based conditioning regimens are currently used, often combined with Cyclophosphamide (according to the European Group for Blood and Marrow Transplantation)[8 OBJ]. However, the risk of severe early and late toxicity related to standard myeloablative conditioning regimens for alloSCT is high [9 10 OBJ].

Therefore, there is a need for conditioning regimen with myeloablative, immunosuppressive and anti-leukemic efficacy, but with lower treatment related toxicity.

Several studies have shown that TBI-containing regimens do not offer any advantage in patients with AML[11, 12]. Busulfan-based regimens represent the «standard of care» in children with AML given allogenic HSCT. In the past, different drugs have been used in combination with busulfan, one of the most frequent combination used in Europe being Busulfan, Cyclophosphamide and Melphan (BuCyMel)[13–15].Despite its potent anti-leukemic effect, BuCyMel is associated with extramedullary toxicities, impacting on long term outcomes.

In recent years, treosulfan has been introduced to conditioning regimens for malignant and non-malignant disorders. Based on the given clinical experience in adults, treosulfan has been widely used as therapeutic alternative in pediatric HSCT conditioning regimens in the past years. A meta-analysis conducted by the EBMT with a total of 604 pediatric patients registered within the EBMT showed that treatment with treosulfan (dose in total: 42 g/m² within three consecutive days) was effective and well tolerated in children of all age groups with malignant as well as non-malignant diseases. The dose of treosulfan had no significant impact on all analysed safety and efficacy parameters[16–18].

1.4 Acute promyelocytic leukemia (APL, AML FAB M3, with and w/o t(15;17)/ PML/RARA)

Acute promyelocytic leukemia (APL, former FAB classification—M3) is now classified according to the WHO as "Acute myeloid leukemia with recurrent genetic abnormalities". Children with APL have good survival rates if they achieve remission. The clinical picture of APL is characterized by a rapidly increasing risk for bleeding due to often marked coagulation disorders and thrombocytopenia [19]. The highest risk for these patients is in the first few days after diagnosis, as most deaths (35%) occurred as a result of bleeding complications with signs of disseminated intravascular coagulation or sepsis in earlier trials. Early treatment with retinoids (all-trans-retinoic acid, ATRA) in the AML-BFM 93/98/04 studies were able to reduce these complications [20, 21]. Recent studies in adults have shown that combined therapy with ATRA and arsenic trioxide (ATO) leads to very good therapeutic outcomes and survival rates, while side effects and toxicities have been significantly reduced [22]. Experiences with children and adolescents with APL support this data [23]. Accordingly, it is recommended to treat APL with a combination of ATRA and ATO in children and adolescents. In patients with high-risk APL (defined as ≥10x10^9 leukocytes/L), cytoreduction is additionally performed with induction chemotherapy. In addition,

the high significance of minimal residual disease and molecular remission could be demonstrated in APL, so that the consolidation therapy and possible therapy intensifications are essentially based on the therapeutic response. The same applies to the occurrence of a molecular relapse.

Patients with acute promyelocytic leukemia have a high risk for bleeding complications at initial diagnosis. No lumbar puncture at diagnosis! For therapy and prophylaxis of bleeding complications, see appendix (emergencies).

1.5 Treatment related AML

Secondary/therapy induced acute myeloid leukemia are the most common secondary malignancies after cytostatic treated of malignant disease [24]. The prognosis is generally unfavorable, whereby the initial response rate is particularly low. Therapy should consider both the cumulative cytostatic dose — in particular that of anthracyclines — and the often already impaired regenerative capacity of the bone marrow. Children who achieved remission (no evidence of leukemia) were successfully treated with double induction followed by allogeneic stem cell transplantation[25].

With this background and dependent on cumulative anthracycline dose of previous treatment, children with secondary leukemia will receive treatment with the induction regimen only. If CR, CRp or at least no-evidence of leukemia (NEL) is achieved, an allo-SCT is recommended. Depending on the pre-treatment (cumulative anthracycline dose), therapy may also be carried out according to recurrence therapy.

In case of blast persistence (>5% prior to conditioning), an allo-SCT cannot be recommended to this patient group.

1.6 AML with multiple dysplasia / AML after myelodysplastic syndrome

According to the WHO classification of myelodysplastic syndromes (MDS) and AML, at least 20% blasts must be present in the bone marrow in order to be defined as AML [26]. In addition to the introduction of MDS-RAEB I (refractory anemia with excess of blasts; 5-10% blasts) and RAEB II (10–20% blasts), RAEB with transformation to AML (RAEB-T) was removed from the classification. Especially in pediatric oncology/hematology, this change was controversially discussed [27], as the determination of blastocyst concentration based on morphological enumeration represents an artificial and arbitrary limit.

To maintain international comparability, AML-protocols/registries are based on the current WHO classification and include all patients who meet the definition of AML. However, the therapy used should not be based solely on the concentration of blasts. In children with AML and a blast concentration of 15%-30%, the characteristics of the disease should be used for therapy stratification as follows:

The presence of relevant lymph node involvement or hepatosplenomegaly or rapid progression indicate AML [28]. If the general condition of the child allows, no therapy is initially administered with regular monitoring of the peripheral blood count. If the results of cytogenetics and molecular genetics do not allow classification, bone marrow cytology should be repeated after 10 to 14 days. In the case of progress with an increase of blasts to >30%, at this point, therapy should be initiated according to the standard protocol for *de novo* AML. For patients with a typical AML aberration such as t(8,21) or others, therapy according to standard protocol for *de novo* AML is recommended.

If blast concentration remains the same, a diagnosis of MDS-related AML should be determined taking into consideration the clinic, genetic findings and previous history.

In addition to a history of MDS and multilineage dysplasia (defined as the presence of 50% or more dysplastic cells in at least 2 cell lines, but only in the absence of NPM1 or biallelic CEBP A), the following list of cytogenetic abnormalities define AML with myelodysplasia-related changes:

- complex karyotype (three or more aberrations including at least one structural aberration))
- unbalanced abnormalities such as -7/del7q, del(5)/t(5q), i(17q)/t(17p), -13/del(13q), del(11q), del(12p)/t(12p), idic(X)(q13)
- balanced abnormalities such as t(11;16)(q23.3;p13.3), t(3;21)(q26.2;q22.1), t(1;3)(p36.3;q21.2), t(2;11)(p21;q23.3), t(5;12)(q32;p13.2), t(5;7)(q32;q11.2), t(5;17)(q32;p13.2), t(5;10)(q32;q21.2), t(3;5)(q25.3;q35.1) [29].

The aim for patients with MDS-related AML (e.g. with genetic alterations monosomy 7 or complex karyotype) is to achieve a blast reduction with reduced intensity treatment. If intensive induction has already been initiated, the therapeutic element should be discontinued. There is a lack of studies that offer detailed information regarding the best treatment schedule for these patients [30]. The therapy (selection of agents/intensity) can only be determined individually.

In this case (MDS-related AML), a reduced AML cytoreductive chemotherapy (such as one cycle of induction, as in ML-DS), a maintenance therapy or the use of azacytidine should be considered. The aim is to reduce the pre-transplant blast-load in the bone marrow. The search for a suitable stem cell donor should be initiated.

1.7 Acute biphenotypic leukemia, bilineage leukemia, acute leukemia of ambiguous lineage (ALAL)/Mixed phenotype acute leukemia (MPAL)

ALAL or biphenotypic acute leukemia (BAL) cannot be clearly diagnosed by morphology and/or immunophenotyping. The disease is characterized by the following:

detection of myeloperoxidase (cytochemistry, less reliable intracellular immunophenotyping) or monoblastic differentiation (NSE, CD11c, CD14, CD64, lysozymes)

- In combination with T-cell differentiation markers (cytoCD3, CD3)

or

- B-cell differentiation markers (cytoCD79a, CD22, CD10)

or

- morphological proof of two independent blast populations (bilineal)

or

- switch to another lineage before CR is reached or at relapse (“lineage switch“).

Overall, the prognosis appears to be less favorable than that of AML. This is particularly attributable to the higher proportion of patients with a translocation t(9;22), an unfavorable 11q23 aberration, and hyperleukocytosis [31].

Therapy should be initiated according to the predominant genetic, cytochemical, immunological, and morphological characteristics, in other words,

a dominant lymphatic ALAL should be treated according to ALL protocol (TEL/AML1 pos., strong cytoCD3/ cytoCD22/ cytoCD79 positive) and

a dominant myeloid ALAL (highly MPO, peroxidase, esterase positive) should be treated according to the standard recommendations of AML-treatment [31]. A recent international cooperative study identified treatment strategies in childhood ambiguous lineage leukemia. It includes treatment recommendations (including immunophenotypic markers and genetic characteristics) and should be taken into consideration for treatment decision of MPAL [32].

Patients with a lineage switch from ALL towards AML should be considered as high-risk leukemia (allo-SCT in CR1).

In the rare cases of an acute undifferentiated leukemia (AUL) (i.e. blast populations that do not fulfill the criteria of B, T, or myeloid lineages), treatment should be initiated according to an AML directed protocol, including the indication for allo-SCT.

1.8 Myeloid leukemia in children with trisomy 21

Children with Down syndrome (DS) have a 150-fold increased risk of myeloid leukemia (ML-DS) before the age of 5 years[3]. The majority of the reported cases show a predominance of megakaryoblasts, corresponding to megakaryoblastic leukemia (AMKL) or FAB-type M7 in non-DS patients[4-6]. ML-DS can be preceded by a period of transient leukemia (TL) or transient abnormal myelopoiesis (TAM)[7]. Both, ML-DS and TAM are associated with mutations (mainly in exon 2) of the hematopoietic transcription factor GATA1,[8] which is causative for leukemogenesis[9,33] .

Approximately 5% to 30% of neonates and infants with Down syndrome (DS) are diagnosed with TAM [34–36]. TAM can also be found in infants with mosaicism. Most of these children are asymptomatic and achieve a spontaneous remission without therapeutic intervention. Nevertheless, ~20% of the patients die within 6 months (referred to as early death) [37–40], which is frequently caused by liver infiltration of blasts and subsequent hepatic failure [37, 39, 41]. Previous studies suggested the benefit of a therapeutic intervention with cytarabine to reduce blast burden in children presenting with symptoms associated with early death, such as high white blood cell (WBC) count, ascites, bleeding diatheses, and preterm delivery [38, 41]. Of the children diagnosed with TAM, 16% to 23% progress to myeloid leukemia (ML-DS) within the first 4 years of life [37–39]

Historically, outcome in children with ML-DS was thought to be poor[10]. To date, excellent cure rates have been achieved for ML-DS using dose-reduced treatment protocols without hematopoietic stem cell transplantation[42]. The excellent response was attributed to the enhanced drug sensitivity of the ML-DS-blasts, especially to cytarabine and anthracyclines[19,20]. Still, despite reduced intensity, many patients suffer from therapy-associated toxicity[11]. This determines therapy-related mortality (TRM) as the main cause of death in this cohort of patients[11-16,21]. However, the prognosis of relapsed ML-DS patients is extremely poor[21-23]. This means that ML-DS treatment schemata have to particularly strive the balance between appropriate chemotherapy dosage to avoid relapses on the one hand and treatment-related toxicity on the other hand.

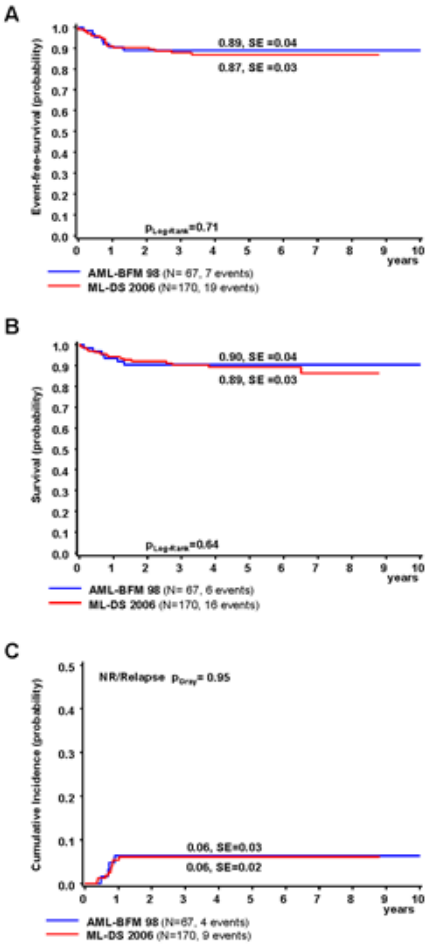


Figure 2 Outcome of ML-DS 2006 patients compared to the historical control arm (AML-BFM 98).

(A) EFS, (B) OS, and (C) Cummulative incidence of relapse/Non respone for ML-DS 2006 patients in comparison to ML-DS children from previous AML-BFM 98 trial (historical control arm). (A-C) 5-year probabilities are given.

170 pediatric patients with ML-DS were enrolled in the prospective, multi-center, open-label, non-randomized ML-DS 2006 trial, conducted by the NOPHO, DCOG and AML-BFM study groups. The ML-DS 2006 trial was based on the reduced-intensity arm for ML-DS patients of the AML-BFM 98 trial. Due to the excellent outcome of the ML-DS patients in the AML-BFM 98 trial (n=67; 5yr-OS: 90±4%, 5yr-EFS: 89±4), the treatment intensity was further reduced in the ML-DS 2006 trial by excluding etoposide from consolidation (reducing the cumulative dose from 950 mg/m² to 450 mg/m²), administering four instead 11 doses of intrathecal CNS-prophylaxis (cytarabine 20-40 mg) and excluding maintenance therapy. Despite this reduction, the outcome in both studies was in a similar range. Especially the CIR was identical in both studies (6%), validating that therapy reduction did not result in a higher relapse risk (Figure 2). The absence of CNS involvement in any of the patients might suggest that the ML-DS blasts cannot home to this niche and explain, why we did not observe an increase in CNS relapses despite reduction of CNS prophylaxis. Although the TRM was not significantly reduced (2.9% vs. 5%; $P_{FishersExact}$= 0.276), excluding etoposide resulted in a fewer severe adverse event after consolidation. However, the non-randomized trial design and the comparison to a historical control is a potential weakness of the study, which was necessary due to the low number

of ML-DS patients per year (expected accrual: 20 patients per year), and the expected low compliance with a more intense treatment arm. Still, the data of the ML-DS 2006 trial may implicate that even further reduction of treatment intensity could be feasible based on prognostic factors.

Despite a general consent about longer treatment intervals and the discouraging role of hematopoietic stem cell transplantation, international study protocols for ML-DS differ substantially (Table 2).[11-17] Especially the role of high-dose cytarabine and the dosing of anthracyclines have yet to be defined. While in most European and North American trials for ML-DS courses with high-dose cytarabine (3g/m²/d) are applied,[11-13,17] Japanese studies (JPSLG AML D05) obtained excellent results (3yr-OS: 88±4% 3yr-EFS: 83±4%) and low TRM (1.4%) using standard-dose cytarabine (100mg/m²/d) only.[16] Together with the results of the Toronto group that used a low-dose cytarabine-based regimen,[14,24] which contained no anthracyclines and no etoposide, these data indicate that subgroups of patients with ML-DS can be cured even with much lower doses than in the current ML-DS 2006 trial. In the ongoing AAML1531 study of COG a classification of standard and high-risk patients according to MRD negativity after the first induction was conducted with the aim of excluding the standard HD-ARAc course. This arm showed a significally reduced EFS a[43]nd underlined the essential role of HD-ARAc for this group of patients[44]. But the identification of clear prognostic factors that would predict which patients are at risk of relapse and need intense therapy remained elusive[25].

Table 2. Comparison of recent ML-DS trials

	Years	N	DNR (mg/m²)	ARA-C (mg/m²)	Etoposide (mg/m²)	TRM (%)	5yr-OS (%)	5yr-EFS (%)	Ref.
ML-DS-06	06-15	170	240	27,400	450	2.9	89	87	1
AML-BFM 98	98-03	67	240	29,400	950	5.0	90	89	11
COG AAML0431	07-13	204	240	27,800	750*	1.0	93	91	17
Al-Ahmari	90-03	34	0	7,400	0	0	77	67	24
JPLSG D05	08-10	72	250	3,500	1,350	1.4	88	83	16

*6-thioguanine: 1,600 mg/m²; L-asparaginase 12,000 IU/m²

The ML-DS 2006 trial showed that the early therapy response – assessed by morphology at the start of the second block – is predictive of treatment outcome and relapse. Future trials will need to show, whether monitoring of minimal residual disease could even increase this predictive value. Furthermore, the study showed that all relapses were in the cytogenetic groups of patients with trisomy 8, aberration of chromosome 16 or isochromosome 7. This means that none of the patients belonging to the other cytogenetic subgroups (n=81) experienced a relapse in the ML-DS 2006 trial. This includes patients with cytogenetic aberrations which were previously proposed to be associated with higher relapse risk, such as monosomy 7 or normal karyotype (i.e. 47,XX/XY,+21c).[15,25] Although the cytogenetic groups are small limiting the confidence of the subgroup analysis, they are in accordance with a recent Japanese study[16] that could neither confirm monosomy 7 nor normal karyotype as poor prognostic factors. Thus, reproducible and consistent data across the study groups imply that monosomy 7 and normal karyotype are not predictive for poor prognosis. Instead, the data of the ML-DS 2006 trial indicate that gain of chromosome 8 to predict a high relapse risk and poor EFS.

Despite the use of high-dose cytarabine, the ML-DS 2006 trial observed a TRM of 2.9% (n=5/170 patients), which is in the same range as the COG A2971 trial [21] (2.3%; n=3/132; $P_{FishersExact}$= 1.000) that also used high-dose cytarabine, and which does not significantly differ from the JPSLG AML D05 trial[16] (1.4%; n=1/72; $P_{FishersExact}$= 0.673) that used standard-dose cytarabine. Three patients died due to infections (Streptococcus mitis sepsis, fungal sepsis and respiratory syncytial virus pneumonia, respectively). Two patients died due to cardiac failure, underscoring the sensitivity of children with DS to cardiotoxic agents. Thus, lowering cardiotoxicity should be an aim for the development of future treatment protocols.

Interestingly the ML-DS 2006 trial demonstrated that ML-DS is not necessarily associated with a high treatment-related toxicity even when compared to non-DS-AML patients. Knowing the high susceptibility of DS patients to cytostatic agents, this observation might be explained by two factors. First, ML-DS patients do not obtain one very intense course (high-dose cytarabine and mitoxantrone [HAM]) and the anthracycline doses are reduced in all courses. Second, the treatment intervals for ML-DS patients are

longer (median interval between first [AIE] and second course [AI/HAM] for ML-DS vs. non-DS-AML: 32 vs. 25 days, P<0.001) giving the patients more time to recover after each course; i.e. a block was only started if the child showed recovery of blood counts and was in a good general condition without clinical signs of an infection, mucositis or fever. In summary, the data of the previous ML-DS 2006 trial showed that therapy-reduction could be achieved in children with ML-DS, without compromising the excellent outcome.

The international ongoing Phase III clinical trial for CPX-351 (EudraCT 2018-002988-25) is a prospective, non-randomized, open-label and multicenter trial for children with myeloid leukemia associated with Down syndrome (ML-DS) aiming to reduce toxicity while maintaining excellent patient outcomes. According to ML-DS 2018, the patients will be stratified based on MRD after the first induction and will be treated with higher or lower (3 vs 1g/m^2) dosis of Cytarabin(HA v.s. hA course). The reduction of treatment-related toxicity is particularly important for children with Down syndrome, who are very vulnerable to the side effects of intense chemotherapy.

2. PATIENT GROUPS

All subgroups of acute myeloid leukemia in children and adolescents defined according to the WHO 2016 classification criteria:

- De-novo AML
- Relapsed/refractory AML
- Acute Promyelocytic Leukemia
- MDS-related AML
- Treatment related AML
- Transient leukemia and Myeloid leukemia in children with trisomy 21/ Down syndrome

2.1 Risk stratification- de novo AML

The patients with de novo AML are assigned to different risk groups including the standard and the high risk in all clinical trials worldwide. In some of the protocols, patients are also assigned to an intermediate risk group. Risk stratification is based on the biological characteristics of leukemia (cyto- and molecular genetics). Moreover, therapy response (evaluated via morphology and immunophenotyping) after the 1st and 2nd induction is used for a subsequent stratification.

In the majority of the study groups the stratification is based initial in the genetic characteristics of the leukemic blasts and a re-evaluation depended on the response after the treatment courses determines the final stratification. On the contrary, in the NOPHO group the initial categorization is based on the response of the therapy with the cytogenetic criteria leading as a second step to the final stratification.

Additionally, patients with high leukemic cell counts (primary WBC >50 Gpt/L) or considerable organ hypertrophies receive a pre-phase with an adapted chemotherapeutic regimen within the first days of treatment. Patients with individual treatment conditions (e.g. severe infections/sepsis, cardiac impairment, acute monoblastic leukemia) require an adapted treatment.

Standard risk group (SR)

As standard risk group are defined the patients with a favorable outcome. In this risk group of AML are assigned patients with t(8; 21), inv(16) and t(1;11) in which the good prognosis is well established since the 90s [19, 45, 46]. Additionally, FAB M2 with Auer rods and t(8;21), the atypical eosinophils in FAB M4 Eo with inv(16) and the FAB M2 with NPM1-mutated AML are the standard examples of the good correlation of cytogenetics with morphology and are defined as independent factors for favorable EFS (> 70%)[5, 47, 48]. In all therapy studies, children and adolescents with AML with the above cytogenetic characteristics after receiving adequate therapy were identified as prognostic favorable subgroups of AML[49]. This also applies to AML with normal karyotype and NPM1 mutation or CEBPA double mutation.

High risk group (HR)

A group of patients is stratified into a high-risk group due to very unfavorable genetic characteristics and/or poor therapy response and qualifies for an allogeneic stem cell transplant during 1st complete remission. Translocations, like t(9;11) and t(11;19) have been showed to correlate with poorer outcomes while patients with t(10;11), t(4;11) and t(6;11) have been related to higher relapse rates in childhood acute myeloid leukemia (AML). The t(9;11)(p22;q23) is the most common *KMT2A* rearrangement in children and is related to poor prognosis[50, 51]. Other fusions, including t(6;11)(q27;q23), t(10;11)(p12;q23), t(4;11)(q21;q23) and t(11;19)(q23;p13.3) are also considered as high risk[4752, 53]. Moreover, adverse characteristics like complex karyotype, del(5q), abn(3q), monosomy 5 and 7 are well established as poor prognostic factors[54–56]. The combination of FLT3-ITD with WT1 represents another group with poor prognosis which is well described by multiple study groups (COG, BFM)[47, 57, 58].

The patients that are not meeting any of the referred criteria are characterized as intermediate risk.

The above data summarize the common criteria for stratification in all study groups for pediatric AML. For detailed information to stratification, please refer to the local guidelines of your study group.

3. DIAGNOSTICS

3.1 Diagnostics-De novo AML

In children and adolescents, diagnostic examination generally requires bone marrow samples and should be based on the findings of morphology, cytochemistry and immunophenotyping.

Indispensable are the following:

- Morphology
- Immunophenotyping
- Cytogenetics/ molecular genetics

In case of a 'punctio sicca' or difficulties to detect the leukemic blasts in the BMA (for example in megakaryoblastic leukemias (AML FAB M7), associated with myelofibrosis) it is recommended to perform a bone marrow biopsy. The diagnosis should be confirmed by two pathologists.

If the BMA does not provide a sufficient percentage of leukemic blasts, genetic analyses may be performed via the bone marrow biopsy in these patients. Please contact the reference study center for more information.

Morphology: Initial morphological assessment should be performed in each participating clinic, followed by the evaluation by the respective reference laboratory.

Cytogenetics/ molecular genetics: Diagnostics should be completed by cytogenetic analysis. The recurrent AML-associated fusion genes that are detected by conventional karyotyping, should be confirmed via a molecular genetic analysis. Additionally, the detection or exclusion of AML specific mutations is required. The analysis should at least cover aberrations that are relevant for risk stratification. For a detailed lists and/or information about the Standard Operating Procedure (SOP), please contact the reference laboratory.

Immunophenotyping: Diagnostics should be completed via immunophenotyping. The selection of markers should follow international recommendations [4, 59] .

CNS involvement: CNS involvement is classified based on an initial CSF cytospin preparation. The unstained cytospin preparations (1000rpm, 5min) should be performed by the respective clinics. Slides will be stained and evaluated by the respective national reference laboratory. If macroscopically contaminated with blood, the documentation of red blood cells and WBC of the peripheral blood and CSF is required to clarify the CNS Status. In addition, clinical findings and/or imaging may influence the CNS status of the patient.

In case of hyperleukocytosis (>50 Gpt/L), diagnosis of CNS involvement will be performed only after a reduction of the cell count to <50 Gpt/L. Do not perform initial lumbar puncture!

In addition, do not perform initial lumbar puncture on patients with APL.

Laboratories:

The reference morphology, immunophenotyping and molecular genetics are performed centrally in the reference laboratory of the appropriate national reference laboratory as defined by each cooperative study group. Please contact the national pediatric AML coordinator.

For further information or any questions, the recently founded European Pediatric Acute Leukemia (EuPAL) foundation, Utrecht, (info@eupal.org) can support the search for an accredited laboratory.

3.1.1 Initial diagnostics

In this section, an overview of the suggested examination at the initial diagnosis is presented. For detailed information about the necessary material, please refer to the local protocol of you study group.

General initial diagnostic work-up: The initial work-up includes a careful documentation of the initial disease characteristics, symptoms, and patient specific details according to the following list:

Medical history	current Symptoms
	pre-existing illnesses
	medical history of patient and family
	signs of tumor predisposition syndrome
Clinical examinations	general health condition
	weight (kg. percentile)
	height (cm. percentile)
	vital parameter
	pulmonary symptoms (dyspnea, tachypnea)
	neurology (orientation, ataxia, vision, sensitivity)
	organ involvement (lymph nodes, spleen, liver, testes)
Blood sampling	blood count (hemogram/CBC)
	differential blood count
	blood gas analysis
	electrolytes
	transaminases
	creatinine/urea
	LDH/uric acid
	Lactate
	CRP
	Coagulation status: Quick, PTT, fibrinogen, D-dimer
	Blood group
Exclusion of other causes	Serology:
	EBV, CMV, HSV, HHV6, PV B19, HIV1/2, HBV, HCV, HAV, VZV
	Vaccination titer:
	MMR, Pertussis, Polio 1/2/3, Diphtheria, Tetanus, HIB

	if necessary, serum in order to establish evidence of vitamin B12
Imaging	Ultrasound (obligatory: abdomen, liver, spleen, testes, mediastinum, kidney) Chest X-ray (obligatory) Cranial CT (optional) Thorax, abdomen-CT (in case of suspected organ infiltration, masses) MRI (optional, in case of neurological symptoms obligatory) ➔ Patients with clinical symptoms of suspected CNS involvement should be evaluated by imaging study for intracranial bleeding, leptomeningeal disease, and mass lesion. ECG (obligatory) Echocardiography (obligatory) Pulmonary function test (record or history of pulmonary symptoms) EEG (obligatory)
Ophthalmologic council	(optional)

Additionally, in order to evaluate the significant value of response peripheral blood and bone marrow will be collected at diagnosis, prior to each treatment element. For more details of the requested material please refer to the local study protocols.

3.1.2 Detection of molecular relapse

For patients with a detectable MRD marker (list of the updated markers available upon request) MRD-monitoring from month 6 until month 24 after diagnosis is recommended to detect a possible molecular relapse. This will be performed on peripheral blood that is collected every 4 weeks. If the MRD-level increases by more than one log, a bone marrow examination is recommended. If molecular relapse can be validated in the bone marrow and a second independent peripheral blood sample, patients can be included into an active clinical trial for the diagnosis and therapy of a molecular relapse. Patients with a molecular relapse can be eligible for the European AMORE 2017 trial.

3.2 Diagnostics- APL

3.2.1 Initial diagnostics

The suspected diagnosis is made by means of the characteristic morphology with hyper granulation and Auer bundles and is validated by specific cytogenetics t(15;17)(q22;q21)—respectively the fusion gene *PML/RARA* or other RARA fusions. In case of morphologically suspected AML M3, therapy should be initiated—even if the genetic analyses are not yet completed.

Exceptions

A small proportion of patients with APL (<5%) have variants with different fusion partners of RARA; some do not respond to ATRA. ATRA-resistant subtypes include the following: *ZBTB16-RARA* (formerly *PLZF-RARA*, t(11; 17)(q23; q21)) and the *STAT5B-RARA* fusion gene (in which a normal chromosome 17 is found with conventional cytogenetics). *ZBTB16-RARA* and *STAT5B-RARA* positive APLs are also likely to be resistant to arsenic trioxide (ATO) [29, 60]. These subtypes in particular have a generally poor long-term prognosis.

Recommendation: Fusion partners of APL should be determined. In addition to cytogenetics, FISH and molecular genetics are necessary. Patients with ATRA-resistant subtypes should be re-stratified into the intermediate-risk group of AML as soon as a STAT5B-RARA fusion gene or ZBTB16-RARA fusion has been detected. The ATRA treatment may be continued. A SCT in CR1 with *STAT5B-RARA*-positive APL may also be considered [61]. Patients with *NPM-RARA* fusion gene should receive a prolonged MRD monitoring (comparable to high-risk patients) due to an increased risk of relapse [62].

The micro granular variant (AML M3v) is a morphologically definable special form of APL, which is predominantly associated with increased leukocyte counts. The disease pattern of this APL is a hematological emergency, which immediately requires a diagnostic evaluation and specific therapeutic measures.

Anamnesis and physical examination (in particular with the consideration of bleeding tendency)

- Blood count and differential blood count
- Bone marrow aspirate with
  - Morphology, cytology and cytochemistry
  - Immunophenotyping
  - FISH: RARA split and/or t(15;17) with fusion gene (PML/RARA) or immunofluorescence (PML)
  - RT-PCR of PML/RARA (and other fusion partners, see above)
  - Conventional cytogenetic analysis
- Coagulation status with Quick, PTT, fibrinogen, D-dimer
- Important: ECG and echocardiogram
- The first lumbar puncture (diagnostic and therapeutic) is recommended on day 10 when the risk of bleeding has subsided.

3.2.2 Diagnostics during therapy

- The determination of the PML-RARA fusion gene has to be done from the bone marrow (BM) and peripheral blood (PB) since—so far—the prognostic relevance at the beginning of the disease is more reliable from bone marrow.
- Examinations during the course (through real-time q-PCR) should be conducted prior to each therapy block until 4 months of treatment, then prior the 4th and after the 5th cycle with ATO. Afterwards, peripheral blood should be collected every 3 months. Examinations should be performed until the 12th month in patients with SR and until the 18th month in patients with HR. In case of inadequate quality/representativeness of bone marrow, the BM sampling should be repeated.
- The PML-RARA transcript is monitored via RQ-PCR. In the case of persistence of the molecular MRD marker (RQ-PCR +) PML/RARA after 4 months, the results need to be confirmed in a second control. If positivity is confirmed, contact the study center; these patients are regarded as high-risk. Intensified therapies are recommended to achieve MRD negativity. If blasts or MRD persist, allogeneic stem cell transplantation should be considered.

3.3 Diagnostics-Transient Abnormal Myelopoiesis (TAM)

Diagnostics are performed in the national reference laboratories including; morphology, immunophenotyping and molecular genetics.

3.3.1 Initial diagnostics

The diagnosis of Transient Abnormal Myelopoiesis (TAM) must be made morphologically, immunophenotypically, cytogenetically and molecularly from peripheral blood:

- All newborns with trisomy 21 should be examined for **symptoms associated with TAM** within the first days of life: organomegaly, hepatopathy (raised transaminases with conjugated hyperbilirubinemia), skin rash, pericardial and pleural effusions, extreme leukocytosis and coagulopathy
- Additionally, a differential blood count as well as blood smear should be performed for every child within the first week of life.
- If abnormalities suspicious of TAM are detected, further diagnostic measures should be taken in the reference laboratory. Here, molecular genetic (*GATA1*-mutation analysis), immunophenotypic (CD34, CD117, CD7, CD13, CD33, CD15, CD36, CD56, CD41, CD42b, CD61) and morphologic examinations are performed. For this, EDTA blood (1 to 5 ml) and blood smears need to be sent.

TAM is diagnosed in neonates with trisomy 21 if

- **>5% myeloid blasts in the peripheral blood and/or a *GATA1*-mutation (or exclusive GATA1 expression in the blasts) are detected.**
- **TL-associated symptoms are present and a *GATA1*-mutation (or exclusive GATA1 expression in the blasts) is detected.**
- **CAUTION: BM NOT REQUIRED**

Summary of initial diagnostics:

- PERIPHERAL BLOOD SAMPLE:
  - Morphology (myelogram should be done from well-spread PB smears preferably stained with May-Grünwald-Giemsa)
  - Immunophenotyping to detect the following antigens: CD34, CD117, CD7, CD13, CD33, CD15, CD36, CD56, CD41, CD42b, CD61
  - Cytogenetics (confirmation of trisomy 21)
  - Molecular genetics: *GATA1*-mutation

3.3.2 Diagnostics during and after treatment-MRD

It is recommended to assess the minimal residual disease (MRD) during the course of TL to estimate the risk of transformation to ML-DS. For MRD peripheral blood samples should be obtained at the times of time of diagnosis (day 0), 4 weeks, 8 weeks, 12 weeks, 6 months, 12 months, 18 months, 24 months, 36 months and 48 months respectively.

3.4 Diagnostics- Myeloid leukemia of Down syndrome (ML-DS)

Diagnostics are performed in the national reference laboratories including; morphology, immunophenotyping and molecular genetics

ML-DS can be diagnosed if the following criteria are met:

- Myeloid Leukemia (ML) or Myelodysplastic Syndrome (MDS), according to WHO
- Trisomy 21: Down syndrome or mosaic
- Age: > 6 months and ≤ 4 years of age with/without GATA1 mutation
  or
  > 4 years of age < 6 years of age with GATA1 mutation
- Morphology/immunophenotyping: FAB M0, M6 or M7

Diagnosis of ML-DS is **excluded** in case of any of the following criteria:

- Children with Transient Abnormal Myelopoiesis (TAM), according to WHO
- Cytogenetics: AML with recurrent genetic abnormalities (WHO 2016)

3.4.1 Initial Diagnostics

Recommendations for diagnostics for ML-DS/ AML according to WHO classification 2016:

- BONE MARROW ASPIRATE:
  - Morphology (myelogram and FAB score should be done from well-spread BM smears preferably stained with May-Grünwald-Giemsa)
  - Immunophenotyping to detect the following antigens: CD34, CD117, CD7, CD13, CD33, CD15, CD36, CD56, CD41, CD42b, CD61
  - Cytogenetics
  - Molecular genetics: *GATA1*-mutation

3.4.2 MRD Diagnostics

It is recommended to assess the minimal residual disease (MRD). For MRD peripheral blood samples and bone marrow aspirates should be obtained at the times of day 0, day 28, and before each intensive therapy element, i.e. on days 56 and 84.

4. TREATMENT

4.1 De Novo-AML first line therapy

Both, risk group stratification and the treatment schedule is similar in most treatment protocol of the cooperative study groups worldwide. This refers to the applied drugs (anthracyclines, cytarabine incl. analogs, etoposide) the number of treatment-blocs and the definition of risk groups.

4.1.1.1 Pre-phase treatment

Exchange transfusion (ET)/leukapheresis (LPh): Patients with hyperleukocytosis are at high risk of bleeding/leukostasis. ET/LPh shows a trend toward reduced ED rate due to bleeding/leukostasis and is recommended at WBC >200 Gpt/L, or monoblastic AML at even lower WBC [63]. Theoretically, exchange transfusion is preferable to leukapheresis (elimination of blasts and toxic metabolites). However, the feasibility and timing must be thoroughly considered (i.e. required blood volume, available intravenous lines, availability of blood transfusion).

The flow chart (Figure 3) serves as a recommendation. Individual patient characteristics need to be considered by the treating physician.

We recommend an exchange transfusion/leukapheresis at a cell count of >200x10^9/L; in monoblastic leukemia (AML FAB M5), at a leukocyte level >100x10^9/L.

Other measures (start of chemotherapy: cytarabine) should not be delayed!

CAUTION! Do not immediately start anthracycline!

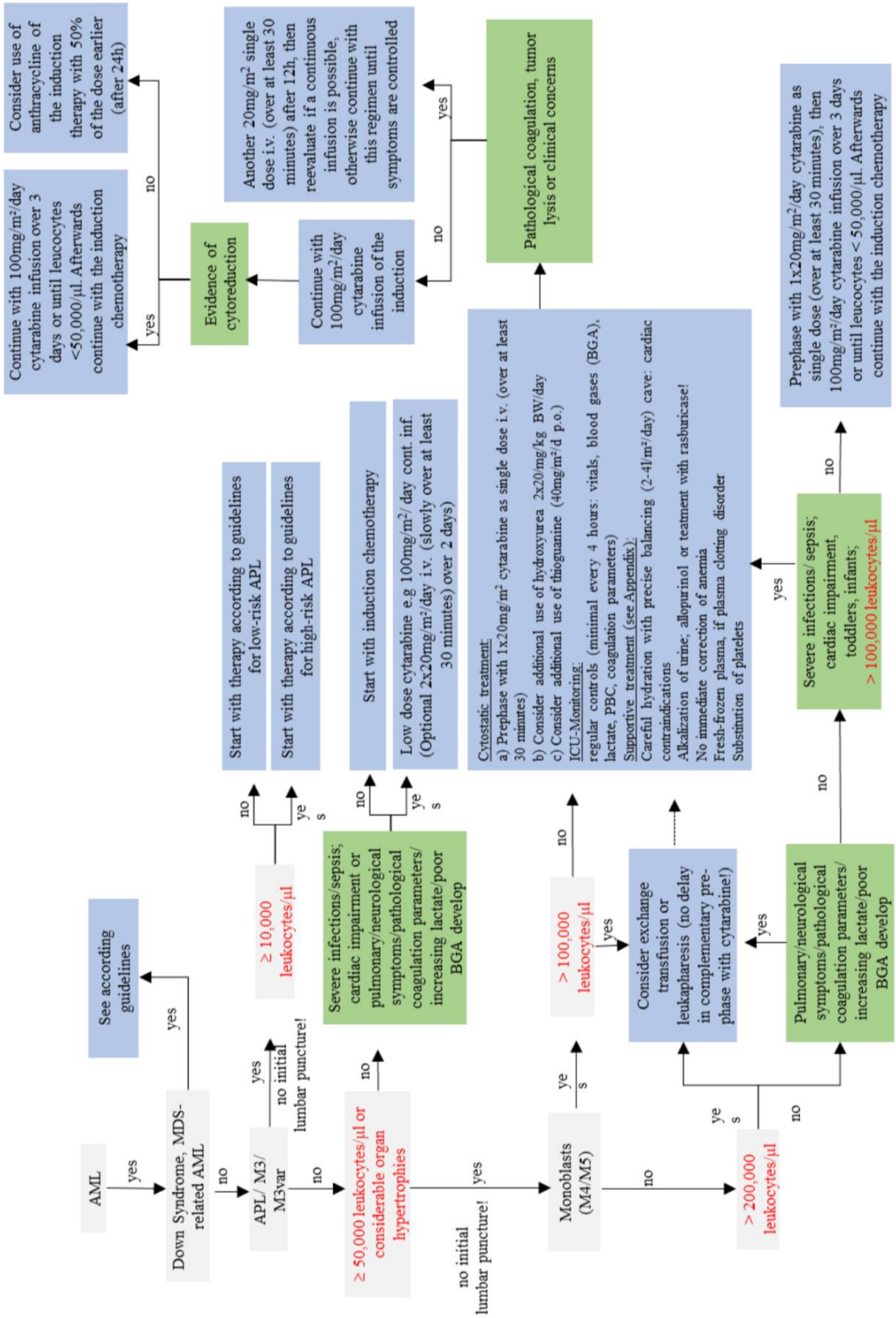


Figure 3 Pre-phase treatment in AML patients with hyperleukocytosis, an example of the AML-BFM protocol.

4.1.2 Therapy overview

The treatment of de novo AML includes two induction courses and two to three consolidation courses. The induction phase aim to achieve complete remission of leukemia. In children with inv(16) the chemotherapy is restricted by the AML-BFM group excluding the second induction of the therapeutic plan. Consolidation begins after the two induction treatments and maintains the leukemic free period till maintenance for the standard risk patients. In the treatment of HR AML is suggested to administrate the initial induction courses followed by two consolidation blocks prior SCT (see section 4.1.3).

The fundamental chemotherapeutic regiments in the treatment for AML are cytarabine (ara-C) and anthracyclines. The induction therapy consists of 3 days of an anthracycline (eg, daunorubicin at least 60 mg/m^2, idarubicin 10-12 mg/m^2, or mitoxantrone 10-12 mg/m^2) followed by 7-10 days of cytarabine (100-200 mg/m^2).The treatment courses last from a week to 10 days and are administrated every four weeks if the regeneration of the blood cells and the physical condition of the patient allows it. The anthracycline analogs, Daunurobicin and Idanuorobicin, have been compared and the randomized trials including AML-BFM 98 and MRC 12/15 showed significant achievements of idarubicin as the most effective anthracycline compared to daunorubicin[64]. Most of the studies conducted worldwide combine these two drugs(one anthracycline with ara-C) with etoposide or thioguanine.

The majority of the study groups deliver high-dose cytarabine, whereas the MRC group administrated high doses of anthracyclines. High-dose cytarabine with mitoxantrone (HAM) and etoposide (HAE) is resommended by the BFM study group.

The last decade targeted agents, such as GO and FLT3 inhibitors, are new therapeutic tools and are administrated depended on the diagnostic genetic characteristics of each patient (+CD33, FLT3-ITD respectively). More details for FLT3-ITD inhibitors are given in section 4.1.3.

Please note that for children with a body weight ≤ 10 kg or age <1 year, dosages for intravenous chemotherapy must be calculated per kg body weight and not according to body surface area.

For detailed information about the chemotherapy courses and the doses please contact the local reference group. If there is no national study group in your country, you can contact the authors for the detailed treatment schedules.

Requirements before the treatment courses:

- Good general condition (Karnofsky/Lansky performance status ≥ 50)
- free of active infection, fever, and mucositis (even if >5% persistent blasts in marrow)
- Recovering (increasing) blood counts
    - Granulocytes >1 x10^9/L
    - Platelets >80 x10^9/L

Diagnostics prior treatment:

- bone marrow exam (MRD and morphology) before start of treatment
- lumbar puncture to be done on day 1
- echocardiography and ECG
- exclusion of ophthalmological infections

4.1.3 FLT3-ITD positive AML

Midostaurin, a FLT3-inhibitor, combined with standard chemotherapy and as a single-agent post-consolidation therapy is being evaluated in a phase II study (NCT03591510) in newly diagnosed pediatric patients with *FLT3*-mutated AML, other than acute promyelocytic leukemia. Gliteritinib is also tested at the moment in children and adolescents in an ongoing phase I trial (NCT04240002). The treatment group includes patients with FLT3-ITD positiv AML in relapse or refractory disease. Due to the few data of the administration of those Inhibitior in the pediatric population, the use and the possible toxicities have to be consindered.

Approach with primary CNS involvement: see details in chapter 4.1.7.

CNS irradiation is only intended for patients with primary CNS involvement and is carried out 4 weeks after completion of the intensive treatment. During irradiation i.th. is only carried out with cytarabine in age-dependent doses. (<1 year: 20 mg; 1-<2 years: 26mg; 2-≤3 years: 34mg; >3 years: 40mg).

4.1.4 CNS involvement

Traumatic lumbar puncture:

For CSF that is macroscopically contaminated with blood, the following procedure is recommended: For CSF that subsequently clears, the administration of intrathecal therapy may be considered.

For persistent macroscopic contamination of CSF, we do not recommend the administration of intrathecal therapy. Upon physician's discretion, LP may be performed immediately from a different site or may be postponed until the next day but should not be delayed for more than 3 days—if feasible.

4.1.4.1 Prophylactic CNS treatment

Patients with no evidence of blasts in CNS.

Intrathecal (i.th.) therapy is carried out according to the national recommendations:

Dosages for i.th. cytarabine administration in cytarabine/methotrexate/prednisolone

	<1 year	1 <2 years	2 <3 years	≥3 years
Cytarabine	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Pred	4 mg	6 mg	8 mg	10 mg

Cave: During irradiation i.th. is only carried out with cytarabine in age-dependent doses.
(<1 year: 20 mg; 1-<2 years: 26mg; 2-≤3 years: 34mg; >3 years: 40mg).

4.1.4.2 Treatment by primary CNS involvement

Patients who have primary CNS involvement receive triple (cytarabine/methotrexate/prednisolone) i.th. in age-dependent doses at weekly intervals; an additional dose is administered subsequent to cerebrospinal fluid clearing. In total, at least three doses should be administered in the first block (day 1, 8 and 15). It is recommended to correct thrombocytopenia and/or coagulation disorders prior to the lumbar puncture according to local standards (e.g. platelet substitution if the platelet count <30 Gpt/L). The subsequent intrathecal therapy follows the guidelines for prophylactic treatment. If the cerebrospinal fluid is not clear, the intrathecal therapy should be continued during the next block of chemotherapy. Please contact the national reference center.

CNS irradiation **is only intended for patients with primary CNS involvement** and is carried out 4 weeks after completion of the intensive treatment. During irradiation i.th. is only carried out with cytarabine in age-dependent doses. (<1 year: 20 mg; 1-<2 years: 26mg; 2-≤3 years: 34mg; >3 years: 40mg).

Irradiation of the spinal canal is not recommended.

If SCT is planned, irradiation is not recommended.

Patients with CNS positivity and SCT will receive cytarabine i.th. in age-dependent doses every 4 weeks until 6 months after SCT.

Guidelines for infants are as follows: Infants younger than 18 months receive a bridging therapy (i.th. triple therapy every 4 weeks) (max 6 times during bridging therapy) before beginning of CNS irradiation.

Irradiation is carried out under mega voltage conditions with a linear accelerator or using proton irradiation. Thereby, the entire neurocranium, including the upper portion of the cervical spine to C2, the retrobulbar areas, and the entire base of the skull are included in the radiation field. This is especially pertinent for the middle cranial fossa area, which is often located deeper, regardless of the mandibular joints.

A single daily dose consists of 1.5 Gy, so that the weekly dose is equivalent to 7.5 Gy; overall duration of CNS irradiation is 2–3 weeks.

Table 1: Dosages for therapeutic CNS treatment

Age	Therapeutic CNS irradiation
<18 months	*
1 1/2> years ≤2	15 Gy
≥3 years	18 Gy

* In infants, CNS irradiation should not be administered until the patient reached the age of at least 18 months (>1 1/2 years). Infants are treated with i.th. triple therapy every 4 weeks (max 6 times) as bridging therapy before CNS irradiation; If the child is still under the age of 18 months, no further treatment should be administered.

4.1.5 Radiotherapy of extramedullary leukemia (excluding CNS)

In positive cases in which one or both **testicles** are affected, local therapy (surgery, irradiation) should be considered, but needs to be carefully discussed on the basis of current literature [a].

In extramedullary, localized affection of other sites, an imaging should be performed after the second and third block of intensive treatment to define treatment response. In case of poor response, local irradiation of up to 30 Gy may be considered. With involvement of the scalp, saturation can be achieved through a boost irradiation, for example with electrons (electro-beam)[b].

4.1.6 Stem Cell Transplantation (SCT)

The recommended therapy for HR patients is allogenic-SCT. The fundamental requirement for transplantation is the achievement of CR or at least no evidence of leukemia. The preferred options are a human leukocyte antigen (HLA)-matched sibling donor and HLA-matched unrelated donor. If neither

[a] This recommendation is based on the poor prognosis of children with testes involvement, aiming to use all available and potentially effective treatment options.

[b] This recommendation is based on case reports and small retrospective series. There is no prospectively confirmed evidence that additional irradiation will improve outcome; therefore, toxicity and long-term sequelae should be taken into account [66].

of these are possible, please contact the national reference study center to consider other options (mismatched unrelated donor, haplo-identical donor). The recommended graft source in the case of HLA-matched donors is primarily bone marrow.

The conditioning regiments for SCT by pediatric AML involve pediatric patients with AML undergoing total body irradiation (TBI) and cyclophosphamide (Cy); busulfan (Bu) and Cy; or Bu, Cy, and melphalan (Mel). In the European Group for Blood and Marrow Transplantation centers it was shown that Bu-Cy-Mel achieved a lower incidence of relapse at 5 years and higher OS and LFS[67]. A higher life-free-survival was noticed in the many study groups after using the regiments BuCyMel and Clo-FluBu compared to BuCy[68]. In first CR, children younger than 12 years old may receive conditioning with **Bu-Cy-Mel**; children and adolescents older or equal than 12 years may receive **Treo-Flud-Thiotepa**.

Therapeutic drug monitoring of busulfan is recommended.

Graft-versus-host disease (GvHD)

For prophylaxis of graft-versus-host disease please refer to general guidelines for SCT: Cyclosporin A and short term methotrexate in MSD HSCT. Add ATG for MUD.

For more details refer to specific protocols/guidelines.

4.2 Treatment-APL

Risk groups

- Standard-risk (SR): initial leukocyte count < 10 000/µl
- High-risk (HR): initial leukocyte count ≥ 10 000/µl

Initial supportive management: coagulation disorders

Initial coagulation disorders are the main reason for the associated high early mortality rate. Monitoring the status of coagulation is carried out via global tests of activated partial thromboplastin time (aPTT), prothrombin time (INR, Quick), fibrinogen levels and platelet counts. Depending on the clinical situation, laboratory controls should be performed at least once a day until coagulation disorders are regulated. For substitution, the use of FFP (fresh frozen plasma), fibrinogen and platelet concentrates is recommended. Fibrinogen levels should reach values > 100–150 mg/dl and platelet values greater than 30–50 Gpt/L [69]. When the factor XIII level is reduced, in individual cases a substitution can help to stabilize coagulation. The administration of anti-fibrinolytics is not indicated. The benefit of low-dose heparin therapy is unclear [69].

Supportive management in case of initial coagulation disorders

- Thrombocyte concentrate should be given in order to maintain the thrombocyte number to > 50 Gpt/L in the first approximately 10 days
- Subsequently, thrombocyte concentrate should only be given when thrombocyte concentration is <20 Gpt/L or in case of bleeding symptoms.
- Erythrocyte concentrate should be given in order to maintain the Hb value to >8 g/dl.

Supportive management during treatment: Leukocytosis/differentiation syndrome

Therapy with ATRA and ATO can lead to Leukocytosis and differentiation syndrome. A prophylactic treatment with prednisone/prednisolone (0,5mg/kg/d) is recommended. Differentiation syndrome is characterized by fever, weight gain, dyspnea, hypotension, pulmonary infiltrates, pleural or pericardial effusion and even renal failure. It is caused by an excessive inflammatory reaction and the release of cytokines which can occur through ATRA and ATO induced maturation of promyelocytes. Syndrome onset was most often observed during day 2 to 10 after treatment started; symptoms may also manifest later. The condition can be reversed with at least 3-days of dexamethasone therapy. Therapy with ATRA or ATO can usually be continued afterwards. Furthermore, as direct side effect of ATRA, patients may experience headaches (pseudotumor cerebri) in the early phases of therapy. This complication will also be treated by a brief interruption of ATRA and an addition of dexamethasone. Treatment with ATRA/ATO may lead to pronounced hyperleukocytosis (leukocytes > 10 – 100 Gpt/L). An antiproliferative treatment with cytarabine (40 mg/m²/day) and/or hydroxyurea (2x40 mg/kg/day) is necessary, if leukocytes are >

10. Gpt/L. Currently, hydroxyurea is already recommended at leukocyte levels of >5.0 Gpt/L. Leukocytosis can occur without symptoms of differentiation syndrome.

- As prophylaxis (prevention) against differentiation syndrome, prednisone/prednisolone 0.5 mg/kg from day 1 to 15 of the therapy begin is recommended.
- After the occurrence of APL differentiation syndrome, 10-15mg/m² dexamethasone is given for at least three days.
- Leukocytosis: If WBC is > 5 Gpt/L: Start with hydroxyurea (2x 20-40mg/kg/day); If WBC is >10 Gpt/L add cytarabine 40mg/m²/day and give at least hydroxyurea 2x40mg/kg/day.
- During ATO-therapy substitution of electrolytes i.v. is recommended to stabilize potassium concentration to > 4 mEq/l and magnesium concentration to >1.8 mg/dl (0.74 mmol/l).

Both risk groups receive 7 courses ATRA and 4 courses of ATO after complete remission. Thus, in general, patients will receive 9 courses of ATRA and 5 courses ATO from diagnosis until end of treatment. For HR patients, an additional initial chemotherapy block is recommended. ATRA and ATO lead to a differentiation of promyelocytes and not to cell destruction [70] [71]. This in turn, leads to an early improvement in coagulation parameters. ATRA is also associated with high rates of remission without chemotherapy but cannot lead to a cure of FAB M3 on its own. Hyperleukocytosis (antiproliferative therapy with cytarabine or hydroxyurea needs to be considered) and differentiation syndrome—which often occurs as a result of ATRA and ATO—are the decisive side effects of ATRA and ATO therapy and must be taken into consideration. Results with ATRA and ATO in adult SR patients compared to ATRA in combination with chemotherapy improved long-term outcome and reduced toxicity [72].

Primary ATO therapy is recommended until at least day 42, or until the peripheral blood is free from blasts. This often coincides with the time point of morphologic remission (bone marrow aspiration to verify CR is performed after a 2-week therapy break at day 56). Furthermore, a 1-week break from ATRA (25 mg/m²/day) after the first 14 days and later breaks for 2 weeks are recommended (pharmacokinetic studies that show intermittent administration of ATRA prevents the development of tolerance) [73]. A primary lumbar puncture is contraindicated due to bleeding risks. Beginning on day 10 (or subsequent to blast reduction), seven intrathecal applications of cytarabine (in age dependent dose) are recommended.

Side effects of arsenic trioxide include—as previously mentioned—differentiation syndrome. The most important additional side effects are leukocytosis (begin hydroxyurea with a WBC> 5.0 Gpt/L) and prolonged QT intervals. ECG changes with prolonged QT intervals and electrolyte shifts, especially of potassium and magnesium, are commonly observed. Potassium values should be above 4 mmol/l and magnesium values should be above 1.8 mg/dl; therefore, **ECG should be monitored before each ATO course** and once weekly during ATO administrations. At a QTc interval of more than 500 msec, therapy is interrupted due to the risk of cardiac arrhythmia (TdP).

Therapy is discontinued when grade 3 toxicities are observed, particularly those that involve prolonged QT intervals, differentiation syndrome, hepatotoxicity, and pseudotumor cerebri.

Additional potential side effects include dry skin (erythema), peripheral neuropathy, hyperglycemia, and skin reactions, fatigue, joint pain (arthralgia), increase of transaminase levels (hypertriglyceridemia) and teratogenic effects.

4.2.1 Treatment-APL- standard-risk group

Immediately following the diagnosis of FAB M3 or FAB M3v (variant), Patients are treated with intermittent therapy with ATRA ((25mg/m²/day oral divided in 2 doses p.o.) due to pharmacokinetics and tolerance development. The treatment is followed by a break for one week. After morphological CR is achieved, the intermittent cycle changes to 14-days of ATRA followed by a 14-day break. In total, patients should receive 7courses of ATRA. The additional therapy with arsenic trioxide (ATO) should begin between day 2 and day 6 after diagnosis: Arsenic trioxide (ATO) should be administered at a dose of 0.15 mg/kg/d (1-2 h. i.v. infusion) until day 42. ATRA/ATO therapy often leads to hyperleukocytosis; therefore, a prophylactic treatment with prednisone/prednisolon (0,5 mg/kg) is recommended.

If differentiation syndrome is observed, treatment with ATRA and ATO should be discontinued and therapy with cytarabine or hydroxyurea is recommended. Dexamethasone treatment is recommended for at least 3 days.

CNS therapy: Overall, 4 courses of cytarabine are recommended intrathecally in age-dependent doses. At the beginning of treatment, intrathecal treatment is recommended on day 10 and day 28 and then in 4-week intervals until day 112 (i.e. 5 times). After day 112, the last two intrathecal therapies are performed together with the respective bone marrow aspirations (before the 4th and after the 5th cycle of ATO).

4.2.2 Treatment-APL-high risk group

In addition to the recommendations for treatment with ATRA and ATO in SR patients, patients with HR criteria receive an induction chemotherapy in the first cycle. Cytarabine/idarubicin (AI) is recommended as induction as illustrated. Alternatively, gemtuzumab ozogamicin (GO) may be considered (3mg/m²).

The simultaneous administration of chemotherapy in patients with high-risk APL is necessary because of the enhanced risk of hyperleukocytosis/leukostasis due to treatment with ATRA. In general, no leukapheresis should be performed.

CNS therapy: Overall, 4 courses of cytarabine are recommended intrathecally in age-dependent doses. At the beginning of treatment, intrathecal treatment is recommended on day 10 and day 28 and then in 4-week intervals until day 112 (i.e. 5 times). After day 112, the last two intrathecal therapies are performed together with the respective bone marrow aspirations (before the 4th and after the 5th cycle of ATO).

4.2.3 Recommendation for relapse therapy in acute promyelocytic leukemia

Acute promyelocytic leukemia is currently considered to be a curable disease. The molecular equivalent of t(15;17) is the PML/RARA fusion gene found in approximately 98% of all patients with M3. Relapses are rare and curatively treatable. A distinction is made among open, morphologically recognizable relapses and molecular relapses.

Hematological relapse: more than 5% promyelocytic blasts/atypical promyelocytes in bone marrow

Molecular relapse: reoccurrences of the PML-RARA fusion transcript after at least 2 negative RQ-PCR-analyses, detected in two consecutive bone marrow examinations and validated in two independent laboratories

Refractory APL: morphological, cytogenetic and/or molecular genetic detected persistence of APL

In contrast to other AML molecular markers, the detection of the PML/RARA rearrangement was of prognostic importance after completion of the induction and consolidation therapies (more than three chemotherapy blocks, 95% of the patients were then negative, the relapse risk was high with PCR positivity and with an initially increased leukocyte count [> 10 Gpt/L]) [74].This relates to day 112 during the ATRA + ATO therapy. It is also known, that a reoccurrence of this marker after prior PCR negativity can predict a relapse within the next three months [22]. Italian studies (GIMEMA/PETHEMA) could show that the chance for survival was explicitly higher when relapse therapy was begun after molecular relapse compared to therapy begun after hematological relapse (92% vs. 44%) [75]

Based on this data, it is recommended that patients should be treated when molecular relapse is detected. Consequently, MRD-diagnostic of the bone marrow of APL patients should be performed every 3 months during an overall duration of 12 months (SR) or 18 months (HR). The suspicion of a molecular relapse should be confirmed with consecutive aspirations within 14 days in two independent laboratories.

Relapse treatment is dependent on the time of relapse (early relapse <18months, late relapse 18–36 months and very late relapse >36 months after diagnosis) and the treatment received at initial diagnosis (Chemotherapy plus ATRA or ATRA/ATO). For early relapse, an allogeneic or autologous SCT may be considered – dependent on response to treatment (ATRA/ATO [+GO]+Chemotherapy). For late relapse (<3years after diagnosis), a treatment with ATRA/ATO is recommended [76].

Very late relapse (>3 years after initial diagnosis)

A treatment according to the guidelines for "de novo" APL is recommended. If PCR is positive after 3 blocks of chemotherapy, an allo-SCT needs to be considered.

4.3 Treatment- TAM and ML-DS

4.3.1 Treatment- TAM

Therapy should be considered for children with TAM and any of the following symptoms, which have been shown to be associated with an increased risk of early death:

- Hyperleukocytosis (>100 x 10^9/L)
- Liver dysfunction: Hepatomegaly in combination with elevated liver enzymes and/or cholestasis
- Ascites
- Hydrops fetalis
- Life-threatening symptoms such as signs of hyperviscosity, hepatosplenomegaly causing respiratory compromise, heart failure (ejection fraction <47% or shortening fraction <27%) not directly the result of a congenital heart defect, renal dysfunction, disseminated intravascular coagulation (DIC) with bleeding [37]

For children without any of these symptoms, no therapy is recommended.

If therapy is applied, the following regimen should be used:

Low-dose cytarabine (1 to 1.5 mg/kg body weight) i.v. (slowly over at least 5 minutes) or subcutaneously for 5 to 7 days.

If no response is achieved an additional course with an interval of at least 5-7 days can be applied.

4.3.2 Treatment- ML-DS

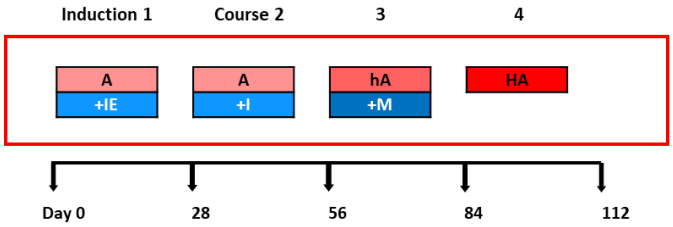
Patients meeting all of the following criteria can be considered for the treatment according to this guideline:

- Myeloid Leukemia (ML) or Myelodysplastic Syndrome (MDS), according to WHO
- Trisomy 21: Down syndrome or mosaic
- Age: > 6 months and ≤ 4 years of age with/without GATA1 mutation OR
  4- 6 years of age with GATA1 mutation
- Morphology/immunophenotyping: FAB M0, M6 or M7
- Lansky performance score at least equal to 50; or Karnofsky performance status at least equal to 50, whichever is applicable

Patients presenting with any of the following criteria **should not be considered** for guideline treatment:

- Children with Transient Abnormal Myelopoiesis (TAM), according to WHO (see chapter 4.5.)
- Cytogenetics: AML with recurrent genetic abnormalities (WHO 2016)
- Previous allogeneic bone marrow, stem cell or organ transplantation
- Evidence of invasive fungal infection or other severe systemic infection requiring treatment doses of systemic/parenteral therapy including known active viral infection with human immunodeficiency virus (HIV) or Hepatitis Type B and C
- Symptomatic cardiac disorders (CTCAE 4.0 Grade 3 or 4)
- Major surgery within 21 days of the first dose
- Any anti-cancer therapy (e.g., intensive chemotherapy, biologics or radiotherapy) for more than 14 days or within 4 weeks before start of therapy, expect TAM patients receiving low-dose cytarabine.

- Concomitant treatment with any other anticancer therapy except those specified in this guideline during the therapy
- History of hypersensitivity to any of the drugs recommended in this guideline or to any drug with similar chemical structure or to any excipient present in the pharmaceutical form of the recommended drugs



Treatment schedule for ML-DS

AIE:

cytarabine 100 mg/m²/d [days 1-2] and 100 mg/m²/12h [days 3-8]

idarubicin 8 mg/m²/d [days 3, 5 and 7]

etoposide 150 mg/m²/d [days 6, 7 and 8]

AI:

cytarabine 500 mg/m²/d [days 1-4]

idarubicin 5 mg/m²/d [days 3 and 5]

haM:

cytarabine 1 g/m²/12h [days 1-3]
mitoxantrone 7 mg/m²/d [days 3-4]

HA:

high-dose cytarabine 3 g/m²/12h [days 1-3]

For the ML-DS patients an international ongoing Phase III clinical trial with CPX-351, a liposomal preparation of cytarabine and daunorubicin, (EudraCT 2018-002988-25) is open since 2018. The single-arm trial will be investigated whether the substituting of course 1 and 2 of standard ML-DS therapy (defined as ML-DS 2006 protocol) with CPX-351 and reduction of treatment intensity in course 4 for patients with a good response (<0.1% blasts in the bone marrow after course 1) result in inferior EFS.

The treatment plan of patients with ML-DS involves a significant lower cumulative dose of chemotherapy regiments in comparison to de-novo AML without DS due to the vulnerability of those patients to toxicities.

CAUTION: Requirement of continuing to the next course of chemotherapy is the good physical condition of the patient.

4.3.2.1 Relapse therapy- ML-DS

Children with ML-DS who suffer from relapse, should be treated according to an individualized schedule, which takes the increased risk of toxicity and potential resistant disease into account. Stem cell transplantation should not be a standard practice, given the high risk of procedure related morbidity and mortality in DS children.

4.4 AML relapse or primary refractory disease

For patients with AML relapse treatment according to our guidelines for relapse therapy or the inclusion into a respective international AML relapse study is recommended.

	Options
early first relapse	1. If eligible, patients should be included in an international relapse trial.
late first relapse	2. If not eligible, but intensive therapy is feasible (consider clinical conditions and pretreatment, as well as duration of remission), treatment with intensive re-induction is recommended within these guidelines.
refractory first AML	
relapse after allo-SCT in 1st CR/refractory AML	
relapse after 2nd CR	3. If no intensive therapy is feasible: Phase I/II studies or salvage therapy and subsequent allo-SCT in CR or NEL (no evidence of leukemia) and good general condition or palliative care.
relapse after SCT in 2nd CR/refractory AML	
others	

Reinduction therapy (intensive): Generally, this therapy is indicated for patients with relapsed AML after intensive chemotherapy, allogeneic stem cell transplantation or refractory AML after primary therapy, if the physical condition of the patient is sufficient.

Idarubicin-FLA

Reinduction

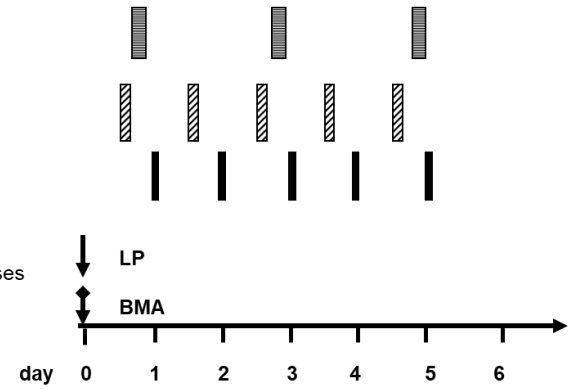
idarubicin[1] 12 mg/m²/d day 1, 3, 5
→ 2h infusion, after start of fludarabine

fludarabine[2] 30 mg/m²/d day 1–5
→ 30min infusion

cytarabine[3] 2000 mg/m² day 1–5
→ 3h infusion, begin 4h after start of fludarabine

cytarabine/pred/MTX[4] day 1
→ intrathecal bolus injection, age-dependent doses

	<1 year	1<2years	2<3years	≥ 3 years
cytarabine	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
pred	4 mg	6 mg	8 mg	10 mg



In case of a body weight ≤ 10 kg and/or ≤ 12 months, calculate dose according to weight as follows: [weight (kg) x dose (per m²)] /30.

[1]Idarubicin 12 mg/m²/day by 4h i.v. infusion on day 1,3 and 5 (3 total doses).
→ start infusion after start of fludarabine.
[2]Fludarabine 30 mg/m²/day by 30min i.v. infusion once every 24h on day 1, 2, 3, 4 and 5 (5 total doses)
[3]Cytarabine 2000 mg/m²/day by 3 hours i.v. infusion once every 24h on 1, 2, 3, 4 and 5 (5 total doses).
→ begin infusion 4h after start of fludarabine.
[4]Triple intrathecal therapy in age-dependent dose on day 1. In case of CNS involvement weekly until 1 week after complete blast clearance of CSF.

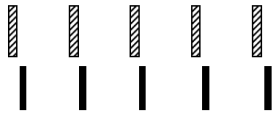
a) **Re-induction FLA:**
The second reinduction course should be started no earlier than 28 days, but no later than 42 days after the start of the first reinduction course. To commence this block of treatment, the patient must have a neutrophil count >0.5 Gpt/L and platelets >50 Gpt/L (without transfusions) and be in good clinical condition. Perform a bone marrow (MRD, morphology and immunophenotype) and lumbar puncture on day 1.

FLA

Reinduction

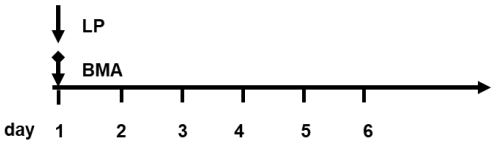
fludarabine[1] 30 mg/m²/d **day 1–5**
30min infusion

cytarabine[2] 2000 mg/m² **day 1–5**
3h infusion



cytarabine/pred/MTX[3] i.th. **day 1**
→ intrathecal bolus injection, age-dependent doses

	<1 year	1<2years	2<3years	≥ 3 years
cytarabine	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
pred	4 mg	6 mg	8 mg	10 mg



In case of a body weight ≤ 10 kg and/or ≤ 12 months, calculate dose according to weight as follows: [weight (kg) x dose (per m²)] /30.

[1]Fludarabine 30 mg/m²/day by 30min i.v. infusion once every 24h on day 1, 2, 3, 4 and 5 (5 total doses)
[2]Cytarabine 2000 mg/m²/day by 3h i.v. infusion once every 24h on 1, 2, 3, 4 and 5 (5 total doses).
→ begin infusion 4h after start of fludarabine.
[3]Triple intrathecal therapy in age-dependent dose on day 1.

b) Bridging high-intensity consolidation cytarabine/etoposide:

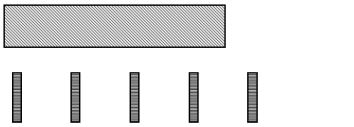
This is to be given as consolidation to patients if SCT is not immediately available, but only to patients who can tolerate a 3rd course of intensive chemotherapy prior to SCT. Perform BMA + LP first! BMA after the 2nd reinduction course no earlier than 28 days after the start of course 2, but if delayed, no later than day 42 and only to patients in good clinical condition. Patients must have a neutrophil count >1.0 Gpt/L and platelets >50 Gpt/L (without transfusions).

AE

High-intensity consolidation

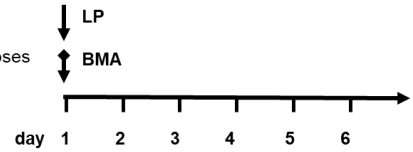
cytarabine 500 mg/m²/d **day 1–4**
96h

etoposide 100 mg/m²/d **day 1–5**
3h infusion



cytarabine/pred/MTX i.th. **day 1**
→ intrathecal bolus injection, age-dependent doses

	<1 year	1<2years	2<3years	≥ 3 years
cytarabine	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
pred	4 mg	6 mg	8 mg	10 mg



In case of a body weight ≤ 10 kg and/or ≤ 12 months, calculate dose according to weight as follows: [weight (kg) x dose (per m²)] /30.

[1]Cytarabine 500 mg/m²/day for 96h, continuous i.v. infusion (day 1–4)
[2]Etoposide 100mg/m²/d at day 1, 2, 3, 4, 5 by 3h i.v. infusion
[3]Triple intrathecal therapy in age-dependent dose on day 1.

c) Bridging Low-intensity consolidation cytarabine/thioguanine

To be given as consolidation to all patients, who cannot tolerate a course of intensive consolidation before SCT and if SCT is not immediately available.

Perform BMA and LP first! BMA after the 2nd reinduction course no earlier than 28 days after the start of course 2, but if delayed, no later than day 42 and only to patients in good clinical condition with

platelets > 50 Gpt/L (without transfusion) and neutrophils > 1.0 Gpt/L.

TC

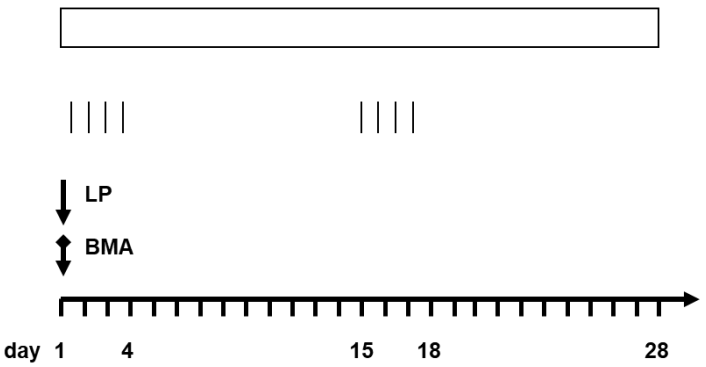
Low-intensity consolidation

thioguanine 100 mg/m²/day
max. 4 weeks 1 daily oral dose (evening)

cytarabine 75 mg/m²/day
1 daily subcutaneous injection
days 1–4 and 15–18

cytarabine/pred/MTX i.th. day 1

	<1 year	1<2years	2<3years	≥ 3 years
cytarabine	16 mg	20 mg	26 mg	30 mg
MTX	6 mg	8 mg	10 mg	12 mg
Pred	4 mg	6 mg	8 mg	10 mg



In case of a body weight ≤ 10 kg and/or ≤ 12 months, calculate dose according to weight as follows: [weight (kg) x dose (per m²)] /30.

Treatment management:

Aim: to reach WBC values between 2.0–3.0 Gpt/L.

Thioguanine (TG):	WBC	>3.0 Gpt/L		150% dose or more
		>2.0 Gpt/L	and ≤3.0 Gpt/L	100% dose
		≥1.0 Gpt/L	and ≤2.0 Gpt/L	50% dose
		<1.0 Gpt/L		0% dose
Cytarabine:	WBC	>2.0 Gpt/L	and platelets	>80 Gpt/L

Treatment procedure: Once a cytarabine block has been started, it should be completed regardless of the leukocyte and platelet counts and should only be interrupted if complications (fever, etc.) occur.

4.4.1 Allogeneic stem cell transplantation in relapse

For all patients with AML relapse/refractory AML, allogeneic stem cell transplantation should be attempted. The preferred options are matched sibling donor and matched unrelated donor. If neither of these are possible, mismatched unrelated donor SCT, haplo-identical donor SCT or cord-blood SCT can be considered. If a previous SCT was performed within the last 6 months, the expected toxicity is high and careful consideration is warranted. AML patients with persistent blasts (morphology) at time of allogeneic stem cell transplantation have an extremely poor prognosis; therefore, this procedure cannot be recommended. A recommendation for this situation should always be in accordance with the patient's clinical condition and a therapy with less side effects is recommended (maintenance therapy).

5. ASSESSMENTS (FOLLOW-UP)

Follow-up should be adapted according to patient-specific and disease-specific characteristics based on the following list:

		After end of therapy					
	therapy end	**1. year**	**2.year**	**3.year**	**4.year**	**5.year**	**from 6.year**
Relapse diagnostics							
general physical examinations + testicles	once	every three months	every six months	annually	annually	annually	annually

CBC/differential blood count*	once	every three months	every six months	annually	annually	annually	annually
Process and late effects diagnostics							
general blood sampling [1]	once	once	–	–	–	–	–
kidney results in serum/urine [2]	once	every six months	every six months	–	–	–	–
ophthalmological examinations	once	–	–	–	–	once	–
ECG/ echocardiogram	once	annually	annually	annually	annually	annually	–
audiometry	once	–	–	–	–	–	–
follow-up radiotherapy [3]	once a year according to APRO guidelines						
neuropsych. testing (motoric, coordination, cognition) from the 4th year of life	once		once			once	
quality of life	once	–	once	–	–	–	once
Endocrinology							
percentiles (body weight, length, BMI) [4]	once	annually	annually	annually	annually	annually	annually
blood pressure	once	annually	annually	annually	annually	annually	annually
thyroidea (palpation and regional lymph nodes)	once	annually	annually	annually	annually	annually	annually
tanner scale[5]	once	every six months	every six months	annually	annually	annually	annually
fertility counseling	on demand, including spermiogram in case of noticeable problems in puberty						
Blood sampling-endocrinology[6]							
-bone metabolism	bone metabolism in symptomatic patients.						
-sexual hormones	at least once before therapy and then at the age of 13 years (female) / 14 years (male). or in case of pathologic findings in puberty development or percentiles; including LH, FSH, testosterone (male), estradiol (female).						
After radiotherapy of head and neck ≥12Gy							
thyreoid hormones (TSH, fT4)	annually until the 10th year of follow-up, then every 2nd year.						
parathyroid gland	in case of hypercalcaemia, monitor parathyroid hormone.						
ultrasound of the thyroid gland	once	afterwards, every 2nd year.					
In addition, after radiotherapy of head and neck ≥18Gy							

weight and heigth	every six months until the age of 8 year (female)/ 9years (male), then annually	
tanner scale	every six months until the age of 8 years (female)/ 9years (male), then annually	
fasting value of blood sugar and lipids (Chol., HDL, LDL, Triglyc.)	once	afterwards, every 2nd year.
fertility counseling	for female patients, including the possibility of impaired fertility	
After radiotherapy of the testicles		
inhibin B, prolactin	at least once before therapy and then at the age of 14 years	
fertility counseling	information about the risk of azoospermia	

* For MRD please see chapter diagnostics.

[1] Blood sampling: hemogram, CRP, Bili, GOT, GPT, □-GT, AP, LDH, α- amylase, ferritin, immunoglobulin.

[2] Kidney results in serum: phosphate, magnesium, natrium, potassium, calcium, creatinine, bicarbonate. Kidney results in urine: urine status, phosphate, creatinine, calcium.

[3] After radiotherapy of the CNS

[4] X-RAY of the bones in the left-hand to determine the bone age in case of noticeable problems.

[5] Beginning at the age of 8 years (female)/9 years (male) until end of puberty. In adult patients including anamnesis of menstrual cycle and libido to identify insufficiency of sexual hormones.

[6] Consider contacting pediatric endocrinologists.

6. ADVERSE EVENTS OF RECOMMENDED TREATMENT

6.1 Cytarabine

Cytarabine is an antimetabolite (pyrimidine antagonist).

Administration options include i.v., s.c., i.m., and i.th. For infusion, the substance may be diluted with any conventional infusion solution. Use only preservative free solution.

ATTENTION: fatal overdose with i.th. injection of 1000 mg vial!

Main side effects include the following: Myelosuppression, oro-intestinal mucosal toxicity (mucositis), enteritis, intestinal wall necrosis, erythema, fever, myalgia, bone and joint pain, facial 'flush', and hepatic dysfunction. Nausea can be relieved by anti-emetics prior to injection. Beware of pre-existing liver damage!

6.2 High-dose cytarabine (HD-Cytarabine)

Ampules of 500 mg and 1000 mg. i.v administration in 5% glucose solution (1 g/50 ml) are infused over 3 hours. Shelf-life of reconstituted infusion is approximately 6 hours. HD-cytarabine therapy can lead to conjunctivitis due to accumulation of the agent in the tears. Eye washes with eye drops (Liquifilm), 1–2 drops in each eye every 6 hours are necessary throughout the first few days. Later, administration of eye drops (2 drops in each eye every 4–6 hours beginning 6 hours before the first dose to 12 hours after the last dose of cytarabine) can be given.

The use of corticosteroid eye drops, which are an effective intervention, presents a risk of secondary infections, such as herpes and keratitis—with prolonged use—there is also a risk of glaucoma and cataracts.

Dose reduction for HD-cytarabine: Reduction, i.e., omission of a 3-hour infusion is indicated in cerebral ataxia and confluent maculopapular exanthema.

Main side effects include: Conjunctivitis, tachycardic arrhythmias, somnolence, cerebellar ataxia, aphasia, nystagmus. Peripheral neuropathy. Immediately stop HD-cytarabine administration if nystagmus and other cerebral disorders occur. Also, immediately discontinue if bilirubin levels increase above >3.0 mg/dl.

Patients who developed grade 4 neurotoxicity following high-dose cytarabine should not receive further high-dose cytarabine but are eligible for continuous lower-dose cytarabine (1 g/m²).

6.3 Etoposide-Phosphate (Etopophos)

The derivative of etoposide has the following advantages for use in children: significantly lower infusion volume, greater stability, and avoidance of benzyl alcohol and other additives. It most likely has lower local toxicity at extravasation and a decreased risk of allergic reactions. The framework for approval corresponds to that of etoposide. Dosages are comparable to dosages of etoposide.

To avoid hypotension and anaphylactic reactions such as local phlebitis, dissolve with 0.9% NaCl solution or 5% glucose solution to a concentration of 11.4 mg/ml etoposide phosphate (equivalent to 10 mg/ml etoposide); give a slow i.v. infusion over 60 min.

Main side effects: myelosuppression, nausea, vomiting, alopecia.

Rare side effects: peripheral neuropathy, mucositis, fever, chills, headache, cholestasis.

Caution! Etopophos contains dextran 40 and must not be used in patients with known allergy to dextran!

6.4 Mitoxantrone (MITOX)

A derivate of anthrachinone, the vials contain 20, 25, or 30 mg. This agent must not be injected undiluted, rather in 50 or 100 ml 0.9% NaCl solution or 5% glucose solution. Cardiologic monitoring, as with daunorubicin and idarubicin, are necessary. Simultaneous administration of mitoxantrone and heparin can lead to precipitation. Urine´s color may be green after 24hs of treatment.

Main side effects: functional heart changes including cardiomyopathy and myelosuppression

Rare side effects: Vomiting and stomatitis.

6.5 Idarubicin (IDR)

Main side effects include: acute and chronic cardiotoxicity with cardiomyopathy. Regular monitoring of ventricular function is required. If patients show any impairment compared to the initial baseline, anthracycline administration should be discontinued. Myelosuppression, tissue necrosis with paravenous application, and phlebitis, oro-intestinal mucosal toxicity, nausea, vomiting and hair loss may occur. Significant liver impairment can cause delayed degradation and increased toxicity.

Note: According to present knowledge, cardiotoxicity seems to be lower compared to other known substances (daunorubicin and adriamycin). The metabolite of IDR–idarubicinol, could be detected in the cerebrospinal fluid in children in cytotoxic concentrations.

6.6 Prednisolone (i.th.)

Solution is available for injection. Intrathecal administration of prednisolone presents no additional risk to children with AML. The side effects of prednisolone as part of i.th. administration cannot be isolated from the overall therapy and seem to be marginal.

6.7 Methotrexate (MTX)

Form: Solution for injection

Systemic toxicity

Severe systemic toxicity (myelosuppression, mucositis, etc.) due to i.th. MTX therapy is very rare but can occasionally occur—especially in patients with Down syndrome. In these cases, the dose of i.th. methotrexate should not be reduced. Instead, a rescue with leucovorin in a single dose of 15 mg/m² (p.o. or i.v.) 48 hours after i.th. injection is indicated in order to avoid the worsening of a pre-existing myelosuppression (neutrophil granulocytes <0.5 Gpt/L) or mucositis. Leucovorin should not be administered solely to prevent a myelosuppression.

Subacute neurotoxicity/MTX encephalopathy:

Subacute neurological symptoms, such as seizures, paralysis/paresthesia, impaired consciousness and aphasia, are associated with i.th. MTX, occur typically 1–2 weeks after administration. Usually, the symptoms spontaneously return to normal within a few hours or a few days. Diagnostic brain imaging (MRI angiography, if not available at short notice, then CCT) is recommended for all patients with neurological symptoms in order to exclude as differential diagnoses; bleeding, SVT, and cerebral infection (abscesses, particularly fungal infections). In subacute neurological MTX-related events, pathological changes in white and/or grey matter (which are often detected initially only in the diffusion weighting) can frequently occur and may appear as fresh infarcts. For safety reasons, it is recommended to wait until the findings of diagnostic imaging return to normal and regression of clinical symptoms occurs, before treatment is resumed.

6.8 Fludarabine

This nucleoside analogue (purine antagonist) is an antimetabolite that is excreted mainly in the urine. After phosphorylation, it is less susceptible to deamination than cytarabine.

Main side-effects include myelosuppression, fever, chills, malaise, nausea, and vomiting. Rarer are auto-immune phenomena such as (hemolytic anemia), pneumonitis, and CNS symptoms such as agitation. Since fludarabine causes profound and long-term lymphocytopenia, irradiation of blood products to prevent graft-versus-host disease and antifungal prophylaxis is strongly recommended from the start of the treatment until 6 months after SCT or if SCT is not being performed, until 6 months after the last administration of fludarabine.

6.9 Arsenic Trioxide and ATRA

For more details see chapter APL (page **Error! Bookmark not defined.**). Side effects include differentiation syndrome. The most important other side effects are: Leukocytosis (begin hydroxyurea with WBC> 5.0 Gpt/L, see above) and prolongation of the QT interval. ECG changes with prolonged QT intervals and electrolyte shifts, especially of potassium and magnesium, are commonly observed. Potassium values should be above 4 mEq/l and magnesium values should be above 1.8 mg/dl; therefore, ECG should be monitored prior to each ATO course and once weekly during ATO administrations. At a QTc interval of more than 500 msec, the therapy is interrupted due to the risk of cardiac arrhythmia (TdP).

Therapy is discontinued when grade 3 toxicities are observed, particularly those that involve prolonged QT intervals, differentiation syndrome, hepatotoxicity, and Pseudotumor cerebri. Moreover, peripheral neuropathy, hyperglycemia, and skin reactions can occur. Additional potential side effects include dry skin (erythema), fatigue, joint pain (arthralgia), increase of transaminase levels (hypertriglyceridemia) and teratogenic effects.

6.10 Radiation therapy

Most predominantly occurring are radiation-induced headaches that can be managed with short-term steroid medication. Following CNS irradiation, specific, non-verbal, intellectual function limitations are described. Caution is advised in children with prior cerebral damage. Additionally, there is a risk of secondary brain tumors.

7. DOSE MODIFICATIONS AND DELAYS

7.1 Adjustments of dosages for young children

In general, infant dosage (children ≤ 12 months of age and children with ≤ 10 kg body weight) should be calculated based on body weight rather than body surface.

Dose reduction in young children is necessary due to a lower deaminase-capacity, reduced cytarabine clearance (due to less deamination to ara-U) as well as higher neurotoxicity (standard HD-cytarabine doses more likely result in peak levels of > 300µM) in these patients.

7.2 Delay of the treatment

In case of delay of the treat due to persistent aplasia or sepsis in granulocytopenia please contact the national reference study group for an individual recommendation. If the delay lasts longer than a week an evaluation of the bone marrow is recommended.

8. SUPPORTIVE TREATMENT

The measures and agents mentioned herein are not part of a study protocol, but are recommendations based on published guidelines, review articles, or the clinical experience of the authors. Chemotherapy for AML is one of the strongest immune and myelosuppressive therapies in pediatric oncology. The treatment of a child with AML should take place exclusively in sites with sufficient experience and the necessary requirements.

Hospitals with little experience are advised to transfer the patient during the initial treatment to a larger site. If this is not possible, close contact the study center.

The treatment causes a long-lasting phase of BM aplasia, which lasts on average 21 days after induction therapy (range, 3–62 days) and is associated with a very high risk of infection and sepsis. About 80% of children develop an infection following a therapy block. The risk is particularly increased for all children who have not achieved remission (partial blast persistence, aplasia with absence of regeneration). Even if, of course, not all complications can be avoided, an optimization of supportive care should be sought, because in addition to the possibly preventable deaths, implementing therapy without delay may contribute to a better overall result.

The following recommendations need to be adapted by the respective clinics. This chapter presents essentially AML-specific information, and for more general recommendations, refer to the relevant published guidelines and review articles.

8.1 Emergencies

8.1.1 Hyperleukocytosis

Risks: bleeding, leukostasis, tumor lysis syndrome

Symptoms: petechiae/ bleeding, dyspnea/tachypnea, renal failure, ataxia/nystagmus, speech disorders, seizures, visual deterioration, priapism

Monitoring: intensive care unit, vital sign monitoring, blood gas analysis, coagulation parameters, renal values, blood count/morphology, electrolytes, chest X-ray

Tumor lysis syndrome prophylaxis: hydratation, urine alcalinization, uric acid synthesis inhibitor, (allopurinol; rasburicase)

Blast elimination: consider/prepare exchange transfusion/leukapharesis in patients with clinical symptoms of leukostasis

Introduction of a “cautious” cytostatic chemotherapy (e.g. hydroxyurea, cytarabine, thioguanine)

CAUTION! Do not immediately use anthracyclines!

CAUTION! It is preferable to avoid erythrocyte substitution[c].

The risk of fatal bleeding and severe coagulopathy or leukostatic syndrome depends on the subtype of AML. In AML FAB M3 low blast concentrations already result in fatal bleeding and severe clotting complications e.g. disseminated intravascular coagulopathy (DIC) when leucocytes are above 10,000 leukocytes/µl. Severe leukostatic syndrome disturbances in the rheology with consecutive ARDS or cerebral infarction/bleeding— is associated with monoblastic leukemia (AML FAB M4/M5) with blast

[c] If clinically justifiable, do not transfuse immediately if Hb is low, as leukostasis is imminent if the cytocrit is elevated. Also, transfusions should not increase Hb above 7-8 g/dl. In special cases with high risk of bleeding (e.g. APL) recommendations can differ (e.g. maintain Hb at 8-9 g/dl).

concentrations >100,000 leukocytes/µl, whilst in myeloblastic leukemias (FAB M1/M2). These complications are mostly observed if leukocytes are above 200,000 leukocytes/µl.

Symptoms of leukostasis are:

- pulmonary insufficiency with tachypnea/dyspnea, cyanosis, diffuse interstitial infiltrates
- CNS: stupor, aphasia, ataxia, nystagmus
- eyes: visual acuity, diplopia, papilledema
- priapism

A decrease in plasminogen below 60% and a blast count >100 Gpt/L were shown as risk factors for an impending brain hemorrhage.

Therapeutically, rapid reduction of the blast concentration must be sought, while a threatening tumor lysis syndrome should be avoided. In this case it is recommended to begin immediately sufficient hydration. Furthermore, the introduction of (1) rasburicase or (2) allopurinol is useful to avoid a pronounced increase in uric acid. The alkalization of the urine leads to an improved solubility of uric acid (not necessary with rasburicase). Simultaneously, to reduce the tumor load, an effective but gentle chemotherapy regimen should be started.

For recommendations of exchange transfusion or leukapheresis please see chapter 2.4.2 “pre-phase”.

Analysis of the previous AML-BFM studies showed no benefits directly related to one of the two methods. This is consistent with other studies that also do not prefer any of the methods specifically. Theoretically, an exchange transfusion could be helpful because of the simultaneous exchange of cell debris. Moreover, the risk of (disseminated intravascular coagulopathy) DIC due to leukapheresis is discussed. In smaller children, leukapheresis may already be difficult for technical reasons, whereas in older children, the exchanged volumes are problematic. In patients with a good general condition, it may be justified to consider the avoidance of the exchange transfusion, especially if coagulation and blood gases are normal. The observation in an intensive care unit with monitoring of vital signs (including signs of dyspnea) and coagulation abnormalities (regular blood gas analysis, determination of lactate level, coagulation parameters) is strongly recommended. According to studies in adults, the serum lactate is an early and meaningful indicator of a possible leukostasis syndrome.

After reduction of the blasts to less than 50 Gpt/L, induction therapy should be started. If there is insufficient blast reduction, intensive therapy (induction) should be started after 5 days at the latest, regardless of the blast count.

Procedure for hyperleukocytosis and risk of bleeding:

1. Coagulation status, including fibrinogen, plasminogen, AT III and global assays
2. Intensive monitoring
3. Careful hydration with exact fluid balance
4. Rasburicase or alkalization of urine plus allopurinol
5. No immediate compensation of anemia (keep Hb <8g / dl)
6. Fresh-frozen plasma in case of decompensation of the plasmatic coagulation
7. Substitution of platelets
8. Cytoreduction
   a. Start: Cytarabine with a single dose of 20 mg/m² slowly (i.v. over at least 30 minutes)
   b. 6 to 12 h: If there is no severe lysis: initiate a 2nd single dose 40mg/m²(i.v. over at least 30 minutes)
   c. 24h after start: with insufficient response increase to cytarabine 100 mg/m²/24h continuous infusion.
   d. 48h after start: in case of non-response begin with the administration of anthracyclines (1st dose 50%)
9. If necessary, exchange transfusion / leukapheresis **!CAVE continue chemotherapy !**
10. Diagnostic LP only after blast reduction

Ad 1: Plasminogen should be determined as early as possible due to therapeutic consequences

Ad 3: Careful oral or parenteral hydration with accurate liquid balance and weight control. Use potassium-free infusion with approx. 3,000 ml/m²/24h in case of large blast number or organomegaly because of the

danger of hyperkalemia by acute cytolysis. In case of insufficient urinary excretion apply furosemide (1-10 mg/kg/24h). Adequate hydration with 2-4 L/m²/day is required to counteract renal failure due to cell destruction and kidney/renal tubular damage due to cytostatics. CAUTION! Attention should be paid to overhydration; electrolytes, balance, and weight must be closely monitored.

Ad 4: After cell decay caused by chemotherapy, acids including uric acid emerge, leading to acidosis and primarily to uric acid crystals in the kidneys. Therefore, in many chemotherapy protocols, alkalization is carried out with sodium bicarbonate in the infusion solution, and a serum pH (normal) and urine pH (7-8) is directed. For the reduction of uric acid, allopurinol or the (more effective) enzyme Rasburicase can additionally be used.
E.g. Alkalization infusion with 3L/m² BSA (infusion with glucose 5% plus 30 ml/L Na^+Cl^- 10%, 40 ml/L Na^+-bicarbonate 8.4%); targeted urine-PH 7-8; Hyperleukocytosis can cause hyperphosphatemia. In this case, increase fluid intake and do not alkalinize (urine pH not> 7.0).

Ad 5: If clinically justifiable, do not transfuse immediately if Hb is low, as leukostasis is imminent if the cytocrit is elevated. Also, transfusions should not increase Hb above 7-8 g/dl. In patients with APL it is justified to maintain the Hb >8g/dl due to the high risk of bleeding.

Ad 6: Try to counterbalance the deficit of pro- and anticoagulant factors (e.g., plasminogen) by plasma delivery. At a daily dose of 30-50 ml/kg/d plasma. The substitutions should be divided into three single doses per day. The administration of PCC should be avoided in this unstable coagulation situation because of the possible thrombogenicity. Since the changes in plasmatic coagulation are multifactorial, individual factors should not be substituted.

Ad 7: For platelet counts <20 Gpt/L, the administration of platelet concentrates is indicated. During exchange transfusions and initial cytoreduction, platelet counts should be kept above 60 x 10^9/L

8.1.2 Tumor lysis syndrome

Especially with a high tumor load, at the begin of the therapy (or spontaneously!), rapid tumor cell lysis and related toxic degradation products can occur.

Hazards: hyperuricemia, hyperpotassemia (CAUTION! cardiac arrhythmia), hyperphosphatemia, hypocalcemia, acute renal failure

Therapy: Therapy of electrolyte imbalances (e.g. if hyperpotassemia occurs, resonium, if applicable, glucose-insulin infusion; in case of symptomatic hypocalcemia or/and normal serum phosphate, consider Ca++ administration). If necessary, consider hemodialysis.

8.1.3 Hypercalcemia

Cause: increased Ca++ reabsorption from the bones (rarely ectopic parathyroid hormone production, especially in adults).

Symptoms: gastrointestinal symptoms, nausea, vomiting, intestinal obstruction, hypotension; adynamia CAUTION! Arrhythmia!

Therapy: diuretics, hydration, phosphate substitution, bisphosphonates (e.g., pamidronate®).

8.1.4 SIADH (Syndrome of Inappropriate Antidiuretic Hormone)

“Inappropriate release of antidiuretic hormone (ADH)”, also known as "Schwartz-Bartter syndrome, leads to a decrease in water excretion (so-called "water intoxication").

Diagnostic indicators include: oliguria/anuria with relative hyponatremia and edema if hypoosmolarity occurs in serum and hyperosmolarity in urine.

Symptoms: oliguria/anuria, edema, if Na+ <125 mmol: CAUTION! Seizures!

Therapy: fluid restriction, possibly Lasix®.

There should be no or only very careful infusion of solutions. Caution is advised in the administration of sodium solutions, as the total content of sodium in the body is unchanged and too little water is excreted. CAUTION! A rapid increase in sodium concentration can also cause seizures!

8.1.5 Upper mediastinal tumor

Mediastinal tumor (relatively rare in AML) can lead to compression of the vena cava with upper body edema, pleural or pericardial effusion, pericardia, heart failure, and shock, as well as to trachea compression with shortness of breath/dyspnea.

CAUTION! Burdening measures should be avoided as far as possible (no CVC placement/only sonography of the mediastinum, no CT or MRI). Consider early start of therapy.

!Monitoring under intensive care conditions is necessary!

8.1.6 Capillary Leakage Syndrome (CLS)

CLS occurs particularly, but not exclusively, in cases of infection. Despite normal hydration, a simultaneous drop in blood pressure and circulatory failure with peripheral edema/lung edema may occur; risk of hypoxia and hypercapnia.

Measures: intensive monitoring of weight, balance, breathing, blood gas analysis, and circulation. If deterioration occurs, administer volumes, catecholamine, intubation, etc. as required.

Corticosteroids may help to reduce the permeability of the vessel walls. Antibiotic therapy should always be started, because the CLS is often exacerbated by infection.

8.1.7 Acute Respiratory Distress Syndrome (ARDS), pulmonary hemorrhage, pneumonia

Pneumonia, pulmonary hemorrhage, CLS, or toxic effects can cause ARDS with rapid lung disorder.

Therefore, a chest X-ray should be done at an early stage. With evidence of ARDS, intensive care should be initiated early, including mechanical ventilation. ARDS in cancer patients is associated with a poor prognosis, especially when therapy is not initiated in the early stages.

8.2 Infections

8.2.1 Infection screening

In newly diagnosed AML, a large proportion of patients have fever, and using current infection parameters, it is very difficult to differentiate between tumor- and infection-related fever. Therefore, diagnostic measures should be taken such as those for a febrile granulocytopenia (see flow chart Table 2). Serological findings prior to chemotherapy initiation should be collected for the following pathogens due to the potential implications for the further course of treatment: EBV, CMV, HIV1/2, HBV, HCV, HAV, and VZV. In the course of intensive chemotherapy for AML, only initially striking serological findings that point to acute infections should be controlled. Microbiological surveillance cultures of catheter blocks, urine, throat, nose, and stool in afebrile patients have no advantage and are therefore not recommended (except initial smearing in sites with high resistance rates or patients from areas with high resistance rates). If open wounds are present (skin ulcers, perianal abscesses or ulcers), microbiological cultures should be performed on a regular basis (e.g. 1–2/ week) for consideration of the corresponding spectrum of pathogens in the initial therapy during a possible febrile episode. Routine radiological controls (e.g., abdominal sonography, chest X-ray) are not required in afebrile, asymptomatic patients, and are only required as a control with prior abnormal findings before initiating a further intensive chemotherapy block. The value of routine checks for infection markers in serum/plasma such as C reactive protein, procalcitonin, interleukin-6, and interleukin-8 outside of febrile episodes is unclear; therefore, they are usually not required in these situations.

8.2.2 Infection prophylaxis:

Non-pharmacological prophylaxis

For detailed information on non-pharmacological prophylaxis (rooms; disinfectant; oral care; requirements for water, air, food, clothing, visitor control, laundry, and transport; measures to control infectious diseases, nosocomial infection, and multidrug-resistant bacteria; reverse isolation) during intensive chemotherapy (protection level 2 to 3) and during maintenance therapy (protection level 1) refer to:

Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective. [77]

Although nonpharmacologic anti-infective measures are widely used and accepted during intensive treatment of AML, there is little evidence of the effectiveness of specific measures. In this regard, a recent study did not demonstrate a significant benefit of any of the restrictions regarding food, social contacts, and pets on the risk of fever, bacteremia, pneumonia, and gastroenteritis in children treated for AML, thus restrictions may be reconsidered and adapted to local circumstances [78].

8.2.2.1 Antibacterial prophylaxis

Intestinal decontamination

Although it is reasonable in theory, the effectiveness of selective intestinal decontamination with non-absorbable antibiotics (e.g., colistin, paromomycin) could not be proven in either adults or children with cancer and therapy-induced granulocytopenia; therefore, a selective intestinal decontamination is usually not recommended. This also applies to selective intestinal decontamination with trimethoprim-sulfamethoxazole.

Antibacterial prophylaxis with systemic antibiotic compounds

Patients with AML have a high rate of infectious complications. In this regard, each patient in the AML-BFM study 93 experienced an average of three infectious complications (e.g., fever of unknown origin, microbiologically or clinically documented infections) during intensive chemotherapy [79], and similar high incidence rates of infections were seen in the AML-BFM 2004 study [80]. In addition to infections caused by gram-negative pathogens, infections caused by alpha-hemolytic streptococci (viridans group streptococci—VGS) are of clinical importance as these infections often have a fulminant and clinically severe course in immunosuppressed children. Various publications report that bacteremia caused by VGS occurs in up to 20% of patients [79], [81], [82], [83], [84] in particular (but not exclusively) in patients with acute myelogenous leukemia, prolonged granulocytopenia and mucositis after cytarabine-based chemotherapy cycles [85], [86], [87] . The mortality caused by VGS is rather low due to the improvement of supportive measures and reaches a maximum of 1% of the patients in these publications. In the interim evaluation of the AML-BFM 2004 study, no patient died as a result of VGS.

In conclusion, the best prophylactic antibacterial strategy in children with AML has currently not been defined and may depend on the local epidemiology. Each decision of the individual centers should be critically questioned on a regular basis, and the detection of the pathogens causing an infection as well as the resistance of invasive isolates to the antibiotics used in prophylaxis should be observed intensively. The administration of antibacterial prophylaxis should be conducted according to the internal guidelines of your clinic or your local study group.

8.2.2.2 Antifungal prophylaxis

a) Pneumocystis jiroveci (pneumocystsis carinii) pneumonia prophylaxis

Prophylaxis against Pneumocystis jiroveci Prophylaxis for *Pneumocystis jiroveci* remains as (previously) recommended(https://aidsinfo.nih.gov/guidelines/html/5/pediatric-opportunistic-infection/415/pneumocystis-jirovecii-pneumonia). The efficacy of prophylaxis with trimethoprim/sulfamethoxacole (TMP/SMX) for the prevention of *Peumocystis jiroveci* (formerly known as *Pneumocystis carinii*) infection was demonstrated in prospective studies in children[88] . Prophylaxis should be continued throughout chemotherapy and for 3 months after completion of therapy. It is known that trimethoprim-sulfamethoxazole may induce skin reactions; additionally, it may increase the risk of *Clostridium difficile*-associated enterocolitis and has known counterindications with several drugs such as MTX [89].

b) Invasive fungal infection prophylaxis

The published incidence of invasive fungal infections in AML patients varies from 10 to 20 %. Due to high morbidity and mortality, a specific chemoprophylaxis in addition to measures for general infection control is considered to be reasonable. According to the recommendation of the European Conference on Infections in Leukemia (ECIL), prophylactic antifungal strategies for children undergoing treatment for AML include: itraconazole (therapeutic drug monitoring (TDM) is advised); voriconazole (TDM is advised); posaconazole (children ≥13 years, TDM is advised); liposomal amphotericin B; or micafungin.

Itraconzale and posaconazole are not approved for use in children and adolescents ≤18 years. Fluconazole can be considered as antifungal prophylaxis at study sites that have a low incidence (<5%) of mold infections in the HR group [90]. The lack of efficacy of voriconazole and itraconazole against mucormycetes as well as the multiple drug-drug interactions of triazoles have to be considered.

8.2.2.3 Antiviral prophylaxis and secondary prophylaxis

A general antiviral prophylaxis in patients with AML cannot be currently recommended. Secondary prophylaxis with acyclovir should be considered in seropositive patients with AML if they have already suffered severe HSV infection during chemotherapy. Furthermore, especially in VZV-seronegative patients, post-exposure prophylaxis with varicella zoster immunoglobulin is recommended, although the measure is effective in only about 70% of patients [91].

A potential strategy may be the use of acyclovir for 7 days from the 8th incubation day, which was described in registries, but randomized trials about the effectiveness are lacking. For children older than 12 years of age, valacyclovir can be given orally, as it has a better bioavailability as acyclovir. Brivudine, does not seem to be in any way inferior to acyclovir, but is neither approved for children nor for this indication [92]. Due to interaction, the simultaneous use of brivudine with 5-fluoropyrimidines must be avoided.

8.2.3 Granulocytopenia and fever

The compiled decision diagram illustrates the diagnosis and treatment steps for patients with fever while in granulocytopenia. Importantly, fever during granulocytopenia is a medical emergency; therefore, patients need to be evaluated and treated immediately.

Table 2: Diagnostic workflow and treatment steps for patients presenting fever during granulocytopenia

First diagnostics and treatment
Initial measures History / physical examination Heart rate—Breathing—Blood pressure—State of awareness (early signs of shock?) **Diagnostic** Blood count; clinical chemistry, Coagulation, CRP (?) blood cultures (aerobe, anaerobe, fungal- In multi-lumen catheters from each lumen) Virus diagnostic if respiratory symptoms Cultures/swaps of suspicious lesions Optional (weak recommendation: consider urinanalysis and urine culture in patients in whom a clean-catch, midstream specimen is readily available) Further diagnosis according to clinical symptoms (E.g., x-ray thoracic in respiratory symptoms) Monitoring the vital parameters, regular clinical evaluation **Treatment** Quick start of empirical antibiotic therapy (before receiving microbiology finding!) Supportive measures by clinical situation or findings
Re-evaluation of diagnostics and treatment
Fever disappeared, stable condition, negative microbiological findings after 48-72 hours:

-de-escalation of antibiotic therapy (discontinuation of glycopeptide and/or aminoglycoside, if given),
-however, continuation of the modified therapy until signs of hematopoietic regeneration

<u>If fever continues after 48 - 72 hours or reappearance of fever after granulocytopenia:</u>
-re-evaluation including CT -thorax
-if necessary, modification of empirical antibiotic therapy (see below)
-empirical antifungal therapy

<u>Increased instability of the patient:</u>
-re-evaluation and modification of the empirical antibiotic therapy (see below)
empirical antifungal therapy

<u>Evidence of a pathogen:</u>
-therapy according to resistogram; in case of fungal infections, contact the study center

8.2.3.1 Empirical antibacterial treatment

The choice of the empirical antibiotic initial therapy is dependent on the department-specific pathogen spectrum and resistance situation as well as specific patient characteristics (e.g. comorbidity).

The therapy regimen should contain active antibiotics against gram-negative bacteria, including *Pseudomonas aeruginosa*. Additionally, previous cytostatic treatment should be considered. After receiving HD-cytarabine, patients have a high risk for infection with VGS, which often leads to severe infection and is highly lethal. In these patients as well as in patients in unstable conditions, a glycopeptide should be added to the initial empirical therapy. It can be discontinued in patients with stable clinical condition if the pathogens were not isolated after 72 hours. Overall, various antibiotics of different substance classes can be recommended as monotherapy or as part of a combinatorial therapy. For monotherapy, piperacillin/tazobactam, ceftazidime, cefipime or imipenem-cilastine, or meropenem from the carbapeneme group can be considered. All these antibiotics as well as ceftriaxone can be used in a combination therapy with an aminoglycoside such as gentamycin, tobramycin, or amikacin [93].

In patients with sepsis syndrome or septic shock, the combination of imipenem or meropenem plus aminoglycoside and possibly vancomycin or teicoplanin provides a broad-spectrum antibiotic regimen. In addition, the use of an echinocandin should be considered in these patients.

The empirical antibiotic initial therapy should be adapted after re-evaluation of diagnostic findings and the clinical condition of the patient according to Table 2. For modifications based on microbiological findings see Table 3.

Finding	Considerations
Mucositis/gingivitis (pronounced)	Additional clindamycin (anerobes); aciclovir (herpes simplex)
Esophageal symptoms	Additional fluconazole (yeast); if refractory: echinocandin, aciclovir if necessary
New focal pulmonary infiltrates	Additional mold-active antifungals; consider BAL or biopsy; if necessary, macrolide or tetracycline (>8 years) (mycoplasma, chlamydia, legionella) and/or quinolone; if necessary, determine mycobacteria

New interstitial pneumonitis	Sputum induction or BAL (CMV, PcP, adenovirus, etc.); empirical start of TMP/SMZ therapy
Sinusitis/intranasal ulcers	Determination of suspected pathogens (e.g., Aspergillus spp vs mucormycetes)
CNS symptoms	Consider pathogens including molds, nocardia, toxoplasma, herpes viruses; imaging (MRI), consider imaging of the lung; consider assessing CSF and/or biopsy

Table 3: Potential modification of the initial antibiotic regimen

8.2.4 Invasive fungal infections

Microbiological pathogen detection is the cornerstone for the diagnosis of invasive fungal infections in the era of therapeutic options in antifungal chemotherapy.

The initial basis for the diagnosis of invasive *candida* infections are blood cultures. In tissue infections, such as chronic disseminated candidiasis (formerly known as hepatosplenic candidiasis), blood cultures are often falsely negative; invasive diagnostic procedures may be indicated. Based on clinical findings, ultrasonography, and magnetic resonance imaging are important tools for diagnosis, monitoring, and potentially planning biopsy.

The early detection of invasive *aspergillosis* is difficult. As the isolation of Aspergillus spp in the blood is problematic, indirect methods such as the detection of galactomannan (GM, Platelia®) are helpful. This molecule can be detected in blood, BAL and cerebrospinal fluid (CSF), but it is important to note that a number of factors can induce false-positivity (e.g., some batches of piperacillin-tazobactam). A galactomannan test may be false-negative, in particular in patients receiving mold-active prophylaxis, and experts no longer recommend the screening of afebrile patients. High-resolution thoracic CT allows earlier detection of pulmonary infiltrates (indicative of invasive fungal infections) than conventional chest X-ray. The finding of pulmonary infiltrates is not specific regarding invasive *aspergillosis*, and a microbiological diagnosis by bronchoalveolar lavage or by a biopsy procedure should be sought. The significance of polymerase chain reaction (PCR) tests on blood samples have not been conclusively clarified; therefore, this method is regarded as experimental. However, it is useful to examine study material from a biopsy with PCR-based methods; during a scheduled biopsy, it is useful to contact the study center to plan the meaningful examination of the material.

8.2.4.1 Antifungal therapy

Early detection of invasive fungal disease is difficult, but early therapy is associated with better outcome. The concept of empirical antifungal therapy in neutropenic patients (see Table 2) uses the symptom of persistent fever or recurrence of fever (despite broad spectrum antibacterial therapy; fever of unknown origin/no pathogen detected) as a surrogate marker for a highest risk of invasive fungal infections. Evaluated and approved substances in this indication are i.v. liposomal amphotericin B (L-AmB) and i.v. caspofungin.

To reduce the patient population receiving antifungal treatment, pre-emptive therapy is given to persistently neutropenic patients who also have a positive galactomannan test or pathological signs in chest CT scan. A recent randomized study in 149 neutropenic febrile children demonstrated that pre-emptive antifungal therapy was as effective as empirical antifungal therapy, and significantly reduced the use of antifungal drugs [94]. However, the precondition for this strategy is the prompt availability of a chest CT scan and of results of galactomannan assays.

Therapy of probable/proven fungal infections

According to the recommendations from the European Conference on Infections in Leukemia, the main agents of choice for invasive candida infections are liposomal amphotericin B or the echinocandins caspofungin or micafunin, respectively. Fluconazole can be used on stable patients when fluconazole-sensitive pathogens are detected. Upon confirmation of candida infection, removal of central catheter or a port is advised.

The substances of choice for first-line therapy of probable or proven invasive aspergillus infections are voriconazole or liposomal amphotericin B. Potential options also include caspofungin or the combination

of voriconazole and an echinocandin or liposomal amphotericin B and an echinocandin, respectively. Importantly, voriconazole is the drug of choice in CNS aspergillosis. The benefit of combination therapy is currently unclear, and a large study in adults failed to reach a significant improvement versus monotherapy.

In case of invasive mucormycosis, surgical debridement and antifungal therapy are the main treatment elements. The substance of choice is liposomal amphotericin B. In patients who are stabilized, a treatment with posaconazole can be considered [95]. Voriconazole is ineffective in mucormycosis.

Assuming stabilization of the clinical and radiological findings, invasive fungal infections are not an absolute contraindication for further intensive chemotherapy. The prerequisite for the continuation of an effective chemotherapy is a secondary prophylaxis.

For therapeutic decisions regarding all other agents for invasive fungal infections, or in case of resistant disease it is advised to contact the responsible study center.

8.3 Further supportive measures: Immunoglobulins and hematopoietic growth factors

The routine prophylactic administration of intravenous immunoglobulins in children who present with AML can generally not be recommended at this time [96]. Studies in patients after SCT could indicate that at very low concentrations of IgG (<400 mg/dl) a substitution of immunoglobulins may be useful [97–99]. Whether this is true for patients with AML is unclear, while there is no data to support this.

The routine use of hematopoietic growth factors in children with AML cannot be recommended, but in certain situations, such as with a severe bacterial infection or a fungal infection during granulocytopenia, individual application should be considered [100]. The randomization of granulocyte colony-stimulating factor (G-CSF) administration in the AML-BFM 98 study showed no benefits in terms of mortality or morbidity and no significant reduction in the incidence of infection in the G-CSF arm [101]. This data was also verified by the NOPHO-AML 2004 and DB AML-01 studies published in 2019 associating G-CSF with increased risk of relapse[102].

8.4 Further supportive measures: Cardioprotection

Current studies have not been able to prove a clear indication of cardioprotection as a result of dexrazoxane in pediatric AML; however, the available data on dexrazoxane in pediatric AML suggest a benefit from using this agent. Thus, the use of dexrazoxane should be considered [103–106].

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