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Recommendations for Diagnosis and Treatment of Children with Transient Abnormal Myelopoiesis (TAM) and Myeloid Leukemia in Down Syndrome (ML-DS)

Empfehlungen für die Diagnose und Behandlung von Kindern mit transienter abnormer Myelopoese (TAM) und myeloischer Leukämie bei Down Syndrom (ML-DS)

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ABSTRACT

Children with Down syndrome are at a high risk of developing transient abnormal myelopoiesis (TAM; synonym: TMD) or myeloid leukemia (ML-DS). While most patients with TAM are asymptomatic and go into spontaneous remission without a need for therapy, around 20 % of patients die within the first six months due to TAM-related complications. Another 20–30 % of patients progress from TAM to ML-DS. ML-DS patients are particularly vulnerable to therapy-associated toxicity, but the prognosis of relapsed ML-DS is extremely poor – thus, ML-DS therapy schemata must strive for a balance between appropriate efficacy (to avoid relapses) and treatment-related toxicity. This guideline presents diagnostic and therapeutic strategies for TAM and ML-DS based on the experience and results of previous clinical studies from the BFM working group, which have helped reduce the risk of early death in symptomatic TAM patients using low-dose cytarabine, and which have achieved excellent cure rates for ML-DS using intensity-reduced treatment protocols.

ZUSAMMENFASSUNG

Kinder mit Down-Syndrom haben ein hohes Risiko, eine transiente abnorme Myelopoese (TAM; Synonym: TMD) oder myeloische Leukämie (ML-DS) zu entwickeln. Während die

meisten Patienten mit TAM asymptomatisch bleiben und ohne Therapie eine Spontanremission erreichen, sterben ca. 20 % der Patienten innerhalb der ersten sechs Monate an TAM-bedingten Komplikationen. Weitere 20–30 % der Patienten zeigen einen Progress von TAM zu ML-DS. Patienten mit ML-DS sind besonders durch therapieassoziierte Toxizität gefährdet. Gleichzeitig ist die Prognose nach einem Rückfall sehr schlecht. Daher muss die Therapieintensität so gewählt werden, dass einerseits Rückfälle verhindert und andererseits therapiebedingte Komplikationen minimiert werden. Diese Richtlinie präsentiert diagnostische und therapeutische Maßnahmen für Patienten mit TAM und ML-DS, die auf den Erfahrungen und Ergebnissen früherer klinischer Studien der BFM-Arbeitsgruppe basieren, welche dazu beigetragen haben, das frühe Mortalitätsrisiko von symptomatischen TAM Patienten unter Einsatz von niedrig dosiertem Cytarabin zu reduzieren und mit intensitätsreduzierten Chemotherapieprotokollen hervorragende Heilungsraten für ML-DS Patienten zu erzielen.

Introduction

Approximately 5 to 10 % of neonates and infants with Down syndrome (DS) present with a transient clonal proliferation of myeloid blasts with megakaryoblastic or erythroblastic features – a phenomenon called transient abnormal myelopoiesis (TAM), transient myeloproliferative disorder (TMD), or transient leukemia [41]. Most of these children are asymptomatic and achieve spontaneous remission without therapeutic intervention. However, 20 % of patients die within 6 months (referred to as early death), commonly due to blasts infiltrating the liver and subsequent hepatic failure [19, 27, 32, 34]. Another 20 to 30 % of patients with TAM progress to myeloid leukemia (ML-DS) within their first 4 years of life [19, 27, 32]. The preceding period of TAM prior to ML-DS development may be clinically apparent or silent [27, 32, 59]. In general, children with Down syndrome have a 150-fold increased risk of developing myeloid leukemia before 5 years of age [24]. The majority of reported cases present with a predominance of megakaryoblasts, corresponding to acute megakaryoblastic leukemia (AMKL) or FAB-type M7 in non-DS patients [20, 31, 57]. Morphologically, TAM and ML-DS blasts are indistinguishable.

Historically, outcome in children with ML-DS was thought to be poor [40]. On one hand, this was due to higher therapy-related mortality (TRM) with intensive treatment protocols [11, 30]. One the other hand, many patients received only symptomatic or no therapy fearing the lower tolerance to chemotherapy [13]. More recently, however, excellent cure rates have been achieved for ML-DS using intensity-reduced treatment protocols without hematopoietic stem cell transplantation [1, 14, 44, 50]. This excellent response has been attributed to the enhanced drug sensitivity of ML-DS blasts, especially to cytarabine and anthracyclines [51, 60]. Yet, despite the reduced intensity, many patients suffer from therapy-associated toxicity [14] , and TRM is the main cause of death in this cohort of patients [1, 44, 45, 50]. Still, the prognosis of relapsed ML-DS patients is extremely poor [25, 45, 47]. This means that, moving forward, ML-DS treatment schemata must strive for balance between appropriate efficacy to avoid relapses and reduction of treatment-related toxicity.

This guideline presents diagnostic and therapeutic strategies for TAM and ML-DS based on the results of previous clinical studies from the Berlin-Frankfurt-Münster (BFM) study group: namely, TMD07 [17] and ML-DS 2006 [53]. In the **TMD07 study**, patients who presented with TAM-related symptoms at diagnosis or minimal residual disease (MRD) 8 weeks after diagnosis received low dose cytarabine [17]. This treatment helped reduce TAM-related mortality compared to the historical control, but was insufficient to prevent progression to ML-DS. In the **ML-DS 2006 study** treatment intensity was further reduced compared to the reduced-intensity arm of the AML-BFM 98 arm [53], which did not impair the excellent prognosis, achieving a 5-year overall survival (5yr-OS) of 89 ± 3 %. Additionally, poor early treatment response and trisomy 8 were identified as independent prognostic factors that predict worse EFS.

Pathogenesis of TAM and ML-DS

The progression from Down syndrome to TAM, and subsequently, ML-DS can be described as a three-step-model, involving trisomy 21, acquired *GATA1* mutations, and – for the development of ML-DS – additional oncogenic mutations.

Trisomy 21 itself is known to perturb fetal and neonatal hematopoiesis even in the absence of *GATA1* mutations. *In vitro* and animal studies demonstrated an increased proliferation of megakaryocyte-erythroid progenitors in fetal livers with trisomy 21 [42, 52]. Meanwhile, megakaryocytic differentiation is impaired and platelet counts are reduced in neonates with Down syndrome, suggesting that trisomy 21 perturbs normal megakaryopoiesis. Other hematological abnormalities in neonates with Down syndrome include increased hemoglobin and hematocrit, increased numbers of leukocytes including neutrophils, monocytes, and basophils, as well as an increase in peripheral blood blasts [41].

In addition to the abnormal proliferation of megakaryocyte-erythrocyte progenitors caused by trisomy 21, somatic mutations of *GATA1* lead to the transformation and clonal expansion of these progenitors, which is defined as the clinical syndrome TAM. TAM is a transient, clonal, neonatal myeloproliferative disorder unique to Down syndrome, characterized by increased circulating blast cells that harbor acquired N-terminal truncating mutations in *GATA1*. In a physiological context, the transcription factor GATA1 acts as a key regulator of normal megakaryocytic and erythroid differentiation [33]. *GATA1* mutations are present in 25–30 % of all neonates with Down syndrome and are found in all cases of TAM or ML-DS [41]. Most acquired *GATA1* mutations occur in exon 2, including insertions, deletions, and point mutations [4], and lead to the exclusive expression of a truncated protein known as GATA1s [38, 54]. The type of *GATA1* mutation does not predict whether patients with TAM will later progress to ML-DS [4]. Importantly, the detected *GATA1* mutations always disappear when TAM or ML-DS patients go into remission [2, 55].

The presence of a *GATA1* mutation and trisomy 21 is necessary but insufficient for the development of ML-DS – additional oncogenic mutations are required. Most frequently, these additional genetic events occur in genes encoding members of the cohesin complex, epigenetic regulators, or the RAS pathway [29, 55]. Novel ML-DS-specific hotspot mutations were also found in the myeloid cytokine receptor subunit *CSF2RB*[29].

Transient abnormal Myelopoiesis (TAM)

Definition of TAM

The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia recognizes TAM as a unique entity without defining specific diagnostic criteria [6].

Therefore, the BFM working group established the following criteria for the diagnosis of TAM in neonates with trisomy 21: [17, 27]

- > 5 % myeloid blasts in the peripheral blood **or** TAM-associated symptoms (▶ **Table 1**) **and**
- detection of a *GATA1* mutation (or exclusive expression of GATA1s in the blasts)

Cases are classified as "silent TAM" when the *GATA1* mutation is detected in the absence of > 5 % blasts or TAM-associated symptoms. Such cases should also be monitored as outlined below.

Diagnosis of TAM

Symptoms and clinical course of TAM

TAM can present with variable clinical symptoms, ranging from asymptomatic to clinically severe. Overall, severe TAM affects 10–30 % of all patients, and is accompanied by disseminated leukemic infiltration, liver failure/fibrosis, ascites, pleural/pericardial effusion, renal failure, and/or coagulopathy [19, 27, 32].

The median age at diagnosis is 3–7 days, and almost all cases are diagnosed within 2 months after birth [19, 27, 32, 34]. Therefore, all newborns with trisomy 21 should be examined for symptoms associated with TAM within the first days of life [5, 12]. The majority of neonates with TAM will show one or more of the clinical features of TAM, which are summarized in ▶ **Table 1**, [17, 27]. Amongst these symptoms, hepatomegaly, splenomegaly and pericardial/pleural effusions are more frequent in neonates with TAM compared to neonates without *GATA1* mutations [7]. Importantly, no single symptom is specific for TAM; e. g. jaundice is common in neonates with Down syndrome with or without TAM. However, one should always consider that delayed onset or prolonged hyperbilirubinemia in neonates with Down syndrome might be the presenting feature of progressive TAM-associated liver fibrosis, which can be lethal [7, 17, 27]. In general, clinical symptoms associated with early death are high white blood cell (WBC) count (> 100×10^9/L), *hydrops fetalis*, ascites, liver dysfunction/failure (including coagulopathy) and preterm delivery/low birth weight [27]. Except for prematurity, where the association with TAM-related early death is less clear, these symptoms – which were defined by the TMD07 study – are in accordance with other studies [19, 31, 32, 34]. Liver dysfunction, especially, with elevated aspartate transaminase (AST) or alanine transaminase (ALT), or elevated direct bilirubin levels, seems to be directly linked to TAM blasts infiltrating the liver and TAM-related mortality.

▶ **Table 1** Clinical and laboratory features in TAM (data based on information from [15, 25]).

Clinical & laboratory features	Present in patients with TAM (%)
Hepatomegaly	50
Splenomegaly	35
Pericardial effusion	15
Pleural effusion	10
Elevated transaminases	25
Pathological coagulation	30
Hydrops fetalis	5
Ascites	10
Cholestasis	20
Hematological features	**Median in patients with TAM**
WBC, x 10^9/L	32.2
Platelets, x 10^9/L	110
Hemoglobin, mmol/L	9.2
Blasts in PB, %	33
Blasts in BM, %	23

Most cases of TAM go into spontaneous remission within the first 3 months of life [19, 27, 32, 34]. Complete remission is often characterized by the normalization of blood counts, including a decline in peripheral blasts followed by the disappearance of clinical symptoms such as hepatomegaly [36]. About 20 % of patients with TAM die from their disease, mostly due to liver failure caused by hepatic fibrosis and blast infiltration [19, 27]. Another 20–30 % of patients subsequently develop ML-DS, either through overt progression from TAM to ML-DS with persistent hematological abnormalities, or after a phase of apparent remission [19, 27, 32]. MRD positivity at week 12 is associated with a higher risk of ML-DS irrespective of prior therapy (46 ± 15 % vs. 13 ± 5 %, $P_{Gray} = 0.01$) [17].

In around 20 % of neonates with Down syndrome without any clinical symptoms, a *GATA1*-mutated clone can be detected – referred to as "silent TAM" [41]. The presence of the *GATA1* mutation means that neonates with silent TAM are at risk of subsequently developing ML-DS if the mutant *GATA1s* clone persists [41].

Hematological and laboratory features of TAM

The main hematological features of TAM are leukocytosis and increased blasts in the peripheral blood [27, 32, 58]. However, there is no international consensus regarding the threshold for the percentage of blasts that reliably identifies all cases of TAM, as blasts can be found in 97.5 % of all neonates with Down syndrome, which can exceed 10 % in the peripheral blood even in the absence of a *GATA1* mutation [19, 27, 34, 41]. Based on our previous studies and experience, we suggest a threshold of > 5 % myeloid blasts in the peripheral blood combined with the detection of a *GATA1* mutations for the identification of TAM [17, 27].

For the detection of *GATA1* mutations, next generation sequencing (NGS) is superior to Sanger sequencing due to its lower limit of detection, especially since multiple *GATA1* clones may be present at the time of diagnosis [2, 41]. In cases where TAM is strongly suspected, but where no *GATA1* mutation can be detected, the exclusive expression of the aberrant GATA1s protein in blasts can be ver-

ified using flow cytometry (intracellular staining) or western blotting [16]. Morphologically, TAM blasts are often described as megakaryoblastic with cytoplasmic blebbing and basophilic cytoplasm. However, their morphology can be highly variable. Similarly, their immunophenotype is also variable: the characteristic co-expression pattern of stem cell markers (CD34 and CD117), myeloid markers (CD33/CD13), and platelet glycoproteins (CD36, CD42, CD61) together with CD56 and CD7 is heterogeneous [8, 21, 26, 31]. Platelet counts can vary, and thrombocytopenia is not more common in patients with TAM than in other patients with Down syndrome [41]. Anemia is uncommon in patients with TAM. Disturbed coagulation is observed in 20–25 % of patients with TAM, and disseminated intravascular coagulopathy (DIC) may occur. The latter is usually associated with severe liver dysfunction due to hepatic infiltration by blast cells. Hepatic dysfunction presents as severe conjugated hyperbilirubinemia and is often accompanied by elevated levels of transaminases [19, 27, 32, 34, 36]. Altogether, patients with TAM present with various hematological and laboratory abnormalities, none of which is specific when considered as a single symptom. Thus, suspicious blast counts as well as other hematological and/or clinical findings must always be validated by molecular testing for *GATA1* mutations.

In rare cases, TAM may occur in patients with trisomy 21 mosaicism [37]. Therefore, trisomy 21 mosaicism should be considered in newborns with increased peripheral blasts or other TAM-related symptoms who present without clinical features of Down syndrome. As with the diagnosis of TAM in patients with Down syndrome, the detection of a *GATA1* mutation or aberrant GATA1s expression likewise confirms the diagnosis of TAM in these cases.

Recommendation for diagnostic procedures

The diagnosis of TAM must be made morphologically, immunophenotypically, cytogenetically (proof of trisomy 21), and molecularly from peripheral blood. A differential blood count as well as a blood smear should be performed for every child with Down syndrome within the first week of life [5, 12]. If abnormalities reminiscent of TAM are detected, further diagnostic measures should be undertaken, including the assessment of molecular genetics (*GATA1* mutation analysis), immunophenotype (recommended surface markers: CD34, CD117, CD7, CD13, CD33, CD15, CD36, CD56, CD41, CD42b, CD61), and morphology. An overview of recommended initial diagnostic procedures, and of further diagnostics prior to starting treatment, is provided in ▶ **Table 2** and ▶ **Table 3**, respectively. Advice from an experienced reference laboratory is strongly recommended.

▶ **Table 2** Recommendation for diagnostic procedures for diagnosis of TAM.

Initial diagnostics for TAM
Peripheral blood sample for ▪ Morphology ▪ Immunophenotyping to measure the following antigens: CD34, CD117, CD7, CD13, CD33, CD15, CD36, CD56, CD41, CD42b, CD61 ▪ Cytogenetics (confirmation of trisomy 21) ▪ Molecular genetics: ***GATA1***-mutation (also as base line for MRD)
Full blood count
Physical examination including: weight/height, organ size (spleen and liver), signs of bleeding
Ultrasound: size/structure of liver and spleen, detection of ascites, pleural or pericardial effusions
Chest x-ray: in case of respiratory symptoms or if infection is suspected

▶ **Table 3** Recommendation for diagnostic procedures prior to treatment or to further guide treatment decision.

Further diagnostics prior to treatment of TAM
Coagulation (INR, aPTT, d-dimers)
Electrocardiography and echocardiography: for LV function and to rule out congenital heart disease
Clinical chemistry (electrolytes [Na^+, K^+, Mg^{2+}, Ca^{2+}, PO_4^-], blood glucose, renal retention parameters [e. g. creatinine], transaminases [ALT, AST, γGT], bilirubin, LDH, fT3, fT4, TSH, others according to clinical findings)
Viral serologies (measles, mumps, rubella, EBV, CMV, HSV, HHV6, Parvovirus B19, HIV 1/2, HAV, HBV, HCV, VZV)
Medical and family history
Blood group

▶ **Table 4** Symptoms of TAM associated with increased risk of early death upon which treatment should be initiated [2].

Indications for treatment of TAM
Hyperleukocytosis ($>100\times10^9$/L)
Liver dysfunction: Hepatomegaly in combination with elevated liver enzymes and/or cholestasis
Ascites
Hydrops fetalis
Life-threatening symptoms including: ▪ hepatosplenomegaly causing respiratory failure ▪ heart failure (ejection fraction < 47 % or shortening fraction < 27 %) not directly the result of a congenital heart defect ▪ pleural or pericardial effusion ▪ renal dysfunction ▪ disseminated intravascular coagulation (DIC) with bleeding

Treatment of TAM

In the TMD07 study, patients who presented with TAM-related symptoms at diagnosis (high WBC count [$>100\times10^9$/L], *hydrops fetalis*, ascites and/or liver dysfunction/failure [defined as hepatomegaly in combination with elevated liver enzymes and/or cholestasis]) or minimal residual disease 8 weeks after diagnosis received low dose cytarabine [17]. In patients who were eligible for treatment because of symptoms (n = 43), we observed a significantly lower cumulative incidence (CI) of early death compared to symptomatic patients in the historical control (n = 45) (12 ± 5 % vs 33 ± 7 %, $P_{Gray} = 0.02$), while no early deaths occurred in children without any of these symptoms [27]. Hence, our definition of symptoms reliably identifies patients at risk of early death. In accordance with our findings, Gamis et al. defined life-threatening symptoms that require therapeutic intervention (▶ **Table 4**) [19].

It has been shown that TAM blasts are highly susceptible to cytarabine;[60] thus, it is commonly used for the treatment of TAM patients. In the TMD07 trial, low dose cytarabine (1.5 mg/kg for 7 days) was applied subcutaneously or intravenously (i. v.). In some patients, typical side effects of cytarabine – such as fatigue, nau-

sea, and hematologic toxicity – were observed. Nevertheless, in general, the treatment was well tolerated [17]. Other studies have applied higher doses of cytarabine (e. g. 3.33 mg/kg/24 h continuous infusion), albeit with severe hematologic toxicity and no clear improvement in EFS or prevention of ML-DS compared to the lower dose [19, 27]. In the TMD07 study, two patients who presented with TAM-related symptoms died before receiving treatment – suggesting that the treatment of TAM cases should be considered and initiated early.

Importantly, the treatment of symptomatic or MRD-positive patients in the TMD07 study did not result in a significantly lower CI of ML-DS (25 ± 7 % [treated] vs. 14 ± 7 % [untreated], P_{Gray} = 0.34 [per protocol analysis]; historical control: 22 ± 4 %, P_{Gray} = 0.55) [17]. This failure to prevent the development of ML-DS suggests that it is currently not possible to entirely eliminate the preleukemic *GATA1* mutated clone using the applied intervention.

Recommendation for treatment

Therapy should be considered for children with TAM who show any of the symptoms that are associated with an increased risk of early death (▶ **Table 4**) [17, 19, 27]. In such children, treatment should be considered as early as possible.

If therapy is applied, we recommend low-dose cytarabine (1.5 mg/kg body weight) i. v. (slowly over at least 5 min.) or subcutaneously for 5 to 7 days (▶ **Fig. 1**). If no response is achieved, an additional course after an interval of at least 5–7 days can be applied.

General preventive chemotherapeutic treatment of children diagnosed with TAM cannot be recommended. Children without any of the symptoms in ▶ **Table 4** should not receive therapy.

Monitoring of peripheral blood counts, liver parameters and renal function is recommended before therapy and after 1, 4 and 10 days of therapy. In the case of severe anemia (Hb < 5.5 mmol/L), thrombocytopenia (< 100 × 10^9/L), or neutropenia (< 0.5 × 10^9/L) that is not caused by TAM (i. e. due to ITP, congenital neutropenia, hemolytic anemia), no treatment should be administered. Treatment with low-dose cytarabine should be stopped if severe toxicities (≥ CTC grade 3) are observed.

Monitoring and follow up of patients with TAM

Due to the risk of subsequent progression to ML-DS, children with TAM should be regularly examined for signs of ML-DS following remission. Children may present with symptoms caused by anemia, thrombocytopenia or neutropenia. As MRD positivity at week 12 is associated with an increased risk for progression to ML-DS, we recommend assessing MRD during the course of TAM to estimate the risk of transformation. MRD can be measured by NGS or flow cytometry [17, 22, 41]. As minor *GATA1s* clones can grow out and give rise to ML-DS, mutation specific quantitative PCR is not recommended [17, 29]. We recommend the following examinations 4 weeks, 8 weeks, 12 weeks, 6 months, 12 months, 18 months, 24 months, 36 months and 48 months after TAM diagnosis: general physical examination, blood count/differential blood count and blood smears as well as MRD-measurement.

Myeloid leukemia associated with Down syndrome (ML-DS)

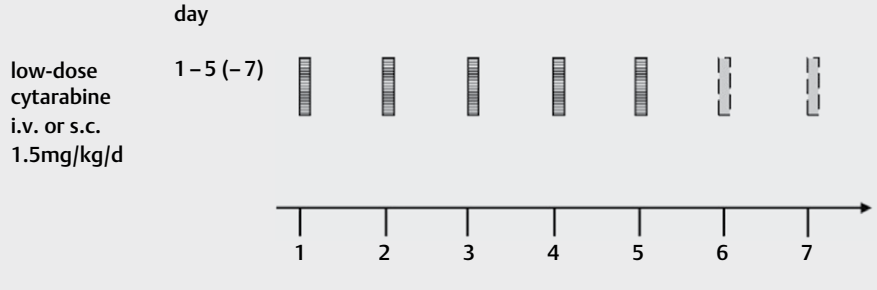
Definition of ML-DS

The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia recognizes ML-DS as a unique entity, characterized by the proliferation of usually megakaryoblastic blasts with *GATA1* mutations and additional mutations such as mutations affecting the JAK-STAT pathway, which primarily occurs within the first 3 years of life in patients with Down syndrome. However, the WHO classification does not define specific diagnostic criteria for ML-DS [6]. Cases with Down syndrome and sporadic AML have to be distinguished from ML-DS, as their prognosis and treatment greatly differs [23]. Therefore, the BFM working group established criteria for the diagnosis of ML-DS as presented in ▶ **Table 5** [53], for which reduced-intensity treatment according to this recommendation can be applied.

Diagnosis of ML-DS

Clinical and hematological features of ML-DS

ML-DS occurs at a median age of 1.6 years and is rare after the age of 4 years [14, 53]. ML-DS often displays an indolent presentation with myelodysplasia and progressive pancytopenia – in particular, thrombocytopenia and leukopenia – and a low percentage of circulating blasts, in contrast to TAM [14, 39, 53]. Since peripheral blast counts are often low in patients with ML-DS, a bone marrow aspirate (or a bone marrow trephine, in cases of “dry tap” aspira-



▶ **Fig. 1** Therapy overview for TAM. Low-dose cytarabine (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.

▶ **Table 5** Criteria for diagnosis of ML-DS.

Diagnostic criteria for ML-DS
Presence of AML or MDS(-EB) according to WHO classification (WHO 2016)
Trisomy 21: Down syndrome or mosaic
Age: > 6 months and ≤ 4 years of age with/without *GATA1* mutation or > 4years of age < 6 years of age with *GATA1* mutation
Morphology/immunophenotyping: FAB M0, M6 or M7
Cytogenetics: no recurrent genetic abnormalities of AML (WHO 2016)
No Transient Abnormal Myelopoiesis (TAM) (see chapter "*Definition of TAM*")

tions due to secondary bone marrow fibrosis, which are frequent) is essential for diagnosis.

ML-DS blasts mostly present with a megakaryoblastic morphology similar to TAM (FAB M7), but can also present with morphology equivalent to the FAB subtypes M0 or M6 [15, 56]. Similarly, ML-DS blasts share the same immunophenotype as their TAM predecessors , with co-expression of stem/progenitor cell markers (CD34, CD117), myeloid (CD33), megakaryocytic (CD42b and CD41) and erythroid markers (CD36 and glycophorin A) as well as aberrant markers (CD7, CD56 and CD11a$^-$). Several karyotypic abnormalities are more frequent in ML-DS than in pediatric AML patients without Down syndrome, including trisomy 8, trisomy 11, trisomy 21, del (6q), del(7p), del(16q) and dup(1p) [18]. Trisomy 8 was identified as an independent prognostic factor for predicting poor outcome [53]. *GATA1* mutations are always present in ML-DS blasts and sequencing usually reveals additional mutations in other oncogenes [29, 35, 55].

Recommendation for diagnostic procedures

An overview of recommended initial diagnostic procedures is provided in ▶ **Table 6**. Advice from the reference laboratory is strongly recommended. The diagnosis of ML-DS must be made morphologically, immunophenotypically, cytogenetically, and molecularly from the bone marrow. A lumbar puncture is necessary to diagnose CNS involvement. An MRI should be performed if the cerebrospinal fluid (CSF) is abnormal, although in the ML-DS 2006 trial, no patients had CNS involvement [53]. The exception is when patients present with hyperleukocytosis ($> 50 \times 10^9$/L). In this case, lumbar puncture is performed after the cell count decreases to $< 50 \times 10^9$/L or after decay of the risk of bleeding. However, in the ML-DS 2006 trial, no children presented with hyperleukocytosis, leading us to expect that this clinical situation will be rare [53].

We recommend assessing MRD by NGS or flow cytometry during the course of treatment. For MRD detection, peripheral blood samples and bone marrow aspirates should be obtained on day 1 and day 28 of treatment, and before each intensive therapy element, i. e. on days 56 and 84.

Treatment of ML-DS

Children with ML-DS have a superior outcome compared to non-DS AML patients, but suffer from higher constitutional susceptibility to cytotoxic drugs. We previously analyzed the outcome of 170 pediatric patients with ML-DS enrolled in the ML-DS 2006 trial [53].

▶ **Table 6** Recommendation for initial diagnostic procedures for ML-DS.

Initial diagnostics for ML-DS
Bone marrow aspirate: ▪ Morphology (myelogram and FAB classification 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 If any of these tests have been omitted on the first diagnostic samples, a second sample should be taken before treatment starts, if clinically possible. Analysis of peripheral blood blasts, and trephine biopsy are required if an adequate marrow sample cannot be obtained by aspiration.
Medical and family history ▪ history of TAM or prior low-dose cytarabine treatment
Physical examination including: ▪ weight/height ▪ organ size (spleen and liver) ▪ signs of bleeding
Lumbar puncture for CSF cytology
Electrocardiography and echocardiography for LV function and to rule out congenital heart disease
Ultrasound: liver, spleen, mediastinum
Chest x-ray
Full blood count and peripheral blood smear for morphology
Clinical chemistry (electrolytes [Na^+, K^+, Mg^{2+}, Ca^{2+}, PO_4^-], blood glucose, renal retention parameters [e. g. creatinine], transaminases [ALT, AST, γGT], bilirubin, LDH, fT3, fT4, TSH others according to clinical findings)
Coagulation (INR, aPTT, d-dimers)
Viral serologies (measles, mumps, rubella, EBV, CMV, HSV, HHV6, Parvovirus B19, HIV 1/2, HAV, HBV, HCV, VZV)
Blood group

The ML-DS 2006 trial was based on the reduced-intensity arm for ML-DS patients as a part of the AML-BFM 98 trial [14]. Given the excellent outcome of ML-DS patients in the AML-BFM 98 trial (n = 67; 5yr-OS: 90 ± 4 %, 5yr-EFS: 89 ± 4), treatment intensity was further reduced in the ML-DS 2006 trial through the exclusion of etoposide from consolidation (reducing the cumulative dose from 950 mg/m^2 to 450 mg/m^2), administration of 4 instead of 11 doses of intrathecal CNS-prophylaxis and exclusion of maintenance therapy. Despite this reduction, the 5-year overall survival (5yr-OS; 89 ± 3 % vs. 90 ± 4 %, $P_{log\text{-}rank} = 0.64$), event-free survival (5yr-EFS; 87 ± 3 % vs. 89 ± 4 %, $P_{log\text{-}rank} = 0.71$) and cumulative incidence of relapse/non-response (CIR/NR; 6 ± 3 % vs. 6 ± 2 %, $P_{Gray} = 0.95$) did not significantly differ between the ML-DS 2006 trial and the historical control arm [53]., validating that therapy reduction did not result in a higher risk of relapse. The absence of CNS involvement in any of the patients could suggest that ML-DS blasts cannot home to this niche, and explain why we did not observe an increase in CNS relapse despite reduction of CNS prophylaxis. Although the reduction of TRM from 5 % to 2.9 % did not reach significance ($P_{FishersExact} = 0.276$), excluding etoposide resulted in fewer severe adverse events after consolidation. The data imply that even further reduc-

tion of treatment intensity may be feasible based on prognostic factors.

International study protocols for the treatment of ML-DS differ substantially, especially regarding the role of high-dose cytarabine and the dosing of anthracyclines (**Supp. Table 1**). While in most European and North American trials for ML-DS, including ML-DS 2006, courses with high-dose cytarabine (3 g/m²/d) are applied [1, 14, 39, 50], Japanese studies (JPSLG AML D05) obtained excellent results (3yr-OS: 88 ± 4 % 3yr-EFS: 83 ± 4 %) and low TRM (1.4 %; n = 1/72) using standard-dose cytarabine (100 mg/m²/d) [48]. However, despite the use of high-dose cytarabine in the ML-DS 2006 trial, TRM did not significantly differ from the JPSLG AML D05 trial ($P_{FishersExact}$ = 0.673).

Together with the results of the Toronto group that used a low-dose cytarabine-based regimen [3, 49], 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. But clear prognostic factors that would predict which patients are at risk of relapse and need intensive therapy remain elusive.

However, there is a general international consent about longer treatment intervals and the discouraging role of hematopoietic stem cell transplantation. Accordingly, as ML-DS patients are susceptible to treatment-related toxicity, treatment intervals in the ML-DS 2006 study were longer, giving the patients more time to recover after each course. A block was only started if the child showed a recovery of blood counts and was in good general condition without clinical signs of infection, mucositis or fever. This resulted in manageable treatment-related toxicity in that trial.

In summary, the data of the ML-DS 2006 trial showed that therapy reduction could be achieved in children with ML-DS, without compromising their excellent outcome. Further therapy reduction for ML-DS patients with good early response will be a subject of investigation in future trials.

Recommendation for treatment

Patients with ML-DS are a particularly vulnerable group of patients, requiring specific attention and monitoring of treatment. Therefore, it is recommended that treatment should only be performed in pediatric oncology centers that are experienced in the treatment of ML-DS. If a trial is opened for these children, taking their needs and the specific features of this entity into account, patients should be enrolled or transferred to a study center, assuring optimal access to tailored treatment protocols and disease monitoring.

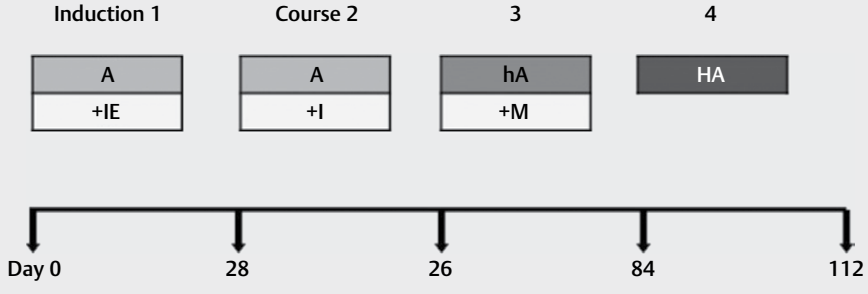
Based on the ML-DS 2006 trial, the recommended chemotherapy for patients with ML-DS consists of 4 cycles at 4-week intervals, namely AIE, AI, hAM and HA (▶ **Fig. 2**). At the beginning of each cycle, a bone marrow puncture (MRD and morphology) and a lumbar puncture with intrathecal administration of cytarabine as CNS-prophylaxis should be performed. Age-related doses of cytarabine i.th. are provided in **Supp. Table 2**. Treatment should only be initiated in children with a Lansky performance score of at least equal to 50, or a Karnofsky performance status of at least equal to 50 – whichever is applicable. Therapy with the next cycle should only be continued in the absence of active infection, fever, and mucositis, and with recovering blood counts with granulocytes > 1×10^9/L and platelets > 80×10^9/L. An echocardiography and ECG are necessary before starting each cycle.

Patients presenting with any of the criteria listed in ▶ **Table 7** should not be considered for treatment according to these recommendations.

Of note, for children who weigh ≤ 10 kg or are < 1 year of age, dosages for intravenous chemotherapy must be calculated per kg of body weight and not according to body surface area (**Supp. Table 3**). An exception to this is the dosage of HD-cytarabine, which is calculated based on an age-dependent, reduced dose according to body surface area (**Supp. Table 4**).

Cytoreductive pre-phase

Hyperleukocytosis (WBC > 50×10^9/L) with severe coagulopathy is a very rare event in children with ML-DS. In cases with hyperleukocytosis, cytoreductive therapy prior to definitive therapy can be considered, as suggested in **Supp. Table 5**. Exchange transfusion or leukapheresis should be considered in cases with severe pulmonary or CNS symptoms, worsened coagulation status, increased lactate, or abnormal blood gas analysis.



▶ **Fig. 2** Overview of ML-DS therapy. 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] and etoposide 150 mg/m²/d [days 6, 7 and 8]); AI (cytarabine 500 mg/m²/d [days 1-4] and idarubicin 5 mg/m²/d [days 3 and 5]); haM (high-dose cytarabine 1 g/m²/12h [days 1-3] and mitoxantrone 7 mg/m²/d [days 3-4]); HA (high-dose cytarabine 3 g/m²/12h [days 1-3]). The cumulative doses are 27,400 mg/m² cytarabine, 450 mg/m² etoposide, 34 mg/m² idarubicin and 14 mg/m² mitoxantrone. Chemotherapy for children <=12 months of age, or weighing <=10 kg is calculated based on body weight, with the exception of HD-cytarabine, which is calculated based on an age-dependent, reduced dose according to body surface area in children <=24 months of age. In addition, patients receive cytarabine intrathecally at the start of each treatment block (4 doses in total, 20-40 mg per dose adapted to age) as a CNS-prophylaxis.

In cases with hyperleukocytosis, an initial diagnostic lumbar puncture should not be performed! The lumbar puncture should be delayed until blast counts are sufficiently reduced.

1. Cycle: Induction – AIE

Prior to the start of induction therapy (AIE), patients should have WBC below 50×10^9/L. AIE consists of cytarabine i. v. (100 mg/m^2), idarubicin i. v., and etoposide i. v.. An infusion plan is provided as **Supp. Fig. 1 and Supp. Table 6**.

2. Cycle – AI

AI therapy commences at least 28 days after the start of course 1 (induction - AIE). AI consists of cytarabine i. v. (500 mg/m^2) and idarubicin i. v.. An infusion plan is provided as **Supp. Fig. 2 and Supp. Table 7**.

3. Cycle – hAM

hAM therapy commences at least 28 days after the start of course 2 (AI). Ophthalmological infections must be excluded before starting hAM. hAM consists of HD-cytarabine i. v. (1 g/m^2) and mitoxantrone i. v. An infusion plan is provided as **Supp. Fig. 3 and Supp. Table 8.** For optional supportive care, diclofenac eye drops can be administered two times a day beginning 4–6 h before the first dose of HD-cytarabine, or artificial tears 2 drops/eye can be administered every 4–6 h beginning 6 h before, and continuing until 12 h after, the last dose of HD-cytarabine.

4. Cycle – HA

HA therapy commences at least 28 days after the start of course 3 (hAM). Ophthalmological infections must be excluded before starting HA. HA consists of HD-cytarabine i. v. (3 g/m^2). An infusion plan is provided as **Supp. Fig. 4 and Supp. Table 9**. For optional supportive care, diclofenac eye drops can be administered two times a day beginning 4–6 h before the first dose of HD-cytarabine, or artificial tears (2 drops/eye) can be administered every 4–6 h beginning 6 h before, and continuing until 12 h after, the last dose of HD-cytarabine.

▶ **Table 7** Exclusion criteria for recommended treatment.

Exclusion criteria for recommended treatment
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 5.0 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, except 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

CNS prophylaxis and CNS treatment

All patients should receive i.th. CNS-prophylaxis at the start of each treatment block (4 doses in total). Monotherapy with cytarabine is recommended. Dosages should be adapted to age, as indicated in **Supp. Table 2**.

Patients with primary CNS involvement must receive weekly intrathecal cytarabine injections until the CSF is cleared of blasts, followed by 1 additional injection. A minimum of three injections should be given. After this initial treatment, further intrathecal cytarabine injections are given according to the prophylactic schedule.

Treatment of relapsed ML-DS

Children with ML-DS who suffer from relapse should be treated according to an individualized schedule, which takes into account the increased risk of toxicity and potential treatment resistance. No general recommendations can be made.

Discussion

TAM and ML-DS are unique clinical hematological diseases, recognized by the WHO classification [6]. Herein we provide diagnostic procedures and criteria as well as treatment recommendations, taking the specific laboratory and clinical features of these entities into account. These guidelines and recommendations are mainly based on the experience and previous trials of the BFM study group, of which the TMD07 [17] and ML-DS 2006 [53] trials were the most recent. These studies were among the largest of their kind and yielded excellent results compared to other international studies [19, 34, 45, 48, 50]. However, it should be noted that other study groups follow different approaches and that not all recommendations have international consensus. However, where applicable, we mention these different views and approaches, allowing the reader to interpret and evaluate our definitions and recommendations.

In particular, the definition of TAM and the indication for its treatment are still under debate. The TMD07 trial showed that upon treatment with low-dose cytarabine, the CI of death within the first 6 months was significantly reduced in patients with severe symptoms (high WBC count [$> 100 \times 10^9$/L], *hydrops fetalis*, ascites and/or liver dysfunction/failure [defined as hepatomegaly in combination with elevated liver enzymes and/or cholestasis]) [17]. No early deaths occurred in children without any of these symptoms [17]. In accordance with this finding, Gamis et al. defined life-threatening symptoms requiring therapeutic intervention, which we incorporated into our treatment recommendations [19].

Another point of debate is the appropriate treatment intensity for ML-DS, which differs substantially between study groups [1, 14, 28, 39, 45, 48, 49]. Whereas most European and North American trials for ML-DS contain courses with high-dose cytarabine [1, 14, 39, 45], Japanese studies obtained excellent results using standard-dose cytarabine [46, 48]. Together with the results of the Toronto group – who used a low-dose cytarabine-based regimen [3, 49] containing no anthracyclines and no etoposide – these data indicate that subgroups of patients with ML-DS can be cured even with much lower doses than those presented in this treatment recommendation. However, clear prognostic factors that can predict patients who are at risk of relapse and need intense therapy remain elusive. The ML-DS 2006 trial identified poor early treatment re-

sponse (> 5 % blasts in the bone marrow), as assessed by morphology after the first therapy cycle, as a marker for poor outcome and relapse. Similar results were obtained in the COG AAML0431 trial, using flow cytometry for measuring residual blasts [50]. Future trials will need to show whether MRD monitoring using molecular techniques, such a next-generation sequencing-based MRD, can increase predictive value.

In the ongoing AML-BFM trial, ML-DS 2018, we introduce MRD-based risk stratification after induction to reduce the dose of cytarabine in course 4 from 3 g/m²/12 h to 1 g/m²/12 h in good responders. Additionally, we substitute idarubicin, cytarabine, and etoposide (course 1 and 2) by CPX-351, a liposomal formulation of cytarabine:daunorubicin encapsulated at a 5:1 molar ratio in order to achieve a favorable efficiency/toxicity profile, in all children with ML-DS (**Supp. Table 1**) [10]. Other possible new approaches towards a less toxic therapy for ML-DS include histone deacetylase inhibitors [43] and wee1 kinase inhibitors [9].

In conclusion, although our knowledge on ML-DS and TAM has substantially increased over the past years – especially with the advent of next-generation sequencing – definitions, treatment indication, disease monitoring and treatment protocols are still undergoing refinement. Nonetheless, we are confident that these treatment guidelines represent the current state-of-the-art, with the capacity to grant patients access to recent advances and provide excellent outcome as well as acceptable treatment-related toxicity.

Contributor's Statement

S. Al-Kershi wrote the manuscript. R. Golnik, M. Flasinski, K. Waack, M. Rasche, U. Creutzig, M. Dworzak, and D. Reinhardt revised the manuscript. J.-H. Klusmann designed the guideline and revised the manuscript. All authors contributed to the development of diagnostic and therapeutic recommendations for TAM and ML-DS.

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Conflict of Interest

D. Reinhardt has advisory roles for Celgene Corporation, Novartis, Bluebird Bio, Janssen, and receives research funding from CLS Behring and Roche. J.-H. Klusmann has advisory roles for Bluebird Bio, Novartis, Roche and Jazz Pharmaceuticals.

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Supplemental Material

	Years	N	DNR (mg/m²)	ARA-C (mg/m²)	Etoposide (mg/m²)	TRM (%)	5yr-OS (%)	5yr-EFS (%)	Ref.
ML-DS 2018	21-	-	145	12,330/ 24,330	0	-	-	-	-
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	[2]
COG AAML0431	07-13	204	240	27,800	750*	1.0	93	91	[3]
Al-Ahmari	90-03	34	0	7,400	0	0	77	67	[4]
JPLSG AML-D11	12-15	72	250	3,500	1,350	1.3	90	87	[5]

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

Supp. Table 1: Comparison of the cumulative doses of treatment elements and clinical outcome in different ongoing and previous international ML-DS trials

Age	Dosage for i.th. cytarabine
< 1 year	20 mg
1 year	26 mg
2 years	34 mg
≥ 3 years	40 mg

Supp. Table 2: Age-dependent dosage for intrathecal cytarabine

body weight	cytarabine 100 mg/m²	500 mg/m²	idarubicin 5 mg/m²	8 mg/m²	etoposide 150 mg/m²	mitoxantrone 7 mg/m²
4.0 kg	13.3	67	0,67	1,07	20	0.9
4.5 kg	15	75	0,75	1,20	22.5	1.1
5.0 kg	16.7	83	0,83	1,33	25	1.2
5.5 kg	18.3	92	0,92	1,47	27.5	1.3
6.0 kg	20	100	1,00	1,60	30	1.4
6.5 kg	21.7	108	1,08	1,73	32.5	1.5
7.0 kg	23.3	117	1,17	1,87	35	1.6
7.5 kg	25	125	1,25	2,00	37.5	1.8
8.0 kg	26.7	133	1,33	2,13	40	1.9
8.5 kg	28.3	142	1,42	2,27	42.5	2.0
9.0 kg	30	150	1,50	2,40	45	2.1
9.5 kg	31.7	158	1,58	2,53	47.5	2.2
10 kg	33.3	167	1,67	2,67	50	2.3

Supp. Table 3: Adjustments of dosages for infants and children ≤ 10 kg of body weight (doses in mg). In infants (children ≤ 12 months of age) and children with a bodyweight ≤ 10 kg, the dosages are usually calculated according to body weight, and not to body surface area. The dosages given per m² are to be divided by 30 to obtain the dose (in mg).

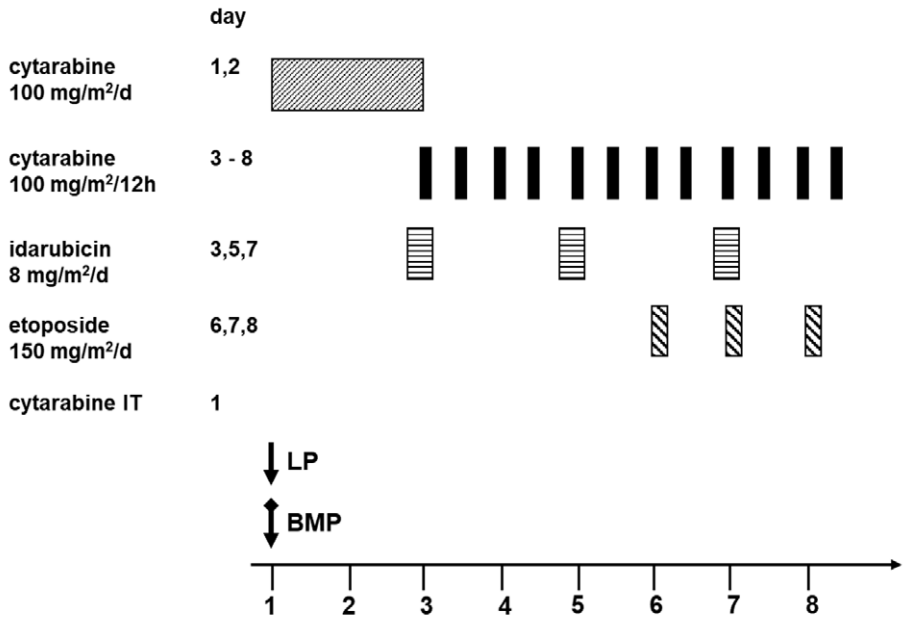
Age (months)	% of dose /m²	HD-cytarabine	
		1 g/m²	3 g/m²
<3	20	0.2	0.6
4 - 5	30	0.3	0.9
6 - 7	40	0.4	1.2
8 - 10	50	0.5	1.5
11 - 13	60	0.6	1.8
14 - 16	70	0.7	2.1
17 - 19	80	0.8	2.4
20 - 24	90	0.9	2.7
>24	100	1.0	3.0

Supp. Table 4: Dose reduction of high-dose cytarabine (hA 1 g/m² and HA 3 g/m²) (doses in g). HD-cytarabine (hA 1 g/m² and HA 3 g/m²) is calculated based on an age-dependent, reduced dose according to body surface area and not according to body weight in children ≤ 24 months of age. Reasons for the dosage reduction of HD-cytarabine are a decreased deaminase capacity, a lower clearance of cytarabine due to a reduced deamination to Ara-U and an increased neurotoxicity.

Cytoreductive pre-phase if WBC > 50 x10^9/L:	Cytoreductive pre-phase if WBC > 100 x10^9/L
cytarabine (40 mg/m^2/d) s.c. or i.v.	50% cytarabine (20 mg/m^2/d) s.c. or i.v.
or 6-thioguanine 40 mg/m^2/d p.o.	
and/or hydroxyurea 20 mg/kg/d p.o.	
Supportive care	
hyperhydration (2-4 L/m^2/d), cave: cardiac contraindications	
	Rasburicase or alkalization of urine plus allopurinol
	intensive monitoring (at least every 4 h): vital signs, ABG, lactate, blood count, coagulations status
	Fresh-frozen plasma in case of decompensation of the plasmatic coagulation
Extended therapy if no WBC reduction or no signs of tumor lysis:	
	4-8h after administration of first dose: cytarabine (40mg/m²) i.v.
12 h after administration of first dose: cytarabine as continuous infusion (100 mg/m^2/24h)	
24 h after administration of first dose: start anthracyclines (50% of the dosis)	

Supp. Table 5: Cytoreductive therapy recommendations

AIE infusion plan:

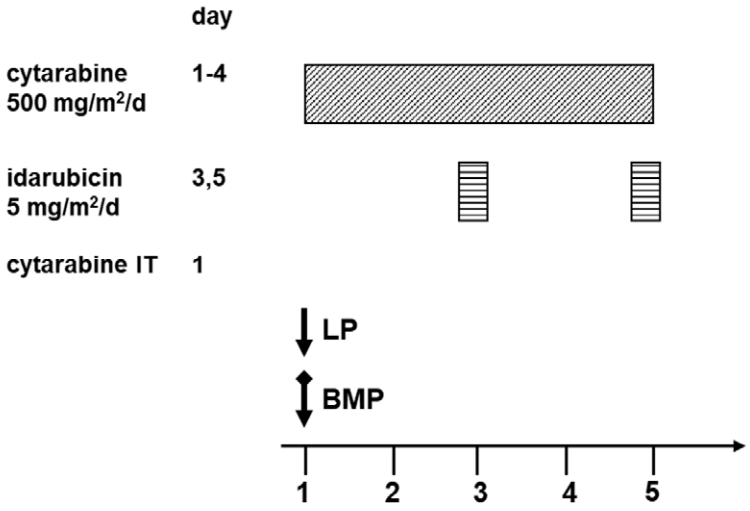


Supp. Fig. 1: Therapy overview for Induction (AIE)

Cytarabine i.v. 100 mg/m²/d:	48 h continuous infusion → 100 mg/m² as 24h infusion each, from day 1 morning to day 3 morning
Cytarabine i.v. 100 mg/m²/12h:	100 mg/m² every 12 h by 30 min infusion (12 doses total)
Idarubicin i.v. 8 mg/m²/d:	4 h continuous infusion on days 3, 5 and 7, immediately prior to cytarabine (3 doses total)
Etoposide i.v. 150 mg/m²/d:	60 min infusion, 6 hours before cytarabine infusions (no. 8,10,12)
Cytarabine i.th.:	Administer in age-related doses (see Supp. Table 2) on day 1 or at the time of the diagnostic LP (in case of hyperleukocytosis and peripheral blasts, LP should be deferred until a satisfactory reduction in blasts)

Supp. Table 6: Chemotherapy dosages for Induction (AIE)

AI infusion plan:

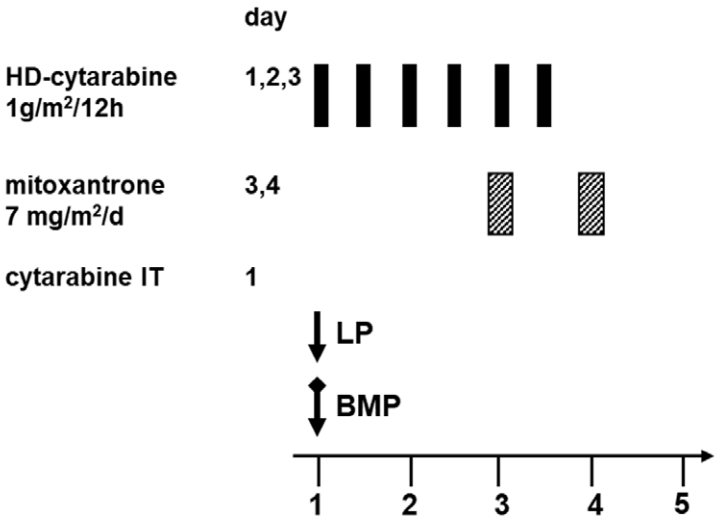


Supp. Fig. 2: Therapy overview for AI

Cytarabine i.v. 500 mg/m^2/d:	96 h continuous infusion → 500 mg/m^2 as 24 h infusion each, from day 1 morning to day 5 morning
Idarubicin i.v. 5 mg/m^2/d:	4h infusion, without interrupting the continuous cytarabine infusion (2 doses total)
Cytarabine i.th.:	Administer in age-related doses (see Supp. Table 2) on day 1

Supp. Table 7: Chemotherapy dosages for AI

hAM infusion plan:

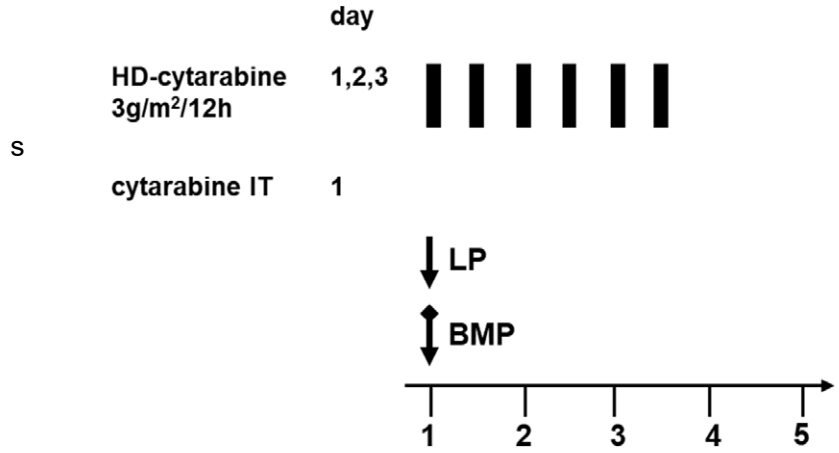


Supp. Fig. 3: Therapy overview for hAM

HD-cytarabine i.v. 1 g/m²/12h:	3h infusion every 12 h (6 doses total), for ≤ 24 months: age-dependent reduced dose (see Suppl Table 4)!
Mitoxantrone i.v. 7 mg/m²/d:	30 min infusion, before HD-cytarabine
Cytarabine i.th.:	Administer in age-related doses (see Supp. Table 2) on day 1

Supp. Table 8: Chemotherapy dosages for hAM

HA infusion plan:



Supp. Fig. 4: Therapy overview for HA

HD-Cytarabine i.v. 3 g/m²/12h:	3h infusion every 12h (6 doses total) for ≤ 24 months: age-dependent reduced dose (see Suppl Table 4)!
Cytarabine i.th.:	Administer in age-related doses (see Supp. Table 2) on day 1

Supp. Table 9: Chemotherapy dosages for HA