Diagnosis and Treatment of Acute Myeloid Leukemia in Children

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Introduction

Acute myeloid leukemia (AML) accounts for 15–20% of acute leukemias diagnosed in children and about 5% of childhood cancer diagnoses. Less than 1000 new cases of AML are diagnosed in children each year in the United States [1]. In the past 30 years, survival rates for children with AML have progressively improved, although not as dramatically as for children with acute lymphoblastic leukemia (ALL) [2]. During this period, the components of AML therapy have remained essentially unchanged. Incremental improvements in outcome are attributable to a number of factors including increased treatment intensity, optimized supportive care, and application of stem cell transplantation. With recent advances in molecular diagnostics and the emergence of targeted approaches, AML therapy is poised to enter a new age of accelerated progress. This chapter focuses on factors specifically relevant to AML in children.

Epidemiology and Pathogenesis

AML is primarily a disease of adults, with the median age at diagnosis in the seventh decade of life. In children, the incidence of ALL far exceeds the incidence of AML [1]. Within the pediatric population, the incidence of AML varies by age with a small peak in the first 2 years of life and then gradual rise after the second decade of life, as depicted in Fig. 19.1.

Although the vast majority of pediatric AML is thought to be sporadic in nature, the contribution of inherited leukemia predisposition has become significantly more appreciated over the past decade as more familial myelodysplastic syndrome (MDS), acute leukemia, and marrow failure syndromes have been described. A large genomic study of germline material from 1120 pediatric cancer patients found that 8.5% of all patients and 4% of leukemia patients had a pathogenic or probably pathogenic mutation in a cancer predisposition gene [3]. Importantly, a positive family history of cancer was only present in 40%, demonstrating that germline predisposition remains an underappreciated and under-evaluated concern, which can impact future risk of relapse, second malignancy and other sequelae, transplant conditioning, and transplant donor selection. Although germline screening for predisposition is not (yet) universally recommended for pediatric AML patients, clinicians should have a high index of suspicion for considering a germline workup in the right clinical context. A comprehensive review of these disorders and their diagnostic workup is beyond the scope of this chapter, but we highlight several important syndromes and concepts.

First, there exist cancer predisposition syndromes in which hematological neoplasms are merely one class of several cancers which are at significantly increased risk. These include Bloom syndrome, ataxia telangiectasia, neurofibromatosis type I, and others. In addition, familial MDS and AML syndromes associated with germline mutations have also been described, which can be organized into three groups. In the first group are those with thrombocytopenia, platelet dysfunction, and an increased risk of myeloid and other hematopoietic neoplasms, including constitutional mutations in RUNX1 [4], ANKRD26 [5], and ETV6 [6]. A second group has increased risk of MDS/AML with associated organ manifestations, which include GATA2, TP53, TERT, and TERC. A final group, which includes mutations in CEBPA, SRP72, and DDX41, has increased risk of MDS/AML and no thrombocytopenia and organ dysfunction. Lastly there are bone marrow failure syndromes, including Fanconi anemia, Shwachman-Diamond syndrome ,and dyskeratosis congenita, which also have an elevated risk for transformation to MDS or AML.

Although not familial, Down syndrome deserves special attention as a germline syndrome associated with increased risk of leukemia. Babies with Down syndrome (DS) exhibit

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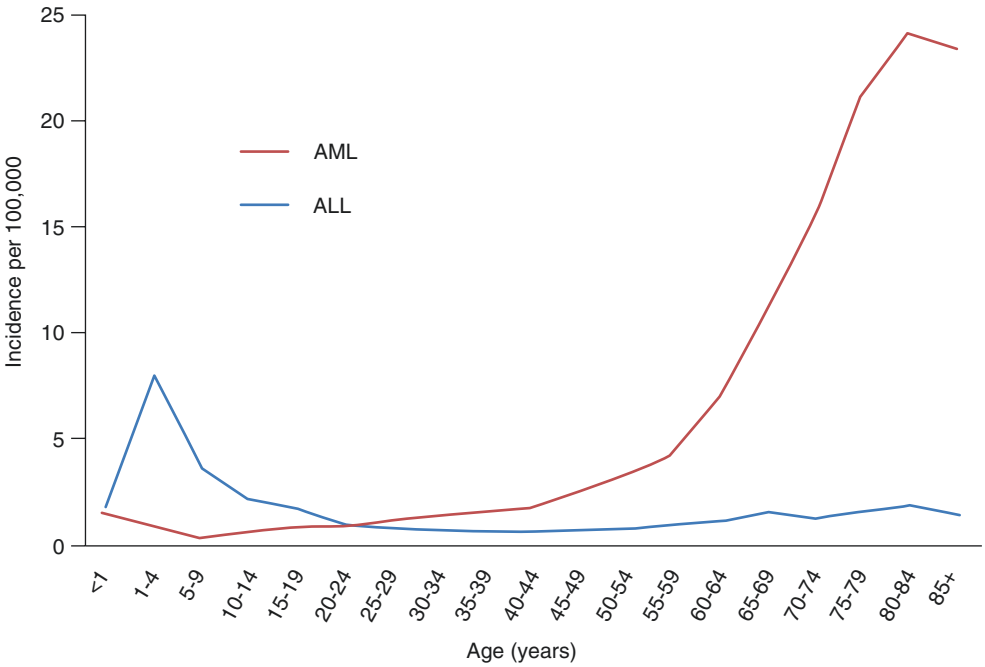

Fig. 19.1 Incidence per 100,000 of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) by age. Data from Surveillance, Epidemiology, and End Results Program (SEER) 1975–2011

a unique pattern of abnormal myelopoiesis in the neonatal period which is usually transient and self-resolving. Myelodysplastic syndrome (MDS) and/or AML (also known as myeloid leukemia of Down Syndrome, or ML-DS) develops later in some of these babies. After the first few years of life, the incidence of AML declines to non-DS baseline levels concomitant with a rise in the incidence of ALL in children with DS (see ML-DS below).

Increased AML risk is also associated with exposure to DNA-damaging agents in the form of chemotherapy or therapeutic, diagnostic, or environmental radiation. As the number of survivors of childhood and adult cancer grows, the population at risk for this complication increases. The risk period for the development of myelodysplasia and overt AML is influenced by the therapeutic class of the genotoxic therapy previously administered. Also, both cumulative dose and administration schedule appear to matter [7]. Secondary leukemias that arise after exposure to topoisomerase II inhibitors, such as epipodophyllotoxins and anthracyclines, characteristically present as overt AML, without a preceding phase of myelodysplasia. These leukemias occur relatively early, peaking at 2–3 years after drug exposure. Balanced translocations, especially rearrangements involving the MLL (KMT2A) gene at chromosome 11q23, are characteristic of AMLs that occur in association with these agents [8]. Frequent, intermittent schedule of administration of etoposide is associated with a higher risk of treatment-related leukemia [9]. In contrast, exposure to alkylating agents is characteristically associated with leukemias that evolve in the setting of MDS occurring more than 5 years after drug exposure. Deletions involving chromosomes 5 and 7 are often observed [8].

Other environmental and lifestyle factors do not appear to contribute strongly to the incidence of AML in children. Studies linking childhood AML to exposure to environmental toxins, pesticides, fetal exposure to cigarettes, drugs or alcohol, parental age, and birth weight have been suggestive but so far inconclusive [10].

Clinical Presentation

Children with acute leukemia, whether AML or ALL, typically present with symptoms related to bone marrow infiltration, including pallor, fatigue, fever, petechiae, and bruising. Hepatosplenomegaly is common. Less often, AML cells can also form solid masses, referred to as myeloid sarcoma or chloroma, or infiltrate tissues such as the gingiva and skin. This may occur with or without concomitant peripheral blood or bone marrow involvement. Extramedullary involvement is associated in particular with young age, monoblastic differentiation, and certain cytogenetic abnormalities [11].

In children with AML, the white blood cell count at presentation may be low, normal, or high and circulating blasts may be present or absent. 15–20% of children present with a white blood cell count of 100,000 cells/μL or more [12–14]. Rarely, circulating blasts are detected on screening blood work performed in the absence of symptoms. When the white blood cell count is extremely elevated, patients may experience signs and symptoms related to impaired tissue perfusion that results from hyperviscosity and microvascular obstruction. Leukostasis is a significant risk in AML patients with white blood cell counts over 100,000. Respiratory

compromise and central nervous system (CNS) manifestations are of primary concern. Concomitant renal dysfunction and metabolic derangement due to spontaneous tumor lysis syndrome and hemorrhagic diathesis due to thrombocytopenia and disseminated intravascular coagulopathy (DIC) exacerbate the problem. As in adults, DIC with or without hyperleukocytosis is a particularly prominent feature of acute promyelocytic leukemia (APL). The role of leukapheresis remains controversial in children with hyperleukocytosis. In conjunction with prompt initiation of cytoreductive chemotherapy and aggressive management of tumor lysis syndrome, the procedure might reduce the risk of early death in selected patients with very high WBC (>200,000) and monoblastic leukemia [15]. Due to risk of bleeding, leukapheresis is discouraged in patients with APL [16].

The diagnosis of AML may be established when leukemic blasts are circulating in the peripheral blood. However, bone marrow aspiration/biopsy is usually recommended to fully characterize the leukemia. In every new case of AML, the possibility of APL must be considered, since this entity demands urgent initiation of specific therapy (see below). Leukemia cytogenetics, FISH, and molecular tests for subchromosomal genetic alterations, such as FLT3-ITD, should be obtained. Although institutional protocols may differ on which exact studies are sent, molecular studies are increasingly important in determining treatment and predicting prognosis. The option to bank leukemia samples for future studies should always be offered when possible.

Examination of CSF is an important part of the diagnostic evaluation in new-onset leukemia, although it may be delayed or deferred in the setting of coagulopathy. CSF sampling is usually performed in conjunction with administration of intrathecal chemotherapy when the diagnosis of acute leukemia has been established. A summary of commonly recommended studies for the diagnostic evaluation of pediatric AML is shown in Table 19.1.

Table 19.1 Diagnostic evaluation of suspected pediatric AML

Initial workup and staging
• History and physical exam, including family history for malignancies and hematological disorders
• Complete blood count with differential
• Electrolytes, including calcium, phosphorous, uric acid, and lactate dehydrogenase
• Liver function tests, including AST, ALT, bilirubin
• Prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR), fibrinogen, d-dimer
• Peripheral blood flow cytometry, if circulating blasts are present
• HLA typing for potential transplant
• Chest radiograph
• Ophthalmology exam
• Echocardiogram, electrocardiogram
• Consider central access options
• Consider fertility-preservation options
• Consider research specimen banking, if available
• Bone marrow aspirate
– Morphology, flow cytometry, karyotype, FISH for prognostic AML cytogenetics including t(8;21), inv. [16], MLL, t(15;17), −7, +8
– FLT3 internal tandem duplication
– PML/RAR if suspected or confirmed APL (consider also from blood)
• Bone marrow biopsy
• If no coagulopathy, diagnostic/therapeutic lumbar puncture

Classification

AML is a morphologically, cytogenetically, and molecularly heterogeneous set of diseases and its classification strongly influences prognosis and therapy. Many classification schema have been developed over time, for example, the French-American-British (FAB) classification system, which used morphologic criteria to divide AML into eight groups (M0: acute myeloblastic leukemia with minimal differentiation, M1: acute myeloblastic leukemia without maturation, M2: acute myeloblastic leukemia with maturation, M3: acute promyelocytic leukemia, M4: acute myelomonocytic leukemia, M5: acute monocytic leukemia, M6: acute erythroid leukemia, and M7; acute megakaryocytic leukemia) [17, 18]. However, with increasing understanding of AML biology, particularly in adults, recurrent genetic and cytogenetic alterations have become integrated with classification. This is reflected in the introduction of World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia in 2001, which was revised in 2008 and again in 2016 (Table 19.2).

Because AML is primarily a disease of adults, it is not surprising that the WHO classification schema has limited applicability to pediatric AML. This is illustrated by the fact that in a study of 639 children with AML, AML-NOS (not otherwise specified) is the largest group [19].

Cytogenetic and Molecular Features of Childhood AML and Their Role in Risk Stratification

In large part, the limited applicability in pediatric AML of the genetic and cytogenetic approach driving the WHO classification is driven by age-related differences in the molecular features of adult and childhood AML. Many of the common cytogenetic abnormalities vary considerably by age (Fig. 19.2), and common pediatric alterations that are uncommon in adult AML are not well represented in the WHO classification. For example, although *KMT2A (MLL)* has over 120 described fusion partners [6, 7] and is translocated in

Table 19.2 World Health Organization 2016 classification of myeloid neoplasms and acute leukemia

Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1
AML with inv. [16](p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11
APL with PML-RARA
AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A
AML with t(6;9)(p23;q34.1);DEK-NUP214
AML with inv. [3](q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1
Provisional entity: AML with BCR-ABL1
AML with mutated NPM1
AML with biallelic mutations of CEBPA
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome
Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
Mixed-phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); BCR-ABL1
MPAL with t(v;11q23.3); KMT2A rearranged
MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS

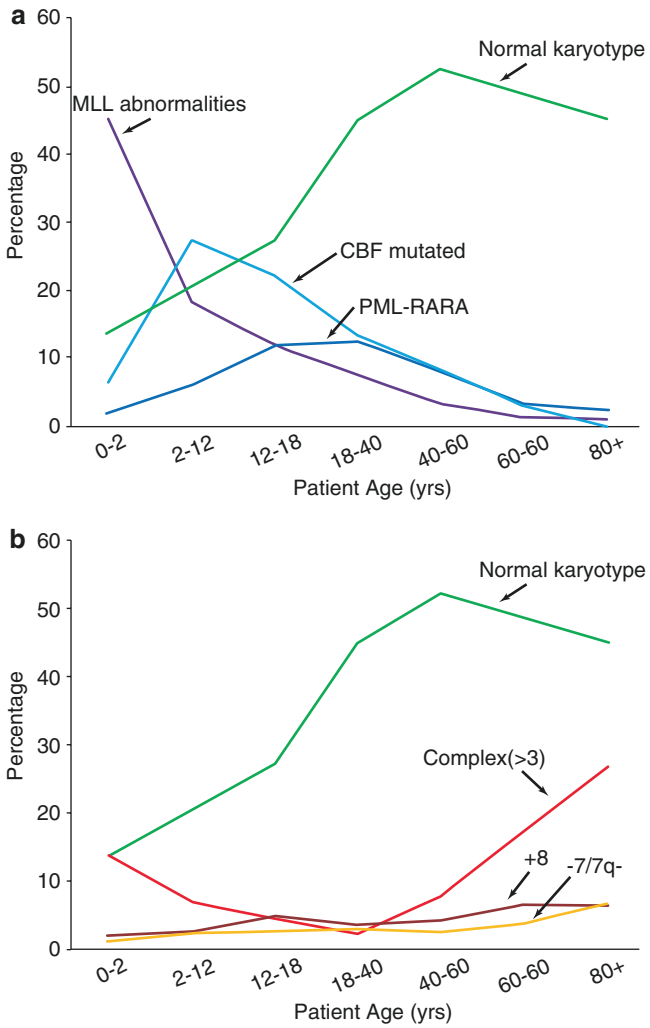

Fig. 19.2 Frequency of molecular abnormalities in AML by age. (**a**) Abnormalities more common in pediatric AML, with normal karyotype AML as a reference. (**b**) Abnormalities more common in adult AML. Data summarized from Cruetzig et al. Cancer 2016

50% of infants and 25% of children with AML, it is uncommon in older adults. As a consequence, the WHO criteria in 2008 and 2016 recognize only the most common fusion protein, t(9;11)(p21.3;q23.3); MLLT3-KMT2A (previously known as MLL-AF9) [8], leading AMLs with other KMT2A translocations to be classified as AML-NOS, despite their differential impact on prognosis.

Other pediatric centric molecular features are recognized however, for example, the favorable risk core-binding factor (CBF) group, with t(8;21) or inv.(16) and t(16;16), is very common (>25%) in childhood AML, while uncommon in older adults with AML. PML-RAR is common (>10%) in adolescents and young adults with AML, while uncommon in young children and older adults over 60. In contrast, cytogenetically normal, complex karyotype and losses of 5q and 7 are more common in older adults.

Although the frequencies of specific cytogenetic abnormalities vary, most abnormalities retain a similar clinical meaning in pediatric compared to adult AML (Table 19.3). However there are a few notable exceptions. Both monosomy 7 and 7q- are poor risk markers in adults, while children with 7q- do relatively well compared to those with monosomy 7 [19–21]. Since 11q23 rearrangement is more common in children, more data is available regarding the prognostic impact of specific KMT2A translocations in pediatric AML, which is variable. The t(6;11)(q27;q23), t(10;11)(p12;q23), and t(10;11)(p11.2;q23) translocations have been specifically associated with unfavorable outcomes, while

Table 19.3 Prognostic cytogenetic alterations in pediatric acute myeloid leukemia

Favorable	
t(8;21)(q22;q22)/RUNX1-RUNX1T1	[21, 24]
Inv(16)(p13.1;q22)/CBFb-MYH11	[21, 24]
Mutated NPM1 without FLT3-ITD	[25, 26]
Biallelic mutations of CEBPA	[27, 28]
t(1;11)(q21;q23)/MLLT11-KMT2A	[22]
t(15;17)/PML-RARA	[24]
Intermediate or unclear	
t(9;11)(p12;q23)/MLLT3-KMT2A	[22]
Other KMT2A fusions	[22]
Unfavorable	
t(6;11)(q27;q23)/MLLT4-KMT2A	22
t(10;11)(p12;q23)/MLLT10-KMT2A	[22]
t(10;11)(p11.2;q23)/ABI1-KMT2A	[22]
t(6;9)(p23;q34)/DEK-NUP214	[29, 30]
t(5;11)(q35;p15.5)/NUP98-NSD1 with FLT3-ITD	[31, 32]
Inv(16)(p13.3q24.3)/CBFA2T3-GLIS2	[33, 34]
FLT3-ITD	[26, 35]
Monosomy 7	[19, 20, 21]
t(9;22)/BCR-ABL	[21]
5q abnormalities	[21]

t(1;11)(q21;q23) is favorable, and others appear to have little impact on prognosis [22]. The prognostic significance of other cytogenetic lesions is emerging in pediatric AML, but is not yet validated [23].

In addition to cytogenetic alterations, nearly all cases of adult AML have multiple pathogenic somatic single-nucleotide variants (mutations) and some copy number variants that can be identified by next-generation sequencing techniques, with the most frequently altered including *FLT3, NPM1, DNMT3A, IDH1, IDH2, TET2, RUNX1, TP53, CEBPA, WT1, KIT*, the Ras pathway (*NRAS*, *KRAS*, *PTPN11*), splicing (*U2AF1, SF3B1, ZRSF2*), and the cohesion complex (*STAG2, SMC3, RAD21*) [36]. Although studies have been limited to small cohorts, often assaying only a few genes, variants in most of these genes have been far less common. For example, *FLT3-ITD* (25% vs. 12%) and mutations in *CEBPA* (35% vs. 8%), *IDH1* or *IDH2* (17% vs. 2%), and *DNMTA* (22% vs. 0%) [37] are all significantly more frequent in adult versus childhood AML patients.

The cell of origin, order, number, and which combinations of mutations are required for AML development remain an active field of investigation, beyond the scope of this chapter. Gilliland and colleagues previously proposed a model in which AML develops from a combination of "class II" mutations, which primarily cause a differentiation arrest and increased self-renewal, with "class I" mutations that cause increased proliferation and survival [38]. Such class I mutations include activating mutations in the Ras pathway as well as constitutive activation of receptor or cytoplasmic tyrosine kinases such as *FLT3* and *KIT*. The contribution of many recently described mutations, such as those in epigenetic regulators, splicing, and cohesin complex, are incompletely understood and their effects on differentiation, self-renewal, and proliferation are under investigation.

Increasing evidence is accumulating that some of these somatic variants, particularly in *DNMT3A*, *TET2*, *ASXL1*, *JAK2*, and *SF3B1*, may be an initial event in the pathogenesis of adult AML. Several studies [39–41] have now documented the existence of detectable, pathogenic, persistent, and typically subclonal variants in these genes in the blood of healthy volunteers, which likely represent the expansion of mutated hematopoietic stem cells and is now termed clonal hematopoiesis of indeterminate potential (CHIP) [42, 43]. These mutations are associated with an increased risk of transformation to a hematopoietic malignancy; they are more frequent in patients with unexplained cytopenias [44] and have been documented in non-leukemic hematopoietic stem cells of leukemic patients [45] and the blood samples of patients prior to the development of AML [46]. The prevalence of CHIP increases dramatically with age, with over 10% affected of those over 70 years or older, but is rare in healthy adults under 40 [39], which is thought to be related to age-related accumulation of DNA damage. The near absence of these variants in healthy young people and low prevalence in childhood AML suggest that most childhood AML may have a very different etiology than most adult AML.

Immunophenotype Analysis at Diagnosis in Pediatric AML

The diagnosis of AML, to be distinguished from ALL and MPAL, has been greatly assisted by the advent of flow cytometry and immunophenotyping. An aberrant immunophenotype can be detected on myeloid blasts in 95% of cases [47] at diagnosis, commonly with expression of myeloid and stem cell markers CD13, CD15, CD33, CD34, CD56, and CD117 as well as lymphoid markers such as CD7, CD10, and CD19. Some specific immunophenotypes are correlated to cytogenetics or outcomes and should be noted.

Minimally differentiated M0 FAB subtype of AML is rare and a poor prognostic marker [48]. The good prognostic t(8;21)(q22;q22) translocation is more frequently found in pediatric patients, in the FAB M2 subtype, and often associated with extramedullary tumors, chloromas, and splenomegaly. They also have a specific immunophenotype, with positivity for the B-cell cell-associated marker CD19, as well as CD13, CD34, CD56, and HLA-DR, while CD2 and CD7 are rarely expressed and CD33 is weak [49].

In FAB M3, acute promyelocytic leukemia (APL) blasts co-express CD13, CD33, and CD9 but are negative for HLA-DR, CD15, CD10, or CD11b. In addition to distinct morphologic features, this immunophenotype will be one of the earliest hints that a newly presenting patient has APL and could benefit from the early initiation of tretinoin (see below).

M6 erythroleukemia is rare in childhood AML but associated with a poor outcome, particularly induction failure and death [50]. The megakaryocytic M7 FAB subtype may be suspected based on morphology or histochemistry, and is confirmed by immunophenotypic analysis (identification of platelet or megakaryocytic antigens CD41 and CD61) [18]. It is frequently associated with Down syndrome, and is in fact the most common type of AML in young children with Down syndrome, where it has a favorable prognosis. In contrast, M7 AML in non-Down syndrome patients is rare and carries a poor prognosis [50].

Loken and colleagues [51] recently described a pediatric restricted high-risk immunophenotype with bright CD56, dim to negative CD45 and CD38, and a lack of HLA-DR, named the RAM phenotype (after a particular patient's initials). These patients were significantly younger (1.26 years old vs. 10.1) and lacked FLT3-ITD, CEBPA, or NPM1 mutations and were all considered standard-risk cytogenetics; however, they were more frequently MRD positive (84% vs. 33%), had lower EFS (16% vs. 51%), and OS (26% vs. 66%) compared to the non-RAM cohort.

Treatment

Treatment of AML in children is similar to adults, but outcomes in children are superior in all risk groups [12, 52]. Overall survival for pediatric AML exceeds 60% with current treatment protocols [23]. Treatment is separated into two phases: remission induction and post-remission consolidation. Approximately 90% of children achieve remission after one or two cycles of intensive induction chemotherapy [53]. The choice of post-remission consolidation is determined by risk group which is, in turn, based on the combination of disease characteristics and treatment response. Broadly speaking, lower risk patients in first complete remission are allocated to receive additional cycles of combination chemotherapy while higher risk patients are typically referred for allogeneic hematopoietic cell transplantation (HCT). Allocation to HCT is not based solely on the existence of a matched family donor, as it was in the past. For AML patients who relapse, HCT in second remission is generally considered the best option for the possibility of cure.

Remission Induction

For decades, the combination of cytarabine plus an anthracycline has remained the mainstay of induction therapy for newly diagnosed AML. Multiple clinical trials in childhood AML have examined different cytarabine doses and schedules, different anthracyclines, and addition of a third agent. Despite these efforts, no clear optimal combination and schedule have been defined. Currently in the United States, induction therapy typically consists of cytarabine, daunorubicin, and etoposide (ADE 10 + 3 + 5) based on the series of trials conducted by the United Kingdom Medical Research Council (MRC) [54, 55]. Bone marrow examination is performed to assess response to chemotherapy around days 21–28 of induction I. If residual leukemia is present, a second cycle of chemotherapy may be immediately initiated. Otherwise, induction II begins when peripheral blood counts recover. Morphologic assessment of early treatment response is notoriously challenging. Application of multiparameter flow cytometry and molecular studies significantly improves the accuracy of the hematopathologists' interpretation [56]. Depending on genetic and clinical factors, response to induction I, and provider preference, a second cycle of ADE or a more intensive combination, such as high-dose cytarabine with mitoxantrone [13], is administered in induction II. After two cycles of induction chemotherapy, about 90% of children with de novo AML will achieve remission, defined as <5% marrow blasts by morphology and/or flow cytometry. Among the subset of patients who do not achieve this milestone, refractory disease is the cause in the majority, in most studies. However, early death due to toxicity is also a significant problem [14, 57, 58].

Monitoring Treatment Response

In addition to the initial immunophenotypic, cytogenetic, and genetic features, response to therapy is a strong predictor for relapse and long-term outcome. Morphologic induction failure, unquestionably, portends a dismal prognosis, but occurs in only a small minority of cases and unfortunately nearly half of patients that achieve complete remission (CR) will eventually relapse. Modern assessment of treatment response involves the measurement of minimal residual disease (MRD), which is typically performed by sensitive multidimensional flow cytometry after induction chemotherapy; however RT-PCR analysis of leukemia-specific transcripts [59] and genomic DNA assessment of mutation clearance have been studied as well.

Flow cytometry for aberrant immunophenotypes post-induction is the most generalizable method and has been shown to be prognostic in childhood AML and predicts relapse risk in multiple studies. For example, in the St. Jude AML02 trial [58], the presence of high MRD by flow after one course of induction was associated with a 3-year cumulative incidence of relapse of 39% compared with only 17% for patients with no detectable MRD. The relapse rate was particularly high for patients with MRD >1% after one course of therapy and for those with any detectable MRD (>0.1%) after two courses of induction chemotherapy.

At relapse, 88% of pediatric AML cases demonstrated an antigenic shift in at least one marker [60]. Because immunophenotype can vary greatly and aberrancy can be subtle, it is important to perform multiparameter flow cytometry, especially in cases of minimal disease detection, which should not be restricted to the immunophenotype detected at presentation. In contrast to adult AML trials, flow-based MRD is well accepted and used by most pediatric cooperative group to determine which patients receive high-risk therapy (in combination with clinical, cytogenetic, and genetic features).

Consolidation Chemotherapy

Consolidation therapy for pediatric AML in first complete remission is determined by risk group. It is generally agreed that the core-binding factor (CBF) leukemias have a reasonably good chance of cure with chemotherapy alone. For these patients with so-called low-risk cytogenetics, the up-front toxicity of HCT in first complete remission is not warranted [61, 62]. On the opposite end of the spectrum, high-risk AML is variably defined among cooperative groups and the definition continues to evolve. General consensus has emerged for many of the more frequent subtypes and scenarios, taking into account both disease features and response to therapy. High-risk characteristics include poor response to initial therapy, AML arising in the setting of MDS or prior treatment, presence of FLT3-ITD with high allelic ratio, and presence of adverse cytogenetics such as monosomy 7, monosomy 5, del5q, and other specific rare translocations [14, 55, 63–65]. HCT from the best available donor is typically, although not universally, considered the treatment of choice for children with high-risk AML in first complete remission, even though a clear benefit from HCT has been difficult to demonstrate in prospective [66] and retrospective trials [67]. A loosely defined intermediate-risk group is comprised of patients with neither low-risk nor high-risk features. For this population, the risk-benefit analysis related to consolidation chemotherapy versus HCT is closer to neutral. Individual factors, such as the availability of a matched family donor, may be taken into consideration in selecting the best consolidation strategy.

For patients who do not proceed to HCT in first remission, post-remission treatment usually consists of additional cycles of intensive consolidation chemotherapy, including some exposure to cytarabine at high dose (≥1 g/m^2/dose) [65, 68]. Although the optimal number of cycles is not determined, a total of four cycles of multiagent chemotherapy is relatively standard in the United States at this time. This is primarily based on results from the MRC AML12 trial which demonstrated that three cycles of consolidation chemotherapy did not improve event-free or overall survival in comparison to two cycles [55]. With current intensive chemotherapy, children with CBF leukemia can expect event-free survival in the range of 80% and overall survival in the range of 90% at 3 years [13, 58]. The influence of risk group on outcome is depicted in Fig. 19.3 [58]. Maintenance chemotherapy has no proven benefit in childhood AML [69, 70], with the exception of APL [71].

Extramedullary Disease

The incidence of central nervous system (CNS) involvement is higher in children with AML compared to ALL. However, the impact that it has on prognosis, and therefore on treatment, is less important. CNS involvement in AML is associated with higher presenting WBC count, younger age, and certain karyotypic abnormalities. CNS-directed therapy in the form of intrathecal chemotherapy, in addition to CNS-penetrant systemic chemotherapy is incorporated into all contemporary treatment protocols for pediatric AML. Prophylactic cranial radiation is not routinely used. CNS3 status, which is defined as ≥5 WBC/μL and blasts present on cytospin, occurs in about 10% of children with AML [72]. For these children, intensification of intrathecal chemotherapy is recommended. The management of CNS2 status (<5 WBC with blasts seen) is variable. Although CNS involvement does not appear to confer a significant impact on overall prognosis, it is associated with a higher risk of CNS relapse [72, 73].

Soft-tissue masses, referred to as myeloid sarcoma or chloroma, are identified occasionally in children with newly diagnosed AML. The pattern and prognostic impact of this are linked to associated karyotypic changes. As mentioned above, cutaneous involvement ("leukemia cutis") is characteristically seen in babies with *KMT2A (MLL)*-rearranged AML (Fig. 19.4). Orbital, parameningeal, and CNS masses

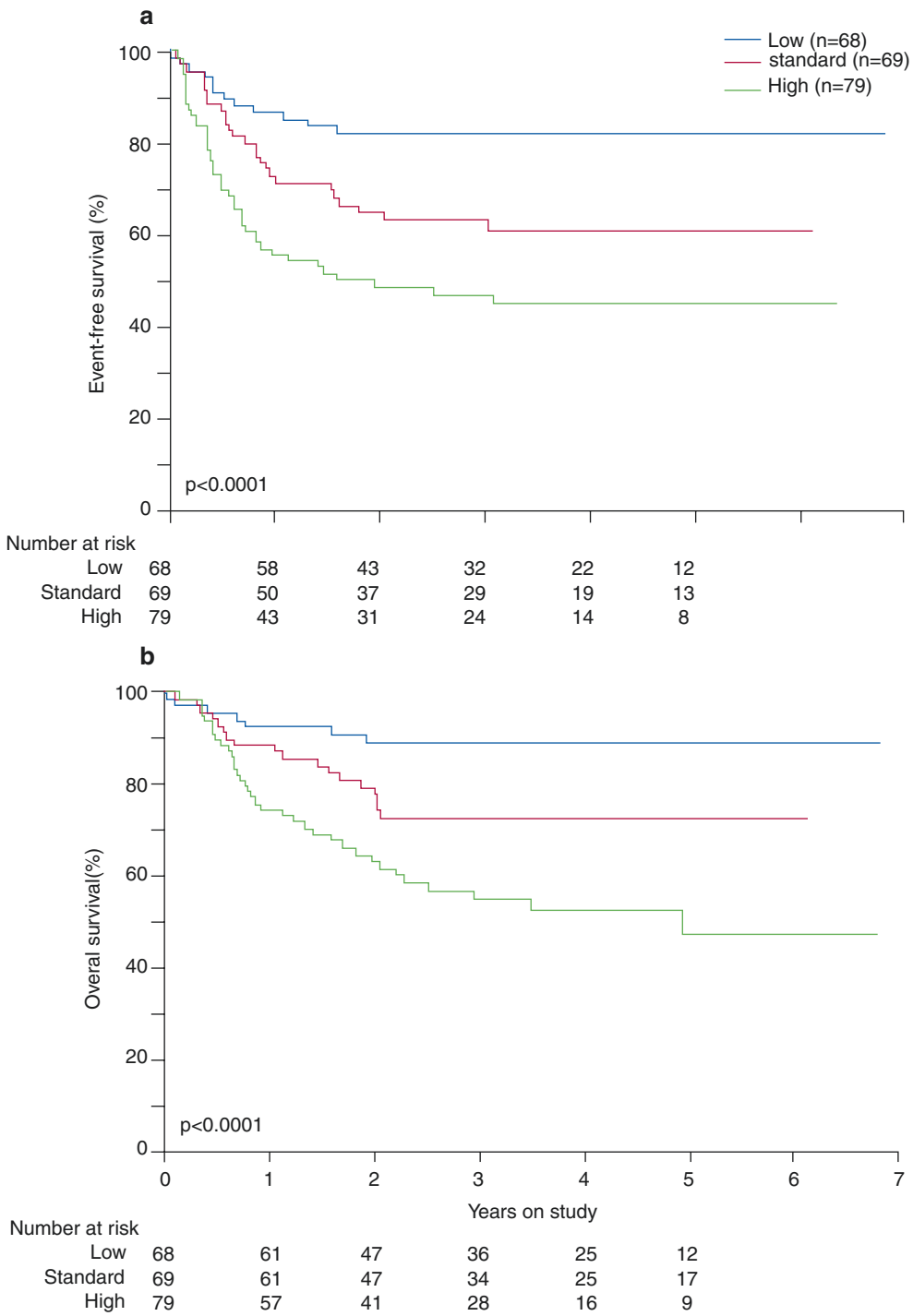

Fig. 19.3 Treatment outcome according to risk group on St. Jude AML02. Reproduced with permission from Rubnitz, et al. Lancet Oncology 2010

are characteristic of AML in older children in association with favorable cytogenetics [74]. Although focal radiation may have a role in selected cases for the acute management of myeloid tumors that threaten permanent consequences due to their location or in the palliative setting, there is no established benefit to addition of focal radiation to myeloid tumors that respond well to chemotherapy [75]. Apparently "isolated" extramedullary disease is viewed as a harbinger of systemic illness both at initial diagnosis and at relapse and should be treated as such [76].

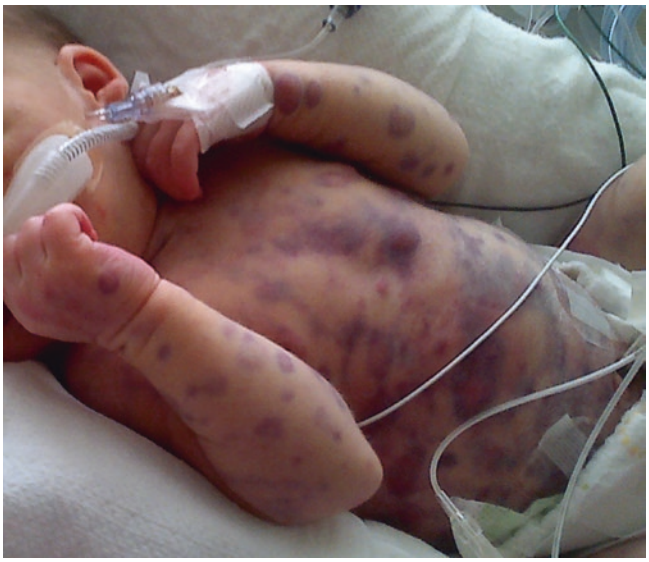

Fig. 19.4 Leukemia cutis in a newborn infant with KMT2A (MLL)-rearranged AML

Newer and Targeted Agents

Due to the suboptimal outcomes in pediatric AML, novel therapies targeting a broad variety of mechanisms are in development. These include second-generation nucleoside analogs, such as clofarabine, which is more resistant to deamination than classical agents such as cytarabine [77], new formulations of traditional chemotherapy, such as CPX-351, a liposomal formulation of daunorubicin and cytarabine, and targeted agents such as kinase inhibitors, monoclonal antibodies, epigenetic modulators, and others. Since AML is much more common in adults, novel therapies are generally studied and often shown to have activity in adults before being used in pediatric studies.

As noted above, the presence of the FLT3 internal tandem duplication is an adverse prognostic feature in children [35, 78] as it is in adults. Several FLT3 inhibitors that range in specificity and potency already exist that have been, or are being, tested in adult AML. These drugs include midostaurin, sorafenib, quizartinib, gilteritinib, and crenolanib [79]. The multikinase inhibitor midostaurin was approved for adults with AML with certain FLT3 mutations in 2017, however data in children is limited at this time. The multikinase inhibitor sorafinab, which is FDA approved for certain solid tumors, also has activity against some FLT3 mutations, with reported clinical activity in relapsed pediatric AML [80] and some use in the post transplant setting [81].

Gemtuzumab ozogamicin is a humanized monoclonal antibody directed against CD33, a protein that is expressed on the surface of a high proportion of AML blasts but not on hematopoietic stem cells. The antibody is covalently linked to an antitumor antibiotic, calicheamicin. The drug was approved in 2017 for AML in patients aged 2 and older [82]. Children's Oncology Group (COG) AAML 0531 [14] demonstrated that addition of gemtuzumab was associated with a statistically significant positive effect on event-free survival through reduction of relapse. High expression of the target antigen, CD33, is associated with response to the drug [83]. Additional immunoconjugates, including vadastuximab talirine (SGN-CD33A) [84], are in development for AML.

Many other classes of agents show promise in AML and are currently under investigation. Epigenetic modifiers are a broad class including drugs that inhibit histone deacetylase (vorinostat) and DNA methyltransferase (azacitidine and decitabine) which are being studied in relapsed pediatric and adult AML. DOT1L inhibition is being studied in children and adults for KMT2A (MLL)-rearranged AML. Other strategies being investigated with new clinical agents include IDH1 and IDH2 inhibition, proteasomal inhibition, exportin inhibition, ubiquitination modulation, and chimeric antigen receptor T-cell therapy [53, 85].

Acute Promyelocytic Leukemia

Acute promyelocytic leukemia (APL) is a unique AML subtype characterized by the presence of reciprocal translocation involving chromosomes 15 and 17 leading to the production of a promyelocytic leukemia (*PML*)-retinoic acid receptor alpha (*RARA*) fusion protein. APL is a disease of older children and young adults. APL accounts for about 10% of pediatric AML and it is distinctly uncommon in children under 10 years. The molecular features of APL are described above and elsewhere in this text. In children as in adults, the treatment of APL differs significantly from other AML subtypes and the overall prognosis is markedly better. But APL is associated with a high risk of early death, due primarily to hemorrhage. Therefore, clinicians must be on alert for this entity because prompt and accurate diagnosis is critical to successful management.

APL is characterized morphologically by the appearance of large hypergranular myeloid blasts with prominent Auer rods. The hypogranular variant (M3v), which is more common in children [86], may be more difficult to distinguish from other AML subtypes. Typically, the blasts co-express CD13, CD33, and CD9 but do not express HLA-DR, CD15, CD10, or CD11b. Blasts of the hypogranular variant typically express CD2 [87]. Demonstration of the molecular fusion of PML gene on chromosome 17 and the RARA gene

on chromosome 15 by RT-PCR, FISH, or karyotype is the key to diagnosis. RT-PCR is the preferred modality since it is rapid, specific, and quantitative.

Disseminated intravascular coagulopathy (DIC) is a significant and potentially life-threatening feature of new-onset APL. Transfusions should be provided to maintain the platelet count ≥30–50 K and fibrinogen level ≥100–150 mg/dL. To address the underlying cause of the bleeding diathesis, emergent initiation of leukemia-directed therapy in the form of tretinoin is recommended at the first suspicion of the disease [16]. Delay in starting tretinoin is associated with a higher risk of early hemorrhagic death [88]. In children, there is an estimated 7.4% risk of death within the first 7 days of APL diagnosis [89].

The introduction of tretinoin into the treatment of APL in the late 1980s transformed the management and the prognosis of this disease. Tretinoin is now a standard component of APL therapy in children. Tretinoin causes differentiation of malignant promyelocytes, sometimes leading to marked increase in the peripheral leukocyte count. Differentiation syndrome occurs in 10–20% of children being treated with tretinoin for APL [90–92]. Higher presenting WBC is a risk factor for the development of this complication of therapy which is characterized by fever, hypotension, pulmonary infiltrates, and renal insufficiency. Aggressive supportive care, administration of dexamethasone, and early introduction of cytotoxic chemotherapy are recommended. Temporary interruption of tretinoin is indicated in severe cases. Pseudotumor cerebri is an important side effect of tretinoin that occurs more commonly in children than in adults [90–92] warranting the use of a lower starting dose of tretinoin in children.

While it is true that APL is associated with an unacceptably high risk of early morbidity and mortality, the overall prognosis for children with APL is significantly better than most other AML subtypes. Treatment with combination of chemotherapy and tretinoin results in event-free and overall survival in the range of 75 and 90%, respectively [90, 91]. As in adults, the intensity of treatment is risk stratified based on presenting white blood cell (WBC) count, with high risk defined as presenting WBC >10,000 cells/dL. Although highly effective, these chemotherapy regimens are lengthy (almost 3 years in duration due to inclusion of a maintenance phase) and associated with potential for significant late effects (especially cardiotoxicity related to high cumulative anthracycline exposure). Exciting recent studies in adults have demonstrated that APL can be cured in many cases with combination of tretinoin and arsenic trioxide with little or no cytotoxic chemotherapy [93]. Arsenic has been shown to be effective and tolerable in children with relapsed APL [94] and in small series of children with newly diagnosed APL [95, 96]. Ongoing clinical trials are examining the use of arsenic and tretinoin as up-front therapy for pediatric APL with concomitant reduction or omission of cytotoxic agents and shortening of treatment duration.

Myeloid Leukemia of Down Syndrome

Children with Down syndrome (DS) are at a 10–20-fold increased risk for developing leukemia. The pattern of disease is unique, comprising neonatal transient abnormal myelopoiesis (TAM), MDS/AML, and ALL. In the first 5 years of life, the risk of myeloid leukemia is about 150 times higher in DS children compared with non-DS children [97].

Myeloid blasts are identified in the peripheral blood smears of approximately 10–15% of newborns with DS. The blasts typically, but not always, express immunophenotypic markers of megakaryoblasts, including CD41, CD42b, and CD61 [98]. Despite the fact that the abnormal cells are morphologically and immunophenotypically indistinguishable from acute megakaryoblastic leukemia (AML M7), they almost always disappear with little or no specific treatment. This process, known as transient abnormal myelopoiesis (also called transient leukemia or transient myeloproliferative disorder) is unique to babies with constitutional and mosaic trisomy 21. In the context of trisomy 21, acquisition of a truncating mutation in the GATA-1 transcription factor in a fetal liver-derived hematopoietic stem or progenitor cell results in expansion of the myeloid blast population. GATA-1 mutation is invariably present in DS babies with clinical TAM. Surprisingly, GATA-1 mutation was also detected in about 20% of DS infants without clinical evidence of TAM. These babies have so-called silent TAM [99].

The main clinical feature of TAM is leukocytosis with circulating blasts. There is currently no defined absolute blast threshold for the diagnosis of TAM. In a systematic review of 48 patients with TAM, the median age at diagnosis was 1 week, median WBC was about 30x10e9/L, and median peripheral blast percentage was 25%. Hepatosplenomegaly was present in more than half and liver dysfunction occurred in about one-third. Even though circulating blasts resolved in nearly all of the patients within a mean of 2 months, almost 20% of the patients died. Mortality in babies with TAM is most often attributed to hepatic fibrosis and liver failure [98]. Survival is associated with the absence of both hepatomegaly and life-threatening symptoms [100]. Treatment with very-low-dose cytarabine appears to decrease the risk of death in babies with severe TAM. However, chemotherapy treatment has never been shown to influence the risk of ML-DS later in childhood [101].

About 20% of TAM patients go on to develop myeloid leukemia before the age of 4 years. As in TAM, immunophenotypic markers of megakaryoblastic differentiation are observed in most cases and GATA-1 mutation is present in all cases. In association with evolution to ML-DS, additional "cooperating" mutations involving genes that encode cohesin complex components, epigenetic regulators, and/or signaling molecules are acquired [102]. In some children with ML-DS, a history of clinical TAM is absent. Presumably, these patients previously experienced undiagnosed "silent" TAM. Taking the incidence of silent TAM into account, it is estimated that progression to ML-DS occurs in about 5–10% of TAM cases [103] (Fig. 19.5).

ML-DS is a subtype of childhood AML that is clinically distinct and warrants distinct treatment. The median age at presentation is in the second year of life and the onset of the disease is often indolent, with gradual progression of cytopenias. ML-DS patients who do not meet the AML threshold of 20% blasts in blood or marrow often fulfill WHO criteria for MDS. In contrast to TAM, successful treatment of ML-DS requires chemotherapy. However, the intensity of chemotherapy necessary to cure ML-DS is less than de novo AML in children without DS. In fact, in an analysis performed by the Children's Cancer Group, higher intensity chemotherapy led to significant treatment-related toxicity in children with ML-DS, substantially offsetting the potential benefit in terms of leukemia control [57]. Since the late 1990s, treatment protocols designed specifically for children with ML-DS have progressively reduced treatment intensity while preserving good results with overall and event-free survival at around 80% [104–106]. Older age, higher presenting white blood cell count, and "normal" karyotype are associated with a significantly worse prognosis [107, 106].

Relapsed and Refractory AML

AML that is refractory to initial treatment or recurs after initial treatment represents a significant therapeutic challenge. Refractory disease occurs in about 5% and relapse affects about 30% [108]. Approximately half of relapses occur within 1 year, and almost all occur within 4 years of initial diagnosis [109, 110]. Long-term disease control can be accomplished in a minority of these patients but, in general, requires attainment of remission followed by HCT. For patients with relapsed AML, favorable cytogenetics [111], longer duration of first complete remission (i.e., ≥1 year from initial diagnosis), and receipt of HCT after relapse are associated with better outcomes [112].

There is currently no standard re-induction protocol for children with relapsed AML. Like up-front therapy, relapsed therapy mainly relies on the use of cytarabine with or without an anthracycline. Newer agents, including the purine analogues fludarabine, clofarabine, and cladribine, are incorporated into some regimens. Several combinations have been tested in children including fludarabine, cytarabine, idarubicin (Ida-FLAG) [113], mitoxantrone, cytarabine [114], fludarabine, cytarabine +/− liposomal daunorubicin [115], clofarabine, and cytarabine [116]. With any combination,

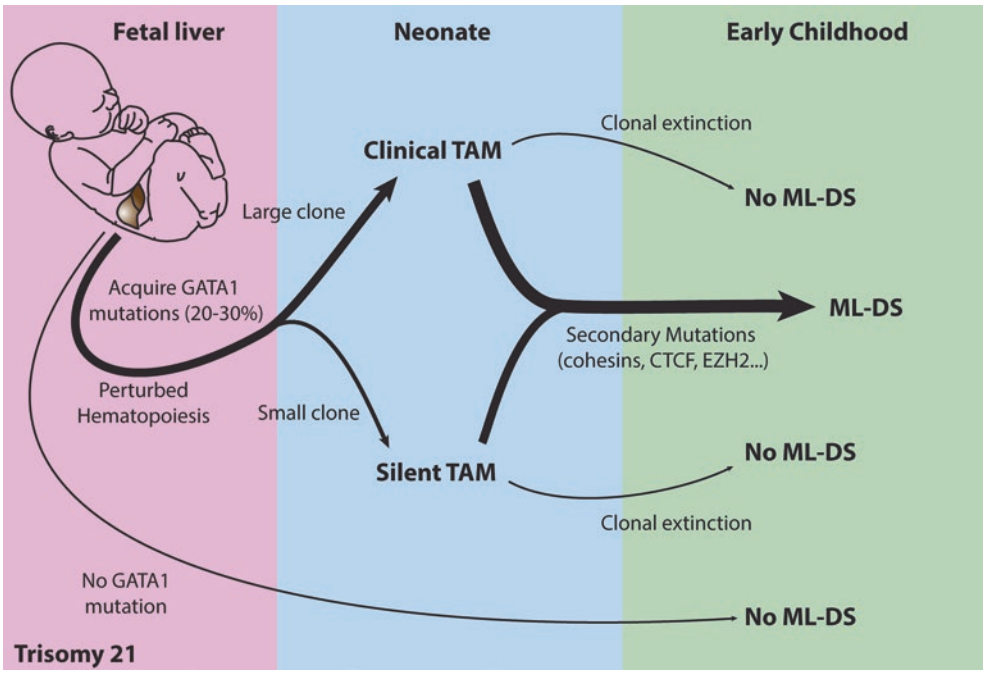

Fig. 19.5 Progression of transient abnormal myelopoiesis to Down syndrome-myeloid leukemia

complete remission is achieved in about 60–70% and many of these patients are able to proceed to HCT. MRD before HCT is a strong predictor of survival [117]. Both subsequent relapse and high treatment-related toxicity are major obstacles to overall survival. Treatment with novel and/or targeted agents, preferably in the context of clinical trials, may be an option for some patients.

The prognosis for patients with relapsed APL is much more favorable than for other relapsed AML subtypes. A role for autologous HCT has been demonstrated for those patients who achieve a second molecular remission [118]. In contrast, relapsed/refractory ML-DS is associated with a dismal prognosis [119].

Treatment Toxicity and Supportive Care

Improved survival in childhood AML is the product of maximizing treatment intensity in coordination with optimizing supportive care. Current treatment protocols appear to be reaching the inevitable limit of dose intensification. The severity and importance of treatment-related risks differ in children compared to adults. In general, children have fewer comorbidities and they tolerate myelosuppression better than adults. However, effects on growth and development matter much more. For children under 1 year of age and those who have a body surface area of less than 0.6 m^2, cytotoxic chemotherapy doses are adjusted, either as percent reduction or by basing calculations on weight instead of body surface area.

All children undergoing treatment for AML experience repeated episodes of prolonged and profound myelosuppression. Infections are the main cause of treatment-related morbidity and mortality. Unsurprisingly, the incidence of infection correlates with treatment intensity [120]. The use of bacterial and fungal prophylaxis is now recommended [121] as this does appear to reduce the rate of severe bacterial and invasive fungal infections [122]. In addition, prophylaxis against pneumocystis pneumonia is advised. The benefit of granulocyte colony-stimulating factor (filgrastim) remains controversial. When addition of prophylactic filgrastim was studied in a randomized fashion in the context of intensive chemotherapy in the Berlin-Frankfurt-Muenster (BFM)-98 study, the duration of neutropenia was shortened but the rate of severe infections was not reduced. However, in an analysis of the COG AAML0531 study, in which infections were collected and monitored prospectively, filgrastim prophylaxis was associated with a statistically significantly lower rate of bacterial infections [123]. However, its use is not without risk. A higher incidence of relapse was observed in children treated with filgrastim whose AML expressed a specific G-CSF receptor isoform [124]. At this time, routine use of filgrastim is not recommended.

Most children treated for AML receive substantial cumulative exposure to anthracyclines, often greater than 360 mg/m^2. As a consequence, survivors are at high risk of long-term cardiac sequelae [125, 126]. Dexrazoxane has not been extensively studied in pediatric AML, but it might reduce cardiotoxicity [127]. Cardiotoxicity is a particular concern for children with DS [128]. After completion of treatment, patients should be monitored for cardiac late effects according to established guidelines [129].

Conclusions

Among pediatricians, it is often said that children are not just small adults. Although pediatric and adult AML share many features, there are important differences in terms of epidemiology, etiology, cytogenetics, and molecular genetics which influence therapy and outcome. Today, childhood AML is a curable disease in more than half of the cases. From the "glass is half-full" perspective, this fact unquestionably represents a major accomplishment. But, much more progress needs to be made to improve outcomes for all children and to reduce the burden of treatment. Progress is needed in many areas including molecular diagnostics, refinement of risk stratification, optimization of chemotherapy, development of targeted agents, and application of conventional and novel transplant strategies. Better outcomes also depend on advances in supportive care and implementation of best clinical practices. The way forward requires cooperation among pediatric oncology providers, scientists, and the pharmaceutical industry and on our patient's ability to access and participate in well-designed clinical trials.

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