Critical Reviews in Oncology / Hematology 138 (2019) 132–138

Contents lists available at ScienceDirect

Critical Reviews in Oncology / Hematology

journal homepage: www.elsevier.com/locate/critrevonc

# The changing scenario of non-Down syndrome acute megakaryoblastic leukemia in children

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ARTICLE INFO

*Keywords:*
Acute megakaryoblastic leukemia
AMKL
FAB M7

ABSTRACT

Pediatric non-Down-syndrome acute megakaryoblastic leukemia (non-DS-AMKL) is a heterogeneous subtype of leukemia that has historically been associated with poor prognosis. Until the advent of large-scale genomic sequencing, the management of patients with non-DS-AMKL was very difficult due to the absence of reliable biological prognostic markers. The sequencing of large cohort of pediatric non-DS-AMKL samples led to the discovery of novel genetic aberrations, including high-frequency fusions, such as *CBFA2T3-GLIS2* and *NUP98-KDM5 A*, as well as less frequent aberrations, such as *HOX* rearrangements. These new insights into the genetic landscape of pediatric non-DS-AMKL has allowed refining the risk-group stratification, leading to important changes in the prognostic scenario of these patients. This review summarizes the most important molecular pathogenic mechanisms of pediatric non-DS-AMKL. A critical discussion on how novel genetic abnormalities have refined the risk profile assessment and changed the management of these patients in clinical practice is also provided.

## 1. Introduction

Childhood acute myeloid leukemia (AML) encompasses a heterogeneous group of malignancies, with great biological heterogeneity. While some subtypes of this aggressive disease are still poorly genetically characterized, some others have been intensely deconvoluted employing genome-wide analysis. This is the case of acute megakaryoblastic leukemia (AMKL), itself also a heterogeneous form of AML, defined by the presence of leukemia megakaryoblastic cells expressing platelet-specific surface glycoprotein (Schweitzer et al., 2015). AMKL is significantly more frequent in children than in adults, accounting for ~ 10% and ~ 1% of AML cases, respectively (Athale et al., 2001). AMKL is particularly frequent in pediatric patients with Down syndrome (DS-AMKL), accounting for the majority (~ 70%) of AML cases in this group of patients (Roy et al., 2009). The disease in DS patients has a founding lesion, namely *GATA 1* gene mutation, and is commonly preceded by a transient myeloproliferative disease (TMD) (Roy et al., 2009; Malinge et al., 2009, 2013; Hitzler et al., 2003; Creutzig et al., 1996). While children with DS-AMKL show a favorable outcome (Roy et al., 2009; Malinge et al., 2009, 2013; Hitzler et al., 2003; Creutzig et al., 1996), AMKL in non-Down syndrome patients (non-DS-AMKL) has historically been associated with a poor prognosis (Athale et al., 2001; Ribeiro et al., 1993; De Rooij et al., 2016; O'Brien et al., 2015). Until recently, only one recurrent genetic aberration in pediatric non-DS-AMKL was known, namely the chromosomal translocation t(1;22)(p13;q13), that results in *RBM15-MKL1* fusion transcript (Carroll et al., 1991; Ma et al., 2001; Mercher et al., 2001, 2002; Mercher et al., 2009) (Table 1). The advent of large-scale, genomic sequencing technologies has completely revolutionized the scenario of pediatric non-DS-AMKL. Novel recurrent cytogenetically cryptic fusion genes have been identified, such as *CBFA2T3-GLIS2,* resulting in inversion on chromosome 16 [inv(16)(p13.3q24.3)] (Gruber et al., 2012; Thiollier et al., 2012) and the *NUP98-KDM5 A* (Gruber et al., 2012; Thiollier et al., 2012; Rooij et al., 2013) transcript, a newly-discovered aberration of the NUP98-recombinome (Table 1). Other less frequent fusion genes, such as *MN1-FLI1* and *GRB10-SDK1*, were also identified and previously known aberrations like *KMT2A* (De Rooij et al., 2016; Rooij et al., 2013; Inaba et al., 2015) and *HOX* rearrangements (Gruber et al., 2012; De Rooij et al., 2017) have been better characterized. This intense effort resulted into crucial insight into the genetic etiology of non-DS-AMKL and rapidly led to a significant contribution in defining the prognosis. While the genetic risk-assessment and consequent outcome prediction of

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https://doi.org/10.1016/j.critrevonc.2019.04.011
Received 28 February 2019; Received in revised form 7 April 2019; Accepted 9 April 2019

**Table 1**
Frequency and prognostic value of non-DS-AMKL with CBFA2T3-GLIS2, NUP98-KDM5 A, KMT2A rearrangements and HOX rearrangements.

| Aberration | Frequency | OS (5 years) | EFS (5 years) | Reference |
|---|---|---|---|---|
| *RBM15-MKL1* | 5–10% | 5 years 65% | 5 years 53% | (20) |
| *CBFA2T3-GLIS2* | 12–27% | 5 years 14–28% | 5 years 8-35% | (16) (18) (20) |
| *NUP98-KDM5 A* | 8–12% | 5 years 22–36% | 5 years 22–36% | (18) (20) |
| *KMT2Ar* | 7–17% | 5 years 27% | 5 years 25–28% | (18) (20) |
| *HOXr* | 14% | 5 years 77% | 5 years 77% | (20) |

pediatric non-DS-AMKL was previously mainly based on the presence of *RBM15-MKL1* fusion and recurrent cytogenetic aberrations, the discovery of novel genetic lesions led to an important re-categorization of this scenario. Recent studies demonstrated and confirmed that these specific aberrations identify different prognostic subgroups (De Rooij et al., 2016; Inaba et al., 2015; De Rooij et al., 2017). We here review the advancement in understanding the genetic complexity and heterogeneity of childhood non-DS-AMKL revealed by recent deep genomic studies and we report how these novel genetic abnormalities have more precisely refined the risk-profile assessment of this disease.

## 2. Main text

### 2.1. RBM15-MKL1 (OTT-MAL)

The t(1;22)(p13;q13) chromosomal translocation is peculiarly associated of AMKL in infants (Carroll et al., 1991; Lion et al., 1992). It was the earliest recurrent aberration described in approximately 12% of pediatric non-DS-AMKL (Carroll et al., 1991; Lion et al., 1992; Baruchel et al., 1991).

The genes involved in the translocation are *RBM15* (also named *OTT*) on chromosome 1 and *MKL1* (also named *MAL*) on chromosome 22 (Ma et al., 2001; Mercher et al., 2001).

RBM15 is closely related to SHARP protein (Ma et al., 2007). SHARP is a component of the transcriptional corepressor complex, recruited by the DNA-binding protein RBP-Jk/CBF1, in the absence of Notch signaling, to repress transcription of its target genes (Oswald et al., 2002).

MKL1 is an SRF coactivator, regulated by Rho-actin signaling pathway (Miralles et al., 2003). Rho GTPases induce actin polymerization, and this event implicates nuclear localization of MKL1 and its interaction with SRF (Miralles et al., 2003), which is an important transcription factor in megakaryocyte differentiation (Halene et al., 2010) (Fig. 2).

As a result, the *RBM15-MKL1* fusion gene is sufficient to induce transformation and proliferation of megakaryocytic cells in consequence of positive regulation of RBPJ transcription factor, mediated by the transactivation domain (TAD) of MKL1 and the RNA recognition motif (RRM) domains of RBM15 (Mercher et al., 2009). Nevertheless, the incidence of AMKL in animals expressing *RBM15-MKL1* alone was very low, suggesting that cooperating mutations are needed for the development of leukemia (Mercher et al., 2009).

The *MPL* and *JAK2* genes are the most common mutated genes in pediatric AMKL (Mercher et al., 2009; Le et al., 2005; Jelinek et al., 2018; Mercher et al., 2018; Pikman et al., 2006) (Fig. 2). Mercher et al (Mercher et al., 2009). demonstrated that the presence of *MPL* mutation in the *RBM15-MKL1* mouse model induced more severe AMKL with characteristic features of the human disease (Mercher et al., 2009).

The t(1;22)(p13;q13) translocation occurs in 10–15% of pediatric non-DS-AMKL (Schweitzer et al., 2015; De Rooij et al., 2016; Rooij et al., 2013; Inaba et al., 2015) (Fig.1).

Children diagnosed with t(1;22)(p13;q13) AMKL are younger (median age ˜ 0.5 years) compared to other AMKL pediatric patients (De Rooij et al., 2016; Carroll et al., 1991; Rooij et al., 2013; Inaba et al., 2015; Lion et al., 1992; Duchayne et al., 2003). Other clinical features of patients with t(1;22)(p13;q13) AMKL are prevalence of females, hypocellularity of bone marrow with myelofibrosis, and massive infiltration of abdominal organs (spleen and liver) by leukemic cells (De Rooij et al., 2016; Carroll et al., 1991; Rooij et al., 2013; Inaba et al., 2015; Lion et al., 1992; Duchayne et al., 2003).

Several study reported conflicting data about the prognostic significance of the t(1;22)(p13;q13) translocation in pediatric non-DS-AMKL (Schweitzer et al., 2015; De Rooij et al., 2016; O'Brien et al., 2015; Carroll et al., 1991; Rooij et al., 2013; Inaba et al., 2015; Duchayne et al., 2003). In particular, many groups (De Rooij et al., 2016; O'Brien et al., 2015; Rooij et al., 2013) reported a better outcome for patients with *RBM15-MKL1*-positive AMKL, compared to other genetic subgroups, reporting a 5-year pOS of ˜ 80% and pEFS of ˜ 70% for these patients (Table 1). Some other studies (Inaba et al., 2015) did not confirm a better prognosis for children with t(1;22)(p13;q13) AMKL, and (Schweitzer et al., 2015) reported that AMKL patients with t(1;22)(p13;q13) had a considerably worse 5-year EFS compared to patients without this translocation. These different outcomes observed by various study groups may be explained in part by disparities in supportive care, which remains essential for children with AMKL and t(1;22)(p13;q13), most of these patients being very young and, thus, particularly vulnerable to the toxic effects of chemotherapy (De Rooij et al., 2016; Inaba et al., 2015).

### 2.2. CBFA2T3-GLIS2

*CBFA2T3-GLIS2* is the most frequent chimeric oncogene identified to date in non-DS-AMKL patients (Gruber and Downing, 2015), being detected in 18–27% of the cases (De Rooij et al., 2016, 2017; Hara et al., 2017) (Fig. 1).

The chimeric transcript results from a cryptic inversion of the telomeric region of chromosome 16 that fuses the 5' portion of *CBFA2T3* in frame with the 3' region of *GLIS2*. The common chimeric *CBFA2T3-GLIS2* transcript is between exon 11 of *CBFA2T3* and exon 3 of *GLIS2* (Gruber et al., 2012; Thiollier et al., 2012; Masetti et al., 2013a). Other rare fusion transcripts have been reported: *CBFA2T3*-ex10/*GLIS2*-ex3 (Gruber et al., 2012), *CBFA2T3*-ex12/*GLIS2*-ex1 (Gruber et al., 2012), and *CBFA2T3*-ex10/*GLIS2*-ex2 (Masetti et al., 2013a). CBFA2T3 is a member of the ETO/CBFA2T1/MTG8 complex, ubiquitously expressed in hematopoiesis, which is functionally essential for hematopoietic stem cells maintenance and self-renewal, and furthermore, it plays a critical role in cellular differentiation during erythropoiesis and megakaryopoiesis (Fischer et al., 2012; Leung et al., 2013; Lopez et al., 2017). *GLIS2* (GLI-similar 2) is a member of the Krüppel-like zinc finger transcription factor group that is closely related to the GLI family of proteins, mediating the transcriptional response to Hedgehog pathway activation (Kim et al., 2007; Lamar et al., 2001). *GLIS2* is highly expressed in the adult kidney and is targeted by a loss-of-function mutation in chronic kidney diseases (Attanasio et al., 2007), but is not expressed in differentiating hematopoietic cells, suggesting that its fusion with *CBFA2T3* leads to ectopic *GLIS2* activity as well as its aberrant transcription activity (Gruber et al., 2012; Thirant et al., 2017a; Dang et al., 2017).

Pediatric AMKL associated with *CBFA2T3-GLIS2* has a peculiar expression pathway. Indeed, several transcription factors, including GATA1, known to interact with CBFA2T3 are down-regulated by the fusion, while the megakaryocytic oncogene *ERG* is up-regulated upon *CBFA2T3-GLIS2* expression. As a result, the *CBFA2T3-GLIS2* gene, in a unique hit, blocks the differentiation of megakaryocytic cells, inhibiting the expression of *GATA1*, and increases self-renewal inducing the expression of *ERG* (Thirant et al., 2017b) (Fig. 2). Moreover the fusion seems to be associated with a distinct immunophenotype characterized by overexpression of CD56 and under-expression of HLA-DR and CD38, similarly to the one described as RAM phenotype (Thiollier et al., 2012; Eidenschink Brodersen et al., 2016). The total burden of somatic mutations associated with *CBFA2T3-GLIS2* is significantly lower than that found in other subgroups of AMKL (7.17 ± 3.60 *versus* 16.60 ± 5.13,

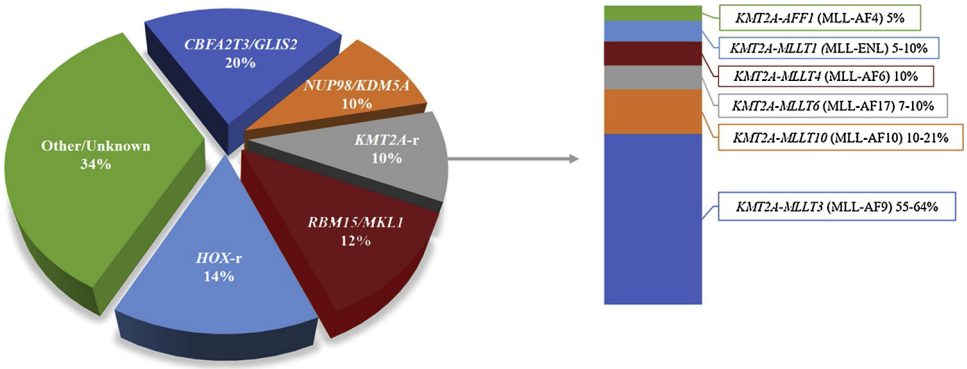


**Fig. 1.** Frequency of chromosomal aberrations reported in pediatric AMKL.

p = 0.009) (De Rooij et al., 2017). When not associated with normal karyotype, *CBFA2T3-GLIS2* was associated with somatic trisomy 21, complex karyotype, and hyperdiploidy (Gruber et al., 2012), as well as activating mutations in Jak/STAT and RAS pathway (De Rooij et al., 2017). Furthermore, a new fusion transcript was recently identified in 40% *CBFA2T3-GLIS2*-rearranged patients involving Desert Hedgehog (*DHH*), a member of the Hedgehog family, and Ras Homologue Enrich in Brain Like 1 (*RHEBL1*), a gene coding for a small GTPase of the Ras family (Masetti et al., 2013b). Despite these peculiar molecular features, the expression of fusion gene in mouse model is not sufficient to induce full leukemia phenotype (Gruber et al., 2012; Dang et al., 2017).

The *CBFA2T3-GLIS2* fusion gene was reported exclusively in pediatric patients, having been never found in adults (Gruber et al., 2012; Thiollier et al., 2012; De Rooij et al., 2017; Masetti et al., 2013a). Fusion-positive non-DS-AKML patients were found to be significantly younger than fusion-negative patients (De Rooij et al., 2016; Hara et al., 2017; Masetti et al., 2013a). In particular, the majority of fusion-positive patients are younger than 5 years of age (Gruber et al., 2012; Masetti et al., 2014), most of these being infants (*i.e.*, with an age < 1 year) (Hara et al., 2017; Masetti et al., 2014). The fusion has been detected not only in non-DS-AMKL, but also in *de novo* pediatric, non-AMKL, cytogenetically normal AML belonging to FAB subgroups M5, M0, M1, M2 and M4 (Masetti et al., 2013a), suggesting that this condition is not restricted to a unique FAB subtype. No significant differences in white blood cells count at diagnosis were found between fusion-positive and fusion-negative AMKL pediatric patients, but *CBFA2T3-GLIS2*-positive patients tended to have a higher percentage of bone marrow blasts at diagnosis compared to fusion-negative patients (De Rooij et al., 2016; Hara et al., 2017). Extramedullary involvement (EMI) is more frequent in *CBFA2T3-GLIS2*-positive patients (25%) compared to the frequency reported for childhood AML in general (Pession et al., 2013). In some of these cases, EMI can be initially confused with a non-hematopoietic tumor, with cranial bone, ribs, and lumbosacral column involvement (Thiollier et al., 2012). Central nervous system involvement is more frequent than in fusion-negative patients, but this does not seem to affect prognosis (Masetti et al., 2013a; Creutzig et al., 2017).

*CBFA2T3-GLIS2*-fusion positive non-DS-AMKL is unanimously recognized as a type of leukemia associated with a grim prognosis, with lower overall survival (OS) and event-free survival (EFS) rates

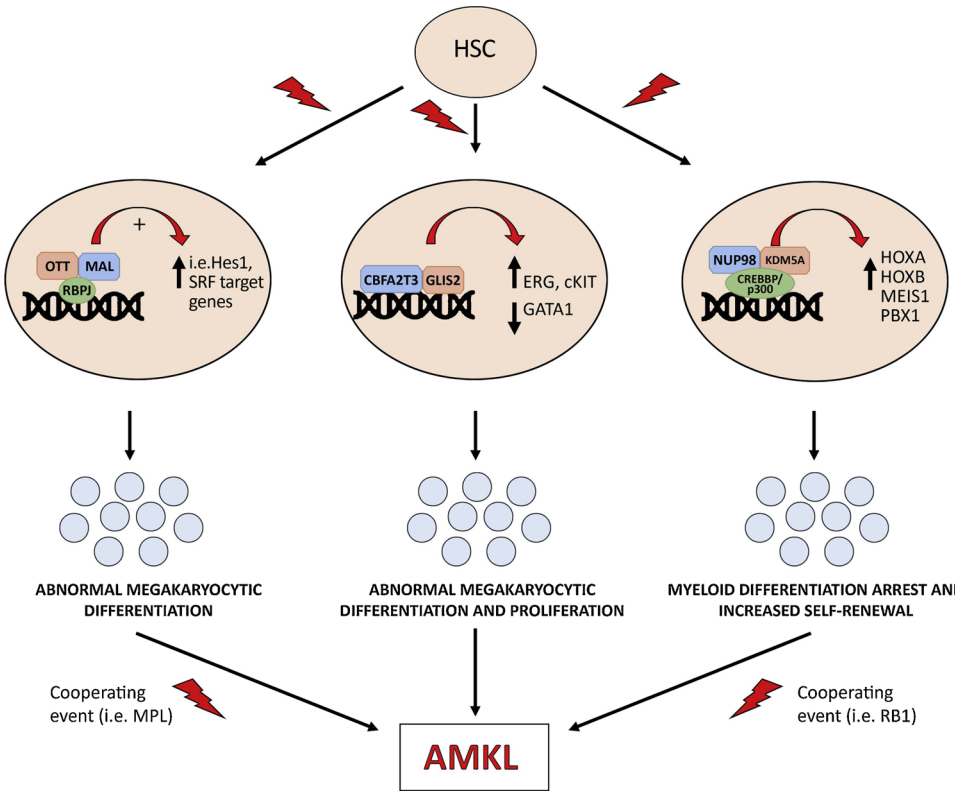


**Fig. 2.** Molecular mechanism representation of common chromosomal aberrations in inducing full leukemia phenotype. Each of common aberrations together with specific cofactors bind specific promotor region of DNA leading to a peculiar expression pathway. This event with or without cooperating mutations drives transformation of hematopoietic stem cells.

compared to fusion-negative AMKL and AML (Gruber et al., 2012; De Rooij et al., 2017; Hara et al., 2017; Masetti et al., 2013a) (Table 1). It is currently allocated to the high-risk group in many protocols of treatment and children carrying this anomaly are candidate to receive allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1) (De Rooij et al., 2017). Recent studies, reporting the clinical outcome of non-DS-AMKL children according to the different specific recurrent genetic abnormalities, showed that *CBFA2T3-GLIS2* has the strongest negative association with survival, the 5-year probability of OS ranging between 15 and 30% (De Rooij et al., 2016, 2017) (Table 1). This dismal outcome is mainly due to both a higher non-response to induction therapy (primary induction failure, PIF) and a cumulative incidence of relapse (ranging between 50 and 86%), compared with other chimeric gene subgroups (De Rooij et al., 2016, 2017; Masetti et al., 2013a). Fusion-positive infants have a worse prognosis than fusion-positive older patients, showing higher frequency of relapse and PIF33].

Given the poor prognosis, it is extremely important to identify molecular targets of the expression pathway induced by the fusion gene, in order to elaborate new target therapy approaches. To date, the efficacy of three different molecules have been demonstrated. Dimethylfasudil (DiMF) is an inhibitor of AURKA (Aurora A Kinase), that efficiently induces differentiation and polyploidization of leukemic blasts and drastically inhibits proliferation *in vitro* and *in vivo* (Thiollier et al., 2012; Wen et al., 2012). Recently two studies attempted specifically inhibiting the transcription activity of the fusion gene targeting the CBFA2T3 protein complex and GLIS2 activity, respectively. A peptide called NC128, consisting of 128 amino acids, can interfere with NHR2 domain of CBFA2T3 disrupting the protein complex (Wichmann et al., 2007). The expression of NC128 decreased proliferation, reduced cell-cycle progression and increased cell death *in vitro* and *in vivo* (Thirant et al., 2017b; Wichmann et al., 2007). On the other hand, GANT61, GLI inhibitor 61, a small molecule inhibiting DNA-binding activity of GLI family proteins, has been widely used in preclinical studies (Wellbrock et al., 2015; Pan et al., 2012; Agyeman et al., 2014; Masetti et al., 2017). As GLIS2 shares a highly homologous zinc finger domain with members of the GLI proteins (Vasanth et al., 2011), it has been hypothesized that GANT61 might be used to specifically target the CBFA2T3-GLIS2 fusion in pediatric AML (Masetti et al., 2017). GANT61 treatment resulted in sensitivity of cell lines and primary AML cells carrying *CBFA2T3-GLIS2* higher than that of AML cells without *GLIS2* fusion, in promoting apoptosis and G1 cell-cycle arrest (Masetti et al., 2017). It also induced down-regulation of some genes directly regulated by the fusion, such as *ERG*, *GATA3*, *DNMT1*, and *DNMT3B*, suggesting the specificity of GANT61 treatment to block the GLIS2 DNA binding (Masetti et al., 2017).

### 2.3. NUP98-KDM5A

The chimeric gene *NUP98-KDM5 A* has been identified for the first time in adult AML in 2006 (van Zutven et al., 2006) and, recently, it has been detected in approximately 8–15% of pediatric non-DS-AMKL patients (De Rooij et al., 2016; Rooij et al., 2013; De Rooij et al., 2017; Gruber and Downing, 2015; Hara et al., 2017) (Fig. 1).

*NUP98-KDM5 A*, also known as *NUP98-JARID1A*, results from the cryptic translocation t(11;15)(p15;q35), leading to the fusion of *NUP98* located on chromosome 11p15 with *KDM5 A*, located on the telomeric end of 12p13 and therefore undetectable with conventional karyotyping. The most common in-frame fusion is between exon 13 of *NUP98* and exon 27 of *KDM5 A,* but another breakpoint of *NUP98* has been detected in exon 14 (Rooij et al., 2013). NUP98 is a member of the nucleoporin complex with transactivation activity. It was initially described to be rearranged in approximately 4% of pediatric AML and is associated with poor prognosis (Struski et al., 2017). NUP98 is known to have many different partner genes (Romana et al., 2006), suggesting that its association with AMKL depends on the specific role of the fusion partners during megakaryocyte development. KDM5 A is a histone lysine demethylase that is overexpressed in several human cancers and plays key roles in tumorigenesis, metastasis, and drug tolerance (Gale et al., 2016). The fusion transcript maintains the ability to recruit the coactivator CREBBP/p300, a histone acetyltransferase that induces the transcriptional activation of different target genes, and to bind H3K4me3 mononucleosomes (Rooij et al., 2013). As a result, ectopic expression of *NUP98-KDM5 A* induces full AML phenotype, leading to upregulation of HOX genes in particular HOXA and HOXB genes (HOXA5, -A9, -A10, -B2, -B3, -B4, -B5 and -B6) (Fig. 2). Similarly to *NUP98-NSD1*, *NPM1* mutations and *DEK-NUP214* fusion, *NUP98/JARID1A* is associated with higher expression of several *HOXA* and *HOXB* genes, suggesting a common mechanism of leukemogenesis in these cases. This expression pattern is distinct from *KMT2A*-rearranged cases, which are characterized by overexpression of *HOXA* genes only (Rooij et al., 2013).

Other target polycomb proteins, including genes upregulated in *KMT2A*-rearranged leukemia, have been detected through microarray analysis and chromatin immunoprecipitation, such as MEIS1 and PBX1 (Thiollier et al., 2012; Wang et al., 2009).

Unlike *CBFA2T3-GLIS2* subgroup, *NUP98-KDM5 A* is strongly associated with *RB1* mutation (Lopez et al., 2017).

The fusion-positive patients present negative association with *FLT3/ITD* and *WT1* mutations, in contrast to other NUP98 fusions (De Rooij et al., 2017), mutually exclusive with other cytogenetic aberrations (Rooij et al., 2013).

Concerning the clinic characteristics, *NUP98-KDM5 A* fusion positive cases show no significant differences from other AMKL subtypes in age at diagnosis, sex or white blood cell count at diagnosis (Rooij et al., 2013).

Several studies analyzed the outcome of fusion-positive cases to evaluate the prognostic significance of the expression of *NUP98-KDM5 A*. Fusion-positive patients are characterized by an extremely poor outcome, with an overall survival ranging between 22 and 36% (De Rooij et al., 2016; Rooij et al., 2013; Hara et al., 2017). According to the results of the cohort studied by De Rooij et al.18], the outcome of fusion-positive patients was poor, but not significantly different from that of fusion-negative AMKL patients (5-year pOS, pEFS and pCIR: 22 ± 14% vs 45 ± 7%, P = 0.22; 22 ± 14% vs 36 ± 6%, P = 0.54; 56 ± 19% vs 54 ± 7%; P = 0.94; respectively) (Table 1). However, more recent studies reported *NUP98-KDM5 A* to confer a poorer outcome compared to other AMKL subgroups and to be an independent prognostic factor of poor outcome, similarly to *CBFA2T3-GLIS2* and *KMT2A*-rearrangement positive AMKL (De Rooij et al., 2016; Hara et al., 2017). The poor outcome was mainly due to the high rate of refractory and relapsed disease (De Rooij et al., 2016). Although these data still need confirmation, NUP98-KDM5 A chimeric gene expression, being associated with a low 4-year pOS, pEFS and pCIR (approximately 36%, 34% and 36%, respectively) (De Rooij et al., 2016), should be classified as high-risk, and allogeneic HSCT should be considered in CR1 (De Rooij et al., 2016, 2017; Hara et al., 2017).

Also in light of this poor outcome, the fusion gene was screened to develop new potential target therapy strategies. DiMF and MLN8237, another AURKA inhibitor, were demonstrated to induce differentiation and increased polyploidization of leukemic blasts, to induce apoptosis detected by Annexin V and cleaved caspase 3, and to drastically inhibit proliferation. These data were demonstrated through *in-vitro* cultures of AMKL blasts from immunodeficient recipient mice, and were confirmed in AMKL cells *in vivo* (Thiollier et al., 2012; Wen et al., 2012). In addition, specific histone acetyltransferase inhibitors may be potential

candidate drugs in all NUP98-rearranged leukemias, and overexpressed HOXA and HOXB genes may represent an alternative potential target (Rooij et al., 2013).

### 2.4. KMT2A-rearrangements

Chromosomal rearrangements involving *KMT2A* are recurrent genomic alterations in pediatric AML (Balgobind et al., 2019). *KMT2A* gene is localized at 11q23 and is involved in chromosomal translocations with more than 70 partner genes (Meyer et al., 2013). KMT2A protein is an H3K4 methyltransferase which regulates the transcription of *homeobox* genes, including *HOXA* cluster genes and *MEIS1*, which are essential for the regulation of normal hematopoiesis (Chen and Armstrong, 2016). A typical feature of *KMT2A*-rearranged AML is overexpression of *homeobox* genes *HOXA* and *MEIS1,* resulting from the activity of the H3K79 methyltransferase DOT1L (Chen and Armstrong, 2016). *Homeobox* gene deregulation is responsible for leukemogenesis, and DOT1L has a key role in the pathogenesis of *KMT2A*-rearranged leukemias (Chen and Armstrong, 2016).

*KMT2A*-rearrangements occur in ˜ 10% of pediatric non-DS-AMKL (De Rooij et al., 2016; Rooij et al., 2013; Inaba et al., 2015) (Fig. 1). Different fusion partners have been described: *KMT2A-MLLT3* (MLL-AF9), resulting from t(9;11)(p22;q23), which is the most common *KMT2A* fusion event, *KMT2A-MLLT10* (MLL-AF10), *KMT2A-MLLT4* (MLL-AF6), *KMT2A-MLLT1* (MLL-ENL), *KMT2A-MLLT6* (MLL-AF17), *KMT2A-AFF1* (MLL-AF4) (De Rooij et al., 2016; Rooij et al., 2013; Inaba et al., 2015). Children with *KMT2A*-rearranged AMKL have been reported to have a poor outcome (4-year pOS and pEFS ˜ 30%) (De Rooij et al., 2016); in particular, patients with t(9;11)(p22;q23) were reported to have a 5-year OS and EFS as low as 17.1% ± 7.8% (Inaba et al., 2015).

The poor prognosis associated to this subgroup of non-DS-AMKL emphasizes the requirement of new target opportunities. The significant role of DOT1L in the onset and the maintenance of KMT2A-rearranged leukemias makes this gene attractive for therapeutic approach. In several *in vitro* studies, the inactivation of the DOT1L gene expression on cells carrying translocations involving KMT2A gene showed antiproliferative effects inducing cell differentiation and apoptosis (Chang et al., 2011; Jo et al., 2011). This evidence suggested the development of DOT1L inhibitors as promising treatment strategy. DOT1L inhibitor EPZ-5676 has been tested in phase I trial in pediatric patients with KMT2A-rearranged refractory or relapsed leukemia (ClinicalTrials.gov Identifier: NCT02141828). EPZ-5676 showed an acceptable safety profile with a recommended phase II dose defined as 70 mg/m$^2$ cIV in children > 1 year and determined transient reductions in peripheral or bone marrow blasts in approximately 40% of the patients (Shukla et al., 2016). However, DOT1L inhibitors need high drug concentration for treatment and has low oral bioavailability, in addition to limited effects on many KMT2A-rearranged cell lines (Morera et al., 2016). A recent study performed shRNA screening to identify combination partners that could improve the antileukemic effect of DOT1L inhibitors. In particular, according to the study results, the association of a DOT1L inhibitor with an inhibitor of the KMT2A–Menin interaction (DOT1L-Menin inhibition) may represent a more effective therapeutic approach (Dafflon et al., 2017).

### 2.5. Other fusion genes

Less frequent novel chimeric genes have been also recently identified, restricted to pediatric non-DS-AMKL (Gruber et al., 2012): *GATA2-HOXA9*, *MN1-FLI1*, *NIPBL-HOXB9*, *GRB10-SDK1*, *C8orf76-HOXA11AS*. Most of the genes involved in these fusion events play an important role in normal megakaryocyte development or have been described to have a role in leukemogenesis (Gruber et al., 2012). (Dang et al. (2017)) confirmed the oncogenic role of the fusion genes *GATA2-HOXA9*, *MN1-FLI1* and *NIPBL-HOXB9*, reporting that all three fusions induced leukemias with AMKL features at different levels (Dang et al. (2017)). In particular, the fusion gene *MN1-FLI1* induced leukemia with a clear megakaryocytic phenotype and a gene expression program very close to megakaryocyte progenitors gene expression signature (Dang et al. (2017)). *NIPBL-HOXB9* and *GATA2-HOXA9* leukemias showed myeloid precursors phenotypic signatures and did not express megakaryocyte surface markers; however, dysplastic megakaryocytes were identified in these leukemias and fusions-positive tumor cells showed elements of megakaryocyte progenitors gene-expression program (Dang et al. (2017)).

A comprehensive recent report of sequencing of pediatric non-DS-AMKL (De Rooij et al., 2017) reported other novel fusion events: *NIPBL-HOXA9* (analogous to *NIPBL-HOXB9*), *GATA2-HOXA10* (analogous to *GATA2-HOXA9*), *EWSR1-HOXB8*, *PLEK-HOXA11AS*, *BMP2K-HOXD10*, *EP300-HOXA7* (De Rooij et al., 2017). These fusion events involve *homeobox* genes, like the low frequency above-described fusions. *HOX*-rearrangements occur in 14% of pediatric non-DS-AMKL, defining a new subtype within pediatric non-DS-AMKL, characterized by favorable outcome (5-year pOS and pEFS 77% ± 12%) (De Rooij et al., 2017). The triple angiokinase inhibitor Nintedanib combined with Azacitidine is currently in phase I trial in adult patients with newly diagnosed and relapsed/refractory acute myeloid leukemia presenting *HOX* overexpression and who are not eligible for intensive chemotherapy treatment (ClinicalTrials.gov Identifier: NCT03513484).

### 2.6. Risk stratification and tailored treatment approach

The prognostic scenario of acute pediatric non-DS AMKL is changing, concomitantly with the evolving genetic landscape. The mutually exclusive fusion oncogenes, which characterize approximately 70% of non-DS-AMKL, can be used for a more refined risk-group stratification of a disease that has been historically associated to poor prognosis (Table 1).

Before the advent of the described genomic characterization, the prognostic risk of pediatric non-DS-AMKL was mainly assessed through the identification of cytogenetic characteristics. However, the prognostic value of recurrent cytogenetic aberrations — such as complex karyotype, trisomy 8, 19, or 21, *KMT2A*-rearrangements, loss of chromosome 7 or 7q-, or der(3q) — has always been controversial (Schweitzer et al., 2015). A clear prognostic impact of cytogenetically defined subgroups has always been limited by the small size of study cohorts. Moreover, in pediatric AMKL, a high incidence of myelofibrosis may complicate a cytogenetic-driven stratification based on conventional karyotyping. In a large-scale retrospective study including 490 non-DS-AMKL patients, Inaba et al (Inaba et al., 2015). proposed to classify pediatric non-DS AMKL in 3 risk groups according to cytogenetic findings. A good risk group including patients carrying 7p abnormalities, a poor risk including cases with monosomy 7, or 9p abnormalities, including *KMT2A-MLLT3*, -13/13q-, and -15 and an intermediate risk group, encompassing all other AKML patients (Inaba et al., 2015). Nowadays a risk stratification based on conventional karyotyping could be inadequate for a proper and refined stratification. The most comprehensive risk group refinement of pediatric non-DS-AMKL should now integrate cytogenetic aberrations together with the following recurrent molecular lesions: *CBFA2T3-GLIS2, NUP98-KDM5 A, RBM15-MKL1* and rearrangements of *KMT2A*. In particular, it can be reasonable to propose a high-risk group composed of *NUP98-KDM5 A, CBFA2T3-GLIS2, KMT2A*-rearrangements and monosomy 7, and a standard-risk subgroup, including *RBM15-MKL1* positive patients, children carrying 7p abnormalities, and all other pediatric AMKL.

Based on this risk-group stratification, questions about the optimal molecularly driven treatment approach arise. In pediatric non-DS-AMKL, the optimal treatment strategy has always been subject to controversy. Whereas some study groups treat non-DS-AMKL globally as very-high-risk disease, recommending allogeneic HSCT in first complete remission[2], other groups obtained superior survival rates with intensive

chemotherapy alone without any advantage being observed employing HSCT during post-remission treatment (Schweitzer et al., 2015; Hama et al., 2008). Although there is clear evidence that allogeneic HSCT has a greater anti-leukemic potential than chemotherapy as post-remission treatment, this advantage can be offset by a higher risk of treatment-related mortality (Hasle, 2014; Passweg et al., 2014; Bastos-oreiro et al., 2014). Despite the lack of robust data showing an undisputable advantage of an allograft in CR1 for all non-DS-AMKL patients, it is reasonable to speculate that, after achieving remission, HSCT could be the best strategy to avoid recurrence at least in high-risk non-DS-AMKL. In light of these considerations, for example, *CBFA2T3-GLIS2*-positive *KMT2A*-rearranged patients, together with those carrying monosomy 7, are currently being considered in the high-risk group in many pediatric treatment protocols and, thus, candidate to HSCT in CR1 (Hasle, 2014). Similarly, *NUP98-KDM5 A* cases warrant close monitoring and for them allogeneic HSCT in CR1 should be considered, as well. On the other hand, standard-risk non-DS-AMKL patients could receive an intensive chemotherapy-based approach, with HSCT in CR1 reserved to those showing a poor response to induction therapy (*i.e.* absence of morphological remission or presence of high levels of minimal residual disease at flow-cytometry analysis).

## 3. Conclusions

A substantial amount of recent findings has significantly changed the perspective on the biology of pediatric non DS-AMKL. A genetic alteration, playing a pivotal role in driving leukemogenesis, can now be identified in almost 65% of pediatric cases of non-DS-AMKL. This deeper knowledge of pathogenic mechanisms associated to specific driver fusion oncogenes is extremely appealing for potential targeted therapy approaches. This scenario is certainly promising for a disease that has historically been associated with a grim prognosis. Moreover, the prognosis of pediatric non-DS-AMKL could now take advantage of a re-categorization of the risk assessment allowed by these recently identified aberrations. A screening panel of pediatric non-DS-AMKL including *CBFA2T3-GLIS2, KMT2A*-rearrangements, and *NUP98-KDM5 A*, combined with conventional karyotyping, could nowadays be a standard for tailoring treatment intensity to the biological characteristics of the leukemic clone. Although the advantage of allogeneic HSCT in CR1 remains controversial, transplantation can be considered as the best post-remission approach for patients carrying a fusion gene associated with poor prognosis and for those with high levels of minimal residual disease at the end of standard induction chemotherapy. Further comprehensive genomic studies are warranted in pediatric non-DS-AMKL cases in which the genetic alterations leading to the malignancy are unknown.

## Conflict of interest

The authors declare that they have no competing interests.

## Acknowledgements

This work was partly supported by the grants from AIRC (Associazione Italiana Ricerca sul Cancro), MFAG2016, Id. 19117 to Riccardo Masetti and by the grants from AIRC (Associazione Italiana per la Ricerca sul Cancro)-Special Project 5 × 1000 no. 9962 to F.L.

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