Pediatric myelodysplastic/myeloproliferative neoplasms and related diseases

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Abstract Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are clonal hematopoietic disorders with myeloproliferative features, varying degrees of dysplasia and cytopenias, and increased propensity for progression to acute myeloid leukemia (AML). MDS/MPN are uncommon in the pediatric age group, and best exemplified by Juvenile myelomonocytic leukemia (JMML), a rare but aggressive leukemia of early childhood. Remarkable progress has been made in understanding the genetic basis of JMML leading to improved diagnostic criteria and better management. It is now understood that JMML is associated with somatic or germ line mutations in *NF1*, *NRAS*, *KRAS*, *PTPN11*, and *CBL* in greater than 90 % of cases with the common downstream mechanism being uncontrolled activation of the RAS/MAPK pathway. More recently, *KRAS* and *NRAS* mutations have also been identified in RAS-associated autoimmune leukoproliferative disorder (RALD), which shares some clinical, hematopathological, and genetic features with JMML, but has an indolent clinical course. Hematopoietic stem cell transplant (HSCT) is currently the only curative therapy for

JMML, although newer therapeutic agents are currently in clinical trials. This review will focus primarily on the current clinical features, diagnostic criteria, pathologic features, and therapy of JMML and provide a brief description of RALD.

Keywords Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) · Juvenile myelomonocytic leukemia (JMML) · RAS-associated autoimmune leukoproliferative disorder (RALD)

Introduction

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are clonal hematopoietic disorders with overlapping features of myelodysplasia and myeloproliferation. The 2008 WHO classification of MDS/MPN includes three defined disorders: chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), and atypical chronic myeloid leukemia, BCR-ABL1 negative (aCML). A fourth category, MDS/MPN-unclassifiable (MDS/MPN, U) is reserved for myeloid neoplasms not meeting diagnostic criteria for the other three [1]. CMML and aCML are typically disorders of adulthood while JMML, as the name implies is seen in children. Myeloproliferation in MDS/MPN is most often a result of aberrant RAS/MAPK signaling with the vast majority of JMML patients exhibiting mutations in *NF1*, *PTPN11*, *CBL*, *NRAS*, *KRAS*, and a subset of CMML and aCML patients showing *NRAS* mutations [2, 3]. The identification of RAS/MAPK pathway protein mutations in patients with rare genetic disorders has also provided insights into leukemogenesis, biomarkers, and therapeutic targets [2]. These studies have also demonstrated the invaluable role of astute clinical observations combined with powerful genomic analysis in understanding the biology of disease.

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Juvenile myelomonocytic leukemia

Epidemiology

JMML is a rare, aggressive myeloproliferative neoplasm that occurs exclusively in children with an approximate annual incidence of 1.2 cases per million from infancy to early adolescence, but the majority of cases are diagnosed in children <3 years with boys affected twice as often as girls. A fraction of cases occurs in children with neurofibromatosis type I (NF1) [1].

Clinical and laboratory features

JMML patients usually present with non-specific constitutional symptoms including fever, cough, tachypnea, and evidence of infection. Hepatosplenomegaly is present, although in rare cases, the spleen is not enlarged at diagnosis. Lymphadenopathy or enlarged tonsils is identified in some cases. Skin lesions are common, including eczematous patches, erythematous papules, and juvenile xanthogranulomas. Café-au-lait spots are noted in children with NF1.

Leukocytosis, anemia, and thrombocytopenia are characteristic of JMML. The median WBC count ranges from 25 to $33 \times 10^9/L$ but can occasionally exceed $100 \times 10^9/L$ secondary to neutrophilia and monocytosis [4, 5]. Absolute monocytosis, $>1 \times 10^9/L$ is required for diagnosis. However, unlike chronic myelomonocytic leukemia (CMML), persistent monocytosis, defined as greater than 3 months duration, is not required for the diagnosis of JMML. Of note, an absolute monocyte count of $1 \times 10^9/L$ or greater can also be seen in healthy infants. The peripheral blood smear often shows left-shifted myeloid cells and monocytes, but blasts are <2 % in approximately half of cases (Fig. 1a). Overt morphologic dysplasia may be lacking.

Anemia is usually normocytic and circulating nucleated red cells can be seen. Thrombocytopenia can range from moderate to life threatening [4].

Most JMML patients have abnormally high hemoglobin F (Hb F) levels. Elevated Hb F levels are not specific for JMML as high Hb F levels can also be seen in other settings of stressed erythropoiesis in children (e.g., MDS, sickle cell disease, hereditary persistence of fetal hemoglobin, and bone marrow failure syndromes). Additionally, interpreting Hb F levels in young children can be challenging due to widely fluctuating levels seen in infancy. A subset of JMML has immune abnormalities including polyclonal hyperglobulinemia and circulating autoantibodies. Myeloid progenitor cells from JMML patients show selective hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF), a feature that is used as a diagnostic test [6]. In cases of suspected JMML, infection with EBV, CMV, and HHV-6 should be excluded in addition to any other infectious process that may present with monocytosis and features overlapping with JMML. Autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus can also present with monocytosis and rarely thrombocytopenia and should be considered in the differential diagnosis.

Histologic features

The bone marrow is hypercellular with varying degrees of myeloid hyperplasia, left-shifted maturation with subtle or absent dysplastic features; however, blasts, by definition, are less than 20 %. Monocytosis and dysgranulopoiesis are often not as prominent in the marrow, as in peripheral blood. Erythroid maturation may appear megaloblastic and megakaryocytes are reduced [4, 7] (Fig. 1b–c).

Fig. 1 a–c JMML-like disorder in an 11-week-old boy with mildly dysmorphic facies, short broad neck, widely spaced nipples, and very enlarged and firm spleen, ultimately found to have Noonan syndrome and a *PTPN11* c.218C>T (p.Thr73Ile) mutation. The peripheral blood smear (**a**) was remarkable for a leukocytosis of nearly $50 \times 10^9/L$ and $36 \times 10^9/L$ platelets. Neutrophils were shifted to immaturity and rare blasts were present, along with almost $9 \times 10^9/L$ monocytes. The trephine biopsy (**b**) showed a virtually 100 % cellular marrow [normal for age would be 80–90 %] with myeloid hyperplasia. The aspirate smears (**c**) similarly demonstrated myeloid hyperplasia, and blasts were mildly increased at 9 %. **d** JMML in a 7-year-old girl with NF1. Initial presentation of JMML was gradually increasing leukocytosis with absolute monocytosis, rare circulating blasts, thrombocytopenia, and hepatosplenomegaly. Hemoglobin F was elevated for age at 15.8 % [normal <2 %]. The bone marrow aspirate (shown) revealed 15 % blasts, 8 % basophils, and 2 % monocytes. An abnormality of chromosome 5 was seen on conventional cytogenetic analysis. She underwent allogeneic stem cell transplantation. Bone marrow examination at day +100 showed similar findings, confirming relapse which had been suspected on the basis of falling chimerism. **e–f** Splenectomy specimen from 22-month-old boy with JMML, status-post cord blood transplant, with primary graft failure. The spleen was markedly enlarged and the red pulp diffusely infiltrated by a myeloid/monocytic proliferation (**e**) Myeloperoxidase immunostain highlighting the myeloid infiltrate is depicted in (**f**). **g–i** MDS/MPN, U with increased blasts in a 17-year-old boy with *RANBP2-ALK* fusion. The peripheral blood (**g**) showed a marked leukocytosis of $114 \times 10^9/L$ but more modest anemia and thrombocytopenia. Neutrophils were dysplastic and shifted to immaturity with 5 % circulating blasts. Thirty-one percent monocytes were present. The trephine biopsy (**h**) demonstrated a virtually 100 % cellular marrow [normal for age would be 40–60 %]. The aspirate smears (**i**) had increased “blast equivalents” (mainly promonocytes) accompanied by 65 % maturing/mature granulocytes and 12–13 % mature-appearing monocytes but was insufficient for a diagnosis of AML. Of note, the patient had no history of prior therapy, as is typically seen in pediatric CMML. The patient’s age was not typical for JMML or adult-type CMML, and the case was considered most consistent with MDS/MPN, U. **j–l** RALD in an 8-year-old girl with splenomegaly, lymphadenopathy, leukocytosis with absolute monocytosis ($1.4 \times 10^9/L$), lymphocytosis, and thrombocytopenia. Onset of symptoms began at the age of 4 years, and a somatic mutation in *KRAS* c.38G>A (p.Gly13Asp) was subsequently identified. The patient has hypergammaglobulinemia and is positive for lupus anticoagulant, anti-nuclear antibodies, and is DAT positive. Peripheral smear shows vacuolated monocytes (**j**) with a significant subset of CD16+ monocytes by flow cytometry (not shown). Bone marrow core biopsy shows a hypercellular marrow (**k**). Aspirate smear is cellular with evidence of granulocytic hyperplasia, mild-to-moderate left shift in myeloid maturation, less than 5 % blasts, and a normal karyotype on cytogenetic analysis (**l**)

Extramedullary involvement is common. Hepatic leukemic infiltrates are seen in a periportal and sinusoidal distribution. Splenic infiltrates expand the red pulp leading to compression of the white pulp (Fig. 1e–f). Tonsillar and cutaneous infiltrates can also be identified.

Cytogenetics

Monosomy 7 is the most common cytogenetic abnormality, seen in approximately 25 % of JMML patients. Other abnormalities are seen in 10 % of cases and 65 % have normal cytogenetics [4, 5].

Genetics

It is now known that uncontrolled activation of the RAS/MAPK pathway plays a central role in the pathogenesis of JMML. Approximately 90 % of JMML patients harbor mutually exclusive mutations in one of five genes—*NF1*, *PTPN11*, *NRAS*, *KRAS*, and *CBL* [2].

The observation that patients with NF1 had a higher incidence of JMML led to the discovery that *NF1* is a tumor suppressor gene that functions by inhibiting the Ras signaling pathway [8]. Mutations in *NF1* account for 10–15 % of JMML cases. Bone marrow analysis of NF1 patients with JMML has shown inactivation of the *NF1* gene locus by either

uniparental disomy (UPD) or compound heterozygous mutations, which are critical events in leukemogenesis [9, 10]. JMML associated with NF1 is typically fatal unless treated with hematopoietic stem cell transplantation (HSCT).

Noonan syndrome (NS) is developmental disorder, which shares some clinical features with NF1, including the increased incidence of JMML and JMML-like transient myeloid proliferations [11–13]. The transient myeloid proliferation (TMP) associated with NS is seen in early infancy and is non-clonal unlike JMML. Most of these patients have spontaneous resolution of their symptoms over a span of months. However, approximately 10 % of patients with NS/TMP acquire a secondary cytogenetic abnormality and progress to JMML. Germ line *PTPN11* mutations, encoding for SHP2, and downstream activation of Ras/MAPK pathway, have been identified in the majority of NS patients [14]. This led to the discovery that somatic *PTPN11* mutations are the most common genetic lesions in sporadic JMML accounting for 35 % of all cases [15, 16]. Functional studies have shown that most of the SHP2 mutations associated with JMML have a stronger gain-of-function effects than those seen in NS, which may explain the transient nature of NS/TMP [17–19]. JMML associated with *PTPN11* mutations is uniformly fatal without HSCT.

Somatic point mutations in *NRAS* or *KRAS* leading to gain-of-function alterations in Ras proteins are identified in up to 20–25 % of JMML cases. Most mutations occur in codons 12, 13, or 61, leading to an active Ras-GTP-bound state and downstream activation [20, 21]. JMML associated with *NRAS* or *KRAS* mutations is aggressive and requires urgent HSCT.

Germ line or somatic mutations in *CBL*, the gene encoding CBL, an E3-ubiquitin ligase have been identified in nearly 10 % of JMML patients. In addition to myeloproliferative disease, germ line *CBL* mutations result in additional deformities, including developmental delay and cryptorchidism, a Noonan syndrome-like phenotype. As in NF1, leukemogenesis is initiated by LOH at the *CBL* locus [22]. Interestingly, many patients with JMML and germ line *CBL* mutations show

spontaneous regression of their leukemia, but suffer multiorgan vasculitides later in life. HSCT in these patients may be directed more towards immune reconstitution and is not indicated unless they show rapid progression of disease [22]. Patients with somatic mutations in *CBL* are non-syndromic and JMML arising in this background tends to have an aggressive clinical course.

Whole exome sequencing has identified mutations in *SETBP1* and *JAK3* mutations in up to 17 % of JMML patients. However, they appear to be secondary mutations and not initiating events [23]. *ASXL1* mutations are identified in a minority of JMML patients but *IDH1/2*, *TET2*, *EZH2*, *DNMT3A*, *SRFS2*, *U2AF1*, and *SF3B1* mutations have not been described [23, 24].

Revised diagnostic criteria

The discovery of genetic mutations and cytogenetic alterations associated with JMML have been incorporated into the new recommended diagnostic criteria for JMML [25, 26], which is more specific compared to the existing WHO 2008 [1] criteria (Table 1). Existing diagnostic assays such as GM-CSF hypersensitivity of myeloid precursors involve techniques that are difficult to reproduce and hence are only available in specialized centers. Newer tests like GM-CSF-mediated hyperphosphorylation of STAT5 protein by flow cytometry show promise but are not yet approved for clinical use [27].

Prognostic features

JMML remains a genetically and phenotypically heterogeneous entity with some patients showing spontaneous regression while others succumb rapidly. Several studies have examined mutational status with phenotypic severity, but results have been varied. One study showed that older age at diagnosis, *PTPN11* mutations, and Hb F > 10 % were poor prognostic factors [28]. Patients who present with NS/TMP are most likely to show spontaneous regression of their disease, and

Table 1 Revised diagnostic criteria for juvenile myelomonocytic leukemia (adapted from [25] and [26])

Category 1 All of the following	Category 2 At least one of the following	Category 3 At least two of the following
<ul style="list-style-type: none"> • Splenomegaly • Absolute monocyte count (AMC) >1000/mL* • Blasts in PB/BM <20 % * • Absence of the t(9;22) <i>BCR-ABL</i> fusion gene* 	<ul style="list-style-type: none"> • Somatic mutation in <i>RAS</i> or <i>PTPN11</i> • Clinical diagnosis of NF1 or <i>NF1</i> gene mutation • Homozygous mutation in <i>CBL</i> • Monosomy 7* 	<ul style="list-style-type: none"> • Circulating myeloid precursors* • WBC >10,000/mL* • Increased fetal hemoglobin (Hgb F) for age* • Clonal cytogenetic abnormality excluding monosomy 7* • GM-CSF hypersensitivity*

The diagnosis of JMML is made if a patient meets all of the category 1 criteria and one of the category 2 criteria without needing to meet the category 3 criteria. If there are no category 2 criteria met, then the category 3 criteria must be met

*Current WHO 2008 diagnostic criteria

the same holds true for the vast majority of patients with germ line *CBL* mutations. A subset of JMML patients with RAS G12S mutations have shown spontaneous hematologic improvement with minimal or no therapy in one study, leading the authors to suggest that this specific mutation leads to a milder phenotype [29]. However, other groups have demonstrated lack of genotype-phenotype correlation in JMML patients with RAS mutations [30].

Gene expression profiling studies have shown that JMML can be segregated into an AML-like group and a non-AML-like group, with a significantly poorer survival in the former [31]. For now, poor prognostic factors include older age (>1.4–4 years), increased fetal hemoglobin (hemoglobin F, >40 %), reduced platelets (<33,000/ μ L), monosomy 7, and mutated *PTPN11* [32].

Therapy

HSCT remains the definitive therapy of choice in JMML. However, 5-year overall survival remains suboptimal at 64 %. Additional therapeutic options for symptomatic patients include pre-HSCT chemotherapy or splenectomy [26]. Targeted therapies against the RAS/MAPK pathway and epigenetic modulators are currently under clinical trials [8].

Pediatric chronic myelomonocytic leukemia

Pediatric CMML is a rare MDS/MPN that is considered a therapy-related myeloid neoplasm seen secondary to chemotherapy or radiotherapy. However, it has also been reported in children in their late teenage years without a history of toxic exposure. The incidence of pediatric CMML is unknown. The current diagnostic criteria are: (i) peripheral monocytosis ($>1 \times 10^9/L$), (ii) absence of (9:22) (*BCR-ABL*) rearrangement, (iii) absence of rearrangement involving *PDGFRA*, *PDGFRB*, or *FGFR1* genes, (iv) <20 % blasts in the bone marrow or peripheral blood, (v) prior therapy, (vi) one of the following—evidence of dysplasia, acquired cytogenetic abnormality, or unexplained monocytosis for at least 3 months [33]. The prognostic factors and genetic lesions underlying pediatric CMML are not well defined.

Myelodysplastic/myeloproliferative neoplasms, unclassifiable

This is a rare subset of MDS/MPN, which does not fulfill criteria for the other specific MDS/MPN entities, including CMML, JMML, and aCML, *BCR-ABL* negative (Fig. 1g–i). The WHO 2008 classification also included refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) as a provisional entity within this subgroup. The incidence MDS/

MPN, U in the pediatric population is not well known. In the largest series published to date, the youngest patient was 22 years old [34].

Related disease of undetermined significance

RAS-associated autoimmune leukoproliferative disorder

Introduction and epidemiology

In 2007, Oliveira et al. described a 49-year-old male with a history of “acute leukemia” in infancy which resolved with minimal therapy, followed by a lifelong history of lymphadenopathy, splenomegaly, multiple autoantibodies, leukocytosis with monocytosis and lymphocytosis, and large B cell lymphoma as a young adult. The patient was presumptively diagnosed with a variant of autoimmune lymphoproliferative syndrome (ALPS). Subsequently, genetic analysis showed a heterozygous somatic mutation (originally misreported as germ line) in codon 13 (p. G13D) of *NRAS* leading to hyperactivation of the *RAS/RAF/ERK* pathway [35]. Somatic gain-of-function *KRAS* mutations (p. G13C, G13D, G12D, and G12S) were identified in other patients being evaluated for ALPS with similar clinical features, including peripheral blood monocytosis, reminiscent of juvenile myelomonocytic leukemia [36]. The term “RAS-associated autoimmune leukoproliferative disorder (RALD)” was adopted and incorporated into the ALPS classification to distinguish it from ALPS harboring mutations in *FAS*, *FASL*, or *CASP10* [37]. RALD is a newly described clinical entity of underdetermined significance, with features overlapping with both JMML and ALPS. The incidence of RALD is currently undefined.

Clinical and laboratory features

RALD typically presents in childhood, most often within the first 2 years of life (ranging from 3 months to 5 years in one study) [38] and persists throughout adolescence with an indolent clinical course. Persistent relative or absolute peripheral blood monocytosis, B cell lymphocytosis, hypergammaglobulinemia, lymphadenopathy, and splenomegaly have been described in virtually all patients. Auto-antibodies, including anti-nuclear antibodies (ANA), anti-cardiolipin (ACA), and lupus anticoagulant are common [35]. Autoimmune cytopenias are common.

Peripheral blood immunophenotyping has shown increased activated CD16+ monocytes, B cell lymphocytosis with polytypic expression of kappa and lambda light chains, a subset of patients with increased circulating CD10+ late precursor B cells, and CD14 (dim) positive granulocytes [38]. Circulating CD4−/CD8−/TCR $\alpha\beta$ T cells are not

typically increased. GM-CSF hypersensitivity of myeloid progenitor cells is positive in a subset of patients. Examination of peripheral blood smear shows monocytosis with predominantly large mature vacuolated monocytes (Fig. 1j).

Histologic features

Lymph node biopsies have shown non-specific features, including follicular hyperplasia, paracortical expansion, and sinus histiocytosis, including features reminiscent of Rosai-Dorfman disease [39]. Bone marrows are mildly hypercellular with left-shifted myeloid maturation and mild or minimal myeloid dyspoiesis [38] (Fig. 1k–l). Bone marrow cytogenetic analyses have demonstrated a normal karyotype in all RALD patients.

Genetics and biology

The first documented case of RALD demonstrated a somatic *NRAS* mutation in codon 13, while subsequent cases have shown somatic mutations in *NRAS* or *KRAS*, involving codons 12 or 13 in myeloid and lymphoid cells [40]. These mutations impart gain-of-function effects by stabilizing RAS proteins in the active GTP-bound state and reduced expression of a pro-apoptotic protein, BCL2-interacting mediator of cell death (BIM). Reduced BIM levels have been documented in leukocytes from RALD patients, suggesting that like ALPS, RALD is a disorder of defective apoptosis [35, 36].

Table 2 Features of RALD and JMML (modified from [38])

	RALD	JMML
Clinical and laboratory findings		
Massive Splenomegaly	Common	Common
Rash and arthralgias	Common	Common
Autoimmune disorders	Common	Reported in subset
Autoantibodies (DAT, ACA, ANA)	Common	Reported in subset
Increased Hb F for age	Yes in subset	Common
Anemia (<10 g/dL)	10 %	Common
Thrombocytopenia (<100 K/uL)	30 %	Common
Infiltration of extramedullary tissues by myelomonocytic cells	No	Common
Clinical course	Indolent	Aggressive
Peripheral blood flow and morphology		
Monocytes		
Monocytosis (>1.0 K/uL)	Nearly 100 %	100 %
CD16 expression (activated phenotype)	82 %	Unreported
Atypical expression of other markers on monocytes		
CD56+, CD13–, CD10+	18 %	Unreported
Circulating immature monos	23 %	Common
Circulating vacuolated monocytes	Common	Variable
Granulocytes and lymphocytes		
Neutropenia (<1.3 K/uL)	30 %	Reported in subset
Neutrophilia (>8.0 K/uL)	20 %	Reported in subset
Atypical CD14+ (dim) expression on granulocytes	64 %	Unreported
Circulating blasts	0 %	Common
Pelgeroid granulocytes	15 %	Common
B cells, polyclonal B lymphocytosis with circulating late hematogones (CD19+, CD10+, CD20+)	64 %	Unreported
Bone marrow cytogenetic abnormalities	0 %	25–35 %
Apoptosis defect (IL2 cytokine withdrawal assay)	100 %	Unknown
GM-CSF hypersensitivity assay	Positive in subset	Positive
<i>KRAS</i> or <i>NRAS</i> mutations	100 %	20–25 %
<i>PTPN11</i> mutations	0 %	~35 %
<i>NF1</i> mutations	0 %	10–15 %
<i>CBL</i> mutations	0 %	~10 %

Current diagnostic and prognostic criteria

RALD is rare but should be considered in the differential diagnosis of JMML and ALPS due to overlapping clinical presentation and laboratory features. The absence of circulating CD4⁺/CD8[−] TCRαβ T cells and lack of mutations in *FAS*, *FASLG*, or *CASP10* need to be confirmed to rule out ALPS [37]. In general, RALD patients are not as acutely ill as patients with JMML. However, distinguishing RALD from JMML on the basis of the 2008 WHO or revised JMML criteria may not be possible in all cases. Occasional patients with fulfilling diagnostic criteria for JMML and with RAS mutations have shown spontaneous resolution of leukocytosis but persistent hepatosplenomegaly, autoimmunity, and RAS clones in their hematopoietic cells, suggestive of RALD [41]. While there are some distinctive features of RALD that help to differentiate it from JMML (Table 2), retrospective studies of JMML patients and long-term follow-up of RALD patients with serial monitoring of hematologic parameters and genetic and cytogenetic markers is required for the development of accurate diagnostic criteria to distinguish these entities.

Current evidence gained from follow-up of small patient cohorts supports an overall indolent clinical course for RALD [36, 38]. Complications arising from autoimmunity and inflammation may be problematic in some patients. A small subset of RALD patients with *KRAS* mutations have had pericarditis and/or pericardial effusions with one fatality presumed due to cardiac failure due to inflammation; malignancy was ruled out by autopsy in that patient [38]. However, a recent report documented aggressive transformation of RALD to severe JMML, 10 years after RALD diagnosis, suggesting that these entities are related and lie within a spectrum of severity [39]. Future studies will be directed towards understanding the natural history and evolution of RALD.

Therapy

Current treatment for RALD is aimed towards alleviation of symptoms using immunosuppressive medications like sirolimus. A small number of patients have undergone splenectomy to obtain relief from pain and discomfort associated with hypersplenism. Close clinical and laboratory monitoring of RALD patients for acquisition of dysplastic or karyotypic abnormalities that may herald malignant transformation is recommended.

Conclusions

Rapid advances have been made in the diagnosis and management of JMML. New diagnostic guidelines with the incorporation of mutation analysis have allowed for more accurate

and early diagnosis and detection of minimal disease. Although JMML remains an aggressive disease with high therapy-related morbidity and mortality, targeted therapies aimed at the RAS/MAPK pathway offer exciting avenues for disease control.

The identification of RALD as a unique clinical entity with a less aggressive clinical course but sharing RAS aberrations with JMML will allow us to study the natural course of these proliferations and identify significant secondary alterations that affect disease progression and overall disease prognosis.

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Compliance with Ethical Standards All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Images of diagnostic peripheral blood, bone marrow, or spleen specimens were taken from clinical samples for which informed consent was obtained prior to procedure.

Conflicts of interest The authors declare that they have no conflict of interest.

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