Juvenile Myelomonocytic Leukemia

Charlotte Marie Niemeyer, MD[*]
Christian Kratz, MD

Address
[*]Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, University of Freiburg, Mathildenstrasse 1, 79106 Freiburg, Germany.
E-mail: niemeyer@kikli.ukl.uni-freiburg.de

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Opinion statement

Juvenile myelomonocytic leukemia is an aggressive neoplasia of early childhood. Only allogeneic stem cell transplantation (SCT) offers a long-term cure. In the absence of an HLA-matched family donor, early SCT from an unrelated donor will be the treatment of choice for most children. With clear evidence of a graft-versus-leukemia effect and a high post-transplant relapse rate, outcome of SCT will depend, in part, on the management of immunosuppression during the procedure. The impact of pretransplant cytoreductive treatment, such as intensive chemotherapy, splenectomy, or 13-cis retinoic acid, is unclear. Hypersensitivity for granulocyte-macrophage colony-stimulating factor and pathologic activation of the Ras/MAPK pathway play an important role in the pathophysiology of juvenile myelomonocytic leukemia and will provide the opportunity for several novel therapy approaches.

Introduction

Juvenile myelomonocytic leukemia (JMML) is a unique clonal hematopoietic disorder of infancy and early childhood characterized by excessive proliferation of the monocytic and granulocytic lineages. Children are diagnosed at a median age of 1.8 years with pallor, fever, infection, bleeding, and cough [1••], and there is generally marked hepatosplenomegaly. A macular-papular skin rash can be noted in one third of the cases. Almost all patients have an increased white blood cell (WBC) count with absolute monocytosis, anemia, and thrombocytopenia. WBC 50×10^9/L or greater is found in 30% of children, and only 7% have a WBC of 100×10^9/L. JMML lacks the Philadelphia chromosome and BCR-ABL fusion gene. To account for myelodysplasia observed in some cases, the World Health Organization included JMML in the category of myelodysplastic and myeloproliferative disorders. The entity incorporates those leukemias previously referred to as juvenile chronic myeloid leukemia and chronic myelomonocytic leukemia of infancy. Cases previously included in the infantile monosomy 7 syndrome are incorporated into this category. Guidelines for diagnosis of JMML have been established by an International JMML Working Group [2].

Chromosomal studies of leukemic cells show monosomy 7 in approximately 25% of patients, other abnormalities in 10%, and a normal karyotype in 65% [1••]. There are no major clinical differences between patients with and without monosomy 7. However, patients with monosomy 7 are diagnosed with normal or only moderately elevated hemoglobin F (HbF), whereas HbF is greatly elevated in patients with normal karyotype.

Juvenile myelomonocytic leukemia arises from a pluripotent stem cell. When cultured in semisolid media without the addition of exogenous growth factors, JMML mononuclear cells of blood or bone marrow give rise to an excessive number of monocyte-macrophage colonies. This so-called spontaneous proliferation of JMML myeloid progenitors is dependent on the endogenous production of interleukin-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), or tumor necrosis factor-α by leukemic monocytes. Tumor necrosis factor-α appears to act through GM-CSF by specifically modulat-

ing its gene expression. However, because JMML hematopoietic progenitors do not produce sufficient GM-CSF to sustain in vitro colony formation, JMML is not an autocrine-driven disease. Myeloid progenitor cells show a distinct hypersensitivity for GM-CSF [3] and are dependent on the production of GM-CSF by adherent cells. GM-CSF hypersensitivity has become the hallmark of the disease and an important diagnostic tool.

The hypothesis that a specific defect in the GM-CSF signal transduction pathway plays a major role in the pathogenesis of JMML has led to studies at the receptor and cytoplasmic signaling transduction. Although GM-CSF receptor mutations could not be demonstrated, abnormalities in the Ras/MAPK pathway became evident. Members of the Ras family of signaling proteins regulate cellular proliferation by cycling between an active guanosine triphosphate (GTP)-bound state (Ras-GTP) and an inactive guanosine diphosphate (Ras-GDP)-bound state. Mutant Ras alleles encode proteins that accumulate in the GTP-bound conformation because of defective GTP hydrolysis. Such oncogenic point mutations in N-Ras and K-Ras are observed in leukemic cells of approximately 15% of children with JMML [4].

The conversion from active Ras-GTP to the inactive Ras-GDP state is facilitated by GTP-ase–activating proteins. Neurofibromin, the protein encoded by the gene for neurofibromatosis type 1 (NF1), functions as GTP-ase–activating proteins and negatively regulates Ras. Fourteen percent of children with JMML carry the clinical diagnosis of NF1 [1••]. In addition, NF1 gene mutations have been detected in approximately 10% to 15% of patients without clinical evidence of NF1 [5]. Consistent with Knudson's "two-hit" tumor suppressor gene model involving germline transmission of an inactive allele and subsequent somatic inactivation of the remaining normal allele, homozygous inactivation of the NF1 alleles has been demonstrated in some children with NF1 and JMML [6••]. In murine model systems, it could be shown that loss of NF1 results in activation of the Ras signaling pathway and aberrant growth in hematopoietic cells in vivo [7••]. In addition, recipients engrafted with double knock-out NF -/- GM-CSF -/- hematopoietic cells demonstrated hypersensitivity to exogenous GM-CSF [8].

Juvenile myelomonocytic leukemia has also been observed in several infants with Noonan syndrome [9], a developmental disorder characterized by cardiac defects, short stature, dysmorphic facial features, and skeletal abnormalities. It has been shown that these children carry germline mutations in PTPN11, the gene encoding the protein tyrosine phosphatase SHP-2 [10]. SHP-2 is a key molecule in intracellular signaling and is necessary for activation of the Ras/MAPK cascade in a variety of growth factors, hormones, and cytokines. Most cases of JMML in Noonan syndrome spontaneously resolve, and the only case tested showed polyclonal hematopoiesis [9]. Therefore, it was surprising that somatic mutations in PTPN11 represent the major molecular event for nonsyndromic JMML. PTPN11 mutations account for approximately 35% of all JMML cases [10]. All mutations identified are predicted to cause gain of function in SHP-2 through preferential occupation of the activated state of the enzyme. The observation that mutations in NF1, PTPN11, and Ras are mutually exclusive further suggests that pathologic activation of the Ras/MAPK cascade plays a central role in growth characteristics of JMML.

Stem cell transplantation (SCT) is the only curative treatment modality capable of curing approximately 30% of patients. If left untreated, JMML is rapidly fatal in most children. Low platelet count, age older than 2 years, and high HbF at diagnosis are the main predicting factors for short survival [1••]. In a retrospective series of 110 children, patients with a platelet count of 33×10^9/L or less had died within a year of diagnosis, whereas patients with a higher platelet count who were younger than 2 years had the longest survival time with a median of 3 years [1••]. Blastic transformation is infrequent, and most untreated patients die from organ and respiratory failure because of infiltration of mature leukemic elements.

Treatment

Diet and lifestyle

- Approximately 10% of children with JMML are diagnosed within the first 3 months of life. Although not formerly proven, it is likely that JMML is a congenital disorder in these infants. It is tempting to speculate that similar mechanisms in leukemogenesis may operate pre- and postnatally. In the absence of epidemiologic studies for this rare disorder, we do not have a clue on potential carcinogens. NF1 is associated with a more than 200-fold increased risk of JMML. In JMML cases with familial NF1, NF1 is inherited from the mother in most patients (personal observation).

Surgery

- Patients with JMML generally have large spleens, and hypersplenism may give rise to increased morbidity with excessive transfusion requirements. Therefore, early splenectomy for amelioration of disease has been recommended [11,12]. In our own retrospective study on 72 children with JMML who received an allogeneic stem cell graft, splenectomy prolonged survival independent of other risk factors (unpublished observation). It has been common practice to remove large spleens before SCT [13•]. However, the impact of splenectomy for post-transplant relapse is unknown. In the ongoing study of the Children's Oncology Group, all clinically stable patients are scheduled for splenectomy, whereas the current SCT study by the European Working Group on Myelodysplastic Syndrome in Childhood (EWOG-MDS) and the European Group of Blood and Marrow Transplantation refers the decision for splenectomy to the local physician. Patients who are splenectomized should receive vaccines against *Streptococcus pneumoniae* and *Haemophilus influenzae* at least 2 weeks before splenectomy (prophylactic penicillin is to be initiated afterward).

Pharmacologic treatment

- Long-term survival has been achieved only with SCT. The role of antileukemic therapy before SCT is unknown. Evaluation of efficacy of different therapies is hampered by the lack of uniform response criteria. In addition, some young children with JMML may experience a longer course characterized by temporary clinical improvement even without therapy.

Low-dose conventional chemotherapy

- 6-Mercaptopurine, often used as single-drug therapy, was first described by Lilleyman *et al.* [14] in 1977. They recorded a clinical and hematologic response to repeated cycles of oral 6-mercaptopurine and subcutaneous cytarabine in three JMML cases. However, there was no influence on length of survival. Lutz *et al.* [12] reported partial remission with regression of organomegaly, and improvement of WBC and platelet counts in seven of 21 children treated with 6-mercaptopurine as a single agent or in combination with cytarabine or etoposide. Responses to 6-mercaptopurine in children younger than 2 years presenting with a normal platelet count have also been reported [15]. Many other single agents and combination-type therapies have been applied but were generally followed by poor response [12,16].

Intensive chemotherapy

- Most approaches of intensive chemotherapy arise from treatment protocols for acute myeloid leukemia (AML). Chan *et al.* [17] reported on four children treated with AML-type combination chemotherapy who achieved clinical remission and had significantly longer survival time (21–32 months) than five children receiving no or minimal chemotherapy. DeHeredia *et al.* [18] studied AML-type chemotherapy in six children, with long-lasting responses (51–145 months) in three. The prospective study CCG 2891 of the Children's Oncology Study Group for treatment of childhood AML, MDS, and JMML included 13 children with JMML [19]. Seven of 12 patients with JMML receiving sequential intensive induction chemotherapy achieved remission. These results may suggest that aggressive chemotherapy can ameliorate the disease in a substantial minority of patients. Therefore, the ongoing JMML protocol of the Children's Oncology Group applies cytoreductive therapy

consisting of fludarabine 30 mg/m^2 and cytarabine 2 g/m^2 daily for 5 consecutive days concomitantly with 13-cis retinoic acid. Other investigators point out that intensive chemotherapy is notably unsuccessful in patients with aggressive disease [12,16,20,21]. It can be complicated by therapy-related death after long-lasting aplasia [20,21]. True remissions are not achievable. Overall survival at 10 years in a group of 72 children with JMML who did not receive an SCT was 6%, with no difference between those patients who did and did not receive intensive chemotherapy [1••]. The current study of the EWOG-MDS does not recommend intensive chemotherapy before allogeneic SCT.

Interferon-α

- The apparently increased sensitivity of JMML to interferon-α prompted some investigators to apply the cytokine in JMML. Besides some non-responses [22–24], several transient responses [1••,12,25] in vivo have been reported. A prospective study of the Pediatric Oncology Group with interferon-α 30,000 units/m^2 subcutaneously daily for 14 days followed by the same dose three times weekly was stopped for excessive toxicity [26]. None of the evaluable patients had achieved a partial or complete response.

13-Cis retinoic acid

- Spontaneous growth of JMML myeloid progenitors in vitro can be inhibited by 10^{-6} to 10^{-8} m 13-cis retinoic acid. Based on these laboratory observations, 10 children with JMML were treated daily with 100 mg/m^2 isotretinoin orally [27]. Response was evaluated by reduction of WBC and decrease of organomegaly. Two children had complete remission (one patient for 83 months), three had partial responses, and one had a minimal response. The remaining four patients had progressive disease (median duration of response, 37 months). The authors concluded that isotretinoin could induce durable clinical and laboratory responses. This interpretation was questioned because the mean age of patients was 10 months, and young age is a favorable prognostic feature in JMML [28]. In a subsequent phase II trial of the Pediatric Oncology Group, 22 evaluable patients could be accrued in which there were five complete and four partial responders [29]. In contrast to these results, many other investigators did not observe significant clinical responses with the drug in their population of patients with JMML [12,28]. Therefore, the value of 13-cis retinoic acid in JMML remains controversial.

Radiation therapy

- Radiation therapy to the spleen does not generally result in a decrease in spleen size [15] or reduction of platelet transfusion requirement.

Stem cell transplantation

- Allogeneic SCT is the only curative approach for JMML, resulting in long-term survival in approximately one third of the patients [30•]. The malignant JMML clone is difficult to eradicate even with SCT, and post-transplant relapse rate is high. Generally, SCT shortly after diagnosis is recommended, and younger age at SCT may predict for improved survival [13•,31]. A wide variety

of pretransplant therapies, donor selection, graft manipulation, conditioning regimens, and graft-versus-host prophylaxis have been applied, giving rise to many open questions.

Donor

- Because long-term survival in children with JMML who are not grafted is less than 10% [1••], matched unrelated donor (MUD) transplants are justified for all children with JMML who lack a matched family donor. In vivo T-cell depletion with antilymphocyte globulin is often used for additional prophylaxis of graft-versus-host disease (GVHD) in MUD transplants. Although relapse rates in MUD and matched family donor transplants are comparable, transplant-related mortality is still significantly higher in MUD grafts. Most transplant-related deaths have infectious causes. Many unrelated umbilical cord transplants have been performed with outcomes similar to those observed in MUD transplants with other stem cell sources.

Conditioning regimen

- The first report on successful SCT in JMML from Seattle used a conditioning regimen of total body irradiation (TBI) and cyclophosphamide [32]. A similar regimen is still being used in the ongoing JMML trial of the Children's Oncology Group. Radiation-induced late effects, such as severe growth retardation and neuropsychologic sequelae, may be especially deleterious for very young children. Therefore, avoiding TBI is particularly attractive in JMML. There had been some concern that conditioning with chemotherapy only may not be sufficient to eradicate the malignant clone. However, several investigators reported comparable outcome for patients conditioned with TBI compared to non-TBI regimens [31,33•]. In a retrospective analysis by the EWOG-MDS, busulfan-based myeloablative therapy offered a greater antileukemic efficacy than TBI [13•]. The EWOG-MDS study assessed a preparative regimen with busulfan, cyclophosphamide, and melphalan.

Graft versus leukemia

- The ability of SCT to eradicate hematopoietic malignancy is based on the antineoplastic effects of the conditioning regimen and graft-versus-leukemia reaction. In JMML, there is clear evidence that graft versus leukemia plays an important role in curing the disease. Children who receive less immunosuppressive therapy for GVHD prophylaxis have a lower relapse rate [13•]. Similarly, acute or chronic GVHD is associated with a lower risk of relapse [13•,31,33•]. Following SCT, re-emerging donor cells and frank relapse have been successfully eradicated by reduction of ongoing immunosuppressive therapy. However, unlike BCR-ABL–positive chronic myeloid leukemia, donor lymphocyte infusion is known to induce subsequent remissions in only a minority of children with JMML [34].

Relapse

- After SCT, the relapse rate may be as high as 50% [30•]. Relapse occurs early at a median of 3.5 months from transplantation [13•] and generally within the first year. Despite aggressive re-emergence of the malignant clone and short interval between the first and second SCT, many children are cured after a second SCT.

Emerging therapies

- GM-CSF and pathologic activation of the Ras/MAPK transduction pathway plays a central role in the pathophysiology of JMML. Several potential therapeutic strategies to interfere with this cytokine signaling pathway have been described. They include interference with the GM-CSF cell interactions at the cell surface, blockage of Ras function by inhibition of post-translational modifications, and suppression of protein expression of downstream Raf-1 by mRNA degradation. It is unlikely that any of these potential therapies can actually abolish the entire malignant clone by themselves. However, they may have an important role in future multimodality therapy concepts.

Targeting the granulocyte-macrophage colony-stimulating factor receptor

- E21R is a GM-CSF analogue with a single amino acid substitution that abolishes the interaction of GM-CSF with the beta chain of its receptor, thus eliminating signaling. E21R acts as an antagonist of GM-CSF function by selective binding to the receptor alpha chain. In addition, it induces apoptosis in cells carrying the GM-CSF receptor. To examine the effect of E21R for inhibiting GM-CSF in vivo, primary JMML were engrafted into irradiated severe combined immunodeficient/nonobese diabetic mice [35]. Administration of E21R at the time of transplantation or 4 weeks after profoundly reduced JMML cell load in the mouse bone marrow and eliminated JMML cells from the spleen and peripheral blood. Studies of mice engrafted simultaneously with cells from normal donors and from patients with JMML showed that E21R preferentially eliminated leukemic cells. In a child with end stage JMML, the administration of two cycles of E21R at a dose of 1 mg/kg per day for 10 days had a transient hematologic and clinical effect lasting approximately 60 days [36]. Overall tolerance of E21R was very good. In another approach, it could be demonstrated that progenitors from JMML patients show an increased sensitivity to GM-CSF fused to a truncated diphtheria toxin in vitro [37]. JMML mononuclear cells exposed to the targeted toxin reduced the number of cells capable of forming colonies in a semisolid medium. These approaches deserve further investigation.

Targeting Ras proteins

- The Ras proteins are synthesized as precursor molecules in the cytoplasm. A series of post-translational modification of the Ras protein is required for localization to the inner surface of the plasma membrane. The first obligatory step in this series is the addition of a farnesyl moiety catalyzed by the enzyme farnesyl protein transferase. Many inhibitors of this enzyme (farnesyltransferase inhibitors [FTI]) have been synthesized and are in clinical trials for various cancers. For JMML, FTIs have been shown to inhibit colony formation in vitro [38•]. One of the FTIs, R115777, is being studied in newly diagnosed patients with JMML in an upfront therapeutic window in the ongoing JMML study by the Children's Oncology Group.
- In the NF1-/- murine model, the FTI L-744,832 induced dose-dependent inhibition of myeloid colony formation, but did not produce responses when used in vivo in the whole mouse with myeloproliferative disease [39]. There may be differences in cellular pathways between the NF1-/- mouse and primary human cells.

Targeting Raf-1 gene expression

- Raf kinases are direct downstream mediators of the Ras proteins. DNA enzymes designed to cleave Raf-1 mRNA can mediate mRNA degradation, suppressing Raf-1 protein expression. Addition of an active DNA enzyme abolished GM-CSF hypersensitivity of JMML myeloid progenitors in vitro [40]. Furthermore, continuous treatment with the active DNA enzyme reduced tumor burden in immunodeficient severe combined immunodeficient/nonobese diabetic mice transplanted with human JMML cells. This approach holds promise as a clinical therapeutic and warrants further clinical trials in JMML.

Inhibition of angiogenesis

- Angiogenesis is essential for growth and metastasis of solid tumors and probably also for hematologic malignancies. In the murine JMML transplant model, continuous treatment with the angiogenic inhibitors decreased microvessel density and leukemic cell mass, but did not alter in vitro proliferation of JMML cells [41]. Antiangiogenic therapy may be an adjunct in future therapy protocols.

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