Available online at www.sciencedirect.com
ScienceDirect
Leukemia Research xxx (2008) xxx–xxx
Leukemia Research
www.elsevier.com/locate/leukres

Meeting Report

Juvenile myelomonocytic leukemia: A report from the 2nd International JMML Symposium

Rebecca J. Chan [a], Todd Cooper [b], Christian P. Kratz [c], Brian Weiss [d], Mignon L. Loh [e,*]

[a] Departments of Pediatrics, The Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, USA
[b] Department of Pediatrics, Division of Pediatric Hematology/Oncology, University of Alabama at Birmingham, Birmingham, AL, USA
[c] Department of Pediatrics and Adolescent Medicine, University of Freiburg, Freiburg, Germany
[d] Department of Pediatrics, Division of Pediatric Hematology/Oncology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[e] Department of Pediatrics, University of California, 513 Parnassus Ave, Box 0519, San Francisco, CA 941143, USA

Received 15 August 2008; received in revised form 15 August 2008; accepted 19 August 2008

Abstract

Juvenile myelomonocytic leukemia (JMML) is an aggressive childhood myeloproliferative disorder characterized by the overproduction of myelomonocytic cells. JMML incidence approaches 1.2/million persons in the United States (*Cancer Incidence and Survival Among Children and Adolescents: United States SEER Program 1975–1995*). Although rare, JMML is innately informative as the molecular genetics of this disease implicates hyperactive Ras as an essential initiating event. Given that Ras is one of the most frequently mutated oncogenes in human cancer, findings from this disease are applicable to more genetically diverse and complex adult leukemias. The JMML Foundation (www.jmmlfoundation.org) was founded by parent advocates dedicated to finding a cure for this disease. They work to bring investigators together in a collaborative manner. This article summarizes key presentations from The Second International JMML Symposium, on 7–8 December 2007 in Atlanta, GA. A list of all participants is in Supplementary Table.
© 2008 Elsevier Ltd. All rights reserved.

Keywords: Juvenile myelomonocytic leukemia (JMML); RAS; PTPN11; NF1; Therapy; Diagnosis

1. Introduction

Juvenile myelomonocytic leukemia (JMML) is a lethal myeloproliferative disease (MPD) of young childhood characterized clinically by overproduction of myelomonocytic cells and by the *in vitro* phenotype of hematopoietic progenitor hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) [1,2]. In contrast to normal subjects, the morphological composition of progenitor colonies from JMML patients is predominantly macrophages and monocytes [3,4]. It is notable, however, that progenitor colonies from JMML patients contain monocytic cells along the full spectrum of differentiation, including blast forms, promonocytes, monocytes, and macrophages. This distinct characteristic indicates that JMML is not a disease induced by a complete block in differentiation as observed in acute leukemias, but instead results from shunting of hematopoietic differentiation toward the monocytic pathway, similar to increased granulocytic differentiation in the chronic phase of chronic myeloid leukemia (Fig. 1). In addition to monocytic cell overproduction, patients often present with anemia, thrombocytopenia, and 50% of patients also present with elevated fetal hemoglobin, hemoglobin F (Hgb F) [5]. JMML patients can progress to blast crisis, usually with French-American-British (FAB) M4 or M5 morphology, but more frequently succumb to disease due to tissue infiltration of myeloid cells. Standard cytotoxic chemotherapy in JMML is ineffective, producing few durable remissions [6]. Even following the rigorous therapy of allogeneic hematopoietic stem cell transplantation (HSCT), the probability of

* Corresponding author. Tel.: +1 415 514 0853; fax: +1 415 502 5127.
E-mail address: LohM@peds.ucsf.edu (M.L. Loh).

0145-2126/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2008.08.022

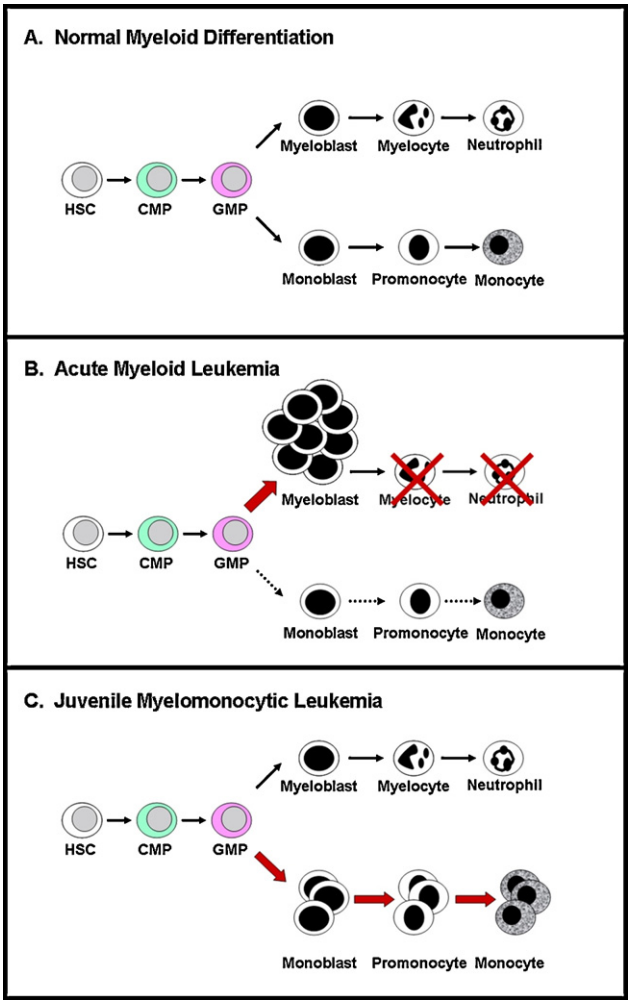

Fig. 1. Schematic diagram of (A) normal hematopoietic differentiation; (B) accumulation of undifferentiated myeloblasts representing acute myeloid leukemia; and (C) increased production of monocytic cells along the full spectrum of differentiation, including blast forms, promonocytes, monocytes, and macrophages, as observed in juvenile myelomonocytic leukemia. HSC = hematopoietic stem cell; CMP = common myeloid progenitor; GMP = granulocyte monocyte progenitor.

event-free survival at 5 years is only 50% [7] and the main cause of treatment failure continues to be leukemia relapse.

Activating mutations of the *NRAS* and *KRAS* genes and disruption of the tumor suppressor gene *NF1* have long been recognized as pathogenic in this disease [8–12]. More recently, somatic mutations in *PTPN11*, which encodes the protein tyrosine phosphatase, Shp2, have been found in 35% of JMML cases [13–15]. Intriguingly, it is now evident that *de novo* JMML, as well as several of the neuro-cardio-facio-cutaneous congenital disorders in which a JMML-like MPD has been associated [16–19], are caused by gene mutations (somatic in the former and germline in the latter) contributing to hyperactivation of the Ras—mitogen activated protein kinase pathway (MAPK, Fig. 2) [20–31].

2. Molecular genetics

2.1. Learning from patients

The clinical presentation of JMML has provided clinicians and researchers with crucial clues to the molecular aberrancies underlying this rare disease. First, young children with the congenital disorder, neurofibromatosis type 1 (NF1), have an increased incidence of malignant myeloid disorders, including JMML [16,17]. The characterization of neurofibromin (encoded by *NF1*) as a Ras GTPase activating protein (GAP) [32,33] that normally restricts Ras activation suggested that dysregulation of Ras activation contributes to the pathological process of JMML. Furthermore, the finding that loss-of-function *NF1* mutations are mostly non-overlapping with gain-of-function *KRAS* and *NRAS* mutations in JMML patients strongly implicates Ras hyperactivation in JMML [12]. Similarly, the predisposition of some children with Noonan syndrome (NS), 50% of whom bear germline *PTPN11* mutations [20,34], to develop a JMML-like MPD [18,19] prompted investigators to test children with non-syndromic, *de novo* JMML for somatic *PTPN11* mutations [13–15]. Taken together, 35% of patients have gain-of-function mutations in *PTPN11*, 35% gain-of-function mutations in *NRAS* or *KRAS*, and another 15% have clinical NF 1. Rarely, patients have been described to harbor dual mutations in these molecules and there is active investigation to determine if these mutations exist in separate hematopoietic progenitor cells.

2.2. Correlating genotype with phenotype

For potential improved prognostication of JMML, investigators are performing studies to examine if *NF1*, *RAS*, and *PTPN11* mutational status yields predictive clinical course information for patients. Increased age (>4 years), increased fetal hemoglobin (HgF, >15%), and reduced platelets (<33,000/μL) consistently correlate with a poor outcome (reduced event free survival and overall survival) of JMML patients following allogeneic HSCT; however, the potential correlation of gene mutational status with clinical outcome is unknown. Matsuda and colleagues made the observation that three children with JMML bearing mutations of *NRAS* or *KRAS* (2 with $NRAS^{G12S}$ and 1 with $KRAS^{G12S}$) demonstrated a mild clinical course with spontaneous remission without HSCT [35]. These findings suggested that certain *RAS* alleles may correlate with favorable clinical outcomes. However, in a follow-up study, Flotho and associates were unable to confirm that certain *RAS* alleles are associated with long-term survival in the absence of HSCT [36]. In another study directed at examining the hypothesis that mutational status may correlate with JMML clinical features and prognosis, Kojima and colleagues evaluated the clinical course and laboratory findings of 49 JMML patients, 32 of whom bore mutations in *NF1*, *KRAS*, *NRAS*, or *PTPN11*. In univariate analyses, *PTPN11* mutations were associated with

Please cite this article in press as: Chan RJ, et al. Juvenile myelomonocytic leukemia: A report from the 2nd International JMML Symposium. Leuk Res (2008), doi:10.1016/j.leukres.2008.08.022

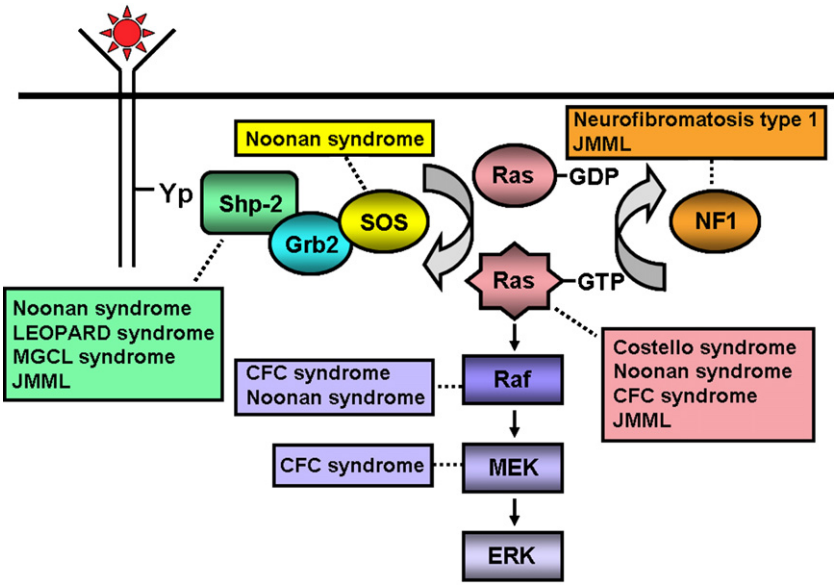

Fig. 2. Schematic diagram showing ligand-stimulated Ras activation, the Ras-Erk pathway, and the gene mutations found to date contributing to the neuro-cardio-facio-cutaneous congenital disorders and JMML. NL/MGCL: Noonan-like/multiple giant cell lesion; CFC: cardia-facio-cutaneous; JMML: juvenile myelomonocytic leukemia.

older age at diagnosis (>24 months), increased HgF (>10%), reduced overall survival, and, importantly, appeared to be an unfavorable prognostic factor predicting relapse following transplantation [37].

2.3. Designing accurate murine models

Definition of key mutated genes in JMML has permitted basic researchers to develop *in vivo* models of JMML. Hematopoietic progenitors from *Nf1*$^{-/-}$ and *Kras*^{G12D} mice demonstrate elevated Ras-GTP, hypersensitivity to GM-CSF, and produce MPD *in vivo* [38–40]. Conditional inactivation of *Nf1* in hematopoietic cells induces MPD with 100% penetrance, hypersensitivity to GM-CSF, and resistance to apoptosis [41]. Likewise, wild type mice reconstituted with fetal liver progenitors from *Nf1*$^{-/-}$ animals develop MPD similar to JMML which is attenuated in mice lacking GM-CSF [42]. Currently, Braun, Shannon, and others are utilizing *Kras*^{G12D} mice and mice bearing a conditional deletion allele of *Ptpn11* [43] to examine the question of whether Shp2 functions upstream, downstream, or in parallel to Ras in GM-CSF-stimulated signaling, in normal hematopoiesis, and in the pathogenesis of JMML. Their studies indicate that Shp2 functions at least partly downstream or parallel to Ras in hematopoietic stem cells, similar to that observed in *Drosophila* [44], although more recent results indicate that Ras is downstream of Shp2 in myeloid progenitors (B. Braun, personal communication). Furthermore, while Shp2 phosphatase activity is needed for leukemogenesis, it is dispensible for normal hematopoiesis [45–47]. An additional *in vivo* model of JMML was developed by Neel and colleagues using retroviral transduction of murine hematopoietic stem cells with gain-of-function *Ptpn11* mutants (Shp2^{D61Y} and Shp2^{E76K}) followed by transplantation into lethally irradiated recipients [46]. Similar to mice bearing loss-of-function *Nf1* or gain-of-function *Kras* mutations, mice expressing gain-of-function Shp2 also developed MPD *in vivo* [46]. To remove some of the uncertainty of variable disease onset following adoptive transfer of retrovirally transduced cells, however, Chan and Neel have developed a conditional knock-in allele of Shp2^{D61Y} (LSL-Shp2^{D61Y}) [48]. Consistent with results obtained from mice transplanted with mutant Shp2-expressing cells, the LSL-Shp2^{D61Y} mice develop MPD by 5–7 months following induced expression of Shp2^{D61Y}. However, unlike the transplanted mice, the LSL-Shp2^{D61Y} mice also develop anemia, thus recapitulating more closely the phenotype commonly observed in JMML patients [48]. Using these sophisticated genetic *in vivo* models, researchers continue to seek unique and novel molecules that can be rationally targeted for improved therapeutic tactics in JMML.

3. Advances in JMML diagnosis and surveillance

3.1. Redefining the criteria for diagnosis

In addition to the basic science studies that are being expanded, improved diagnostic, prognostic, and relapse detection techniques are currently under development. The current diagnostic criteria for JMML include absolute monocyte count > 1000/μL, <20% blasts in the bone marrow, and absence of the *BCR-ABL* fusion gene plus two of the four following criteria: (1) circulating myeloid precursors; (2) WBC > 10,000/μL; (3) increased fetal hemoglobin (HbF) for age; or (4) hematopoietic progenitor hypersensitivity to GM-CSF (Table 1). However, based on the identification of

Please cite this article in press as: Chan RJ, et al. Juvenile myelomonocytic leukemia: A report from the 2nd International JMML Symposium. Leuk Res (2008), doi:10.1016/j.leukres.2008.08.022

Table 1
Current JMML diagnostic criteria.

All of the following	At least 2 of the following
Absence of the t(9;22) BCR/ABL fusion gene	Circulating myeloid precursors
Absolute monocyte count > 1000/μL	White blood count > 10,000/μL
<20% blasts in the bone marrow	Increased fetal hemoglobin (HgF)
	GM-CSF hypersensitivity

multiple gene mutations in JMML, alternative diagnostic criteria were devised at the JMML Working Group Meeting in Geneva, Switzerland in September, 2006. These proposed criteria have incorporated *NF1*, *RAS*, and *PTPN11* mutational status or the identification of monosomy 7 into the diagnostic assessment (Table 2).

While hematopoietic progenitor hypersensitivity to GM-CSF has traditionally been used as a minor diagnostic criteria for JMML (Table 1), it is a time-consuming and cumbersome assay to perform using conventional cell culture methods. To address this problem, Kotecha, Nolan, and Loh have evaluated the correlation between GM-CSF-stimulated activation of STAT5, detected using phosphoflow cytometry for phospho-STAT5, with GM-CSF-stimulated hematopoietic progenitor colony growth in JMML patients and healthy controls [49]. The GM-CSF-stimulated phospho-STAT5 assay yielded results very similar to that of the traditional GM-CSF-stimulated hematopoietic progenitor assay, providing a novel diagnostic test that is 95% specific and 91% sensitive and that reduces the diagnosis time from weeks to days. Additionally, differences in GM-CSF-stimulated phospho-STAT5 levels might be utilized to identify targeted agents with potential efficacy as well as to follow response to treatment, relapse, or transformation to acute myeloid leukemia, which may be particularly relevant in patients receiving experimental or traditional chemotherapeutic agents.

3.2. Redefining the criteria for response

In the past, response to chemotherapy for JMML has been defined using a complicated assessment of white blood cell count, platelet count, and organomegaly (Table 3a). However, in 2006, the International JMML Working Group meeting in Geneva discussed this topic. First, there was general agreement on the importance of developing new agents that may be biologically active in the treatment of JMML. The goals of testing these agents would be to determine the biologic or clinical activity as well as to define the toxicity profile. Therefore, the group advocated simplification of the criteria and removal of the marginal response. Also, in order to evaluate new agents in an upfront window it was important for patients to have measurable disease. Therefore, the group agreed that in order to be enrolled on future clinical trials to evaluate new agents, patients would need to exhibit a WBC count > 20,000/μL and splenomegaly greater than 2 cm below the costal margin. Clinical findings from the European Working Group on MDS in Childhood (EWOG-MDS) and the North American JMML Project (NAJP) indicated that spleen size and WBC count were the most significant response parameters to evaluate during pre-transplant chemotherapy. Dr. Cooper reviewed these definitions during the 2007 Symposium, as summarized in Table 3b.

Table 3a
Current JMML response criteria.

	WBC	Organomegaly
Complete response	Normal	None
Partial response	>50% reduction	>50% reduction in size
Marginal response	>25% ≤ 50% reduction	>25% ≤ 50% reduction in size
	>50% reduction	No change
	No change	>50% reduction in size
Stable disease	≤25% reduction	≤25% reduction in size
Progressive disease	>25% increase	>25% increase in size

Following allogeneic stem cell transplantation, response to therapy and detection of minimal residual disease can be assessed by following donor chimerism; however, no good method exists for tracking molecular responses to therapy in children who receive traditional chemotherapy or experimental therapies prior to transplantation. To address these limitations, Archambeault and Loh have developed a novel method of detecting minimal residual disease to

Table 3b
Proposed JMML response criteria.

	Complete clinical response	Partial clinical response
White blood count	<20 K	<50% of initial WBC but total still greater than 20 K
Splenomegaly	Normalization of spleen size	25% decrease from initial size

Table 2
Proposed JMML diagnostic criteria.

Category 1	Category 2	Category 3
All of the following	At least 1 of the following	At least 2 of the following
Splenomegaly	Somatic mutation in RAS or PTPN11	Circulating myeloid precursors
Absolute monocyte count > 1000/μL	Clinical diagnosis of NF1 or NF1 gene mutation	WBC > 10,000/μL
Blasts in PB/BM < 20%	Monosomy 7	Increased fetal hemoglobin (HgF) for age
Absence of the t(9;22) BCR/ABL fusion gene		Clonal cytogenetic abnormality excluding monosomy 7
Age less than 13 years		

Please cite this article in press as: Chan RJ, et al. Juvenile myelomonocytic leukemia: A report from the 2nd International JMML Symposium. Leuk Res (2008), doi:10.1016/j.leukres.2008.08.022

follow response to therapy by merging TaqMan chemistry with a mismatched amplification mutation assay (MAMA), referred to as TaqMAMA [50]. This method is based on the preferential amplification of the mutant allele compared to the wild type allele. These assays were developed to follow the most common *PTPN11* (226G > A, 214G > A, 227A > G, and 1508G > C), *KRAS* (38G > A and 35G > A), and *NRAS* (38G > A and 37G > C) mutations found in JMML, were adequately sensitive to detect the mutant alleles, and were very specific for seven of the eight mutant alleles examined. Importantly, detection of the mutant allele from peripheral blood cells correlated very well with detection from bone marrow cells, suggesting that tracking disease burden by peripheral blood draws rather than by multiple bone marrow aspirations is feasible using this method. Furthermore, the TaqMAMA method detected disease relapse either earlier or simultaneous to disease relapse detection determined by the conventional autologous cell chimerism method. Collectively, these findings suggest that the TaqMAMA methodology provides a potentially powerful tool for following response to upfront novel therapies and for detecting minimal residual disease following transplantation in children with JMML.

4. Current therapy

Unfortunately, JMML has proved to be resistant to essentially all chemotherapy regimens examined including single agents as well as combination chemotherapy typically utilized in acute leukemias [6]. The only curative therapy is allogeneic stem cell transplantation which is able to cure approximately 50% of patients; however, the leading cause of death following transplantation continues to be leukemia relapse [7,51,52]. Some patients who suffer from relapse are cured from a secondary allogeneic transplant [53]. Two main retrospective studies have been published evaluating the efficacy of allogeneic transplantation in JMML. First, the Japanese Society of Pediatric Hematology (JSPH) published a series of 27 patients diagnosed with JMML based on criteria outlined by the International JMML Working Group. Although the preparative regimens and graft sources varied widely, this retrospective evaluation demonstrated an overall survival of 57.9% and an event free survival of 54.2% at 4 years after allogeneic transplantation [54]. A second trial with 100 children conducted by the European Working Group on Childhood MDS (EWOG-MDS) and the European Blood and Marrow Transplantation (EMBT) Group used a preparative regimen consisting of busulfan (16–20 mg/kg over 4 consecutive days), cyclophosphamide (60 mg/kg/d for 2 consecutive days), and melphalan (140 mg/m^2, single dose). Graft sources varied from bone marrow, peripheral blood, or cord blood. Similar to the JSPH study, children undergoing allogeneic transplantation achieved an overall survival of 64% and event free survival of 52% [7]. Other analyses from this study revealed no significant difference in event free survival between children receiving grafts from siblings v. unrelated donors or between splenectomized v. non-splenectomized patients at the time of transplant. Event free survival was significantly increased in males and in children < 2 years of age at the time of JMML diagnosis, consistent with the finding of increased survival in children <1 year at the time of diagnosis demonstrated in the JSPH study. However, findings from the EWOG/EBML trial revealed no difference in event free survival between patients with normal vs. karyotypic abnormalities while the JSPH trial demonstrated a significant reduction in overall survival in patients with karyotypic abnormalities [7,54]. Two subsequent studies have been performed by the JPHS. Patients on trial MDS 99 received busulfan (140 mg/m^2 × 4 doses), cyclophosphamide (60 mg/kg × 2 doses), and high dose cytarabine (3 gm/m^2 × 4 doses). In this study, the investigators observed a 50% disease free survival. Upon subgroup analysis, disease free survival was better in individuals with a normal karyotype as well as patients experiencing either acute or chronic graft vs. host disease. In a second trial, a conditioning regimen including busulfan (140 mg/m^2 × 4 doses), fludarabine (30 mg/m^2 × 4 doses), and LPAM (90 mg/m^2 × 2 doses) produced a disease free survival of 75% at 6 years following transplant. In short, although a rigorous intervention, allogeneic stem cell transplantation provides a real chance of cure for children suffering from JMML. However, scientists and clinicians continue to study JMML aggressively in basic research labs in a concerted effort to define novel therapeutic targets and to develop effective, less toxic, therapeutic interventions.

5. Experimental therapies

Although allogeneic transplant does provide a curative modality for JMML, there are significant morbidities associated with this aggressive intervention; thus, improved and less toxic therapies are currently under evaluation. Given the importance of Ras hyperactivation in the pathophysiology of JMML, agents designed to target Ras activity are being evaluated. Ras is first activated at the cell membrane via the addition of a farnesyl group to the newly translated protein. Farnesyl transferase inhibitors (FTIs) prevent Ras translocation to the plasma membrane by inhibiting Ras isoprenylation, thus leading to downregulation of Ras-activated cellular pathways [55]. It was thus hypothesized that inhibiting farnesyl transferase activity may inhibit Ras activation, despite the knowledge that an alternative pathway to Ras activation occurs by geranylgeranylation. *In vitro* studies demonstrated significant growth inhibition of JMML patient samples by the peptidomimetic FTIs, L-739,749 and L-744,832 [56]. Based on these *in vitro* findings, Castleberry, Emanuel, and co-workers recently completed a phase II window clinical trial (AML0122) designed to evaluate the dosing and potential effectiveness of R115777, a non-peptidomimetic FTI, in newly diagnosed, previously untreated JMML patients.

Please cite this article in press as: Chan RJ, et al. Juvenile myelomonocytic leukemia: A report from the 2nd International JMML Symposium. Leuk Res (2008), doi:10.1016/j.leukres.2008.08.022

Patients received two courses of R115777 (200 mg/m^2 or 300 mg/m^2 by mouth each day for 21 days) followed by two courses of cis-retinoic acid, cytarabine, and fludarabine, splenectomy, and allogeneic transplantation. Following administration of R115777, response to therapy was assessed by white blood cell counts and organomegaly based on current guidelines for JMML response criteria (Table 3a). A total of 11 patients received 200 mg/m^2 for two courses. The therapy was well tolerated and six patients achieved a partial response, one patient achieved a marginal response, two maintained stable disease, and one demonstrated progressive disease. A total of 36 patients received 300 mg/m^2 for two courses. Among this group, two patients achieved a complete remission, 14 achieved a partial response, seven a marginal response, two maintained stable disease, and three demonstrated progressive disease. Taken together, 81% of patients demonstrated a clinical response to R115777 (including all with complete, partial, and marginal responses at both doses administered). The main toxicity induced by R115777 was bone marrow suppression. Response or lack of response to the drug was not correlated with patient mutational status (*NF1*, *KRAS*, *NRAS*, *PTPN11*, or monosomy 7), nor was inhibition of farnesyl transferase activity as measured by the surrogate prenylation of the heat shock molecule, HDJ2 [57]. Unfortunately, preliminary findings presented at the 2nd International JMML Symposium indicated that event-free survival did not appear to be favorably impacted by the use of R115777 compared to the outcomes of other trials; however, this trial is ongoing and the data has not matured. Thus, the event-free survival and overall survival of patients receiving R115777 prior to allogeneic transplantation continues to be evaluated.

In addition to directly targeting Ras, agents that target Ras effectors within the MAPK cascade, such as RAF-1 and MEK, are also being evaluated in myeloid leukemias. Previous *in vitro* and *in vivo* studies demonstrated that treatment of JMML cells with a DNA enzyme designed to target RAF1 mRNA, thus reducing RAF1 expression, resulted in reduced hypersensitivity of JMML cells to GM-CSF and reduced mortality of NOD-SCID mice transplanted with JMML cells, respectively [58]. Based on these findings, a phase II window clinical trial is under development to evaluate response rate and acute toxicity of JMML patients to sorafenib (BAY 43-9006), a RAF kinase inhibitor.

Additionally, Lauchle and colleagues have evaluated the effect of CI-1040, a selective MEK inhibitor, on *Nf1*$^{-/-}$ MPD [41] and *Nf1*$^{-/-}$AML (induced by infection of *Mx1-Cre, Nf1*$^{flox/flox}$ mice [41] with a retroviral insertional mutagen, MOL4070LTR) [59]. While *Nf1* mutant MPD cells were not more sensitive to CI-1040 inhibition compared to WT cells, the *Nf1* mutant AML cells demonstrated significant sensitivity to CI-1040 inhibition. Similarly, *in vivo* administration of CI-1040 to *Nf1* mutant mice with MPD did not improve survival; however, CI-1040 treatment of mice transplanted with *Nf1* mutant AML cells demonstrated significantly increased survival compared to vehicle-treated mice. Interestingly, AML clones that demonstrated resistance to CI-1040 were found to have novel retroviral integration sites, suggesting that cooperating mutations can modulate response to CI-1040 [59].

6. Conclusions

Tremendous clinical and scientific advances have been made in JMML over the last 20 years; however, regrettably, up to 50% of JMML patients continue to succumb to this lethal malignancy. The goals continue to be improved understanding of the disease at a molecular level in an effort to identify rational therapeutic targets and efficient and successful translation of our mechanistic understanding into superior, less toxic therapeutic modalities for JMML. Furthermore, ideally, JMML will serve as a model disease leading to improved therapies for leukemias and solid tumors, in general, bearing Ras hyperactivation as a central pathogenic mechanism.

Conflict of interest

None.

Acknowledgements

This work was supported by the U.S. National Institutes of Health (5RO1HL082981-03, RJC; 3R13CA132568-02, The Office of Rare Diseases, and 5K22CA113577-03, MLL) and the Leukemia and Lymphoma Society (LLS 2157-08, MLL). MLL is a Clinical Scholar of the Leukemia Lymphoma Society. The authors gratefully acknowledge the administrative assistance of Linda S. Henson.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.leukres.2008.08.022.

References

[1] Emanuel PD, Bates LJ, Castleberry RP, Gualtieri RJ, Zuckerman KS. Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood 1991;77:925–9.

[2] Freedman MH, Cohen A, Grunberger T, Bunin N, Luddy RE, Saunders EF, et al. Central role of tumour necrosis factor, GM-CSF, and interleukin 1 in the pathogenesis of juvenile chronic myelogenous leukaemia. Br J Haematol 1992;80:40–8.

[3] Estrov Z, Zimmerman B, Grunberger T, Chao J, Teshima IE, Chan HS, et al. Characterization of malignant peripheral blood cells of juvenile chronic myelogenous leukemia. Cancer Res 1986;46:6456–61.

[4] Altman AJ, Palmer CG, Baehner RL. Juvenile chronic granulocytic" leukemia: a panmyelopathy with prominent monocytic

involvement and circulating monocyte colony-forming cells. Blood 1974;43:341–50.

[5] Emanuel PD. Juvenile myelomonocytic leukemia. Curr Hematol Rep 2004;3:203–9.

[6] Bergstraesser E, Hasle H, Rogge T, Fischer A, Zimmermann M, Noellke P, et al. Non-hematopoietic stem cell transplantation treatment of juvenile myelomonocytic leukemia: a retrospective analysis and definition of response criteria. Pediatr Blood Cancer 2007;49:629–33.

[7] Locatelli F, Nollke P, Zecca M, Korthof E, Lanino E, Peters C, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood 2005;105:410–9.

[8] Brodeur GM. The NF1 gene in myelopoiesis and childhood myelodysplastic syndromes. N Engl J Med 1994;330:637–9.

[9] Side L, Taylor B, Cayouette M, Conner E, Thompson P, Luce M, et al. Homozygous inactivation of the NF1 gene in bone marrow cells from children with neurofibromatosis type 1 and malignant myeloid disorders. N Engl J Med 1997;336:1713–20.

[10] Shannon KM, O'Connell P, Martin GA, Paderanga D, Olson K, Dinndorf P, et al. Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. N Engl J Med 1994;330:597–601.

[11] Sheng XM, Kawamura M, Ohnishi H, Ida K, Hanada R, Kojima S, et al. Mutations of the RAS genes in childhood acute myeloid leukemia, myelodysplastic syndrome and juvenile chronic myelocytic leukemia. Leukemia Res 1997;21:697–701.

[12] Kalra R, Paderanga DC, Olson K, Shannon KM. Genetic analysis is consistent with the hypothesis that NF1 limits myeloid cell growth through p21ras. Blood 1994;84:3435–9.

[13] Tartaglia M, Niemeyer CM, Fragale A, Song X, Buechner J, Jung A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet 2003;34:148–50.

[14] Loh ML, Vattikuti S, Schubbert S, Reynolds MG, Carlson E, Lieuw KH, et al. Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood 2004;103:2325–31.

[15] Kratz CP, Niemeyer CM, Castleberry RP, Cetin M, Bergstrasser E, Emanuel PD, et al. The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease. Blood 2005;106:2183–5.

[16] Bader JL, Miller RW. Neurofibromatosis and childhood leukemia. J Pediatr 1978;92:925–9.

[17] Shannon KM, Watterson J, Johnson P, O'Connell P, Lange B, Shah N, et al. Monosomy 7 myeloproliferative disease in children with neurofibromatosis, type 1: epidemiology and molecular analysis. Blood 1992;79:1311–8.

[18] Bader-Meunier B, Tchernia G, Mielot F, Fontaine JL, Thomas C, Lyonnet S, et al. Occurrence of myeloproliferative disorder in patients with Noonan syndrome. J Pediatr 1997;130:885–9.

[19] Choong K, Freedman MH, Chitayat D, Kelly EN, Taylor G, Zipursky A. Juvenile myelomonocytic leukemia and Noonan syndrome. J Pediatr Hematol Oncol 1999;21:523–7.

[20] Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001;29:465–8.

[21] Schubbert S, Zenker M, Rowe SL, Boll S, Klein C, Bollag G, et al. Germline KRAS mutations cause Noonan syndrome. Nat Genet 2006;38:331–6.

[22] Carta C, Pantaleoni F, Bocchinfuso G, Stella L, Vasta I, Sarkozy A, et al. Germline missense mutations affecting KRAS Isoform B are associated with a severe Noonan syndrome phenotype. Am J Hum Genet 2006;79:129–35.

[23] Roberts AE, Araki T, Swanson KD, Montgomery KT, Schiripo TA, Joshi VA, et al. Germline gain-of-function mutations in SOS1 cause Noonan syndrome. Nat Genet 2007;39:70–4.

[24] Tartaglia M, Pennacchio LA, Zhao C, Yadav KK, Fodale V, Sarkozy A, et al. Gain-of-function SOS1 mutations cause a distinctive form of Noonan syndrome. Nat Genet 2007;39:75–9.

[25] Pandit B, Sarkozy A, Pennacchio LA, Carta C, Oishi K, Martinelli S, et al. Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. Nat Genet 2007;39:1007–12.

[26] Razzaque MA, Nishizawa T, Komoike Y, Yagi H, Furutani M, Amo R, et al. Germline gain-of-function mutations in RAF1 cause Noonan syndrome. Nat Genet 2007;39:1013–7.

[27] Aoki Y, Niihori T, Kawame H, Kurosawa K, Ohashi H, Tanaka Y, et al. Germline mutations in HRAS proto-oncogene cause Costello syndrome. Nat Genet 2005;37:1038–40.

[28] Niihori T, Aoki Y, Narumi Y, Neri G, Cave H, Verloes A, et al. Germline KRAS and BRAF mutations in cardio-facio-cutaneous syndrome. Nat Genet 2006;38:294–6.

[29] Rodriguez-Viciana P, Tetsu O, Tidyman WE, Estep AL, Conger BA, Cruz MS, et al. Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. Science 2006;311:1287–90.

[30] Sarkozy A, Conti E, Seripa D, Digilio MC, Grifone N, Tandoi C, et al. Correlation between PTPN11 gene mutations and congenital heart defects in Noonan and LEOPARD syndromes. J Med Genet 2003;40:704–8.

[31] Keren B, Hadchouel A, Saba S, Sznajer Y, Bonneau D, Leheup B, et al. PTPN11 mutations in patients with LEOPARD syndrome: a French multicentric experience. J Med Genet 2004;41:e117.

[32] Basu TN, Gutmann DH, Fletcher JA, Glover TW, Collins FS, Downward J. Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. Nature 1992;356:713–5.

[33] DeClue JE, Papageorge AG, Fletcher JA, Diehl SR, Ratner N, Vass WC, et al. Abnormal regulation of mammalian p21ras contributes to malignant tumor growth in von Recklinghausen (type 1) neurofibromatosis. Cell 1992;69:265–73.

[34] Tartaglia M, Kalidas K, Shaw A, Song X, Musat DL, Van Der Burgt I, et al. PTPN11 mutations in Noonan Syndrome: molecular spectrum, genotype–phenotype correlation, and phenotypic heterogeneity. Am J Hum Genet 2002;70:1555–63.

[35] Matsuda K, Shimada A, Yoshida N, Ogawa A, Watanabe A, Yajima S, et al. Spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia with specific RAS mutations. Blood 2007;109:5477–80.

[36] Flotho C, Kratz CP, Bergstrasser E, Hasle H, Stary J, Trebo M, et al. Genotype–phenotype correlation in cases of juvenile myelomonocytic leukemia with clonal RAS mutations. Blood 2008;111:966–7 [author reply 967–968].

[37] Yoshida N, Yagasaki H, Yoshimi A, Takahashi Y, Xu Y, Tanaka M, et al. Correlation of clinical features with the mutational status of GM-CSF signaling pathway-related genes in children with juvenile myelomonocytic leukemia. Blood 2007;110:457a.

[38] Braun BS, Tuveson DA, Kong N, Le DT, Kogan SC, Rozmus J, et al. Shannon KM. Somatic activation of oncogenic Kras in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. Proc Natl Acad Sci USA 2004;101:597–602.

[39] Largaespada DA, Brannan CI, Jenkins NA, Copeland NG. Nf1 deficiency causes Ras-mediated granulocyte/macrophage colony stimulating factor hypersensitivity and chronic myeloid leukaemia. Nat Genet 1996;12:137–43.

[40] Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, Thompson P, et al. Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. Nat Genet 1996;12:144–8.

[41] Le DT, Kong N, Zhu Y, Lauchle JO, Aiyigari A, Braun BS, et al. Somatic inactivation of Nf1 in hematopoietic cells results in a progressive myeloproliferative disorder. Blood 2004;103:4243–50.

[42] Birnbaum RA, O'Marcaigh A, Wardak Z, Zhang YY, Dranoff G, Jacks T, et al. Nf1 and Gmcsf interact in myeloid leukemogenesis. Mol Cell 2000;5:189–95.

Please cite this article in press as: Chan RJ, et al. Juvenile myelomonocytic leukemia: A report from the 2nd International JMML Symposium. Leuk Res (2008), doi:10.1016/j.leukres.2008.08.022

[43] Fornaro M, Burch PM, Yang W, Zhang L, Hamilton CE, Kim JH, et al. SHP-2 activates signaling of the nuclear factor of activated T cells to promote skeletal muscle growth. J Cell Biol 2006;175:87–97.
[44] Allard JD, Chang HC, Herbst R, McNeill H, Simon MA. The SH2-containing tyrosine phosphatase corkscrew is required during signaling by sevenless, Ras1 and Raf. Development 1996;122:1137–46.
[45] Schubbert S, Lieuw K, Rowe SL, Lee CM, Li X, Loh ML, et al. Functional analysis of leukemia-associated PTPN11 mutations in primary hematopoietic cells. Blood 2005;106:311–7.
[46] Mohi MG, Williams IR, Dearolf CR, Chan G, Kutok JL, Cohen S, et al. Prognostic, therapeutic, and mechanistic implications of a mouse model of leukemia evoked by Shp2 (PTPN11) mutations. Cancer Cell 2005;7:179–91.
[47] Braun BS, Cheung LS, Chan GG, Yang WA, Archard JA, Neel BG, et al. A noncatalytic Ras-independent function of SHP-2 is essential in hematopoietic progenitors. Blood 2007;110:34a.
[48] Chan G, Kalaitzidis D, Mohi MG, Yang W, Kutok JL, Neel BG. Inducible expression of leukemia-associated Shp2 (Ptpn11) affects multiple stages of hematopoiesis and causes a fatal myeloproliferative disorder (MPD) in mice. Blood 2007;110:457a.
[49] Kotecha N, Flores NJ, Irish JM, Simonds E, Sakai DS, Archambeault S, Diaz-Flores E, Coram M, Shannon KM, Nolan GP, Loh ML. Single cell profiling identifies aberrant stat5 activation in myeloid malignancies with specific clinical and biologic correlates. Cancer Cell 2008, doi:10.1016/j.ccr.2008.08.014.
[50] Archambeault S, Flores NJ, Yoshimi A, Kratz CP, Reising M, Fischer A, et al. Development of an allele-specific minimal residual disease assay for patients with juvenile myelomonocytic leukemia. Blood 2008;111:1124–7.
[51] Koike K, Matsuda K. Recent advances in the pathogenesis and management of juvenile myelomonocytic leukaemia. Br J Haematol 2008.
[52] Niemeyer CM, Kratz CP. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia: molecular classification and treatment options. Br J Haematol 2008;140:610–24.
[53] Yoshimi A, Mohamed M, Bierings M, Urban C, Korthof E, Zecca M, et al. Second allogeneic hematopoietic stem cell transplantation (HSCT) results in outcome similar to that of first HSCT for patients with juvenile myelomonocytic leukemia. Leukemia 2007;21:556–60.
[54] Manabe A, Okamura J, Yumura-Yagi K, Akiyama Y, Sako M, Uchiyama H, et al. Allogeneic hematopoietic stem cell transplantation for 27 children with juvenile myelomonocytic leukemia diagnosed based on the criteria of the International JMML Working Group. Leukemia 2002;16:645–9.
[55] Rowinsky EK, Windle JJ, Von Hoff DD. Ras protein farnesyltransferase: a strategic target for anticancer therapeutic development. J Clin Oncol 1999;17:3631–52.
[56] Emanuel PD, Snyder RC, Wiley T, Gopurala B, Castleberry RP. Inhibition of juvenile myelomonocytic leukemia cell growth in vitro by farnesyltransferase inhibitors. Blood 2000;95:639–45.
[57] Castleberry RP, Loh ML, Jayaprakash N, Peterson A, Casey V, Chang M, et al. Phase II Window study of the farnesyltransferase inhibitor R115777 (Zarnestra®) in untreated juvenile myelomonocytic leukemia (JMML): a children's oncology group study. Blood 2005; 106:727a.
[58] Iversen PO, Emanuel PD, Sioud M. Targeting Raf-1 gene expression by a DNA enzyme inhibits juvenile myelomonocytic leukemia cell growth. Blood 2002;99:4147–53.
[59] Lauchle JO, Le DT, Kim D, Akagi K, Gorman MF, Tran M, et al. Mutations That cooperate with Nf1 inactivation in leukemogenesis influence therapeutic response to MEK inhibition. Blood 2007; 110:183a.

Please cite this article in press as: Chan RJ, et al. Juvenile myelomonocytic leukemia: A report from the 2nd International JMML Symposium. Leuk Res (2008), doi:10.1016/j.leukres.2008.08.022