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To the editor:

Genotype-phenotype correlation in cases of juvenile myelomonocytic leukemia with clonal RAS mutations

In a recent issue of *Blood*, Matsuda et al reported 11 children with juvenile myelomonocytic leukemia (JMML) and clonal *NRAS* or *KRAS* mutations.¹ Three patients showed improvement of various clinical and laboratory features over a 2- to 4-year period without chemotherapy or hematopoietic stem cell transplantation (HSCT). The authors correlate the comparatively mild course with a specific mutation predicting a glycine-to-serine substitution at position 12 in the *NRAS* or *KRAS* protein (G12S), and suggest that “no chemotherapy may be a recommended management” for JMML patients with *NRAS/KRAS*^{G12S}.

We have some reason to believe that these conclusions are premature. Available data do not support that *RAS*^{G12S} has weaker oncogenic activity than substitutions with valine, arginine, or aspartic acid. Interestingly, the authors show that myeloid progenitor cells from their patients with G12S respond to granulocyte macrophage–colony stimulating factor (GM-CSF) in a comparable manner as other mutants (Figure 1B in Matsuda et al¹). Others reported that *HRAS*^{G12S} led to focus

induction in NIH3T3 cultures with a similar potency as substitutions with arginine or aspartic acid.²

We argue that the clinical course observed in the 3 children is not uncommon in JMML cases with similar hematologic features and age. The European Working Group of Myelodysplastic Syndromes in Childhood (EWOG-MDS) has previously shown that platelet count $33 \times 10^9/L$ or more and hemoglobin F less than 15% at diagnosis identifies a prognostically favorable subgroup in JMML with a 40% to 70% probability of survival at 2 to 4 years without HSCT.³ The relatively favorable course in the Matsuda patients is also because all 3 were less than 1 year old at diagnosis.⁴ It is known that infants with JMML without severe thrombocytopenia at diagnosis may experience transient improvement even without treatment.⁵

To examine whether *RAS*^{G12S} is overrepresented in JMML patients with less aggressive disease (defined as survival ≥ 3 years without HSCT), we reviewed the clinical and molecular data of 216 cases collected in the EWOG-MDS registry, excluding patients

Table 1. Clinical characteristics of patients with JMML and clonal RAS mutations surviving long-term without HSCT

Case number	Mutation	Age, y	Sex	Liver, cm	Spleen, cm	WBC, 10 ⁹ /L	Mono, 10 ⁹ /L	Hb, g/L	Plt, 10 ⁹ /L	HbF, %	Outcome (time from diagnosis)
SC047	<i>NRAS</i> c.G35T (codon 12 Gly > Val)	0.1	M	0	7	23.7	5.2	102	54	10	alive without HSCT (4.5 y)
I 013	<i>NRAS</i> c.G38A (codon 13 Gly > Asp)	1.7	M	2	2	22.2	2.4	103	40	1.3	alive without HSCT (5.5 y)
D 175	<i>NRAS</i> c.G35C (codon 12 Gly > Ala)	0.7	F	4	7	18.6	1.3	96	75	3.1	alive without HSCT (8.8 y)
D 028	<i>NRAS</i> c.G35A (codon 12 Gly > Asp)	0.4	M	3	5	57.4	8.0	112	192	8.3	alive without HSCT (21.5 y)
CZ011	<i>NRAS</i> c.G35A (codon 12 Gly > Asp)	0.5	M	5	2	57.6	11.5	100	162	6.0	dead without HSCT (3.3 y)
D 278	<i>KRAS</i> c.C181A (codon 61 Gln > Lys)	0.5	M	2	4	62.5	13.1	87	68	n.d.	alive with HSCT (7.0 y), HSCT given at 3.3 y from diagnosis
Matsuda 9	<i>NRAS</i> c.G34A (codon 12 Gly > Ser)	0.8	M	4	5	29.4	4.9	105	113	0.5	alive without HSCT (4.2 y)
Matsuda 10	<i>NRAS</i> c.G34A (codon 12 Gly > Ser)	0.8	M	5	10	31.8	6.4	54	100	1.7	alive without HSCT (3.5 y)
Matsuda 11	<i>KRAS</i> c.G34A (codon 12 Gly > Ser)	0.3	F	4	1	21.2	1.7	110	52	8.8	alive without HSCT (2.5 y)

Liver and spleen sizes are given in cm below the costal margin. Leukemic clones in all patients had a normal karyotype. WBC indicates white blood cell count; Mono, monocytes; Plt, platelet count; Hb F, fetal hemoglobin; and nd, not done.

with Noonan syndrome. Fifty patients were not given HSCT within the first 3 years from diagnosis. Of these, 17 survived this period. Six of 17 carried a clonal RAS mutation (Table 1). It is evident that there is no appreciable difference in clinical features between the 3 patients reported by Matsuda (included in Table 1 for convenience) and our 6 patients. However, all 6 patients in our group had substitutions other than G12S. Overall, only 1 of 216 children had a G12S mutation. This patient received early HSCT, so the evaluation of his spontaneous course is precluded. However, he presented with 60% hemoglobin F, which is usually associated with an aggressive form of the disorder.

We agree that sporadic patients with JMML enjoy long-term survival without intervention. However, in the absence of reliable markers that prospectively identify those rare cases, and in view of the clear superiority of HSCT over other treatment modalities (probability of 10-year survival 0.39 vs 0.06 without HSCT),^{3,6} the EWOG-MDS recommends prompt HSCT for every patient with JMML, except children with Noonan syndrome.⁷ Importantly, the relapse incidence dramatically increases with age (from 18% in children \leq 2 years old to 73% in patients \geq 4 years old).⁶ Although we are not convinced that NRAS/KRAS^{G12S} is a useful marker for treatment decisions, we thank Matsuda and colleagues for stimulating this discussion and illustrating the need to continue the search for accurate prognostic markers in JMML.

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Response:

Evaluation of relationship between the genetic abnormalities and disease phenotype is required in juvenile myelomonocytic leukemia

In their letter, Flotho et al comment on our recently published paper on spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia (JMML) with NRAS or KRAS2 glycine to serine (Gly12Ser) mutation.¹

Our 3 patients with Gly12Ser mutation in the RAS gene continue hematologic and clinical improvement despite no chemotherapy 3 to 5 years after diagnosis. Based on no significant difference in proliferative response of granulocyte-macrophage (GM) progenitors to low doses of GM colony-stimulating factor between the patients with Gly12Ser mutation and the other RAS mutants, Flotho et al claim that our dose-response study does not support weaker oncogenic activity of RAS Gly12Ser compared with the activity of the other substitutions. However, more importantly there is marked reduction in numbers of circulating GM progenitors during the clinical course in patients with the RAS Gly12Ser substitution. Taken together with substantial decreases in leukocyte counts and spleen size during a 3- to 5-year follow up, their leukemic burden appears to become smaller.

The European Working Group of Myelodysplastic Syndromes in Childhood (EWOG-MDS) previously showed that platelet count $33 \times 10^9/L$ or more, hemoglobin F less than 15%, and age younger than 2 years at diagnosis identify a prognosti-

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cally favorable subgroup in 72 JMML patients receiving no allogeneic hematopoietic stem cell transplantation (HSCT).² However, their genetic background was unclear. Some of the subjects enrolled in the study might have inactivation of the *NFI* gene or mutations in the *NRAS*, *KRAS2*, or *PTPN11* genes. Flotho et al presented that 4 patients with *NRAS* mutation (codon 12 Gly > Val, codon 12 Gly > Ala, codon 12 Gly > Asp, codon 13 Gly > Asp) survive 4.5 to 21.5 years without HSCT. Coupled with our 3 patients with the RAS Gly12Ser mutation, JMML patients carrying RAS mutations may be heterogeneous with respect to the hematologic progression.

Flotho et al recommend prompt HSCT for every patient with JMML, except children with Noonan syndrome, based on the superiority of HSCT over other treatment modalities. In recent reports,^{3,4} approximately 50% of JMML patients who received such transplants were alive and leukemia-free after 4 to 5 years. The major cause of treatment failure is high incidence of leukemia recurrence after HSCT. In the results of the EWOG-MDS/European Blood and Marrow Transplantation (EBMT) Group trial,⁴ age younger than 2 years at diagnosis was associated with a better probability of event-free survival after HSCT. Nevertheless, it remains to be determined whether interval between diagnosis and HSCT influences the outcome. Therefore, we