Mutation analysis of the *BRAF* oncogene in juvenile myelomonocytic leukemia

Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative/myelodysplastic disorder associated with mutations in the *Ras-Raf-MEK-ERK*-signaling pathway. B-Raf plays a central role in this pathway. In 65 screened JMML patients we identified no *BRAF* mutations and we conclude that this gene is unlikely to play a role in the pathogenesis of JMML.

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JMML is a rare myelodysplastic/myeloproliferative disease of early childhood, accounting for less than 3% of all hematologic malignancies in children. JMML is characte-rized by prominent hepatosplenomegaly, absolute monocytosis, presence of myeloid precursors in peripheral blood, low platelet count, frequent skin involvement and *in vitro* granulocyte-macrophage colony stimulating factor (GM-CSF) hypersensitivity.[1] GM-CSF hypersensitivity in JMML results from continuous activation of the GM-CSF-receptor-Ras-Raf-MEK-ERK signal transduction pathway due to activating mutations of *RAS*[2] or *PTPN11*.[3] These mutations occur in 25% and 35% of JMML cases respectively. Furthemore, approximately 11% of cases of JMML cases occur in individuals with neurofibromatosis type 1 (NF1) and JMML cells show bi-allelic inactivation of the *NF1* tumor suppressor gene encoding for neurofibromin, a GTPase activating protein for Ras. Loss of *NF1* leads to activation of RAS signaling.[4] The remaining cases presumably carry somatic mutations of other yet undefined genes of the Ras signaling pathway.

The Ras effector B-Raf plays a central role in transmitting growth signals from Ras to downstream molecules including MEK and ERK. Somatic mutations of *BRAF* occur at high frequency in numerous human cancers.[5] The *BRAF*^{V600E} mutation results in increased kinase activity and accounts for more than 90% of the mutations.[5] Germline mutations of *BRAF* cause cardio-facio-cutaneous syndrome (CFC), a dominant disorder characterized by short stature, cardiac defects, distinct facial features, developmental delay, and ectodermal abnormalities.[6] CFC shares many features with other dominant syndromes including Costello syndrome (CS) and Noonan syndrome (NS).[7] CS and NS are caused by germline mutations in genes of the Ras pathway, including *HRAS* (mutated in CS) and *PTPN11*, *KRAS*, or *SOS1* (mutated in NS).[7] Interestingly, infants with NS are predisposed to develop a myeloproliferative disease resembling JMML. So far, a *BRAF* mutational screen of JMML specimens has not been conducted.

In this study, we screened 65 JMML specimens for somatic mutations of *BRAF*. The diagnosis of JMML was based on criteria described by Hasle *et al.*[8] and specimens were treated according to the guidelines for JMML of the European Working Group on Childhood MDS (EWOG- MDS).

DNA was extracted from mononuclear cells derived from cryopreserved primary bone marrow, peripheral blood, or spleen at diagnosis. Sequence analysis was performed using forward and reverse primers for *BRAF* exon 15: forward 5'- AGC CCC AAA AAT CTT AAA AG- 3' and reverse 5'- CTC AGG GCC AAA AAT TTA AT- 3'. In a subset of patients the entire coding sequence of *BRAF* was analyzed. Details on the used primers are available on request. Of the 65 patients with JMML included in this study, 26 had no clinical diagnosis of NF1 and lacked mutations of *KRAS*, *NRAS* or *PTPN11*. Six patients were diagnosed with NF1, 12 had a somatic mutation of NRAS or KRAS, and 12 patients had a somatic mutation of *PTPN11*. No data on the molecular background were available for nine patients. No V600E mutation of the *BRAF* gene was found in any of the 65 patients. Furthermore, in a subset of 15 patients without a clinical diagnosis of NF1 and without mutations of *PTPN11* or *RAS*, the entire coding region of BRAF was analyzed. No mutations were identified in this group of patients.

JMML is regarded as a model disease that is associated with hyperactive Ras signaling. Mutant proteins of the Ras-Raf-MEK-ERK pathway play an important role in the pathogenesis of JMML and explain the GM-CSF hypersensitivity observed *ex vivo*. In the majority of cases of JMML, these mutations are mutually exclusive and affect *NRAS, KRAS, NF1*, or *PTPN11* genes. Additionally, mutations in this pathway function as an important diagnostic tool. They are a potential marker of minimal residual disease[9] and an attractive new potential therapeutic target.

Our hypothesis was that B-Raf might play an important role in JMML as it is an important downstream effector of Ras. Somatic *BRAF* mutations occur frequently in other types of human cancer and recently, *BRAF* mutations were found in leukemia. We excluded the occurrence of BRAF mutations in a large cohort of JMML patients. Therefore, additional screening of genes of the Ras pathway will be necessary to identify genetic aberrations in cases without known mutations.

Andrica C.H. de Vries, Ronald W. Stam,* Christian P. Kratz,° Martin Zenker,# Charlotte M. Niemeyer,° Marry M. van den Heuvel-Eibrink,*@ on behalf of the European Working Group on childhood MDS (EWOG-MDS)*

**Department of Pediatric Oncology/Hematology, Sophia Children's Hospital, Erasmus University Medical Center, Rotterdam, The Netherlands; °Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, University of Freiburg, Germany; #Institute of Human Genetics, University of Erlangen-Nuremberg, Germany; @Dutch Childhood Oncology Group, The Hague, The Netherlands*

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Correspondence: Marry M. van den Heuvel-Eibrink, MD, PhD, Erasmus MC-Sophia Children's Hospital, Dept. of Pediatric Hematology/Oncology, Room Sp 2568, Dr. Molewaterplein 60, 3015 GJ Rotterdam, The Netherlands. Phone: international +31. 10.4636691. Fax: international +31.10.4636801. E-mail: m.vandenheuvel@erasmusmc.nl

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. 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.
3. 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.
4. Side LE, Emanuel PD, Taylor B, Franklin J, Thompson P, Castleberry RP, et al. Mutations of the NF1 gene in children with juvenile myelomonocytic leukemia without clinical evidence of neurofibromatosis, type 1. Blood 1998;92:267-72.
5. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54.
6. Rodriguez-Viciana P, Tetsu O, Tidyman WE, Estep AL, Conger BA, Santa Cruz M, et al. Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. Science 2006;311:1287-90.
7. Kratz CP, Niemeyer CM, Zenker M. An unexpected new role of mutant Ras: perturbation of human embryonic development. J Mol Med 2007;85:223-31.
8. Hasle H, Niemeyer CM, Chessells JM, Baumann I, Bennett JM, Kerndrup G, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia 2003;17:277-82.
9. Archambeault S, Yoshimi A, Kratz CP, Reising M, Fischer A, Noellke P, et al. Development of an allele-specific minimal residual disease assay for patients with Juvenile Myelomonocytic Leukemia-moving beyond clinical assessment. Blood 2006;108:[ASH Annual Meeting Abstracts].