News Release

Title

**Integrated molecular profiling of juvenile myelomonocytic leukemia**

Key Points

- **We identified ALK/ROS1 tyrosine kinase fusions (DCTN1-ALK, RANBP2-ALK, and TBL1XR1-ROS1) in patients with juvenile myelomonocytic leukemia (JMML) who lacked canonical RAS pathway mutations. Crizotinib, potent ALK/ROS1 inhibitor, was administered to a chemotherapy-resistant patient with the RANBP2-ALK fusion who subsequently achieved complete molecular remission.**
- **We identified the hypermethylation profile as a novel poor prognostic factor for JMML. The hypermethylation profile was stronger predictor than most established risk factors for JMML.**
- **Molecular targeted therapy and risk stratification using methylation profile are expected to improve the outcome.**

Summary

Prof. Yoshiyuki Takahashi, Dr. Hideki Muramatsu, Dr. Norihiro Murakami, and emeritus Prof. Seiji Kojima in Department of Pediatrics, Nagoya University Graduate School of Medicine, Prof. Hiroyuki Aburatani in Genome Science Division, Research Center for Advanced Science and Technology, The University of Tokyo, Prof. Seishi Ogawa in Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Director Hiroyuki Mano in National Cancer Research Institute, and their colleagues performed an integrated molecular profiling of juvenile myelomonocytic leukemia.

Juvenile myelomonocytic leukemia (JMML), a rare and aggressive myelodysplastic/myeloproliferative neoplasm occurring in infants and early childhood, is characterized by excessive myelomonocytic cell proliferation. More than 80% of patients harbor germline and somatic mutations in RAS pathway genes, and preceding studies have identified several biomarkers associated with poor prognosis. We performed an integrated molecular analysis of samples from 150 JMML patients.

As a result, we identified ALK/ROS1 tyrosine kinase fusions (DCTN1-ALK, RANBP2-ALK, and TBL1XR1-ROS1) in 3 of 16 patients who lacked canonical RAS pathway mutations. Crizotinib, an ALK/ROS1 inhibitor, markedly suppressed ALK/ROS1–fusion positive JMML cell proliferation in vitro. Therefore, we administered crizotinib to a chemotherapy-resistant patient with the RANBP2-ALK fusion who subsequently achieved complete molecular remission. We also identified a “hypermethylation profile” as novel poor prognostic factor for JMML, and it was stronger predictor than most established risk factors.

## Research Background

Juvenile myelomonocytic leukemia (JMML) is a rare myelodysplastic/myeloproliferative neoplasm that occurs in infants and during early childhood, characterized by excessive myelomonocytic cell proliferation and granulocyte-macrophage colony-stimulating factor (GM-CSF) hypersensitivity. More than 80% of JMML patients harbor mutually exclusive somatic and/or germline mutations in canonical RAS pathway genes, such as PTPN11, NF1, NRAS, KRAS, and CBL. Preceding studies identified several biomarkers as poor prognostic factors for JMML, such as an older age at diagnosis, low platelet count, and secondary mutations.

## Research Results

We conducted an integrated analysis of genome-wide methylation, whole exome sequencing, and RNA-sequencing in 150 patients with JMML. We identified three in-frame fusions involving receptor tyrosine kinases (DCTN1-ALK, RANBP2-ALK, and TBL1XR1-ROS1 **Figure 1**) in 3 of 16 patients who lacked canonical RAS pathway mutations. ALK and ROS1 fusion genes have been reported in other non-hematological and hematological malignancies, including lung cancer and malignant lymphoma, and crizotinib, a potent ALK/ROS1 inhibitor, yielded obvious clinical responses in patients with ALK/ROS1-aberrated malignancies. Crizotinib significantly suppressed proliferation of JMML cells obtained from a patient with RANBP2-ALK *in vitro*. Based on these results, we translated these findings into clinical application in a patient with RANBP2-ALK. Although the patient with RANBP2-ALK was refractory to conventional AML-type chemotherapy, she achieved complete molecular response 1 month after start of crizotinib (**Figure 2**). She was successfully bridged to hematopoietic stem cell transplantation and survived without disease recurrence for about two years after transplantation. During genome-wide methylation analysis, we identified the hypermethylation profile as novel risk factor for JMML (**Figure 3**). The hyper methylation profile was stronger predictor than most of established risk factors.

## Research Summary and Future Perspective

We identified recurrent activated ALK/ROS1–fusions in JMML patients without canonical RAS pathway gene mutations and the hypermethylation profile as novel risk factor for JMML. Our findings should contribute to diagnostics and to the development of therapeutic approaches by providing a model of precise risk stratification.

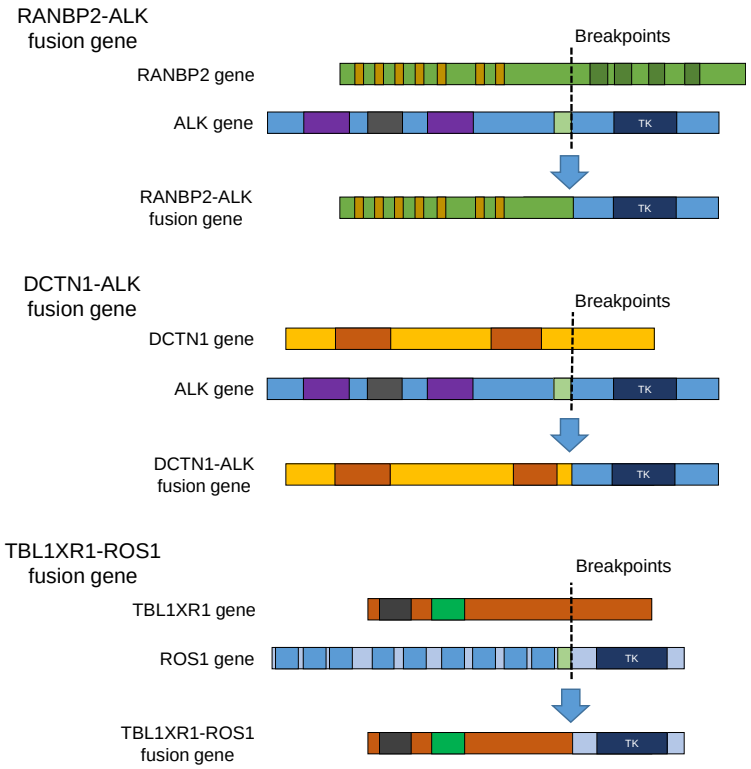


Figure 1. Tyrosine kinase fusion genes identified in JMML.

All detected fusions have a common structure in which the back part of ALK/ROS1 tyrosine kinase and front part of fusion partners are fused. TK, tyrosine kinase;

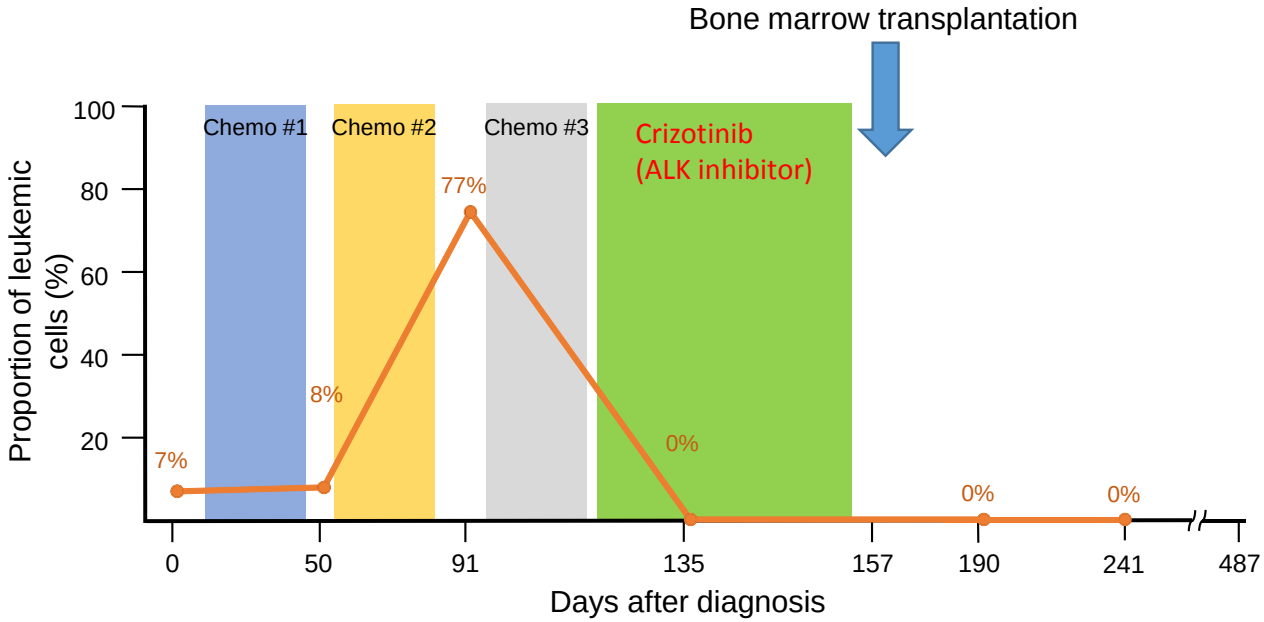


Figure 2. Targeted therapy for a patient with RANBP2-ALK

Although the patient with RNABP2-ALK fusion gene was refractory to conventional chemotherapy, leukemic cells of this patient were disappeared after targeted therapy using crizotinib which was a potent ALK inhibitor.

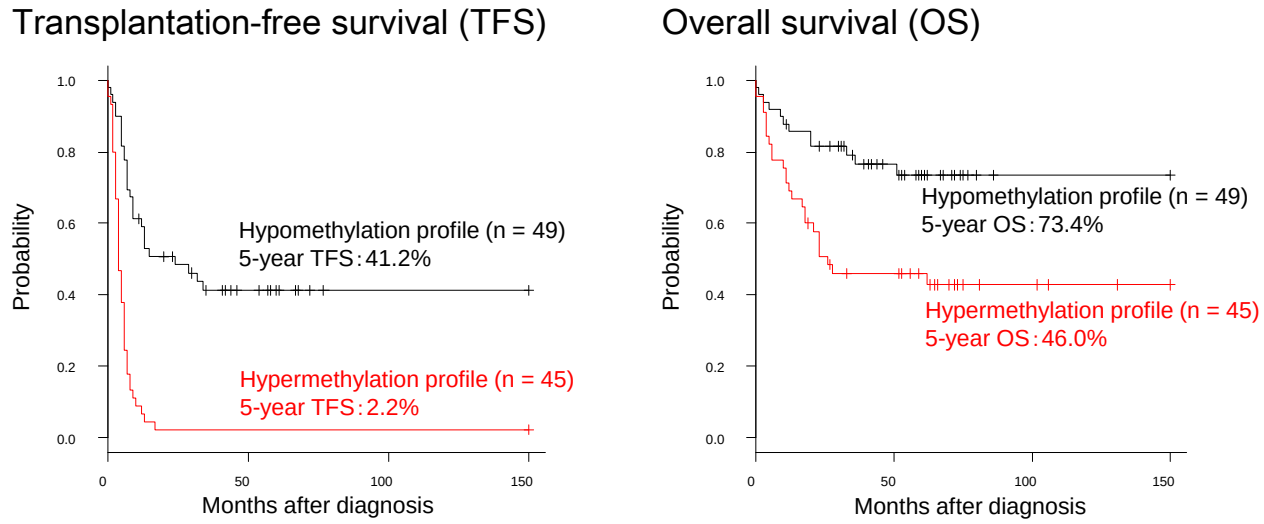


Figure 3. Comparison of survival rate by methylation status.

The hypermethylation profile had poorer survival rate compared with hypomethylation profile.

## Publication

Murakami N, Okuno Y, Yoshida K, Shiraishi Y, Nagae G, Suzuki K, Narita A, Sakaguchi H, Kawashima N, Wang X, Xu Y, Chiba K, Tanaka H, Hama A, Sanada M, Ito M, Hirayama M, Watanabe A, Ueno T, Kojima S, Aburatani H, Mano H, Miyano S, Ogawa S, Takahashi Y, Muramatsu H. Integrated molecular profiling of juvenile myelomonocytic leukemia, Blood,

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Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Blood_20180205.pdf