ELSEVIER

Blood 142 (2023) 3199–3200

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

634.MYELOPROLIFERATIVE SYNDROMES: CLINICAL AND EPIDEMIOLOGICAL

Error-Corrected Next-Generation Sequencing Provides a Comprehensive Overview of the Subclonal Mutation Landscape and Its Prognostic Implications in Juvenile Myelomonocytic Leukemia

Daichi Sajiki [1], Manabu Wakamatsu, MDPhD [1], Hideki Muramatsu, MD PhD [1], Yusuke Tsumura, MD [1], Daiki Yamashita, MD [1], Ryo Maemura, MD [1], Ayako Yamamori, MD PhD [1], Kotaro Narita, MD PhD [1], Shinsuke Kataoka, MD PhD [1], Atsushi Narita, MD PhD [1], Noriko Shimasaki, MD PhD [1], Nobuhiro Nishio, MD PhD [1,2], Yoshiyuki Takahashi, MD PhD [1]

[1] Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan
[2] Department of Advanced Medicine, Nagoya University Hospital, Nagoya, Japan

Introduction: Juvenile myelomonocytic leukemia (JMML) is a rare pediatric myelodysplastic/myeloproliferative neoplasm characterized by mutations in genes activating the RAS pathway (*PTPN11*, *NF1*, *NRAS*, *KRAS*, and *CBL*). Approximately 20% of patients with JMML have secondary mutations in *SETBP1* and/or *JAK3*, and recent droplet digital PCR (ddPCR) analyses revealed that mutations in these two genes, including very rare mutations (variant allele frequency [VAF] < 0.05) that are difficult to detect by conventional next-generation sequencing (NGS), are associated with poor prognoses. Meanwhile, the landscape of low VAF subclonal mutations of other genes in patients with JMML has not been adequately evaluated. Therefore, we utilized error-corrected NGS, which can reduce the systematic sequencing error rate of conventional NGS by incorporating molecular barcodes (MBCs).

Methods: We performed error-corrected NGS in 93 patients with JMML and 11 patients with JMML-like myeloproliferative disorder with Noonan syndrome (NS/MPD) using a small panel associated with myeloid malignancies. Samples for error-corrected NGS were prepared using a SureSelect XT HS2 DNA Library Preparation Kit (Agilent Technologies), including unique dual index and duplex MBCs. A unique MBC is incorporated into each DNA fragment, and the incorporated MBC can differentiate true and false duplications, allowing for highly accurate sequencing that can detect mutations with VAF > 0.001. We used the HiSeq X platform (Illumina) and SureCall v.4.2 application (Agilent Technologies) for analysis. In addition, DNA methylation analysis was performed on the same cohort using the Infinium HumanMethylation 450K BeadChip and/or Digital Restriction Enzyme Analysis of Methylation as previously described (Murakami et al. 2018;131:1576-1586, Kitazawa et al. Blood Adv. 2021;5:5507-5518). This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine.

Results: In the cohort of 104 patients with JMML or NS/MPD, 160 mutations were identified, of which 30 (19%) had VAF < 0.05 (eight in *JAK3*, seven in *NF1*, five in *ASXL1*, three in *NRAS*, two each in *PTPN11* and *SOS1*, and one each in *SETBP1*, *SH2B3*, and *ZRSR2*). Subclonal mutations in *SETBP1* p.D868N and *JAK3* p.R657Q identified by error-corrected NGS were confirmed using ddPCR, and consistent results were obtained for both assays ($R^2 = 0.9977$ and 0.9919 respectively). Of 93 patients with JMML, 30 (32%) had subclonal or clonal secondary mutations, whereas no such mutations were detected in patients with NS/MPD ($P = 0.031$).

Of 104 patients, 23 (22%) had secondary mutations with VAF ≥ 0.05 (Major group), 7 (7%) only had secondary mutations with VAF < 0.05 (Minor group), and the remaining 74 (71%) had no secondary mutations (Negative group). We assessed the associations of subclonal mutations detected by error-corrected NGS with the DNA methylation profile, the most important prognostic factor in JMML. Whereas 22 of 23 (96%) and 5 of 7 (71%) patients in the Major and Minor groups, respectively, had high methylation (HM) profiles, only 20 of 74 (27%) patients in the Negative group featured HM profiles ($P < 0.001$). The 4-year overall survival (OS) rates were significantly lower in the Major (52.2% [95% confidence interval {CI} = 30.5%-70.0%], $P = 0.01$) and Minor groups (42.9% [95% CI = 9.8%-73.4%], $P = 0.03$) than in the Negative group (76.3% [95% CI = 64.6%-84.5%]). In a subgroup analysis of 30 patients with secondary mutations, patients with secondary mutations in RAS pathway genes (n = 18) had significantly lower 4-year OS rates than those with secondary mutations in other genes (n = 12; 32.4% [95% CI = 12.7%-54.0%] *vs.* 75.0% [95% CI: 40.8%-91.2%], $P = 0.047$).

Conclusions: We successfully performed error-corrected NGS to assess comprehensive subclonal secondary mutational profiles, including very low VAF variants. The presence of subclonal mutations, particularly in RAS pathway genes, was associated

with poor OS. These findings provide important information for appropriate risk stratification, which will contribute to the implementation of precision medicine for patients with JMML.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-189160