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ORIGINAL ARTICLE

Detection of *NPM1* Mutations in Acute Myeloid Leukemia by using Drop-Off Droplet Digital PCR and its Clinical Application

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SUMMARY

Background: **Nucleophosmin 1 (*NPM1*) mutations, which occur in 25 - 30% of acute myeloid leukemia (AML) and 50 - 60% of AML with normal karyotype, have been identified as an important marker for stratification of prognosis in AML. This study aimed to establish a new quantitative polymerase chain reaction (PCR) technique, the drop-off droplet digital PCR (ddPCR), for rapid and sensitive detection of *NPM1* mutations in AML.**
Methods: **We established the drop-off ddPCR system and verified its performance. *NPM1* mutations were screened in 130 AML patients by drop-off ddPCR and were validated by Sanger sequencing and next-generation sequencing (NGS). Then, the *NPM1* mutation burden was dynamically monitored in five patients.**
Results: **The limit of blank (LOB) of drop-off ddPCR established for *NPM1* mutation was 3.36 copies/μL, and the limit of detection (LOD) was 5.00 - 5.37 copies/μL in 50 ng DNA, and the sensitivity was about 0.05%, which had good linearity. Drop-off ddPCR identified 33/130 (25.4%) *NPM1* mutated cases, consistent with Sanger sequencing. In 18 *NPM1* positive cases selected randomly, NGS identified fourteen with type A mutation, two with type D mutation, and two with rare type mutations. The mutation burden of *NPM1* mutation analyzed by NGS was consistent with the drop-off ddPCR. The sequential samples were detected for measurable residual disease (MRD) monitoring in 5 patients showed that the *NPM1* mutation burden was consistent with clinical remission and recurrence. Compared with traditional ddPCR, drop-off ddPCR was also suitable for MRD monitoring.**
Conclusions: **In this study, we established a drop-off ddPCR method for detecting three common mutations in AML with good sensitivity and repeatability, which can be used to screen mutations in newly diagnosed AML patients and for MRD monitoring after remission to guide treatment.**
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Supplementary Data

Table S1 Patient characteristics.

Patient characteristics	130 newly diagnosed AML patients	5 patients for MRD monitoring				
		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age, years		56	59	59	65	52
Median	57					
Range	18 - 85					
Gender (male/female)	71/59	male	female	male	female	female
WBC count, × 10^9/L		49.6	70.8	27.4	4.0	22.7
Median	21					
Range	0.8 - 528					
FAB		M2	M5	M5	M4	M2
M0	1					
M1	8					
M2	65					
M4	35					
M5	18					
M6	3					
Karyotype		Intermediate	Intermediate	Unknown	Unknown	Intermediate
Favorable	14					
Intermediate	91					
Adverse	19					
Unknown	6					
NPM1 mutated	33	+	+	+	+	+
FLT3 mutated	25	-	-	-	-	-
CEBPA mutated	11	-	-	-	-	-
IDH1 mutated	9	-	-	-	+	-
IDH2 mutated	16	+	-	-	-	-
U2AF1	4	-	-	-	-	-
SRSF2	4	-	-	-	-	-
SF3B1	2	-	-	-	-	-
CKIT mutated	3	-	-	-	-	-
NRAS mutated	9	-	-	-	+	-
KARAS mutated	5	-	-	-	-	-
DNMT3A mutated	10	-	-	-	-	-
TP53 mutated	3	+	-	-	-	-

Prognostic group standard according to chromosomal abnormalities: Karyotype associated with favorable prognosis include t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22); Karyotype associated with intermediate prognosis include t(15;17)(q22;q12), normal cytogenesis, +8; Karyotype associated with adverse prognosis include complex karyotype consisting of ≥ 3 abnormalities, t(6;9)(p23;q34), abnormal 11q23 excluded t(9:11), del(5q), -5, del(7q), -7, t(9:22).
WBC - white blood cell, FAB - French-American-British criteria.