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803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

One-Pot Era-CRISPR/Cas12a System for Rapid and Sensitive Detection of Multiple NPM1 Mutations

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Nucleophosmin 1 (NPM1) gene c.863_864 4-bp insertion mutations occur in 30% of adult acute myeloid leukemia (AML) patients, which is associated with a unique pathological phenotype and good prognostic significance. The detection of NPM1 mutations is important for risk stratification, treatment guidance, and minimal residual disease monitoring in AML. However, the current detection methods have their limitations in terms of equipment requirements, sensitivity, turnaround time, price, etc. Moreover, simultaneous detection of multiple NPM1 mutation types is challenging. Here, we developed a one-pot ERA-CRISPR/Cas12a-based assay in which only one guide crRNA is needed to detect hundreds of NPM1 gene variants, covering over 97% of NPM1-mutated AML cases. By optimizing multiple parameters, this assay finally achieved a sensitivity of as low as 0.01% within 30 min detection under constant 39°C, without wild-type related cross signal. In clinical detection, 21 NPM1 mutation positive cases from 70 AML patient were successfully screened out using our method, including two cases not detected by Sanger sequencing. This method is one-step, rapid, sensitive, specific, inexpensive, and without the need of Sequencer or temperature cycler. It holds the potential to assist clinicians in point of care testing.

Disclosures No relevant conflicts of interest to declare.

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