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FLT3

getITD for FLT3-ITD-based MRD monitoring in AML

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Measurable residual disease (MRD) measurement is performed to assess response to treatment and serves as an independent prognostic indicator in patients with acute myeloid leukemia (AML). Along with the assessment of clinical, cytogenetic and molecular data assessed at diagnosis, it is important in planning treatment and for risk stratification. A number of assays have been established for several recurrent leukemic markers.[1,2]

Internal tandem duplications in the *FLT3* gene (*FLT3*-ITDs) are commonly recognized as poor prognostic markers in AML. However, their heterogeneity makes conventional PCR methods laborious or intensive.[3] Therefore, novel methods to detect FLT3-ITD are needed to improve monitoring of these high-risk patients.

In their letter to the editor, Tamara J. Blätte, Department of Hematology, Charité University Medicine, Berlin, DE, and colleagues developed a method[4] based on targeting high-coverage NGS using a two-step PCR to amplify and sequence the affected *FLT3* exons. The team also developed an open-source analysis software program, *getITD*. The team sequenced three human AML cell lines, two healthy volunteers, and 57 samples from 28 AML patients, in the AMLSG BiO study (NCT 01252485).

Key analysis

Assay specificity

- Three *FLT3*-ITD negative control samples (peripheral blood from 2 healthy volunteers and 1 AML cell line HL-60)

Assay sensitivity

- Serial dilutions of *FLT3*-ITD positive DNA from AML cell lines (MOLM-14 and PL-21) were analyzed
- variant allele frequency (VAF) estimates were accurate and decreased linearly
- The most diluted sample (1:10,000) was found to harbor the ITD at 0.0067% VAF ($6.7x10^{-5}$), indicating the current limit of detection

Assay accuracy

- *FLT3*-ITD bone marrow samples of 28 AML patients were sequenced and compared with previously obtained fragments and Sanger sequencing analyses
- Through PCR- and capillary electrophoresis-based fragment analysis (FA), 34 ITDs were identified
- These 34 ITD were also detected by the developed NGS-assay, with insertion sites identical to those identified by Sanger sequencing
- In 19/28 samples the developed assay identified one or more additional ITDs that were not detected by FA
- 75/105 ITDs detected by the assay and not FA were present at lower VAFs, and thus below the detection limit of conventional methods

- Reproducibility: 14/28 samples were independently re-sequenced and resulted in detection of identical insertion sites and lengths

MRD in *FLT3*-ITD positive AML patients

- Serial samples sequenced for five patients who relapsed during therapy, tested ITD positive at the time of relapse with *getITD* at very low VAFs (0.06% and 0.2%). In contrast, FA identified only three samples as ITD positive, with two patients considered to have lost *FLT3*-ITD
- Follow-up samples for five patients who remained in complete remission (CR) tested FLT3-ITD negative at all sampled time points using *getITD*

Conclusions

The team found *getITD* to accurately and precisely detect ITDs at broad range of lengths, insertion sites and VAFs. The maximum detectable ITD read length of the assay is currently 250 bp with a minimum insert length of 6 bp. As the method does not require manual analysis it can be applied to routine clinical monitoring. The open source software can be found online.

This technique has increased sensitivity in comparison to conventional FA, which could be increased further by analyzing more than 50ng of sample DNA, however the researchers determine that closer patient monitoring would be better suited to enable earlier MRD detection. Using *getITD*, ITD lengths, integration sites and sequences can all be identified in one assay, which allow for better monitoring of separate ITD clones within a sample.

References

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