Panel testing for gene mutations in acute myeloid leukaemia by next-generation sequencing

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Introduction
Genomic techniques in recent years have allowed the identification of many mutated genes that are important in the pathogenesis of acute myeloid leukaemia (AML). Together with cytogenetics aberrations, these gene mutations are powerful prognostic markers in AML and can be used to select patients for optimal post-remission therapy. Emerging data also show that these mutations may serve as predictive markers of treatment dose or choice upfront. The mutated genes hold promise as therapeutic targets themselves.

We aim to evaluate the applicability of a gene panel for the detection of AML mutations in a diagnostic molecular pathology laboratory.

Materials & Methods
A total of 31 AML samples (bone marrow = 25 and peripheral blood = 6) were accrued for the study. They comprised 11 males and 20 females at a median age of 58 years. The diagnoses were M0 = 1, M1 = 6, M2 = 7, M3 = 1, M4 = 1, M5 = 2, M6 = 2, NOS = 2, AML transformed from MDS or MDS/MPD = 4, therapy-related AML = 1 and refractory AML = 2. DNA was extracted from the corresponding specimens. Germline DNA from buccal swab was available in one patient. A total of 54 genes (full coding exons of 15 genes and exonic hotspots of 39 genes) were targeted by 568 amplicons (length range: 225 – 275bp) (Fig. 1a). The combined coverage was 141kb.

Bringing bioinformatics pipeline, which mainly consisted of BWA, SAMtools, Ensembl Variant Effect Predictor (Illumina, USA) with reagent kit v3. Sequences obtained were sequenced and is usually soft-clipped and ignored by variation callers. The mean count of sequencing reads obtained per sample was 3.25 million (range 2.02 – 4.57 million) and the mean sequencing depth was over 3000X (Fig. 1c). Thirty-eight mutations in 17 genes were detected in 20 of 31 samples (65%) (Fig. 2). On average 1.9 mutations (range 1 – 5) were detected per sample. Mutations were detected in the following genes: FLT3 (n = 7), NPM1 (n = 7), IDH2 (n = 3), RAS2, SF3B1, STAG2, DNM3TA, TET2, IDH1, KIT (all n = 2), NRAS, ET3/TEL, CEBPA, IKZF1, TP53, SFB31 and SMCD (all n = 1). Mutations detected by NGS were compared with previous results from conventional methods if available. NGS results were not just concordant with conventional results but also more sensitive in detecting additional mutations at low allelic burden. Detection of long insertion mutations (e.g. >70bp) in short amplicon sequencing was challenging and required new bioinformatics algorithm (Fig. 3).

Results
The mean count of sequencing reads obtained per sample was 3.25 million (range 2.02 – 4.57 million) and the mean sequencing depth was over 3000X (Fig. 1c). Thirty-eight mutations in 17 genes were detected in 20 of 31 samples (65%) (Fig. 2). On average 1.9 mutations (range 1 – 5) were detected per sample. Mutations were detected in the following genes: FLT3 (n = 7), NPM1 (n = 7), IDH2 (n = 3), RAS2, SF3B1, STAG2, DNM3TA, TET2, IDH1, KIT (all n = 2), NRAS, ET3/TEL, CEBPA, IKZF1, TP53, SFB31 and SMCD (all n = 1). Mutations detected by NGS were compared with previous results from conventional methods if available. NGS results were not just concordant with conventional results but also more sensitive in detecting additional mutations at low allelic burden. Detection of long insertion mutations (e.g. >70bp) in short amplicon sequencing was challenging and required new bioinformatics algorithm (Fig. 3).

Conclusion
Comprehensive 54-gene panel testing revealed a high frequency and a diverse spectrum of mutations in AML. Gene panel testing by NGS approach in a diagnostic molecular pathology laboratory allows timely, sensitive and accurate detection of actionable AML gene mutations to individualize patient management.