Fusion genes in acute myeloid leukemia: do acute myeloid leukemia diagnostics need to fuse with RNA-sequencing?

by Felicitas Thol

Haematologica 2021 [Epub ahead of print]

Citation: Felicitas Thol. Fusion genes in acute myeloid leukemia: do acute myeloid leukemia diagnostics need to fuse with RNA-sequencing?
Haematologica. 2021; 106:xxx

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
In this issue of Haematologica, Kerbs and colleagues present a detailed analysis of fusion gene detection by RNA-sequencing in acute myeloid leukemia (AML). Fusion genes, i.e. genes made by joining parts of two different genes, occur frequently in carcinogenesis. These fusion transcripts can take place within one chromosome as well as between two chromosomes (inter-chromosomal) and include translocations, deletions, insertions as well as inversions. Thousands of chromosomal aberrations and fusion genes have been identified as occurring recurrently in cancer. Interestingly, in pediatric acute lymphocytic leukemia (ALL) it was shown that many fusion genes already originate in utero and that a further transformation occurs in early childhood through secondary mutations. The discovery of BCR-ABL1 in patients with chronic myeloid leukemia (CML) as well as gene fusions involving Neurotrophic Tyrosine Receptor Kinase (NTRK) and neuregulin-1 (NRG1) in solid cancers are examples of how the identification of fusion genes have not only revolutionized the diagnosis but also the therapy of a disease. In AML, it is estimated that fusion genes occur in 30% of patients. It has been recognized that certain recurrent chromosomal and genetic aberrations describe distinct types of AML. In this line, the current WHO classification of AML describes seven types of recurrent genetic abnormalities in AML as well as the provisional entity “AML with bcr-abl1”. Furthermore, the WHO classification lists recurrent genetic aberrations that classify the disease as “AML with myelodysplasia-related changes”. This underscores the relevance of identifying chromosomal aberrations and fusion genes in AML patients. The European Leukemia Net (ELN) recommends cytogenetics and screening
for certain gene mutations for all AML patients at the time of diagnosis.\textsuperscript{8} Furthermore, screening for gene rearrangements (e.g. with fluorescence in situ hybridization (FISH) or reverse transcription polymerase chain reaction (RT-PCR)) should be performed in special circumstances (e.g. poor quality of chromosome morphology, need for rapid information). However, while cytogenetic analysis is still the gold standard, many smaller aberrations can be easily missed with this technique. RNA-sequencing is a next generation sequencing (NGS) technique that detects the presence and quantity of RNA and thus describes the transcriptome. Additionally, it gives information about alternative splicing, mutations, changes in gene expression, fusion genes etc. We can expect additional information that we would not be able to obtain with routine cytogenetic analysis. Thus, RNA-sequencing can give us a deeper and a dynamic picture of what is happening in cells. Kerbs and colleagues have applied RNA-sequencing to a cohort of 806 AML samples that also underwent conventional diagnostics with karyotyping and molecular diagnostics (RT-PCR and FISH) (Figure 1). This allows a direct comparison between results obtained by these different techniques. Special attention was paid to the detection of fusion genes. Here, 90\% of fusion genes reported by conventional diagnostics were also detected by RNA-sequencing. Nevertheless, the fact that some fusion genes were not recognized by RNA-sequencing indicates a weakness of this novel technique. Some fusion genes can be missed by RNA-sequencing if only low or inhomogenous coverage is achieved. Thus, a high read depth of samples is essential for RNA-sequencing in order to detect all fusion genes. Furthermore, some affected genes might be more easily missed if they are not highly expressed (i.e. RNA-sequencing is highly dependent on gene expression). However, RNA-sequencing allowed the authors to detect 26 cases with known recurrent fusion events that failed to be detected by routine diagnostics. Furthermore, Kerbs and colleagues also detected two novel recurrent fusion genes by RNA-sequencing. The fusion gene NRP1-MIR99AHG occurred in 1.1\% of patients, the fusion gene LTN1-MX1 in 0.25\% of patients. These are just examples that demonstrate that many more recurrent fusion genes will be detected if RNA-sequencing is being employed more widely in AML. It is therefore very likely that we currently still underestimate the occurrence of fusion genes in AML with conventional diagnostics. These encouraging results also raise the question whether RNA sequencing should be applied to all AML patients at the time of diagnosis and whether it can replace conventional diagnostics. Currently, there are still a few obstacles for routinely performing RNA-sequencing in all AML patients. Besides costs and labor consuming aspects of this technique, analysis of results and data interpretation are very challenging. Especially, the high rate of false positive calls for fusion genes raises concerns and requires awareness. So far, interpretation of RNA-sequencing data has not been standardized and over 20 algorithms for fusion gene detection by RNA-sequencing have been described.\textsuperscript{9} However, standardization is a critical requirement for using this
technique more widely and in routine clinical practice. In this sense, it is encouraging as well as it is important that Kerbs and colleagues have developed and report a workflow with integrated filtering strategies for the identification of robust fusion gene candidates by RNA-sequencing. In the future, similar steps need to be undertaken in order to implement RNA-sequencing in routine diagnostics. The outlook of using RNA-sequencing more widely in AML is likely to have positive impacts regarding diagnostic classification as well as prognostic stratification. No less relevant, RNA-sequencing can open the door for novel therapeutic strategies in individual AML patients. Gene fusions involving the \textit{NTRK} gene is such an encouraging example. NTRK fusions rarely occur in AML and are easily missed with routine diagnostics.\textsuperscript{10} Larotrectinib is a targeted therapy that has shown efficacy in a broad spectrum of NTRK fusion positive cancers. In the same light, many fusion genes occurring through chromosomal translocations encode tyrosine kinases that are involved in signal transduction. Here, the development of tyrosine kinase inhibitors (TKIs) represent attractive therapeutic strategies and RNA-sequencing will be essential for identifying these patients. It is therefore very likely that the increase in detection of recurrent gene fusions will also expand opportunities for targeted therapies in AML. RNA-sequencing can also have further implications for measurable residual disease (MRD) monitoring in AML. Fusion genes are ideally suited for molecular MRD assessment as they can be detected by RT-PCR as well as NGS very sensitively.\textsuperscript{11} Of note, in solid cancers RNA-sequencing is evolving as an upcoming standard as well.

In summary, this study is an important step towards implementing RNA-sequencing into routine AML diagnostics. No doubt, further work is required (especially regarding standardizing data analysis). However, the manuscript by Kerbs and colleagues already gives an encouraging outlook of how RNA-sequencing can translate into clinical applications.
References


Figure 1:

Design and outlook of an integrated analysis that includes routine diagnostics (karyotyping, molecular diagnostics) as well as RNA-sequencing in 806 AML samples.
806 AML samples

Cohorts:
- AMLCG
- BEATAML
- DKT
- FIMM

RNA-seq

Routine Diagnostics:
- Karyotyping, MDx

True fusions

Karyotyping

MDx

Known fusions

Novel fusions

Outlook of RNAseq in AML:
- Allows detection of more & novel fusion genes
  - Expands options for targeted therapies
  - Potential prognostic implications

Development of a workflow with integrated filtering activity

MDx = molecular diagnostics