Long noncoding RNA profiling for prognostication in adult acute myeloid leukaemia: abridged secondary publication

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KEY MESSAGES

1. Whole transcriptome sequencing identified a 10-gene long noncoding RNA (lncRNA) prognostic score that has prognostic effects independent of standard European LeukemiaNet risk stratification in acute myeloid leukaemia (AML).
2. The lncRNA prognostic score facilitated more accurate risk stratification in favourable-risk and intermediate-risk AML patients.
3. External validity of the lncRNA prognostic score was demonstrated using large external datasets from Beat-AML and TCGA-LAML.
4. A clinical-grade capture-seq assay was devised and validated to facilitate cost-effective implementation of the lncRNA prognostic score in clinical laboratories.

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Acute myeloid leukaemia (AML) is a neoplastic disorder of stem cells of the myeloid lineage in the haemopoietic system, with diverse pathogenic mechanisms and highly variable clinical outcomes. Prognostication and guidance on clinical management are suboptimal in a large number of AML patients. We established an independent way to stratify risk among patients with AML by using long noncoding RNAs (lncRNA) expression profile measured by massively parallel RNA sequencing (RNA-seq). lncRNA is defined as a group of non-protein-coding RNAs, with transcripts longer than 200 nucleotides. Although lncRNA has been reported to have significant prognostic roles in AML, the implementation of gene expression profiles for AML prognostication has been hampered by the suboptimal external validity of the clinical trials, as studies tend to yield non-overlapping sets of prognostic lncRNA that may not be applicable outside the setting of the original trials. To circumvent this, this study included local discovery and validation datasets, while leveraging large external datasets for validation, to develop a reliable set of lncRNA biomarkers for patient prognostication applicable to patients with AML in Hong Kong.

The discovery cohort comprised 185 patients with newly diagnosed adult AML presented between 2007 and 2018. Ribosomal RNA-depleted and globin mRNA-depleted deep whole transcriptome sequencing was performed to delineate the lncRNA expression profiles in these patients. A 10-gene lncRNA signature was shortlisted by penalised regression from the discovery cohort and was shown to be prognostically significant on top of standard prognostic indicators such as patient age, white cell count at diagnosis, and European LeukemiaNet (ELN) risk stratification by genetic status.[1] This observation was confirmed by independent external data of large cohorts from The Cancer Genome Atlas (TCGA)[2] and Beat-AML.[3]

In clinical practice, one difficulty in managing patients with AML lies in the inaccuracy of predicting those patients with less favourable outcome in the ELN favourable-risk and intermediate-risk groups using current risk stratification tools for AML. For example, when our study cohort and the cohorts of Beat-AML and TCGA-LAML were risk-stratified by ELN 2017, non-significant difference in overall survival was shown between ELN intermediate-risk and adverse-risk groups. Less aggressive treatment strategies were often selected for these patients with underestimated disease risks, resulting in disease relapse and subsequent non-salvageable disease. Because the lncRNA prognostic score has independent prognostic significance on top of ELN risk stratification, a new classification system was devised based on a combination of ELN and the lncRNA score. Patients with AML were first classified by ELN 2017. If the lncRNA score was above a given threshold, patients would be categorised to a less

favourable prognostic category than the original ELN 2017 risk. Using this new classification, we successfully obtained a favourable-risk group that has clinical outcome consistently better than the ELN favourable-risk group across all three cohorts. Similarly, a better segregation was observed between the new intermediate-risk and adverse-risk groups, compared with the ELN intermediate-risk and adverse-risk groups.

In view of the significant clinical utility of our findings in the risk stratification of patients with AML, a capture-seq panel for massively parallel sequencing was designed to study the 10 lncRNA with prognostic relevance, along with reported fusion genes in leukaemias, genes with clinical significant expression profiles in haematological malignancies, and housekeeping genes. This panel provides a cost-effective one-stop testing platform for the diagnosis and prognostication of AML. In a correlation cohort (using 21 samples from the initial discovery cohort), the capture-seq assay had excellent correlations with the expression levels measured by whole transcriptome sequencing (r=0.63-0.93). To independently demonstrate the clinical utility of the capture-seq assay to measure the 10 lncRNA for prognostication in AML and calculate the lncRNA prognostic score, samples from 71 consecutive patients with AML who presented between 2019 and 2021 after the initial discovery cohort were retrospectively retrieved for testing. To use the original lncRNA prognostic score calculation, the lncRNA expression profile was converted from capture-seq scale to transcriptome scale by means of the regression coefficients obtained from the correlation cohort. The prognostic significance of the lncRNA class measured by capture-seq in AML was successfully validated. This provided evidence to substantiate the use of capture-seq for cost-effective measurement of the 10 lncRNA and calculation of the lncRNA score in Hong Kong for better risk stratification of patients with AML.

This study supports the clinical usefulness of the lncRNA prognostic score, which paves ways for the introduction of expression profiling of lncRNA in clinical laboratories.

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References

1. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424-47.
2. Cancer Genome Atlas Research Network; Ley TJ, Miller C, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368:2059-74.
3. Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of acute myeloid leukaemia. Nature 2018;562:526-31.