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POSTER ABSTRACTS

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Diagnostic Utility of Comprehensive RNA-Seq Analysis in Adult B-ALL

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Introduction

Adult B-cell acute lymphoblastic leukemia (B-ALL) comprises 75% of adult ALL cases. The classification and risk stratification of patients have conventionally relied on cytogenetics analysis. However, cytogenetics has limitations due to its reliance on metaphase cells, which can mitigate classification accuracy. In recent years, transcriptome sequencing (RNA-seq) of large pediatrics ALL cohorts has expanded the number of recurrent gene fusions recognized as disease defining events and informed risk stratification [Brady et al. 2022, Mullighan et al. 2017]. This study aims to evaluate the diagnostic utility of RNA-seq for clinical subtype classification in adult B-ALL in a large representative cohort.

Methods

The study cohort comprised 338 adult patients (25-65 years) with newly diagnosed ALL (UKALL14, ISRCTN66541317, NCT01085617). Paired-end RNA-seq data at 60 Million reads per sample were generated. Analysis focused on the detection of disease-defining gene fusions and gene expression signatures. Three RNA fusion callers (FusionCatcher, FuSeq, and STAR-Fusion) and three expression-based classifiers (ALLSorts, ALLspice, and ALLCatchR) were used for consensus fusion calling and expression-based classification, respectively [Nicorici et al. 2014, Vu et al. 2018, Haas et al. 2019, Schmidt et al., Mäkinen et al., Bader et al. 2022]. Integration of classification results from expression-based classifiers and the consensus gene fusions provides 4 levels of RNA-derived subtype information, denoted as "RNA evidence" in this study. These RNA-seq based classification results were compared with clinical cytogenetics findings.

Results

Out of 338 samples in this cohort, cytogenetics unequivocally classified 73% (246/338) of the samples. By integrating RNA expression-based classification with fusion subtypes, we confidently classified 87% (295/338) of the cohort with at least 2 levels of RNA evidence. In total, consensus gene fusions were identified in 74% (249/338), while expression-based classifiers assigned a confident subtype to 82% (319/338). Subsequently, we compared cytogenetics with RNA-seq derived classification and found a high concordance of 98% (240/246) between the two approaches. Only 6 (<2%) samples with cytogenetics subtypes were not classified by RNA-seq, attributed to specific subtype characteristics (near-haploid) or lower RNA purity/quality.

27% (92/338) of subjects were not classified by cytogenetics (31 missing/failed and 61 indeterminate cytogenetics). Of the 31 samples with failed/missing cytogenetics, RNA-seq confidently assigned a class in 74% (23/31). For the remaining 61 cases with indeterminate cytogenetic (complex/other), RNA-seq resolved classification in 74% (45/61). Taken together, this improved the classification for 20% (68/338) of the entire cohort that was previously unclassified through cytogenetics. Notably, RNA-

seq-exclusive findings were enriched in subtypes like DUX4, PAX5alt, and ZNF384, which are challenging for cytogenetics to discern.

Importantly, integration of RNA-seq with cytogenetics led to a confident subtype classification for 93% of the cohort (314/338), a significant improvement over the initial 73% based on cytogenetics alone.

Conclusions

In conclusion, our study highlights the relevance of integrated RNA-seq analysis for reliable and comprehensive subtype classification in adult B-ALL. The high concordance between cytogenetics and RNA-derived subtypes underscores their complementary roles, providing valuable insights into disease mechanisms and guiding personalized treatment strategies.

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