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Acute Leukemias

INTEGRATING NGS AND OPTICAL GENOME MAPPING TO IMPROVE GENOMIC CHARACTERIZATION OF ADULT BCR:ABL1-NEGATIVE B-ALL

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Introduction: Adult B-cell acute lymphoblastic leukemia (B-ALL) lacking the BCR:ABL1 rearrangement represents most of adult cases (70–80%) and shows marked genetic heterogeneity. This complexity makes comprehensive characterization challenging with conventional diagnostic techniques, often resulting in long turnaround times and many “not otherwise specified” (NOS) cases. High-throughput genomic technologies may enhance patient stratification, alignment with guidelines, and deepen understanding of the disease genomic landscape.

Methods: Bone marrow and peripheral blood samples underwent clinical, morphological, and conventional cytogenetic evaluation at diagnosis. A targeted RNA-based NGS assay (FUSIONPlex™ ALL Panel), detecting known and novel fusions and sequence variants across 81 ALL-relevant genes, was applied to all cases. Samples remaining unclassified after NGS were further analyzed, when material was available, by Optical Genome Mapping (OGM), which enables high-resolution, genome-wide detection of structural variants, including deletions, insertions, balanced/unbalanced rearrangements, and copy number variations.

Results: Seventy-five adult BCR:ABL1-negative B-ALL patients diagnosed between 2009 and 2025 in two Italian centers were retrospectively analyzed (median age 46, IQR 27–65). Standard cytogenetics classified 61/75 (81%) patients as B-ALL, NOS, with risk assessment based only on clinical features. NGS identified 68 clinically relevant alterations in 48/75 cases (64%), most frequently affecting FLT3, NRAS, and PAX5. Among B-ALL, NOS cases, 37 (58.7%) harbored classifying alterations, allowing refined stratification into BCR:ABL1-like, PAX5 P80R, PAX5 altered (provisional entities) and other rare subtypes. In 8 patients, only non-classifying variants were detected (Figure 1). Despite the limited cohort size, molecular reclassification showed improved prognostic discrimination for overall survival (p= 0.0059). OGM confirmed previously detected abnormalities and expanded genomic characterization in all analyzed cases, including those negative by other methods. Complex karyotypes (≥3 events) were resolved in 6/9 patients. OGM also revealed cryptic lesions such as KMT2A focal amplification with isochromosome 17, a rare t(8;14) (q24;q32) translocation involving CEBPD:IGH, and also classifying alterations: the UBTF:ATXN7L3/PAN3, CDX2 (“CDX2/UBTF”) and a rare ZNF384 rearrangement.

Conclusions: These findings support integrating NGS and OGM for improved genomic characterization of adult BCR:ABL1-negative B-ALL. NGS efficiently detects sequence-level variants, while OGM uncovers all kinds of structural alterations, providing a genome-wide view valuable for complex and atypical cases. This combined approach refines molecular stratification and disease characterization and may facilitate the identification of novel biomarkers and therapeutic targets. Validation in larger cohorts will confirm its clinical relevance and promote routine adoption

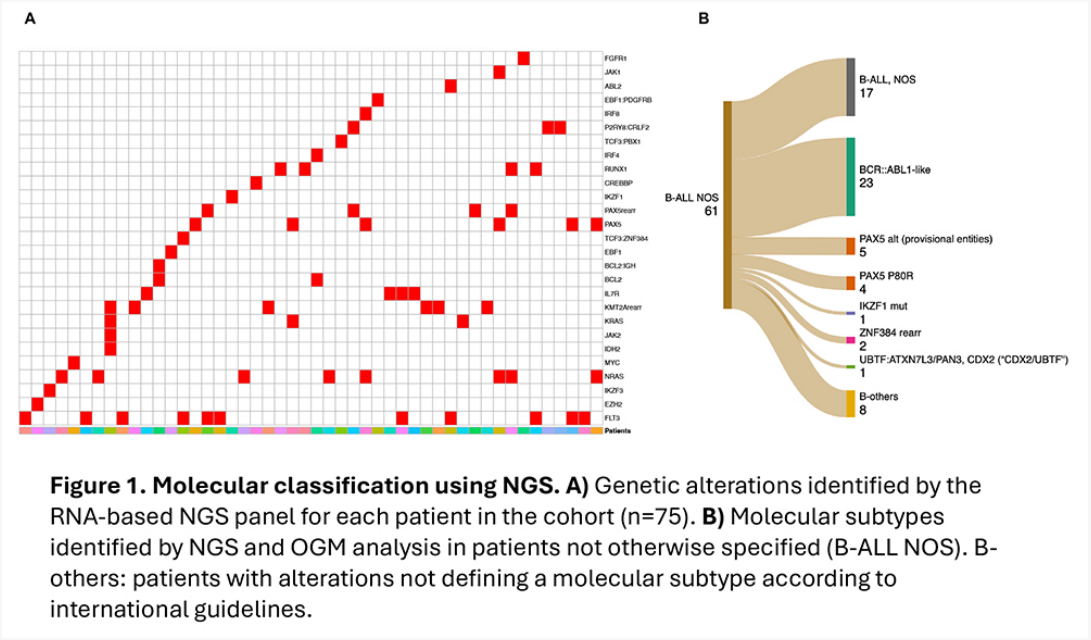

Figure 1. Molecular classification using NGS. A) Genetic alterations identified by the RNA-based NGS panel for each patient in the cohort (n=75). **B)** Molecular subtypes identified by NGS and OGM analysis in patients not otherwise specified (B-ALL NOS). B-others: patients with alterations not defining a molecular subtype according to international guidelines.