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Lymphomas

COMPARATIVE ASSESSMENT OF TARGETED NEXT-GENERATION SEQUENCING PANELS AND ALGORITHMS FOR OPTIMAL MOLECULAR SUBTYPING OF DIFFUSE LARGE B-CELL LYMPHOMA IN CLINICAL PRACTICE

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Background: Molecular classification of diffuse large B-cell lymphoma (DLBCL) represents a critical step toward precision medicine, enabling the identification of biologically and clinically distinct subtypes associated with specific genetic programs and therapeutic vulnerabilities. Among available classifiers, LymphGen—based on whole-exome sequencing (WES)—is considered the reference framework for molecular taxonomy but remains difficult to implement in diagnostic settings due to cost, turnaround time, and computational complexity. In contrast, LymphPlex, a targeted NGS-based algorithm recently adopted in the ongoing phase 3 randomized GUIDANCE-2 trial (NCT0535134), provides a more practical approach for clinical laboratories. However, its concordance with WES-based classifiers and its reproducibility across different targeted gene sets remain to be comprehensively assessed.

Methods: We evaluated three targeted NGS panels including 54, 73, and 60 lymphoma-associated genes, respectively, against the LymphGen (WES-based) and LymphPlex algorithms. Analyses were restricted to overlapping genomic regions. Concordance and subtype assignment accuracy were assessed using Cohen's Kappa, confusion matrices, and sensitivity/specificity analyses in two large, clinically annotated DLBCL cohorts (n = 574 and n = 664), representing the corresponding gold-standard datasets for LymphGen and LymphPlex, respectively.

Results: When compared with LymphGen, the 73-gene panel showed poor agreement (K = 0.03) with reduced sensitivity for EZB (21%) and MCD (20%), while maintaining high specificity (>90%). The 54-gene panel yielded similar concordance (K = 0.035), with sensitivities of 21.3% (EZB) and 20% (MCD) but specificity >90% for most classes. Likewise, the 60-gene panel achieved very low agreement (K = 0.01) and limited sensitivity (<30%) across all molecular subtypes, despite high specificity. In contrast, comparison with LymphPlex demonstrated substantial concordance across all panels (K = 0.48–0.75). The 73-gene panel achieved high sensitivity and specificity for EZB (95%) and MCD (83%), with K = 0.73; the 54-gene panel showed K = 0.66, performing best for EZB (91%) and MCD (73.5%); and the 60-gene panel reached K = 0.75, reflecting balanced performance across subtypes. A direct LymphGen–LymphPlex comparison confirmed low inter-algorithm consistency (K = 0.08).

Conclusions: Targeted NGS assays demonstrate high reproducibility and excellent specificity for the main DLBCL molecular classes, particularly EZB and MCD, when benchmarked against targeted reference classifiers. The limited concordance with WES-based algorithms underscores the importance of expanding and refining the current targeted gene content of LymphPlex to incorporate additional subtype-defining lesions. This optimization will be essential to achieve more accurate, harmonized, and clinically reproducible molecular stratification of DLBCL in routine practice.