Review paper

New technologies in the genomic evaluation of lymphomas

Magdalena Tyburczy

Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Lymphomas represent a heterogenous group of lymphoid neoplasms with a broad spectrum of clinical presentations and challenges in therapy resistance and relapse. Response to treatment and prognosis vary between and within lymphoma subtypes. The introduction of high-throughput molecular profiling methods and next-generation sequencing technologies has significantly enhanced our understanding of lymphomagenesis and improved the description of the tumour subtypes at the molecular level. Still, the current diagnosis of lymphomas is mostly based on morphological evaluation and immunophenotyping. This article describes how newly developed molecular assays already complement clinical diagnoses and have an impact on disease classification. Also, their contribution to risk stratification, therapy prediction, and disease monitoring for certain categories of lymphomas is discussed.

Key words: circulating tumour DNA, lymphomas, next-generation sequencing, whole-genome sequencing, spatial transcriptomics, single-cell RNA-sequencing.

Introduction

Lymphomas comprise a biologically diverse group of neoplasms arising from B-cells, T-cells, or innate lymphoid cells and involving the lymphatic system. More than 100 lymphoma entities are recognised, with marked variation in clinical presentation, management, treatment response, and prognosis [1]. At a broad clinical level, lymphomas are divided into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Diffuse large B-cell lymphoma (DLBCL), the most frequent high-grade NHL in adults, illustrates this diversity particularly well, showing substantial interpatient and intratumoral heterogeneity at both clinical and molecular levels. Accurate classification by lineage, cell of origin, and tumour biology is therefore central to therapeutic decision-making [2]. Large-scale molecular profiling and next-generation sequencing (NGS) have expanded knowledge of lymphomagenesis and have helped identify determinants of clinical outcome [3]. Although morphology and immunophenotype remain the foundation of lymphoma diagnosis, genomic features are increasingly used to refine classification, for risk assessment, diagnostic work-up, and prediction of treatment response [4].

Available NGS-based approaches include targeted gene panels, whole-exome sequencing (WES), whole-genome sequencing (WGS), and whole-transcriptome sequencing, commonly referred to as RNA sequencing (RNA-seq). Targeted panels interrogate selected genes at greater sequencing depth than genome-wide assays, thereby increasing sensitivity for subclonal alterations and making them useful in samples with low tumour purity and for measurable residual disease (MRD) monitoring [5]. Gene expression profiling using DNA microarrays was instrumental in defining lymphoma subgroups and cell-of-origin signatures [6]. RNA-seq provides a broader and more quantitative transcriptomic readout than microarrays, including the ability to detect previously unrecognised transcripts, and it may therefore become increasingly applicable to fresh-frozen (FF) and formalin-fixed,

paraffin-embedded (FFPE) lymphoma samples [7]. Beyond tumour genetics, the tumour microenvironment (TME) is an important contributor to lymphoma biology and treatment response. Single-cell RNA sequencing (scRNA-seq) enables high-resolution assessment of gene expression and cell-cell interactions, but it requires tissue dissociation and therefore loses spatial architecture [8]. Spatial transcriptomics addresses this limitation by combining transcriptomic information with tissue localisation, allowing investigation of tumour-TME relationships and immune escape mechanisms in their histological context [2].

This review discusses genomic and transcriptomic technologies increasingly used in lymphoma research, with an emphasis on their potential roles in classification, diagnosis, risk stratification, prediction of therapeutic response, and post-treatment monitoring. Particular attention is given to WGS, circulating tumour DNA (ctDNA) analysis, single-cell approaches, and spatial transcriptomics as tools that may support precision medicine in lymphoma.

Molecular subclassification of diffuse large B-cell lymphoma

Molecular subclassification of DLBCL is important because clinically defined groups do not fully explain differences in treatment response. Gene expression profiling initially divided DLBCL into cell-of-origin categories: germinal centre B-cell-like, activated B-cell-like, and unclassified disease. Activated B-cell-like DLBCL is generally associated with poorer outcomes after first-line R-CHOP immunochemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) [9]. However, clinical trials have shown that cell-of-origin status alone is insufficient for selecting or predicting the benefit of all therapeutic strategies [10, 11]. More detailed molecular models were subsequently developed in independent studies using combinations of WES, RNA-seq, DNA copy-number profiling, and targeted sequencing. These analyses identified recurrent mutational, copy-number, and structural-variant patterns that converge into several biologically distinct DLBCL subgroups in both FFPE and FF biopsy material [12–14]. Chapuy *et al*. [14] proposed five clusters (C1-C5), whereas Wright *et al*. [13] defined seven genetic categories: MCD, BN2, N1, ST2, A53, and EZB with or without *MYC* rearrangement. In the Chapuy classification, C1 and C4 tumours showed more favourable responses to R-CHOP, while C3 and C5 were associated with inferior outcomes. C2 was characterised by *CDKN2A* loss, biallelic *TP53* inactivation, genomic instability, and the poorest outcome after R-CHOP [14]. These molecular categories highlight targetable oncogenic pathways. For example, adding the Bruton tyrosine kinase inhibitor ibrutinib to R-CHOP appeared beneficial in MCD and N1 tumours but not in BN2 disease [15]. The LymphGen algorithm assigns DLBCL cases to seven genetic subtypes, although approximately 37% remain unclassified [13]. By contrast, the neural network-based DLBclass classifier assigned all tested cases to C1-C5 groups with 89% accuracy [16]. Incorporating genetic profiling into clinical trials will be necessary to define which molecular features are predictive rather than merely prognostic, and to determine how the results of WES/WGS, RNA-seq, and TME-directed analyses should be integrated into personalised lymphoma care.

Circulating tumour DNA analysis in lymphomas

Circulating tumour DNA is the tumour-derived fraction of cell-free DNA found in body fluids such as blood, saliva, urine, and cerebrospinal fluid [17]. In high-grade lymphomas, ctDNA levels increase with tumour burden [18]. Because ctDNA carries lymphoma-associated single-nucleotide variants, translocations, insertions/deletions, and copy-number alterations, it offers a minimally invasive source of genomic information [19]. This makes it attractive for serial sampling, assessment of spatial tumour heterogeneity, real-time monitoring, and longitudinal follow-up during treatment. In selected settings, such as inaccessible tumour sites or potentially unrepresentative biopsies, ctDNA may also complement tissue-based diagnostics [5]. Molecular clustering approaches originally developed for tissue biopsies have also been applied to ctDNA; for example, LymphGen-based classification showed 95.8% concordance between ctDNA and tissue biopsy in one study [20]. Thus, ctDNA genotyping may supplement and, in selected circumstances, partially replace tissue-based genomic testing, particularly for detecting newly emerging alterations after therapy [5].

Circulating tumour DNA is being investigated in multiple clinical trials as a biomarker for response assessment and MRD detection after first- or second-line therapy in aggressive lymphomas [21]. Standard response evaluation is primarily imaging-based, using positron emission tomography/computed tomography (PET/CT) or magnetic resonance imaging. Still, imaging has limited specificity and is not typically performed serially for routine surveillance [22, 23]. Circulating tumour DNA monitoring offers a repeatable, minimally invasive alternative that avoids radiation exposure. In Hodgkin lymphoma, analysis from the phase III SWOG S1826 trial suggested that ctDNA-based molecular tumour burden can identify residual disease below the detection threshold of PET/CT and may reveal relapse even in PET-negative patients [24].

Despite this promise, several barriers currently limit routine clinical implementation of ctDNA testing. Assays differ in design, analytical sensitivity, and specificity, and standardised thresholds, prognostic cut-offs, sampling time points, and quality-control procedures are not yet fully established. Without harmonised methodology and reporting, results may be difficult to compare across laboratories. Translation into daily practice will therefore require collaboration between clinicians, pathologists, and molecular laboratories, to define clinically meaningful indications, technical standards, reporting formats, and decision algorithms [25].

Whole-genome sequencing

Whole-exome sequencing has provided important information on mutations in protein-coding regions relevant to lymphoma biology, but WGS extends analysis beyond exons and can detect structural variants, copy-number changes, and noncoding alterations. These additional layers may help explain mechanisms of lymphomagenesis and drug resistance that are not captured by narrower approaches. Several modern lymphoma subtyping systems depend on genomic features such as driver mutations, structural variants, copy-number alterations, and somatic hypermutation patterns. Structural variants that juxtapose oncogenes with active regulatory elements, such as *BCL2*, or create fusion genes, such as *NPM1::ALK*, may have diagnostic, prognostic, or predictive significance [5].

Whole-genome-sequencing-based studies have also explored predictors of progression or relapse after autologous CD19-directed chimeric antigen receptor T-cell therapy (CAR-T19) in relapsed/refractory DLBCL [26, 27]. Jain *et al*. [26] reported that complex structural variants, APOBEC mutational signatures, and oxidative damage signatures were associated with CAR-T resistance; deletion of 3p21.31, including the *RHOA* tumour suppressor locus, was enriched among patients with treatment failure. A practical limitation was the need for viable lymphoma tissue, which is often difficult to obtain in routine practice. Cherng *et al*. [27] used low-pass WGS of ctDNA, requiring only peripheral blood, to detect tumour-specific copy-number alterations. Because the approach uses low genome coverage (0.1–1.0×), it may be a more accessible and cost-conscious method for genomic risk assessment. In that study, a high focal copy-number variation score, reflecting genomic instability, was associated with lower complete response rates and inferior overall and progression-free survival. These findings require validation in independent cohorts, but they illustrate how tumour-specific genomic features may help identify patients at increased relapse risk after CD19 CAR-T therapy who could benefit from combination or consolidation strategies.

Single-cell analyses

Single-cell analysis (SCA) is particularly useful in lymphomas because malignant cells coexist with complex immune and stromal populations in the TME. Current SCA platforms can interrogate the genome, transcriptome, epigenome, and proteome [5]. Single-cell RNA sequencing has been applied to define tumour heterogeneity, identify expression programs linked to progression or drug resistance, and characterise immune-cell composition, functional states, and tumour-immune interactions [2]. Compared with bulk RNA-seq, it resolves cell states rather than averaging signals across mixed populations, improves detection of rare cell types, and can reveal dynamic cellular programs. Its disadvantages include higher cost, more complex workflows, specialised computational requirements, and the need for sufficient sequencing depth to capture low-abundance transcripts [28]. Current platforms can be applied to fresh, frozen, and FFPE samples. In DLBCL, scRNA-seq has identified cancer stem cell-like B-cells and exhausted T-cell populations, clarified TME dynamics, and suggested mechanisms of immune escape and treatment resistance [2]. Integration with single-cell multiomics and spatial methods is expected to further refine mechanistic understanding and support precision medicine in lymphoma.

Spatial transcriptomics complements scRNA-seq by measuring gene expression while retaining information about where transcripts are located in the tissue. Platforms may be image based or sequencing based: the former relies on microscopic detection, whereas the latter uses sequencing to quantify spatially resolved transcripts. The GeoMx Digital Spatial Profiler (DSP; NanoString) is an established platform that can profile selected regions of interest in FF or FFPE tissue sections using probes that bind to transcripts which are next sequenced at the specific regions of interest [2]. In lymphoma research, Stewart *et al*. [29] used spatial profiling to identify recurrent tissue niches in classic HL lymph nodes that were enriched for mononuclear phagocytes and associated with a tumour-tolerant microenvironment and early treatment failure. This observation suggests potential applications in risk stratification and microenvironment-directed therapy. In DLBCL, Liu *et al*. [30] described eight spatially distinct tumour-associated macrophage subsets with different biological properties, including pro-tumour immunoregulatory signatures that may be therapeutically relevant.

Because spatial transcriptomic platforms can use FF and FFPE material, they may enable retrospective studies of archived lymphoma tissue and provide

spatially resolved single-cell-level information on lymphoma subtypes and the TME. Coupling these datasets with deep learning models could improve classification and help identify diagnostic, prognostic, or predictive markers. At present, however, single-cell and spatial technologies are not sufficiently standardised for routine clinical recommendations. Costs are high compared with immunohistochemistry or immunofluorescence, and preanalytical handling, sample preparation, image/region selection, and computational analysis remain technically demanding. In addition, section-based spatial methods may undersample tumour heterogeneity if the selected tissue area does not adequately represent the full lesion [2].

Conclusions

High-throughput NGS-based technologies have substantially improved our understanding of lymphoma heterogeneity, resistance mechanisms, prognostic biomarkers, and TME interactions. Nevertheless, clinical implementation remains constrained by practical limitations. Fresh surgical biopsies and liquid biopsies provide high-quality nucleic acids, but routine lymphoma diagnostics rely heavily on FFPE tissue and small biopsies; therefore, clinically useful assays must be robust in these materials. Targeted gene panels, WES, RNA-seq, and selected single-cell approaches are increasingly feasible in FFPE samples, whereas WGS remains more vulnerable to reduced data quality and fixation-related artifacts [5]. Liquid biopsy and ctDNA analysis are not replacements for tissue diagnosis, but they may complement it when biopsy is difficult to obtain or when serial monitoring is needed. The short half-life and minimally invasive nature of ctDNA make it suitable for dynamic assessment of tumour burden during and after therapy [21]. In patients with positive end-of-treatment (EOT) PET/CT, a negative ctDNA result has been associated with a low progression risk; accordingly, the National Comprehensive Cancer Network guidance supports considering ctDNA-based MRD testing when EOT PET/CT is positive and biopsy is not feasible before pursuing additional therapy [31].

Further obstacles include technical complexity, particularly for spatial transcriptomics, and the need for specialised downstream computational analysis. Standardisation is still required for sample processing, analytical pipelines, diagnostic thresholds, prognostic cut-offs, and quality-control measures. High-throughput assays also remain expensive and unevenly available, which limits broad clinical adoption. Future progress will depend on prospective clinical trials, interlaboratory collaboration, and consensus workshops to formulate standardised protocols for molecular assays with the aim of incorporating them into personalised treatment decisions in lymphoma.

Disclosures

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Address for correspondence

Magdalena Tyburczy
Institute of Hematology and Transfusion Medicine
Warsaw, Poland
e-mail: mtyburczy@ihit.waw.pl