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Immunohistochemical and Molecular Biomarkers in Diffuse Large B-Cell Lymphoma: A Systematic Review of Diagnostic, Prognostic, and Therapeutic Implications

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Systematic Review

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Abstract

Background

Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive B-cell non-Hodgkin lymphoma worldwide. Although R-CHOP immunochemotherapy has improved outcomes, relapse and refractory disease remain substantial challenges. Emerging immunohistochemical and molecular biomarkers may improve risk stratification and precision management. This systematic review synthesized evidence published between 2021 and 2026 on emerging biomarkers in DLBCL, focusing on their diagnostic, prognostic, predictive, and therapeutic relevance and whether they provide clinically meaningful information beyond conventional cell-of-origin classification and the International Prognostic Index.

Methods

PubMed/MEDLINE, Web of Science Core Collection, and Cochrane CENTRAL were systematically searched, supplemented by Consensus-guided literature discovery, grey-literature searching, and manual reference screening. Forty-three studies were synthesized narratively using the Synthesis Without Meta-analysis (SWiM) framework.

Results

Cell-of-origin classification remained clinically relevant, but did not consistently outperform the International Prognostic Index. Loss of BCL6 expression and a Ki-67 proliferation index above 70% showed independent prognostic value. Next-generation sequencing identified seven molecular subtypes with distinct biological and prognostic profiles. Tumour-microenvironment markers, including VSIG4- and CD163-positive macrophages, CD8-positive lymphocytes, CD11c-positive dendritic cells, and immune-checkpoint ligands, were associated with survival. Liquid-biopsy markers, particularly early molecular response assessed by circulating tumour DNA, demonstrated promising predictive value.

Conclusion

Integrated biomarker profiling combining immunohistochemistry, molecular classification, tumour-microenvironment characterization, and liquid-biopsy monitoring may enhance risk stratification and precision management of DLBCL. Prospective validation is required before routine clinical implementation.

1. Background

Diffuse large B-cell lymphoma (DLBCL), the major subtype of non-Hodgkin lymphoma (NHL), is responsible for about 30–40% of adult cases and the worldwide annual number of new cases is thought to be more than 150,000 (1–4). DLBCL features involve rapidly dividing large B cells and great clinical and biological heterogeneity (5). While the R-CHOP treatment plan (consisting of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) results in long-term remission for 60–65% of patients, about 30–40% will relapse or be refractory, and their survival outlooks are rather dismal, with a median overall survival (OS) of less than 12 months for those who are ineligible for transplant (5–8).

The molecular heterogeneity of DLBCL was first elucidated through landmark gene expression profiling (GEP) studies, which identified the two principal cell-of-origin (COO) subtypes: germinal centre B-cell-like (GCB), associated with a more favourable prognosis, and activated B-cell-like (ABC), associated with inferior survival following R-CHOP therapy (9, 10). Although these seminal studies predate the predefined 2021–2026 eligibility window and were therefore not included in the systematic review, they provide the biological framework upon which subsequent biomarker research has been built. The Hans immunohistochemistry (IHC) algorithm, relying on CD10, BCL6, BCL2, MYC and MUM1, later became the most popular method for COO determination (2, 11–19). Nonetheless, its agreement with GEP is only around 78–82% and recent multivariate

analyses indicate that IHC-based COO has no independent prognostic value beyond the International Prognostic Index (IPI) (13, 20).

Between 2021–2026, we saw significant progress in four major biomarker areas. To begin with, the dark zone signature (DZsig) which was originally described as the double-hit signature (DHITsig) and subsequently refined into the dark-zone signature (DZsig), has now been validated through large real-life cohorts and is able to detect a high-risk GCB-DLBCL subset (about 21% of GCB cases) having very poor prognosis (2-year OS 57%) which cannot be identified by the standard FISH-based double-hit test (21, 22). Secondly, the LymphPlex 38-gene NGS panel, which was developed based on the LymphGen algorithm, currently divides DLBCL into seven molecular subtypes that are distinct from each other MCD, BN2, EZB ± MYC, ST2, A53, N1 and TP53-mutant each of which is associated with distinct biological characteristics, prognostic outcomes, and potential therapeutic vulnerabilities. This classification has been tested on large patient cohorts of over 1,000 individuals (17, 23). Third, providing detailed profiling of the tumour microenvironment (TME) has led to the discovery that among different immune cells, only VSIG4^{+} macrophages, CD163^{+} tumour-associated macrophages, CD8^{+} and TIL-B tumour-infiltrating lymphocytes, CD11c^{+} dendritic cells, and immune checkpoint molecules (PD-L1, LAG3) are separate prognostic factors (13, 18, 24–30). Fourth, among liquid biopsy techniques ctDNA monitoring using the EuroClonality-NDC panel and extracellular vesicle (EV) profiling stand out as ways for non-invasive, real-time assessment of tumour load and minimal residual disease (MRD) (31, 32).

In addition, multi-omics studies have identified many new molecular biomarkers such as FCRL1, BAFF, SH3GL1/FTH1, SubSigLnc-17, RFC5, FLOT2, BEND4, and plasma-protein signatures using Mendelian randomisation (7, 34–44). These are potential biomarkers but they need to be validated thoroughly before they are used in a clinical setting (18, 34). In light of the above, significant clinical knowledge gaps remain regarding diagnostic accuracy, prognostic value, and therapeutic relevance. Accordingly, this systematic review evaluated emerging immunohistochemical and molecular biomarkers in DLBCL and assessed their diagnostic, prognostic, predictive, and therapeutic relevance. The review addressed three questions:

1. How do emerging biomarkers improve diagnostic accuracy and molecular subtyping compared with conventional immunohistochemistry?.
2. What additional prognostic information do these biomarkers provide beyond the International Prognostic Index in patients treated with R-CHOP?.
3. To what extent can biomarker-defined molecular subgroups inform therapeutic stratification and predict response to standard or targeted therapies?

2. Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement. The study protocol was registered on PROSPERO with registration no CD420261408617.

2.1 Eligibility Criteria

Eligible studies included:(1) adult patients (≥ 18 years) who had a histological diagnosis of DLBCL-NOS or variants of DLBCL recognised by WHO;(2) ≥ 1 immunohistochemical or molecular biomarker (genetic, transcriptomic, proteomic, or liquid biopsy, based) used for assessment;(3) outcomes of diagnostic (sensitivity, specificity, concordance with GEP), prognostic (OS, PFS, EFS), or treatment-response presented. Language and type of publications were limited to peer-reviewed articles in English (2021–2026). Paediatric-only studies, conference abstracts without a full article, case series < 20 patients, mixed-NHL studies without DLBCL-specific data, and non-original publications were excluded. Both retrospective and prospective cohort studies were eligible for inclusion. Systematic reviews and meta-analyses were consulted to identify additional relevant references and provide contextual background but were not included in the qualitative synthesis.

2.2 Information Sources and Search Strategy

A comprehensive literature search was conducted in PubMed/MEDLINE, Web of Science Core Collection, and the Cochrane Central Register of Controlled Trials (CENTRAL) to identify studies evaluating biomarkers in diffuse large B-cell lymphoma (DLBCL). To maximize identification of potentially relevant evidence, an additional literature discovery process was performed using the Consensus academic search platform through 28 iterative searches organised into six predefined biomarker domains: (i) biomarker landscape, (ii) cell of origin, (iii) molecular subtypes, (iv) tumour microenvironment signatures, (v) liquid biopsy platforms, and (vi) clinical application framework.

To minimize publication bias and identify supplementary evidence, grey literature was searched, including conference proceedings from the American Society of Hematology (ASH) and the European Hematology Association (EHA) published between 2021–2026. The reference lists of all included studies and relevant review articles were also screened manually to identify additional eligible publications not retrieved through the electronic searches. All searches were completed in March, 2026.

The PubMed/MEDLINE search strategy combined controlled vocabulary (Medical Subject Headings [MeSH]) with free-text keywords related to diffuse large B-cell lymphoma, biomarkers, molecular classification, liquid biopsy, tumour microenvironment, diagnosis, prognosis, and predictive biomarkers. Searches were restricted to studies published between 1 January, 2021 and March, 2026. The complete PubMed/MEDLINE search strategy is provided in Supplementary Material 1.

Equivalent search strategies were adapted for the Web of Science Core Collection and CENTRAL using database-specific indexing terms and search syntax while maintaining comparable concepts and eligibility limits. The Consensus platform was used solely as a supplementary literature discovery tool to identify potentially relevant studies that may not have been captured through conventional database searching.

2.3 Study Selection and Data Extraction

Two independent reviewers performed duplicate removal, title and abstract screening, and full-text eligibility assessment using Covidence. Disagreements between reviewers were resolved through discussion and, when necessary, adjudication by a third reviewer. Inter-reviewer agreement was excellent agreement (Cohen's $\kappa = 0.82$) Data extraction was performed using a standardized data collection form, including study characteristics, biomarker type, detection method, reference cut-off values, treatment regimen, disease stage, follow-up duration, and reported diagnostic, prognostic, or predictive outcomes together with corresponding 95% confidence intervals. The first reviewer extracted all data, and a second reviewer independently verified the extracted information

2.4 Risk of Bias and Evidence Certainty

Risk of bias was assessed independently using the Quality in Prognostic Studies (QUIPS) tool for prognostic biomarker studies and the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool for studies evaluating diagnostic performance. The overall methodological quality of the included studies is summarized in Table 1 and Fig. 1. Owing to substantial methodological heterogeneity in biomarker platforms, laboratory assays, study populations, outcome definitions, and study designs, quantitative meta-analysis was considered inappropriate. Findings were therefore synthesized narratively in accordance with the Synthesis Without Meta-analysis (SWiM) reporting guideline (38). Given the diversity of biomarkers and outcome measures, formal GRADE assessment of the overall certainty of evidence was not undertaken.

Table 1
Summary of Risk of Bias Assessment of Included Studies Using the QUIPS

QUIPS Domain	Overall Judgment	Summary of Assessment
Study participation	**Moderate risk**	Most studies enrolled clearly defined DLBCL populations; however, many were retrospective single-centre cohorts with relatively small sample sizes, limiting representativeness.
Study attrition	**Low risk**	Most studies reported complete survival analyses or clearly described follow-up, with little evidence of substantial loss to follow-up affecting outcome assessment.
Prognostic factor measurement	**Low risk**	Biomarkers were generally measured using validated laboratory techniques (e.g., immunohistochemistry, molecular profiling, transcriptomics, ctDNA assays, spatial transcriptomics), although assay standardization varied between studies.
Outcome measurement	**Low risk**	Clinical outcomes, including overall survival (OS), progression-free survival (PFS), event-free survival (EFS), and treatment response, were generally well defined using accepted clinical criteria.
Study confounding	**Moderate risk**	Adjustment for important prognostic variables (e.g., age, stage, IPI, treatment regimen, cell-of-origin) was inconsistent across studies, particularly in retrospective observational cohorts.
Statistical analysis and reporting	**Moderate risk**	Most studies used appropriate survival analyses (Kaplan–Meier and Cox regression), but several small exploratory and bioinformatics studies lacked comprehensive multivariable adjustment or external validation.
Overall risk of bias	**Low to Moderate risk**	Overall methodological quality was considered acceptable. Most studies demonstrated low risk in biomarker and outcome measurement but moderate risk related to retrospective study design, confounding, and statistical reporting.

2.5 Study Selection Flow

Overall, 2,731 records were identified, including 2,462 records retrieved from the three electronic databases (PubMed/MEDLINE, Web of Science Core Collection, and Cochrane CENTRAL) and 269 additional records identified through supplementary searches, comprising the Consensus academic search platform, grey literature, conference proceedings, manual reference screening, and other supplementary sources. After removal of 1,105 duplicates, 1,626 unique records underwent title and abstract screening. A total of 1,328 records were excluded at this stage, leaving 298 articles for full-text eligibility assessment. Following full-text review, 255 articles were excluded for prespecified reasons, and 43 studies fulfilled the eligibility criteria and were included in the qualitative synthesis (Fig. 2).

3. Results

3.1 Characteristics of Included Studies

A total of 43 studies met the eligibility criteria and were included in the qualitative synthesis. The evidence base was dominated by retrospective cohort studies (n = 27), alongside six prospective cohort studies and one randomized phase II clinical trial. Across these study designs, eight studies incorporated translational molecular analyses, while two primarily used bioinformatics approaches with subsequent clinical validation. The studies originated from 18 countries, with the largest contributions from China, followed by the United States, Canada, Japan, and several European nations. Collectively, the primary clinical cohorts included approximately 11,498 patients with diffuse large B-cell lymphoma (DLBCL), with sample sizes ranging from 30 to 1,194 participants (median, 157). Most studies evaluated patients treated with R-CHOP or R-CHOP-like immunochemotherapy, whereas translational investigations incorporated complementary genomic, transcriptomic, spatial transcriptomic, and liquid biopsy platforms. The biomarkers investigated encompassed immunohistochemical markers, molecular genetic subtypes, tumour microenvironment signatures, circulating tumour DNA (ctDNA), extracellular vesicles, gene-expression signatures, artificial intelligence-assisted pathology, and integrated multi-omics approaches (Table 2).

Reported follow-up durations ranged from 18 months to 8 years, with overall survival (OS) and progression-free survival (PFS) representing the most frequently reported clinical outcomes.

Table 2
Novel and Emerging Biomarkers in Diffuse Large B-Cell Lymphoma Reported Between 2021–2026

Biomarker	Classification	Biomarker Category	Biological/Clinical Role	Potential Clinical Application	Level of Evidence	Representative Study
SH3GL1	Novel	Ferroptosis-related gene	Regulates ferroptosis and tumour progression	Prognostic risk stratification	Moderate	Duan et al. (2025)
FTH1	Novel	Ferroptosis-related gene	Iron metabolism and oxidative stress	Prognostic biomarker	Moderate	Duan et al. (2025)
RFC5	Novel	DNA replication gene	Promotes cell-cycle progression and proliferation	Prognostic biomarker	Low–Moderate	Tian et al. (2025)
FLOT2	Novel	Membrane signalling protein	Facilitates tumour growth and invasion	Prognostic biomarker	Low	Zhang et al. (2024)
BEND4	Novel	Epigenetic regulator	Chromatin regulation and tumour progression	Prognostic biomarker	Low	Wang et al. (2025)
BAFF mRNA	Novel	Immune transcriptomic marker	B-cell activation and survival signalling	Diagnostic and prognostic biomarker	Moderate	Al-Amodi et al. (2025)
FCRL1 mRNA	Novel	Immune receptor transcript	B-cell receptor regulation	Diagnostic and prognostic biomarker	Moderate	Al-Amodi et al. (2025)
VSIG4	Emerging	Tumour microenvironment	Tumour-associated macrophage marker mediating immune suppression	Prognostic biomarker	Moderate	Ma et al. (2025)
PD-L1⁺ extracellular vesicles	Emerging	Liquid biopsy	Reflects systemic tumour immune evasion	Diagnostic and prognostic biomarker	Moderate	Li et al. (2021)
Tumour-informed ctDNA	Emerging	Liquid biopsy	Dynamic monitoring of tumour burden	MRD assessment and treatment monitoring	Moderate	Narkhede et al. (2024)
ctDNA molecular clustering	Emerging	Liquid biopsy	Molecular subtype identification using plasma DNA	Prognostic stratification	High	Moia et al. (2025)

Abbreviations: AI: artificial intelligence; ARID1A: AT-rich interaction domain-containing protein 1A; BAFF: B-cell activating factor; circulating tumour DNA (ctDNA); double-hit signature (DHITsig); diffuse large B-cell lymphoma (DLBCL); dark-zone signature (DZsig); extracellular vesicle (EV); FN1: fibronectin-1; FCRL1: Fc receptor-like protein 1; IPI-G, International Prognostic Index–Genetic; LYZ: lysozyme; MRD: minimal residual disease; PD-L1, programmed death-ligand 1; TIL-B, tumour-infiltrating B cell; VSIG4, V-set and immunoglobulin domain-containing protein 4.

***Level of evidence** reflects the overall strength of evidence within the included studies based on study design, validation, and consistency and is intended as a narrative synthesis rather than a formal GRADE assessment.

Biomarker	Classification	Biomarker Category	Biological/Clinical Role	Potential Clinical Application	Level of Evidence	Representative Study
LymphType	Emerging	Molecular subtyping	Refined genetic classification of DLBCL	Prognostic modelling	Moderate	Mi et al. (2025)
LymphPlex	Emerging	Molecular subtyping	Genetic subtype-guided precision classification	Prognostic and predictive biomarker	High	Shen et al. (2023); Zhang et al. (2023)
DZsig / DHITsig	Emerging	Molecular profiling	Identification of dark-zone biology and high-risk disease	Risk stratification	High	Alduaij et al. (2023); Urata et al. (2023)
MacroSig (Macrophage spatial signatures)	Emerging	Spatial transcriptomics	Characterises macrophage spatial organisation	Prognostic biomarker	Moderate	Liu et al. (2024)
Spatial immune niches	Emerging	Spatial biology	Defines immune-cell architecture within the tumour microenvironment	Prognostic biomarker	Moderate	Dai et al. (2025)
Spatial immune-cell interactions	Emerging	Spatial biology	Evaluates cellular cross-talk within the tumour microenvironment	Prognostic biomarker	Moderate	Autio et al. (2022, 2025)
LYZ⁺ fibroblasts	Emerging	Spatial transcriptomics	Stromal remodelling and immune regulation	Prognostic biomarker	Low–Moderate	Dai et al. (2025)
FN1⁺ macrophages	Emerging	Spatial transcriptomics	Macrophage-mediated extracellular matrix remodelling	Prognostic biomarker	Low–Moderate	Dai et al. (2025)
Tumour-infiltrating B cells (TIL-Bs)	Emerging	Tumour microenvironment	Anti-tumour immune response	Prognostic biomarker	Moderate	Xu-Monette et al. (2023)
CCL8	Emerging	Tumour microenvironment	Macrophage recruitment and immune modulation	Prognostic biomarker	Moderate	Lou et al. (2022)
ARID1A-integrated signature	Emerging	Multi-omics	Integrates genomic and transcriptomic alterations	Prediction of early treatment failure	High	Wenzl et al. (2024)
Integrated transcriptomic signature	Emerging	Multi-omics	Multi-dimensional molecular risk prediction	Prognostic biomarker	Moderate	Li et al. (2026)

Abbreviations: AI: artificial intelligence; ARID1A: AT-rich interaction domain-containing protein 1A; BAFF: B-cell activating factor; circulating tumour DNA (ctDNA); double-hit signature (DHITsig); diffuse large B-cell lymphoma (DLBCL); dark-zone signature (DZsig); extracellular vesicle (EV); FN1: fibronectin-1; FCRL1: Fc receptor-like protein 1; IPI-G, International Prognostic Index–Genetic; LYZ: lysozyme; MRD: minimal residual disease; PD-L1, programmed death-ligand 1; TIL-B, tumour-infiltrating B cell; VSIG4, V-set and immunoglobulin domain-containing protein 4.

***Level of evidence** reflects the overall strength of evidence within the included studies based on study design, validation, and consistency and is intended as a narrative synthesis rather than a formal GRADE assessment.

Biomarker	Classification	Biomarker Category	Biological/Clinical Role	Potential Clinical Application	Level of Evidence	Representative Study
9-lncRNA signature	Emerging	Transcriptomics	Long non-coding RNA-based prognostic model	Prognostic biomarker	Moderate	Wang et al. (2022)
AI-based PD-L1 quantification	Emerging	Artificial intelligence	Automated digital pathology for biomarker assessment	Diagnostic and prognostic support	Moderate	Yan et al. (2024)

Abbreviations: AI: artificial intelligence; ARID1A: AT-rich interaction domain-containing protein 1A; BAFF: B-cell activating factor; circulating tumour DNA (ctDNA); double-hit signature (DHITsig); diffuse large B-cell lymphoma (DLBCL); dark-zone signature (DZsig); extracellular vesicle (EV); FN1: fibronectin-1; FCRL1: Fc receptor-like protein 1; IPI-G, International Prognostic Index–Genetic; LYZ: lysozyme; MRD: minimal residual disease; PD-L1, programmed death-ligand 1; TIL-B, tumour-infiltrating B cell; VSIG4, V-set and immunoglobulin domain-containing protein 4.

***Level of evidence** reflects the overall strength of evidence within the included studies based on study design, validation, and consistency and is intended as a narrative synthesis rather than a formal GRADE assessment.

3.2 Immunohistochemical Biomarkers and Cell-of-Origin (COO)

The Hans algorithm is still the most widely used IHC method worldwide for the COO classification because it is low-cost and available in almost all laboratories (15). Across several studies, patients with GCB-DLBCL generally demonstrated modestly better survival than those with ABC/non-GCB disease.Strong multivariate analyses including a 425-patient population-based cohort and a 928-patient registry study show that IHC-based COO is not an independent predictor of survival beyond the IPI which limits its clinical utility without molecular subtyping (29, 39). Of the IHC markers, Only two markers consistently demonstrated independent prognostic value: loss of BCL6 which predicts worse OS/EFS regardless of COO; Ki-67 > 60, 70%, linked to worse OS/EFS (2, 13). Other markers have effects depending on the context: MUM1/IRF4 is often but not always linked to poor outcomes (8, 16, 19). CD5 positivity delineates a very aggressive variant with more extranodal disease, CNS relapse, and worse survival. As per WHO-HAEM5 (2022), MYC and BCL2/BCL6 rearrangement testing is required when MYC IHC is > 40%, in recognition of the fact that IHC is a poor surrogate for molecular classification (45). Although conventional IHC remains widely used, NanoString Lymph2Cx assay is a method of COO classification by GEP from FFPE samples which is getting more and more use by academic centers.

3.2.1 Dark Zone Signature (DZsig) : A High-Risk GCB Subgroup

One of the most significant developments from 2021–2026 is the confirmation of the dark zone signature (DZsig) as a separate, high-risk subgroup within GCB-DLBCL. Originally, described as the double-hit signature (DHITsig) and subsequently refined as the dark-zone signature (DZsig). to highlight the biological similarity to dark zone germinal centre B cells (21). In a British Columbia study of 1,149 unselected patients, DZsig was found in 21% of GCB cases and these patients had a two-year OS of just 57%, whereas the figure was 89% for DZsig-negative GCB and 71% for ABC (21). What's more, ≈ 60% of DZsig-positive tumours did not have MYC/BCL2 rearrangements by FISH, so conventional double-hit testing would have missed these high-risk patients. DZsig was linked to MYC/BCL2 protein co-expression and decreased microenvironmental infiltration. The presence of DZsig was confirmed in a Japanese cohort of 1,050 patients using the NanoString DLBCL 90 assay, who found 6% DZsig-positive cases with a two-year OS of 66%, which also supports cross-ethnic reproducibility (22). These discoveries have set the scene for considering DZsig as a component of future trial stratification and treatment-intensification strategies.

3.2.2 Double-Expressor Lymphoma (DEL) and Double-Hit/Triple-Hit Disease

The co-expression of MYC (> 40–75%) and BCL2 (> 50–85%) identifies double-expressor lymphoma (DEL), which occurs in about 25–30% of newly diagnosed DLBCL. DEL is a strong predictor of worse survival after R-CHOP, with HR around 1.5-2.0 based on strict IHC cutoffs (22). DEL is more common in ABC/non-GCB tumours and genetic backgrounds resembling MCD. Using IHC for rapid screening of cases suitable for FISH is a practical strategy: MYC IHC > 40% level has about 80% sensitivity

for MYC rearrangements. The cases confirmed by FISH as double-hit (HGBCL-DH-BCL2) and triple-hit lymphomas are the groups with the worst prognosis among DLBCL, with median OS of 12–18 months under R-CHOP (47, 48). The latest important breakthrough is a decision-tree model that combines MYC/BCL2 IHC with CD11c+ dendritic cell density, which yields 84% sensitivity and 79% specificity for predicting the DH/TH genotype, much better than IHC alone (27). This represents a feasible, cost-efficient strategy for laboratories without routine FISH capability.

3.3 Molecular Classification Using Next-Generation Sequencing (NGS)

Recent NGS studies have enabled classification of DLBCL into biologically defined molecular subtypes that extend beyond conventional cell-of-origin (COO) classification. Two major approaches, LymphGen/LymphPlex and the Haematological Malignancy Research Network (HMRN) classifier, show reasonable concordance across independent datasets and molecular panels (23).

3.3.1 LymphGen/LymphPlex: Seven Genetic Subtypes

The LymphPlex 38-gene classifier, developed using whole-exome/whole-genome and RNA-sequencing data from 337 newly diagnosed DLBCL cases and subsequently validated in 1,001 additional cases, identified seven reproducible genetic subtypes (23). The TP53-mutant subtype was characterized by disruption of the p53 pathway, PI3K activation, and immune-deficiency signatures and exhibited the poorest survival, with a reported 5-year overall survival (OS) of approximately 30–35%. The MCD-like subtype, defined by MYD88^L265P, CD79B, PIM1, and IRF4 alterations, accounted for approximately 14% of cases and was characterized by chronic active B-cell receptor signalling and NF-κB activation. It was predominantly derived from the activated B-cell (ABC) group and was frequently associated with extranodal disease, particularly primary central nervous system and sinonasal DLBCL. MYD88 mutations occur in up to 74% of such cases, frequently with concurrent CD79B mutations (23). This biological profile provides a rationale for the development of Bruton tyrosine kinase (BTK) inhibitor-based regimens. The MCD-like subtype had a reported 5-year OS of approximately 35–40%.

The BN2-like subtype, characterized by BCL6 fusions and NOTCH2, CD70, and TNFAIP3 mutations, accounted for approximately 22% of cases. Its biological features resemble those of marginal-zone lymphoma, and it is largely ABC-derived; within the ABC group, it has been associated with relatively favorable outcomes (23). The EZB-like subtype, characterized by BCL2 fusions and EZH2, TNFRSF14, and KMT2D alterations, accounted for approximately 19% of cases and was predominantly GCB-derived, including many cases of HGBCL-DH-BCL2. EZB-MYC-positive tumours have been associated with an immunosuppressive microenvironment characterized by increased PD-L1 expression and reduced tumour-infiltrating lymphocyte density, suggesting a potential rationale for checkpoint-inhibitor combinations. Conversely, EZH2-mutant, MYC-negative EZB tumours may represent candidates for tazemetostat-based strategies (23).

The ST2-like subtype, characterized by SGK1, TET2, SOCS1, and DDX3X alterations, accounted for approximately 8% of cases and was GCB-derived, associated with a stromal-1 programme, and characterized by the most favourable prognosis, with a reported 5-year OS of approximately 80–85%. The N1-like subtype, defined primarily by NOTCH1 alterations, accounted for approximately 3% of cases, was predominantly ABC-derived, and was associated with poor prognosis. Cases that could not be genetically classified were designated molecular NOS and represented up to approximately 50% in some series.

Subtype-specific therapeutic strategies represent an important potential application of molecular classification because tumour genotype may influence biological phenotype and treatment sensitivity. Supporting this approach, the GUIDANCE-01 trial (NCT04025593) evaluated subtype-matched targeted agents combined with R-CHOP and reported improved antitumour activity in MCD and EZB cohorts using the predecessor 20-gene GenClass algorithm (23, 47). The HMRN classification independently identified clinically distinct groups, including MYD88-mutant groups associated with poor prognosis, NOTCH2-mutant groups with intermediate prognosis, BCL2-rearranged groups with favourable prognosis, and a SOCS1/SGK1 group with PMBCL-like features and excellent outcomes (39). However, some smaller studies have not demonstrated prognostic value of genetic subtype independent of the IPI, reinforcing the need for adequately powered, uniformly treated cohorts for biomarker validation.

3.4 Tumour Microenvironment (TME) Biomarkers

The tumour microenvironment (TME) in DLBCL influences prognosis through mechanisms that extend beyond COO classification and genetic subtyping. The composition of stromal and immune-cell populations has been associated with patient survival across several independent cohorts. A conserved stromal/fibroblast-associated gene signature has been associated with better OS and may reveal intratumoural heterogeneity not captured by existing classifiers, while high densities of CD8 + and CD4 + tumour-infiltrating lymphocytes (TILs) have been associated with improved survival at both the protein and transcript levels (49–51).

Conversely, a predominance of CD163 + tumour-associated macrophages (TAMs) has been consistently associated with poorer outcomes and has subsequently been characterized using spatial transcriptomics, which has identified distinct macrophage gene-expression programmes (MacroSigs) with independent prognostic significance for OS (52). Immune-checkpoint interactions further modulate the TME. Autio et al. identified a lymphoma microenvironment subtype characterized by increased PD-L1 and TIM-3 expression on CD163 − macrophages at the macrophage–T-cell interface and associated with poor prognosis, suggesting that spatial context, rather than marker positivity alone, contributes to checkpoint-associated prognostic effects (53).

Multimodal spatial transcriptomic analyses have further identified spatially distinct immune niches with different T-cell and macrophage compositions, whose organization independently predicts survival in ways that may not be detected by bulk transcriptomic approaches (54). Collectively, these findings suggest that an immune-hot, stromal-preserved TME may be associated with more favourable outcomes, whereas macrophage-dominant, checkpoint-engaged, and spatially disorganized immune microenvironments characterize higher-risk DLBCL.

3.5 Circulating Biomarkers (ctDNA and Liquid Biopsy)

Circulating tumour DNA (ctDNA) has emerged as one of the most promising non-tissue prognostic biomarkers in DLBCL. High baseline ctDNA concentration (≥ 2.5 log hGE/mL) was associated with shorter progression-free survival (PFS) and OS independently of IPI, COO, and genetic subtype (55). Early molecular response (EMR), defined by ctDNA clearance after the first treatment cycle, was independently associated with improved event-free survival (EFS) and OS and outperformed the revised IPI in multivariable analysis. EMR also predicted complete response approximately 97 days earlier than standard imaging assessment (56).

End-of-treatment minimal residual disease (MRD) detected by PhasED-Seq in a cohort of 150 patients was strongly associated with inferior 2-year PFS (28% vs 88%; HR, 9.7; $p < 0.0001$) and OS (50% vs 97%; HR, 10.6; $p < 0.0001$) (57). Similarly, combining ctDNA molecular remission (MMR; ≥2.5-log reduction) after cycle 2 with interim PET/CT produced a three-tier risk stratification with 2-year PFS rates of 84%, 17%, and 0%, respectively ($p < 0.001$), significantly outperforming either approach alone (41).

3.6 Integrated Prognostic Models

Many researchers have suggested a combined model that includes clinical, molecular, and tumour microenvironment (TME) markers. Models holding ctDNA + genetic subtype, DZsig + microenvironmental signature, or NGS subtype + MTV had the greatest prognostic accuracy and surpassed IPI and COO in all the comparative analyses (55). Refining COO with the DZsig and integrating genomic, clinical models (molecular subtype + IPI) can better differentiate risk and pinpoint high-risk GCB-DLBCL cases that standard DEL/FISH definitions miss (58, 59). Integrated models are still being recommended more and more for future trials but they are not yet incorporated into standard practice due to issues of cost and availability.

4. Discussion

This systematic review of 43 studies published between 2021 and 2026 demonstrates a major shift in DLBCL biomarker research from a predominantly binary immunohistochemistry (IHC)-based GCB/ABC classification toward an integrated framework incorporating dark-zone signature (DZsig), NGS-based LymphGen/LymphPlex molecular subtyping, quantitative tumour microenvironment (TME) profiling, and circulating tumour DNA (ctDNA)-based minimal residual disease (MRD) monitoring. Five principal findings emerged from the four major biomarker domains evaluated. First, multivariate studies consistently showed that IHC-based cell-of-origin (COO) determination using the Hans algorithm does not provide prognostic

information beyond the International Prognostic Index (IPI), whereas BCL6 and Ki-67 remain among the more consistently informative IHC markers (2, 13). Therefore, COO classification may be more appropriately considered alongside molecular and TME characteristics rather than used as a sole prognostic biomarker.

Second, DZsig identifies approximately 21% of GCB-DLBCL patients who are FISH-negative but have outcomes comparable to those of patients with HGBCL-DH-BCL2, with a reported 2-year overall survival (OS) of 57% (21, 22). These findings suggest that a clinically important subset of patients classified as standard-risk GCB-DLBCL by conventional approaches may harbour biologically high-risk disease and could potentially be considered for biomarker-guided clinical trials. Third, LymphGen/LymphPlex molecular subtyping provides biological and potentially therapeutic information that cannot be adequately captured by conventional IHC or FISH. MCD-like disease may identify patients who could benefit from BTK inhibitor-containing regimens, whereas EZB-MYC-positive tumours may be candidates for immune-checkpoint-based strategies, and EZH2-mutant EZB tumours may represent potential candidates for tazemetostat-based approaches. TP53-mutated/A53 disease may similarly warrant evaluation of novel targeted or combination therapies. Evidence from multiple cohorts, including the GUIDANCE-01 trial, supports the potential value of genetic subtype-guided treatment selection and clinical-trial stratification (23, 39, 47).

Fourth, ctDNA-based molecular MRD assessment after two cycles of R-CHOP emerged as one of the strongest prognostic biomarkers identified in this review. Molecular response was associated with marked differences in 2-year progression-free survival (PFS), with reported rates of 76% versus 0% according to molecular response status (41). Integration of ctDNA molecular response with interim PET/CT further improved risk discrimination, producing three distinct risk groups with 2-year PFS rates of 84%, 17%, and 0% (41). These findings support the incorporation of ctDNA into prospective biomarker-adaptive treatment strategies. Fifth, VSIG4-positive tumour-associated macrophages emerged as a consistently adverse biomarker and a potential macrophage-directed therapeutic target (18). In addition, PD-L1-positive extracellular vesicles may provide a functional surrogate of checkpoint activity in patients with negative tissue IHC, potentially expanding the population identifiable through circulating checkpoint-related biomarkers (31). However, the clinical utility of these approaches requires further prospective validation. Overall, the findings indicate that no single biomarker adequately captures the biological and clinical heterogeneity of DLBCL. Instead, integration of conventional IHC, molecular classification, TME characteristics, and dynamic liquid-biopsy biomarkers may provide a more informative framework for risk stratification and treatment selection.

The principal limitation of the evidence base was the predominance of retrospective and observational studies, which limits causal inference. Considerable heterogeneity in biomarker assays, antibody clones, IHC cut-offs, molecular platforms, outcome definitions, and statistical modelling approaches also prevented quantitative meta-analysis for most biomarker-outcome relationships. Consequently, the findings were synthesized narratively in accordance with the SWiM framework. Publication and language restrictions may have introduced bias and potentially resulted in overestimation of biomarker effects. In addition, changes associated with the WHO-HAEM5 classification during the review period may have contributed to diagnostic and classification heterogeneity. The generalizability of the evidence is also limited because most included studies were conducted in academic centres in high-income countries. This may restrict the applicability of emerging biomarker strategies to resource-constrained settings where access to NGS, advanced IHC panels, ctDNA testing, and other molecular platforms remains limited. Several emerging biomarkers, including VSIG4, FCRL1, SubSigLnc-17, SH3GL1/FTH1, RFC5, FLOT2, and BEND4, were primarily evaluated in single-centre or relatively small studies and have not yet undergone sufficient independent multicentre validation. Accordingly, these biomarkers should currently be regarded as hypothesis-generating rather than practice-changing.

Five major priorities should guide future research. Prospective ctDNA-adaptive trials, including SAKK 38/19, SHORTEN-ctDNA, and LymphPlex-guided GUIDANCE-02, are needed to establish whether biomarker-informed treatment improves outcomes. International harmonization of IHC cut-offs, NGS panels, DZsig platforms, and ctDNA methods is essential for reproducibility. Research in low- and middle-income countries should identify biomarker combinations that are clinically meaningful and feasible in resource-constrained settings. Integrated models combining IHC, molecular subtyping, TME profiling, and ctDNA should be evaluated for improved clinical decision-making. Finally, emerging targets, particularly VSIG4-associated macrophage pathways and SH3GL1/FTH1 ferroptosis, require further investigation to establish their biological relevance and therapeutic potential.

Evidence reviewed from 2021 to 2026 indicates that DLBCL biomarker research is moving toward a hierarchical, multimodal approach. COO classification provides limited prognostic information beyond IPI but may be complemented by BCL6, Ki-67, DZsig, and LymphGen/LymphPlex subtyping. DZsig identifies high-risk GCB-DLBCL subsets missed by FISH, while MCD and TP53-mutant subtypes may support targeted therapy. VSIG4-positive tumour-associated macrophages represent an adverse biomarker and potential therapeutic target, whereas ctDNA response after two R-CHOP cycles provides prognostic value and may facilitate adaptive treatment. PD-L1-positive extracellular vesicles may extend circulating biomarker utility beyond IHC. Standardization, prospective validation, improved access, and multimodal integration are essential for precision DLBCL care.

Declarations

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References

1. Papageorgiou SG, Thomopoulos TP, Katagas I, Bouchla A, Pappa V (2021) Prognostic molecular biomarkers in diffuse large B-cell lymphoma in the rituximab era and their therapeutic implications. Ther Adv Hematol 12:20406207211013987. 10.1177/20406207211013987
2. Balikó A, Szakács Z, Kajtár B, Ritter Z, Gyenesei A, Farkas N et al (2023) Clinicopathological analysis of diffuse large B-cell lymphoma using molecular biomarkers: a retrospective analysis from 7 Hungarian centers. Front Oncol 13:1224733. 10.3389/fonc.2023.1224733
3. Berhan A, Almaw A, Damtie S, Solomon Y (2025) Diffuse large B cell lymphoma (DLBCL): epidemiology, pathophysiology, risk stratification, advancement in diagnostic approaches and prospects: narrative review. Discov Oncol 16(1):184. 10.1007/s12672-025-01958-w
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al (2021) Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3):209–249. 10.3322/caac.21660
5. Sehn LH, Salles G (2021) Diffuse large B-cell lymphoma. N Engl J Med 384(9):842–858. 10.1056/NEJMra2027612
6. Liang XJ, Song XY, Wu JL, Liu D, Lin BY, Zhou HS et al (2022) Advances in multi-omics study of prognostic biomarkers of diffuse large B-cell lymphoma. Int J Biol Sci 18(4):1313–1327. 10.7150/ijbs.67892
7. Duan ZW, Wang WT, Wang Y, Wang R, Hua W, Shang CY et al (2025) SH3GL1-activated FTH1 inhibits ferroptosis and confers doxorubicin resistance in diffuse large B-cell lymphoma. Clin Transl Med 15(3):e70246. 10.1002/ctm2.70246
8. Zerehpoosh F, Farahmandfar M, Sharifi G, Rezaei O, Gachkar L (2025) Immunohistochemical evaluation of CD10, BCL6, BCL2, MUM1 and MYC in diffuse large B-cell brain lymphoma: diagnostic and prognostic significance. Immunopathol Persa 11(2):e34472. 10.34172/ipp.2025.34472
9. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A et al (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403(6769):503–511. 10.1038/35000501
10. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI et al (2002) The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346(25):1937–1947. 10.1056/NEJMoa012914
11. Okap I, Tayel H, El-Latif A, Nafea D, Mashali N (2023) Correlation between germinal center differentiation and double/triple hit score in the classification of diffuse large B-cell non-Hodgkin lymphoma: a clinicopathological approach. Asian Pac J Cancer Care 8(1):77–82. 10.31557/APJCC.2023.8.1.77-82
12. Dhar L, Singh S, Jain SL, Vindal A, Sinha P, Gautam R (2023) Cell of origin classification of diffuse large B-cell lymphoma. J Microsc Ultrastruct 12(4):193–198. 10.4103/jmau.jmau_66_22

13. Halcu G, Evsei-Seceleanu A, Tapoi DA, Cerbu M, Barta C, Ceausu MC (2025) Correlations between immunophenotypic markers and clinical progression in Romanian patients diagnosed with diffuse large B-cell lymphoma. Med (Kaunas) 61(6):948. 10.3390/medicina61060948
14. Yimpak P, Bumroongkit K, Tantiworawit A, Rattanathammethee T, Aungsuchawan S, Daroontum T (2024) Immunohistochemistry-based investigation of MYC, BCL2, and Ki-67 protein expression and their clinical impact in diffuse large B-cell lymphoma in upper Northern Thailand. PLoS ONE 19(7):e0307253. 10.1371/journal.pone.0307253
15. Ananthamurthy A, Murali M (2024) An immunohistochemical study of diffuse large B-cell lymphoma with molecular subtyping based on Hans algorithm. Indian J Pathol Microbiol 67(3):564–568. 10.4103/ijpm.ijpm_683_22
16. Yozgat A, Kasapoğlu B, Akyürek N, Üner A (2021) Expression of CD10, BCL-6 and MUM-1 markers and their effects on prognosis in diffuse large B-cell lymphoma. Acta Oncol Turc 54(2):189–197. 10.5505/aot.2021.35403
17. Shimkus G, Nonaka T (2023) Molecular classification and therapeutics in diffuse large B-cell lymphoma. Front Mol Biosci 10:1124360. 10.3389/fmolb.2023.1124360
18. Xu-Monette ZY, Wei L, Fang X, Au Q, Nunns H, Nagy M et al (2022) Genetic subtyping and phenotypic characterization of the immune microenvironment and MYC/BCL2 double expression reveal heterogeneity in diffuse large B-cell lymphoma. Clin Cancer Res 28(5):972–983. 10.1158/1078-0432.CCR-21-2949
19. Ahmed AM, Abdel-Hakeem SS, Amine MAF, Yassin EH, Badary FAM (2021) Prognostic value of CD10, BCL6 and MUM1 in diffuse large B-cell lymphoma. SVU Int J Med Sci 4(2):128–140. 10.21608/svuijm.2021.82242.1185
20. Miljkovic M, Dragos VS, Gasljevic G, Novakovic S, Boltezar L, Novakovic BJ (2025) Are there clinically relevant prognostic factors in diffuse large B-cell lymphoma beyond International Prognostic Index? Radiol Oncol 59(4):607–616. 10.2478/raon-2025-0028
21. Alduaij W, Collinge B, Ben-Neriah S, Jiang A, Hilton LK, Boyle M et al (2023) Molecular determinants of clinical outcomes in a real-world diffuse large B-cell lymphoma population. Blood 141(20):2493–2507. 10.1182/blood.2022018248
22. Urata T, Naoi Y, Jiang A, Boyle M, Sunami K, Imai T et al (2023) Distribution and clinical impact of molecular subtypes with dark zone signature of DLBCL in a Japanese real-world study. Blood Adv 7(24):7459–7470. 10.1182/bloodadvances.2023010402
23. Shen R, Fu D, Dong L, Zhang MC, Shi Q, Shi ZY et al (2023) Simplified algorithm for genetic subtyping in diffuse large B-cell lymphoma. Signal Transduct Target Ther 8(1):145. 10.1038/s41392-023-01358-y
24. Ma D, Wang Y, Shen Q, Liu X, Lu W, Li S et al (2025) VSIG4 as a tumor-associated macrophage marker predicting adverse prognosis in diffuse large B-cell lymphoma. Front Immunol 16:1567035. 10.3389/fimmu.2025.1567035
25. Xie YL, Ke LF, Zhang WW, Kang F, Lu SY, Wu CY et al (2025) Comprehensive analysis of tumor microenvironment and PD-L1 expression associations with clinicopathological features and prognosis in diffuse large B-cell lymphoma. Blood Lymphat Cancer 15:167–179. 10.2147/BLCTT.S545717
26. Syarifuddin F, Lubis AM, Harahap AS, Shatri H, Rajabto W, Subekti I et al (2026) Prognostic role of absolute monocyte count, $CD163^+$ macrophages, and $CD8^+$ lymphocytes in diffuse large B-cell lymphoma treated with R-CHOP. F1000Res 14:814. 10.12688/f1000research.168760.2
27. Yuan CT, Chuang SS, Cheng PY, Chang K, Wang H, Tsai JH et al (2022) Decreased CD11c-positive dendritic cells in the tumor microenvironment predict double-hit/triple-hit genotype and survival in diffuse large B-cell lymphoma. J Pathol Clin Res 8(5):436–447. 10.1002/cjp2.283
28. William MI, Tantawy DA, Elsergany AR, El-Hawary AK, Yussif SM (2025) Immunohistochemical expression of PD1, LAG3, and CTLA4 in diffuse large B cell lymphoma, clinicopathological correlation, and prognostic value. J Egypt Natl Cancer Inst 37(1):47. 10.1186/s43046-025-00303-0
29. Li JW, Shi D, Wan XC, Hu J, Su YF, Zeng YP et al (2021) Universal extracellular vesicles and PD-L1 + extracellular vesicles detected by single molecule array technology as circulating biomarkers for diffuse large B cell lymphoma. Oncoimmunology 10(1):1995166. 10.1080/2162402X.2021.1995166
30. Yan F, Da Q, Yi H, Deng S, Zhu L, Zhou M et al (2024) Artificial intelligence-based assessment of PD-L1 expression in diffuse large B cell lymphoma. NPJ Precis Oncol 8:76. 10.1038/s41698-024-00577-y

31. Aghayan AH, Bayani A, Abbaspour M, Gharehchahi F, Soleimani SH (2025) Extracellular vesicle-based biomarkers in diffuse large B-cell lymphoma: a systematic review and meta-analysis. Crit Rev Oncol Hematol 215:104884. 10.1016/j.critrevonc.2025.104884
32. Decruyenaere P, Daneels W, Morlion A, Verniers K, Anckaert J, Tavernier J et al (2024) Characterizing the cell-free transcriptome in a humanized diffuse large B-cell lymphoma patient-derived tumor xenograft model for RNA-based liquid biopsy in a preclinical setting. Int J Mol Sci 25(18):9982. 10.3390/ijms25189982
33. Tian Z, Liu S, Weng C, Ren M (2025) Identification of RFC5 as a potential prognostic biomarker in diffuse large B-cell lymphoma: a combined bioinformatics and immunohistochemical study. Discov Oncol 17(1):65. 10.1007/s12672-025-04199-z
34. Zhang Y, Chen Y, Guo Q, Zhang Y, Liu A (2024) Analysis and investigation of bioinformatics and epigenetics reveal the underlying mechanisms by which FLOT2 modulates the progression of diffuse large B-cell lymphoma. Discov Med 36(182):621–631. 10.24976/Discov.Med.202436182.59
35. Wang Y, Zhang Z, Xi L, Wang H, Dong F (2025) BEND4: a novel prognostic biomarker in diffuse large B-cell lymphoma. Discov Oncol 16:674. 10.1007/s12672-025-02519-x
36. Al-Amodi HS, Bedair HM, Gohar S, Mohamed DA, Abd El Gayed EM, Nazih M et al (2025) FCRL1 and BAFF mRNA expression as novel diagnostic and prognostic biomarkers in diffuse large B-cell lymphoma: expression signatures predict R-CHOP therapy response and survival. Int J Mol Sci 26(3):1269. 10.3390/ijms26031269
37. Yousefı Z, Sharifzadeh S, Zare F, Eskandari N (2023) Fc receptor-like 1 (FCRL1) is a novel biomarker for prognosis and a possible therapeutic target in diffuse large B-cell lymphoma. Mol Biol Rep 50(2):1133–1145. 10.1007/s11033-022-08104-7
38. Campbell M, McKenzie JE, Sowden A, Katikireddi SV, Brennan SE, Ellis S et al (2020) Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. BMJ 368:l6890. 10.1136/bmj.l6890
39. Runge HFP, Lacy S, Barrans S, Beer PA, Painter D, Smith A et al (2021) Application of the LymphGen classification tool to 928 clinically and genetically characterised cases of diffuse large B cell lymphoma (DLBCL). Br J Haematol 192(1):216–220. 10.1111/bjh.17132
40. Xu-Monette ZY, Li Y, Snyder T, Yu T, Lu T, Tzankov A et al (2023) Tumor-infiltrating normal B cells revealed by immunoglobulin repertoire clonotype analysis are highly prognostic and crucial for antitumor immune responses in DLBCL. Clin Cancer Res 29(23):4808–4821. 10.1158/1078-0432.CCR-23-1554
41. Alcoceba M, Stewart JP, García-Álvarez M, Díaz LG, Jiménez C, Medina A et al (2024) Liquid biopsy for molecular characterization of diffuse large B-cell lymphoma and early assessment of minimal residual disease. Br J Haematol 205(1):109–121. 10.1111/bjh.19458
42. Khanmohammadi S, Masrour M, Fallahtafti P, Hasani F (2025) MicroRNA as a potential diagnostic and prognostic biomarker in diffuse large B-cell lymphoma: a systematic review and meta-analysis. Cancer Rep (Hoboken) 8(1):e70070. 10.1002/cnr2.70070
43. Li C, Han X, Hu X, Du S, An P, Wang D (2026) Exploring potential biomarkers of diffuse large B-cell lymphoma through multi-dimensional data. Clin Transl Oncol. 10.1007/s12094-026-04295-8
44. Wang X, Lu Y, Liu Z, Zhang Y, He Y, Sun C et al (2022) A 9-lncRNA signature for predicting prognosis and immune response in diffuse large B-cell lymphoma. Front Immunol 13:813031. 10.3389/fimmu.2022.813031
45. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E et al (2022) The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: lymphoid neoplasms. Leukemia 36(7):1720–1748. 10.1038/s41375-022-01620-2
46. Ennishi D, Jiang A, Boyle M, Collinge B, Grande BM, Ben-Neriah S et al (2019) Double-hit gene expression signature defines a distinct subgroup of germinal center B-cell-like diffuse large B-cell lymphoma. J Clin Oncol 37(3):190–201. 10.1200/JCO.18.01583
47. Zhang MC, Tian S, Fu D, Wang L, Cheng S, Yi HM et al (2023) Genetic subtype-guided immunochemotherapy in diffuse large B cell lymphoma: the randomized GUIDANCE-01 trial. Cancer Cell 41(10):1705–1716e5. 10.1016/j.ccell.2023.09.004

48. Lou X, Zhao K, Xu J, Shuai L, Niu H, Cao Z et al (2022) CCL8 as a promising prognostic factor in diffuse large B-cell lymphoma via M2 macrophage interactions: a bioinformatic analysis of the tumor microenvironment. Front Immunol 13:950213. 10.3389/fimmu.2022.95021
49. Dai Y, Kizhakeyil A, Chihara D, Li X, Liu Y, Sainz Zuniga TP et al (2025) Multi-modal spatial characterization of tumor immune microenvironments identifies targetable inflammatory niches in diffuse large B-cell lymphoma. Nat Genet 57(11):2715–2727. 10.1038/s41588-025-02353-5
50. Autio M, Brück O, Pollari M, Karjalainen-Lindsberg ML, Beiske K, Jørgensen JM et al (2025) Characterization of tumor microenvironment and cell interaction patterns in testicular and diffuse large B-cell lymphomas. Haematologica 110(6):1339–1350. 10.3324/haematol.2024.286267
51. Merdan S, Subramanian K, Ayer T, Van Weyenbergh J, Chang A, Koff JL et al (2021) Gene expression profiling-based risk prediction and profiles of immune infiltration in diffuse large B-cell lymphoma. Blood Cancer J 11(1):2. 10.1038/s41408-020-00404-0
52. Liu M, Bertolazzi G, Sridhar S, Lee RX, Jaynes P, Mulder K et al (2024) Spatially-resolved transcriptomics reveal macrophage heterogeneity and prognostic significance in diffuse large B-cell lymphoma. Nat Commun 15(1):2113. 10.1038/s41467-024-46220-z
53. Autio M, Leivonen SK, Brück O, Karjalainen-Lindsberg ML, Pellinen T, Leppä S (2022) Clinical impact of immune cells and their spatial interactions in diffuse large B-cell lymphoma microenvironment. Clin Cancer Res 28(4):781–792. 10.1158/1078-0432.CCR-21-3140
54. Dai L, Lou N, Huang L, Li L, Tang L, Shi Y et al (2025) Spatial transcriptomics reveals prognostically LYZ+ fibroblasts and colocalization with FN1 + macrophages in diffuse large B-cell lymphoma. Cancer Immunol Immunother 74(4):123. 10.1007/s00262-025-03968-7
55. Moia R, Talotta D, Di Terzi L, Almasri M, Dondolin R et al (2025) Molecular clustering on ctDNA improves the prognostic stratification of patients with DLBCL compared with ctDNA levels. Blood Adv 9(7):1692–1701. 10.1182/bloodadvances.2024014136
56. Narkhede M, Tomassetti S, Iqbal M, Tin A, Rivero-Hinojosa S, George GV et al (2024) Tumor-informed ctDNA assessment as a valuable prognostic and predictive biomarker in diffuse large B-cell lymphoma. Front Oncol 14:1407003. 10.3389/fonc.2024.1407003
57. Wang S, Nijland M, Strobbe L, Oosterveld M, Boersma R, Koene HR et al (2025) Prospective validation of end of treatment ctDNA-MRD by PhasED-Seq in DLBCL patients from a national trial. J Clin Oncol 43(16 Suppl):7000. 10.1200/JCO.2025.43.16_suppl.7000
58. Mi L, Deng J, Qin J, Zhang C, Liu L, Yang S et al (2020) Genetic subtype-based International Prognostic Index prognostic model in diffuse large B-cell lymphoma. MedComm 2025;6(7):e70190. 10.1002/mco2.70190
59. Wenzl K, Stokes ME, Novak JP, Bock AM, Khan S, Hopper MA et al (2024) Multiomic analysis identifies a high-risk signature that predicts early clinical failure in DLBCL. Blood Cancer J 14(1):100. 10.1038/s41408-024-01080-0

Figures

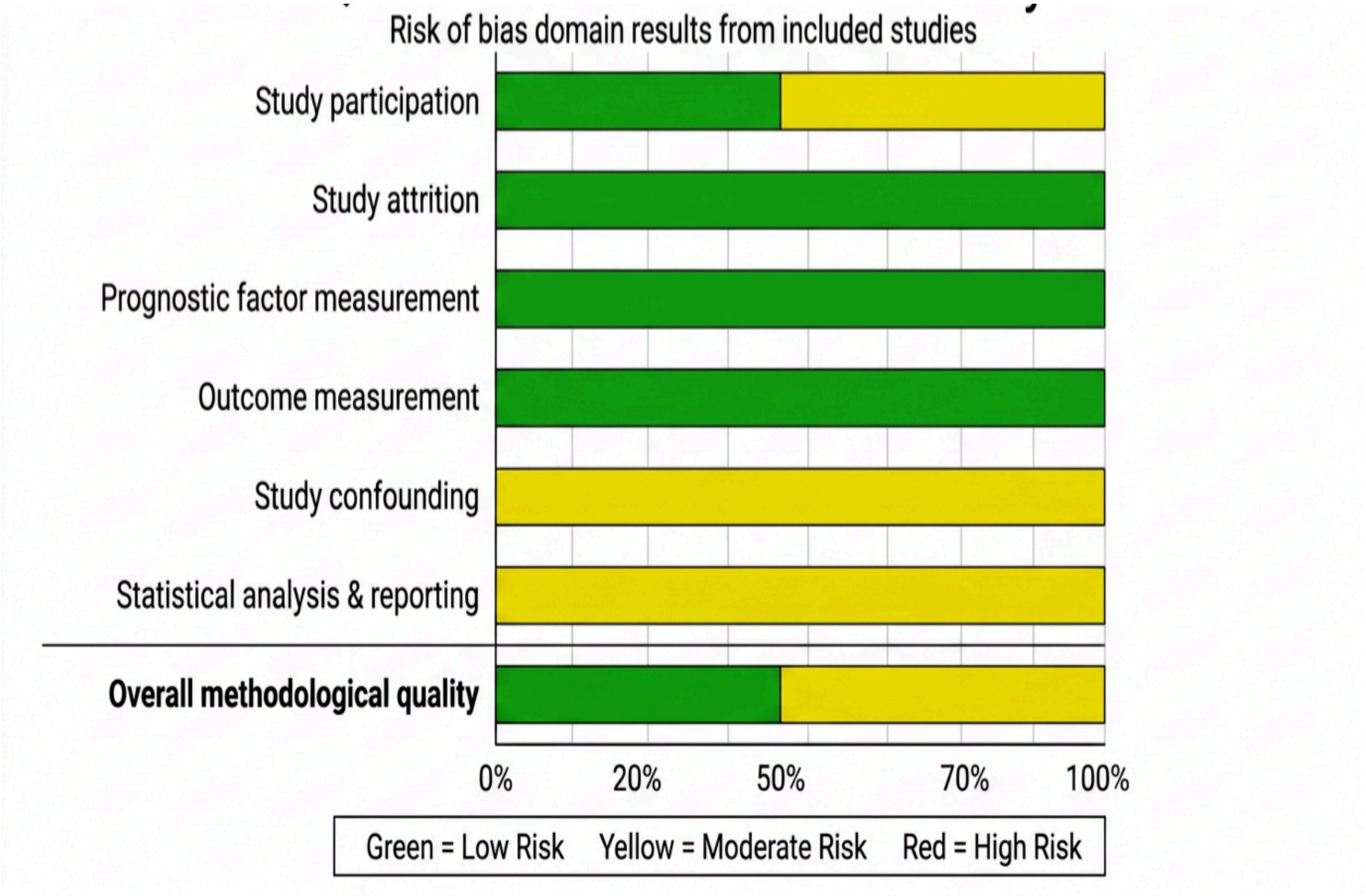

Figure 1

Risk of bias domain results from included studies

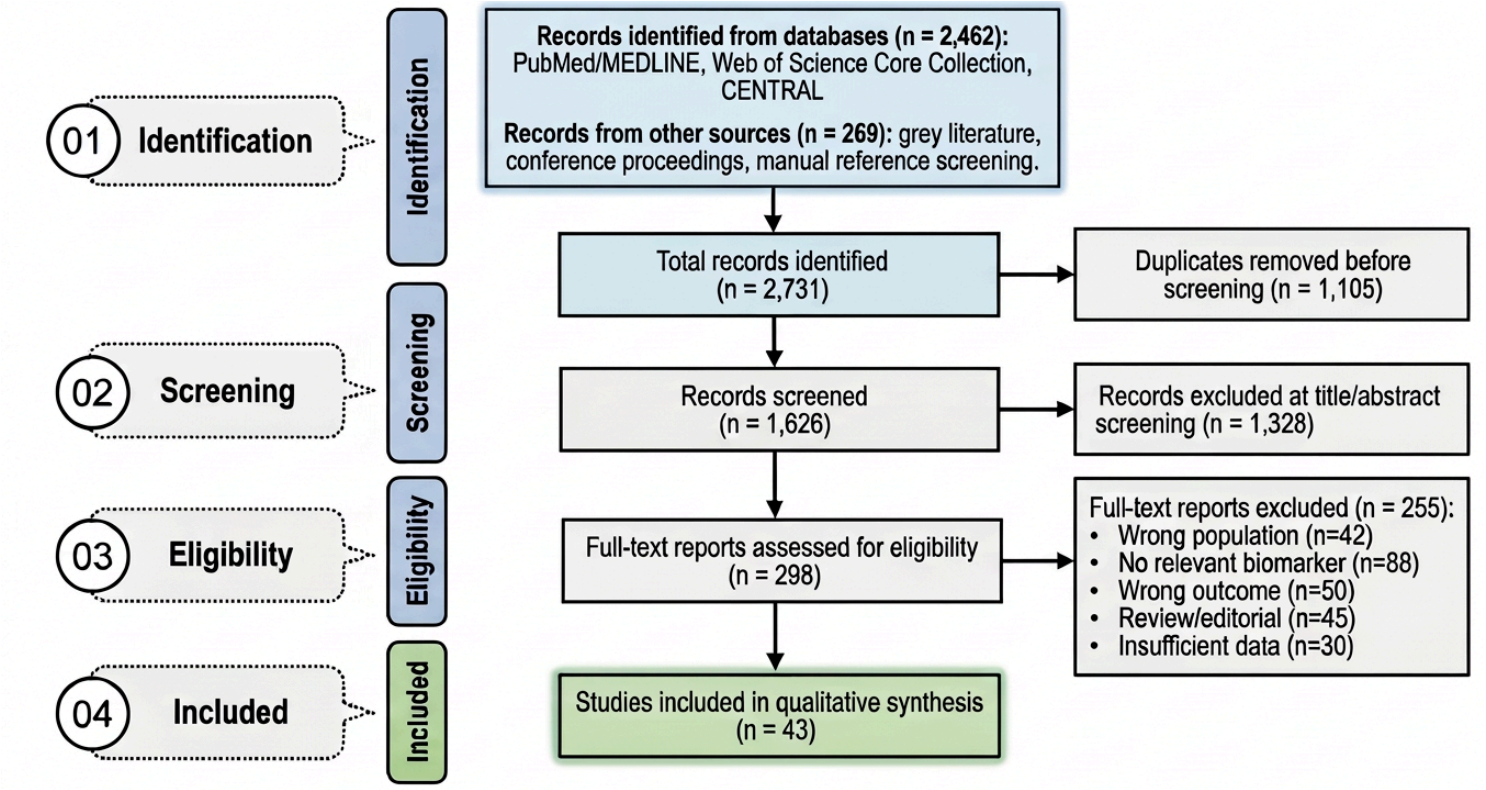

Figure 2

PRISMA flow diagram illustrating the study selection process. Systematic searches were conducted across PubMed/MEDLINE, Web of Science Core Collection, CENTRAL, supplemented by grey literature and manual screening. PRISMA=Preferred Reporting items for Systematic reviews and Meta-Analyses.

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