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Chapter

Diffuse Large B-Cell Lymphomas: From Morphology to Genomic Profiling

Giovanni Insuasti-Beltran and Eric D. Hsi

Abstract

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma in the western world. The classification of these lymphomas has been and continues to be one of the most challenging aspects of this entity. DLBCLs are clinically and morphologically very heterogeneous diseases presenting a barrier to successfully developing adequate classification systems with significant clinical, prognostic and therapeutic relevance. Recent gene expression profiling and next-generation sequencing advances have improved our understanding of the disease. This review will present an up-to-date overview of traditional and modern classification systems in DLBLC, emphasizing newly proposed subgroups based on integrating gene expression profiling and sequencing data.

Keywords: diffuse large B-cell lymphoma, DLBCL, genetics, gene expression profile, classification systems

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) represents the vast majority of cases among large B-cell lymphomas. It constitutes the most common type of non-Hodgkin lymphoma in the western hemisphere [1]. Classification of DLBCL has evolved along with the availability of ancillary techniques. Initial classifications were based purely on morphologic features. Subsequently, immunophenotyping (such as flow cytometry and immunohistochemistry) and cytogenetic techniques improved the classification systems. Finally, advanced molecular methods have become available in recent years that have improved our understanding of the pathophysiology behind these entities, allowing for further refinements in classification and the identification of potential new therapeutic targets. Despite all these significant advancements, our understanding of DLBCL remains incomplete due to its highly heterogeneous nature. In the present chapter, a comprehensive review of past, present, and important future characteristics of DLBCL will be discussed, emphasizing different classification modalities.

2. Morphology

DLBCLs are characterized by partial or complete effacement of the normal architecture (nodal or extranodal) by medium to large-sized lymphoid cells with vesicular chromatin. Large size is defined compared to adjacent macrophage (same size or larger) or residual normal lymphocyte (greater than twice the size) nuclei. Mitotic activity is usually high, with variable amounts of apoptotic debris, tingible body macrophages, and necrosis. All cases of DLBCL will show background small T-cells and histiocytes in different amounts. Other features, such as fibrosis and sclerosis, may also be present. Traditionally, based on morphologic features alone, three subtypes have been recognized (**Figure 1**) [2, 3]:

- The centroblastic subtype (about 80% of cases) is characterized by cells with round to oval nuclei, vesicular chromatin, variable amounts of cytoplasm, and small nucleoli usually distributed adjacent to the nuclear membrane. Cases may be exclusively composed of centroblasts (>90%) or have a more polymorphic composition with a mixture of centroblasts (<90%), large centrocytes, and immunoblasts.

- The immunoblastic subtype represents about 10% of cases and shows predominance (>90%) of large immunoblasts with moderate to abundant basophilic cytoplasm and regular nuclei with a single centrally located nucleoli. Cytologic variability may cause decreased intra and inter-observer reproducibility in diagnosing this variant [4]. Plasmacytic differentiation may be present in some cases.
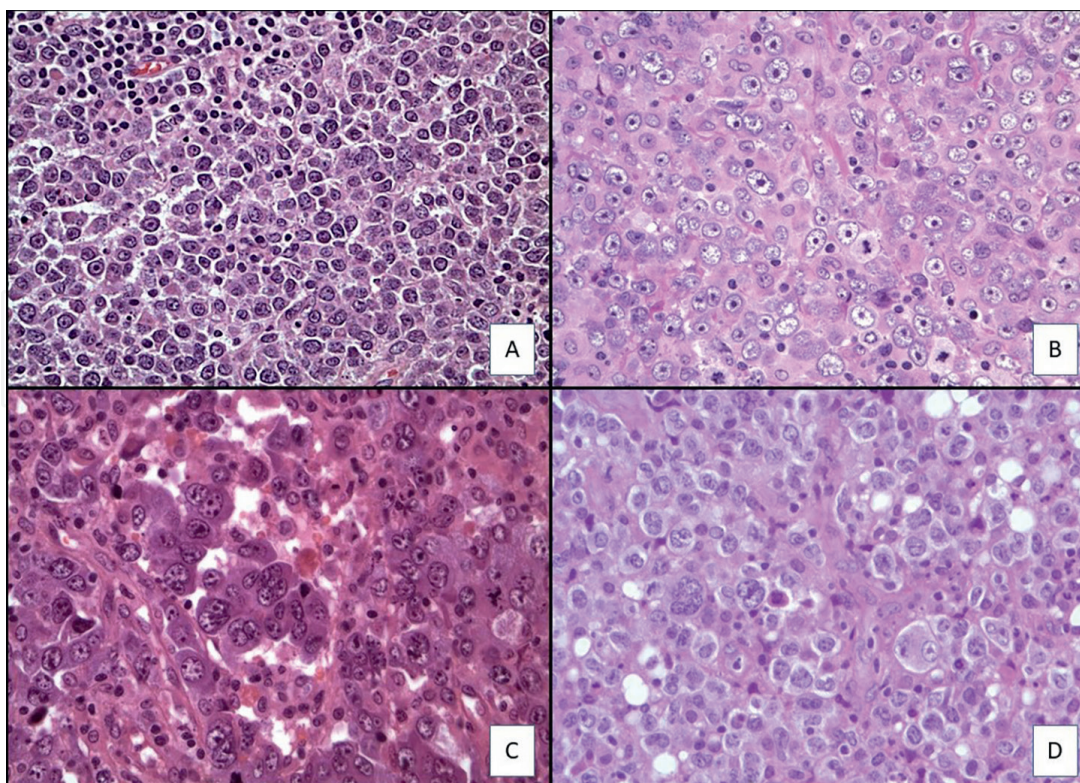

Figure 1.
Examples of the different morphologic subtypes of DLBCL, centroblastic morphology (A), immunoblastic morphology (B), and anaplastic morphology (C, D).

Even if classic immunoblasts represent <90% of cells, cases with a predominance of immunoblasts and plasmacytoid cells (>90%) belong to this category, assuming less than 10% centroblasts. *IGH::MYC* translocations are frequent in this variant [5, 6].

- The anaplastic subtype is the least common of the morphologic variants (~3%) and is usually composed of prominent irregular, pleomorphic or bizarre nuclei with moderate to abundant cytoplasm. These cells may mimic Reed-Sternberg cells of Hodgkin lymphoma, other lymphomas with atypical large cells (such as anaplastic large cell lymphoma), or undifferentiated carcinomas. Partial or extensive sinusoidal infiltration may be seen. This variant frequently expresses CD30 and has increased *TP53* mutations [7, 8].

- Other less frequent (<1%) variants have been described, including cells with a signet ring or spindled-cell morphology, mimicking carcinomas or sarcomas.

While recognition of morphologic variants helps understand the spectrum of DLBCL, there are no independent clinical implications regarding therapy or outcome.

3. Gene expression profiling

From the genomic standpoint, DLBCLs are a group of complex heterogeneous entities with intricate molecular backgrounds. Gene expression profiling (GEP), a technique that allows the determination of the pattern of genes expressed, at the transcriptional level, to provide a global determination of cellular function, has been extensively used to try to understand the pathobiology of DLBCL.

One of the initial attempts to understand the molecular composition of DLBCL happened in 2000 when Alizadeh et al. [9], using DNA microarray-based technology, identified two distinct molecular clusters with either germinal center B-cell (GBC) or activated B-cell like (ABC) phenotypes, allowing for a what has been known as "cell of origin" (COO) subclassification. GBC cases were associated with a genetic programming characteristic of germinal center differentiation, including genes encoding for cell-surface molecules such as CD10 and CD38, nuclear factor A-myb and the DNA repair protein 8-oxoguanine DNA glycosylase (OGG1), as well as alterations in *BCL6, LMO2,* and *BCL7A* genes. Cases with the activated B-cell-like phenotype showed alterations associated with a post-germinal center environment, including *IRF4, BLC2,* and *FLIP* (*CFLAR*) genes. This dual COO-based classification was able to subtype approximately 80–85% of DLBCLs. Significant differences in overall survival (OS) and event-free survival were recognized, with GCB lymphomas showing better outcomes. An additional study identified a potential third discriminating subtype (called type 3) that clinically behaved similarly to the ABC DLBCLs; these two groups were referred to as non-GCB subtypes [10]. These studies were the first attempt to do a molecular subclassification of DLBCL, recognizing COO as key in lymphomagenesis. Numerous additional studies have validated and tried to optimize this COO classification, including using formalin-fixed paraffin-embedded (FFPE) tissues based on the NanoString technology (Lymph2Cx panel), allowing for more testing flexibility [11–13]. A microarray-based assay called B-cell associated gene signature (BAGS) allowed the further subdivision of the COO subtypes into centrocytes, centroblasts, memory B-cells, and plasmablasts, with an associated significant difference in progression-free survival (PFS) and OS among the different subgroups [14]. An adaptation of this panel for the NanoString

platform (BAGS2CLINIC) [15] demonstrated comparative results to the original BAGS assay and more comprehensive, detailed stratification compared to the Lymph2Cx assay. Survival analysis of the memory B-cell and plasmablastic subgroups, defined as ABC lymphomas, showed the former showed inferior PFS and OS. Among GCB types, the centroblastic group demonstrated decreased PFS compared to the centrocytic group but no significant differences in OS.

GEP studies have also recognized the influence and importance of the tumor microenvironment (TME) in association with the behavior of DLBCL. An early pioneer study [16] focusing on TME profiling reported a "consensus clustering" classification. This classification separated tumors into B-cell associated profiles characterized by oxidative phosphorylation and B-cell receptor signaling and proliferation, and a profile showing increased T-cell mediated immune responses and classical complement pathway activation.

In 2008, Lenz et al. [11] identified two significant clusters, stromal-1 and stromal-2, influenced by the TME. The stromal-1 signature included genes usually expressed by mesenchymal tissues associated with generating and modifying the extracellular matrix. Notably, proteins associated with fibrotic reactions, such as fibronectin and CTGF, were overexpressed in this group. Another characteristic of this signature was increased infiltration by myeloid cells like tumor-associated macrophages, myeloid-derived suppressor cells, and Tie2-expressing monocytes. This first stromal signature was associated with better PFS and OS. The stromal-2 signature had a strong angiogenic profile and was therefore associated with increased tumor-blood vessel density and decreased clinical outcomes. Subsequent studies validated these findings and emphasized the importance and influence of the microenvironment in DLBCL [17, 18].

GEP also has identified particular signatures associated with known driver oncogenes in DLBCL. An MYC activity classifier based on an 80-gene panel was used by Carey et al. [19] in order to try to categorize aggressive B-cell lymphomas according to MYC biological activity. DLBCLs were segregated into high (MYC score > 0.5) and low (MYC score < 0.5) risk groups with significant survival differences, with patients in the high-risk group showing decreased OS. An optimized version of this classification with a larger gene panel (104 genes) identified a gene expression signature that detected GCB DLBCLs harboring both *MYC* and *BCL2* rearrangements (so-called DHITsig) [20]. This assay separated GCB-DLBCL into DHITsig-positive and negative groups. Of note, this signature recognized both "double hit" (*MYC* and *BCL2* co-rearranged cases with poor prognosis) as well as GCB-DLBCLs without a "double hit" but with similar poor prognosis. The DHITsig-positive group had a signature corresponding to cells originating within the intermediate zone of the germinal center, a transitional place for B-cells migrating between the light and dark zones. These lymphomas showed overexpression of MYC and E2F targets and genes associated with oxidative phosphorylation and MTORC1 signaling, decreased expression of pro-apoptotic genes, and lower immune and inflammatory signatures. DHITsig-positive lymphomas were associated with strong cell-autonomous survival, proliferative signals, and reduced dependence on the microenvironment. PFS and OS were significantly worse in the DHIT-positive group compared to the DHIT-negative counterpart.

Subtyping based on the cell of origin concept, including the numerous significant improvements in GEP-based methodologies and COO/TME-related lymphoma signatures, is still considered one of the essential breakthroughs leading to the molecular understanding of DLBCLs. However, inconsistent clinical responses have challenged its utility in an era where targeted therapies are more widely available, evidencing the need for additional differentiating factors.

4. The sequencing era

DLBCL is a genetically heterogeneous disorder, with a relatively high frequency of mutations, somatic copy number alterations (CNAs), and structural variants (SVs), averaging 7.8 driver mutations per case, with the number increasing with separated by COO (31 mutations in GCB and 23.5 mutations in ABC subtypes) [21–24]. Using next-generation sequencing (NGS) based technologies or a combination of GEP and NGS, the complexity of DLBLCs has been further highlighted, with numerous studies identifying newer and potentially clinically and therapeutically relevant subclasses.

Chapuy et al. [25] performed whole exome sequencing in 304 patients with newly diagnosed DLBCL. They used a consensus clustering approach to identify five groups or "clusters" with outcome-associated genetic signatures (coordinated genetic signatures C1-C5) and an additional subset without significant detectable alterations (C0). The main characteristics of these clusters are:

- Cluster 1 (C1): These cases showed *BCL6* structural variants associated with mutations of components of the NOTCH2 signaling pathway, including mutations in the *NOTCH2* gene PEST-domain and its negative regulator (SPEN). Mutations of important NF-κB pathway components, such as BCL10 and TNFAIP3, and FAS, also characterized this group. These alterations have been associated with low-grade and transformed marginal zone lymphomas (MZL), suggesting a potential common extrafollicular B-cell precursor. In addition, these cases showed low or absent activation-induced cytidine deaminase (cAID), further suggesting an extrafollicular origin. In a subset, $MYD88^{non-L265P}$ mutations were also present. Most DLBCLs in this cluster corresponded to the ABC subtype by transcriptional profiling.

- Cluster 2 (C2): DLBCLs in this group were characterized by increased CNAs, resulting in gains or losses of critical regulatory elements. Importantly, *TP53* inactivation by mutations and *17p* copy losses were significant in this cluster. Additional copy losses of *9p21.13/CDKN2A* and *13q14.2/RB1* were present, leading to altered chromosomal stability and cell cycle dysregulation. Copy gains of 1q23.3/MCL1 and 13q31.31/miR-17-92. This cluster included both GBC and ABC-type DLBCLs.

- Cluster 3 (C3): This group was characterized by structural variants that juxtaposed *BCL2* and the *IGH* enhancer and *BCL2* mutations. Additional mutations in chromatin modifier genes such as *KMT2D*, *CREBBP*, and *EZH2* were also identified. Present also was inactivation of *PTEN* via focal 10q23.31/*PTEN* loss and truncating mutations. This myriad of alterations have been described in B-cell lymphomas originating in the germinal center (like follicular lymphoma and GCB-type DLBCLs); therefore, most lymphomas in this cluster were classified as GCB type.

- Cluster 4 (C4): These cases displayed mutations in linker and core histone genes, immune evasion molecules, BCR/Pi3K signaling intermediates (including *RHOA*), NF-κB modifiers, and components of the JAK/STAT pathway (*BRAF*, *STAT3* mutations). DLBCLs in this cluster were primarily GCB-type. Alterations in linker and core histones, mainly H1, have also been described in follicular lymphomas.

- Cluster 5 (C5): This group showed near-uniform *18q* gains, likely associated with known driver genes in this region, such as *BCL2* and *MALT1*. Frequent co-occurrent mutations in *CD79B* and $MYD88^{L265P}$ were also present. Additional alterations in the C5 group included gains of *3q, 19q13.42*, inactivation of *PRMD1*, and mutations in *ETV6, PIM1, GRHPR, TBL1XR1*, and *BTG1* genes. The high AID contribution and associated aberrant somatic hypermutation indicated that these tumors have transited through the germinal center. Accordingly, this cluster classified most DLBCLs as ABC-type by COO designation. This signature seems to be associated with extranodal disease, with a predilection for immune-privileged sites like CNS and testes.

   Regarding the prognostic significance of this new subclassification, patients with DLBCLs in clusters C0, C1 and C4 had more favorable outcomes than patients with lymphomas in clusters C3 and C5. Of interest is that ABC enriched clusters had different outcomes, with C1 being favorable and C5 unfavorable. GCB enriched clusters C3 and C4 had unfavorable and favorable outcomes, respectively. Thus, these clusters improved upon the prognostic stratification of the COO concept.

   A similar approach was used by Schmitz et al. [26] with a multiplatform analysis of structural genomic abnormalities and gene expression in 574 DLBCL biopsy samples. Using the GenClass algorithm, four distinct subtypes were identified:

- MCD: This subgroup was characterized by $MYD88^{L265P}$ and *CD79B* mutations, with co-occurrent alterations happening in about 40% of cases. Additional mutations in essential tumor suppressor genes, including *CDKN2A, ETV6, BTG1*, and *BTG2*, were identified. These alterations activate BCR and toll-like receptor pathways, leading to increased NF-κB activity. DLBCLs in the group had the most robust ABC expression signature, corresponding to the COO ABC-type. These genetic features also overlapped with those reported in extranodal lymphomas, including primary CNS lymphoma, primary testicular lymphoma, and primary breast lymphoma [27, 28].

- BN2: The principal alterations included NOTCH pathway aberrations and *BCL6* fusions. The former included *NOTCH2* mutations or amplification and mutations in *SPEN* and *DTX1* (a NOTCH target gene). Another prominent feature of these cases was alterations targeting regulators of the NF-κB pathway, including loss of *TNFAIP3*, gain for *BCL10*, and 3′-untranslated region (3'UTR) mutations leading to enhance expression of NFKBIZ. Based on COO classification, most BN2 cases represented a mix of ABC-, GCB-type or unclassified.

- N1: Cases in this group showed a predominance of activating mutation of the oncogene *NOTCH1* and alterations targeting transcriptional regulators of B-cell differentiation (like *IRF4, ID3*, and *BCOR*). Concerning TME signatures, these cases showed significant signals from T-cells, myeloid cells, and follicular dendritic cells. DLBCLs within this group were mainly classified as ABC-type.

- EZB: Alterations in this subtype included *BCL2* translocations, *EZH2* mutations, and *REL* amplifications, as well as inactivation of tumor suppressors and histone modifiers such as TNFRSF14, *CREBBP, EP300*, and *KMT2D*. Most of these molecular events have been associated with lymphomas derived from a germinal center environment. Other studies have shown that this mutational profile strongly matches that seen in follicular lymphomas (FL) [29], leading to the possibility that EZB DLBCLs may

arise from a concurrent FL. Consequently, the majority of DLBCLs in this category were classified as GCB-type. In addition, given the identification of an EZB-MYC+ subgroup within this cohort, the EZB subtype appears to be the primary genetic background of double-hit lymphomas (with *MYC* and *BCL2* rearrangements).

These four subtypes significantly differed in terms of PFS and OS, with BN2 and EZB subtypes having much more favorable outcomes than MCD and N1.

Comparing the Chapuy [25] and Schmitz [26] findings, overlapping features were present between C1 and BN2, C3 and EZB, and C5 and MCD subgroups. The C2 and C4 clusters did not correlate with any of the Schmitz subgroups, likely due to differences in bioinformatic pipelines.

In a subsequent study, Wright et al. [30] developed the LymphGen algorithm aimed to classify tumors based on the probability of belonging to a particular genetic subtype, allowing for the possibility of additional alterations during evolution. Six genetic subtypes were revealed, including the four identified by Schmitz et al., supplemented by two additional groups (ST2 and A53). The ST2 subgroup was characterized by recurrent mutations in *SGK1* and *TET2* genes. Inactivation of *SOCS1, DUSP2*, and *STAT3* activating mutations promote JAK/STAT signaling in ST2 tumors. Most DLBCLs in this group were classified as GCB-type. The A53 subgroup demonstrated the predominance of aneuploidies and *TP53* mutations and deletions. Additionally, the presence of homozygous deletions and mutations of *TP53BP1* contributed to chromosomal gains and losses in this group. A mixture of GCB-, ABC-types and unclassified DLBCLs were included in this category.

Finally, a study from the UK Hematological Malignancy Research Network (HMRN) applied targeted sequencing in a 293-gene panel to 928 cases of DLBCL [31]. Clustering was based mainly on mutation data, with CNAs data applied only to a small group of genes. Five genomic clusters were identified and applied to about 80% of cases; leaving the remaining cases as "not elsewhere classified" (NEC):

- MYD88 cluster: Dominated by *MYD88*[L265P], *PIM1, CD79B*, and *ETV6* gene mutations and frequent loss of *CDKN2A*. Alterations within this group recapitulated the MCD and C5 subtypes. Most cases belonged to the ABC-type. Interestingly, the majority of lymphomas primary to CNS, testicular, and breast locations mapped to this cluster.

- BCL2 cluster: Showed frequent mutations in *EZH2, BCL2, CREBBP, TNFRSF14, KMT2D*, and *MEF2B* genes. Additionally, *MYC* rearrangements were most enriched within this cluster. When correlated with fluorescence in-situ hybridization (FISH) data, cases with double and triple hit alterations mapped to this category. Gene expression revealed a predominance of GCB-type DLBCLs in this group. This cluster mapped to the previously described EZB and C3 clusters.

- SOCS1/SGK1 cluster: Mutations in this group included *SOCS1, CD83, SGK1, NFKBIA, HIST1H1E*, and *STAT3*. Cases in this cluster were usually classified as GCB-type and showed similarities to the C4 cluster. *SOCS1* mutations are also commonly present in primary mediastinal B-cell lymphomas, suggesting a degree of biological similarities with this entity.

- TET2/SGK1 cluster: Characterized by mutations including *TET2, SGK1, KLHL6, ZFP36L1, BRAF, KRAS*, and *MAP2K1*. DLBCLs in this cluster were GCB-type
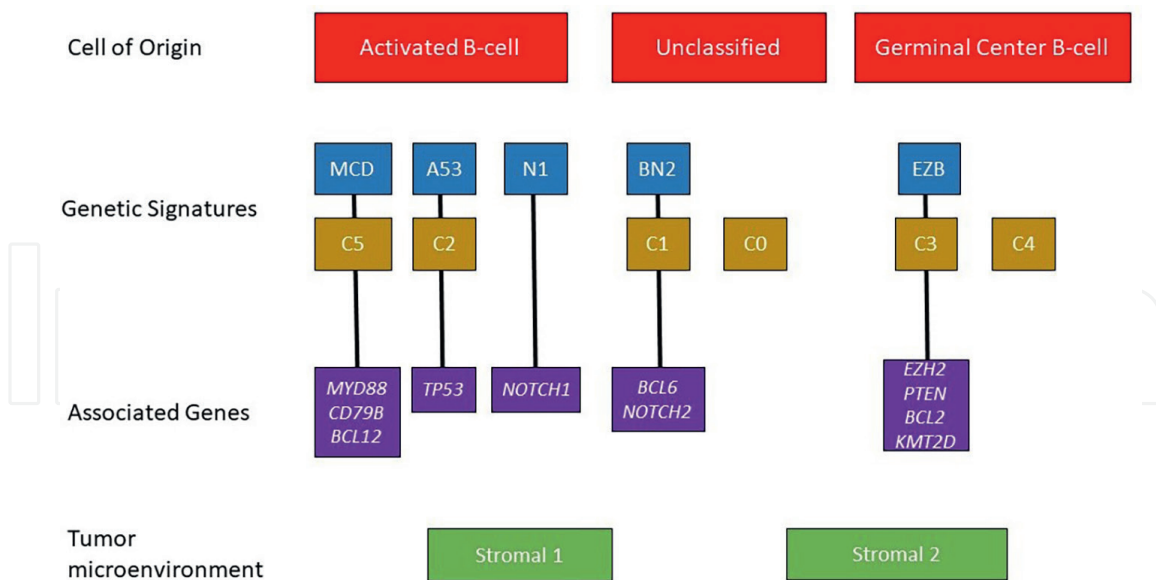
Figure 2.
Summary of different classification systems.

and appeared to represent a subset of the C4 cluster. Alterations in multiple components of the ERK pathway were associated with the enrichment of gene expression signatures of RAS and ERK.

- NOTCH2 cluster: Mutations in this group included NOTCH2, BCL10, TNFAIP3, CCND3, SPEN, TMEM30A, and CD70. This landscape suggests a biological similarity to marginal zone lymphomas (MZL), likely representing an extranodal origin or transformation from a similar entity. Gene profiling identified cases in this cluster as GCB, ABC, and unclassified DLBCLs. This cluster corresponds closely to the BN2 or C1 subtypes.

Additional two subgroups, the BCL2-MYC and NOTCH1, were identified using a modification of the HMRN classification using the presence of *MYC* hotspots and *NOTCH1* PEST domain mutations, respectively. This modified classifier demonstrated high concordance with the LymphGen classifier [32].

GEP and NGS technologies have vastly improved understanding of the complex molecular pathways behind DLBCL and other high-grade lymphomas, distinguishing several COO and TME-based subtypes with prognostic and potential therapeutic relevance (**Figure 2**). Ultimately, classifications based on genomic signatures provide a way to rationalize the heterogeneity of these tumors into subtypes that share a common biological pathogenesis and, therefore, may respond similarly to specific therapies. Unfortunately, despite the overall similarities among signature clusters generated to date, no unified concept for consensus clusters and their significant genetic drivers has been officially established, precluding the definition of a single unified genetic framework for DLBLCs.

5. Immunohistochemistry

Despite the recognized importance of molecular signatures for the subclassification of DLBCs, GEP and NGS are tools that are not widely available for use in routine

laboratories, especially in underdeveloped nations. Therefore, standard and routinely available tools must be developed and implemented for any classification system to be widely adopted. In this regard, immunohistochemistry (IHC) plays a vital role in bridging the gap between morphologic and genetic classifications. The advantages of using IHC are the ability to use FFPE tissues, rapid turn-around time, correlation with corresponding morphologic architecture, implementation across most laboratories, and familiarity with end-user interpretation. Disadvantages include the potential inclusion of artifacts due to preanalytical variants (such as fixation time and use of decalcifying solutions), variations in staining strength, and inter-observer variability in the interpretation.

Multiple algorithms to replicate the GEP/NGS classifications have been described. In a seminal paper, Hans et al. [33] confirmed the utility of IHC to classify DLBCLs based on COO into GCB and non-GBC-types (the latter corresponding to the ABC-type and unclassified cases by GEP), utilizing CD10, BCL6 and MUM1 antibodies. Positivity was defined as staining 30% or more of the tumor cells. The first step in the algorithm was assessing the expression of CD10. CD10+ cases would be classified as GCB-type. If negative, the expression of BCL6 would then be determined. If CD10- and BCL6- the cases were considered non-GBC. If CD10-/BCL6+, MUM1 expression will be determinant of the final classification, with MUM1+ cases being considered non-GCB and MUM- GCB-type. The Hans criteria concerning GEP-derived GCB or ABC classification concordance was approximately 80%.

Muris et al. [34] developed an ICH algorithm based on BCL2, CD10, and MUM1 staining that showed the ability to stratify patients with primary nodal DLBCL into favorable (group 1) and unfavorable (group 2) prognostic groups. Positivity was defined as equal to or greater than 30% of staining for CD10 and BCL6 and 50% for BCL2. All BCL2 negative cases were assigned to group 1. BCL2+ cases expressing CD10 and BCL2+ cases not expressing CD10 or MUM1 were also assigned to group 1. BCL2+ cases, CD10 negative, and MUM1+ were assigned to group 2. Significant differences were observed, with group 1 patients having better PFS and OS.

Choi and colleagues proposed another IHC-based algorithm [35] that incorporated GCET1 and FOXP1, in addition to the described CD10, BCL6, and MUM1 by the Hans algorithm, with better approximation to the COO GEP classification (93% concordance). GCET1, a marker associated with GC activity, was followed by an assessment of MUM1 and CD10. GCET1+/MUM1- cases were classified as GCB, whereas cases GCET1+/MUM+ were ABC. If GCET1 was negative but CD10+, those cases were classified as GCB. If both GCET and CD10 were negative, then BCL6 and FOXP1 were evaluated. BCL6 negative cases and BCL6+/FOXP1+ cases were classified as ABC-type, while BCL6+/FOXP1- were determined to belong to the GCB subtype. Positivity cutoffs used on this algorithm were 30% or more for CD10 and BCL6 and 80% or greater for GCET, FOXP1, and MUM1.

An algorithm focusing on markers related to the activated B-cell-like phenotype was proposed by Nyman et al. [36] Markers used by this group were MUM1 and FOXP1, with ABC-type cases defined as those positive for MUM1 or if MUM1 negative, then FOXP1 positive. Thirty percent or more staining was considered positive. This study helped establish the ABC-type DLBCL as an adverse risk factor in immunotherapy-treated patients.

Despite the attempts to generate meaningful algorithms that can reproduce profiling and sequencing subgroups, data is still conflicting, and several studies have not been able to confirm their prognostic impact on survival. The World Health Organization (WHO) Classification of Tumors of the Hematopoietic and Lymphoid

tissues supports the Hans criteria classification for cell of origin subtyping, recognizing the utility of other GCB- and ABC-associated makers studied by the other groups [37, 38].

Beyond COO classification, immunohistochemistry has also been used to assess other potential significant prognostic variables, including tumor-infiltrating lymphocytes, microenvironment proteins, tumor suppressor expression, and immune checkpoint modifiers [39–42].

6. Cytogenetics and fluorescence In-situ hybridization (FISH)

Despite the multiple highly advanced sequencing and profiling techniques, conventional cytogenetic and FISH methods still play an essential role in evaluating DLBLCs. Assessment of genes like *MYC, BCL2*, and *BLC6* is essential to classify cases as double or triple hit (DH/TH). Different FISH testing algorithms have been proposed. The College of American Pathologists (CAP) and Royal College of Pathologists issued recommendations to test DLBCLs with GBC phenotype for *MYC* rearrangements with or without concurrent testing for *BCL2* rearrangements [43, 44]. Scott et al. [45] recommended *MYC* rearrangement testing in all cases with DLBCL morphology, with further testing for *BCL2* and *BCL6* if *MYC* positive, in order to detect all high-grade B-cell lymphomas (HGBL) DH/TH. Since HGBL-DH/TH with *BCL2* rearrangement occur almost exclusively in GCB-type DLBCLs, triaging screening of tumors for FISH testing by COO can decrease the need for FISH testing, optimizing resources in limited practices. Differences in individual laboratory practice will depend on workflow, turn-around time requirements, and tolerance for misclassification.

Additionally, cytogenetic and FISH methods can be applied to assess IRF4 (6p25.3) abnormalities diagnostic of large B-cell lymphoma with IRF4 rearrangement and 11q abnormalities present in high-grade B-cell lymphomas with 11q aberrations.

7. Conclusions

The classification of DLBCL has seen tremendous advancements in the last twenty years. Modern molecular methods have been pivotal in generating data that has allowed a better understanding of the underlying pathobiology of these neoplasms, with subsequent diagnostic, prognostic, and therapeutic implications. As further data is generated, classification systems will continue to evolve, and with them will come more effective, biologically targeted therapies to improve the survival of patients affected by these conditions. This review has presented a comprehensive overview of the different attempts to subclassify these lymphoid tumors from a different perspective.

Conflict of interest

The authors declare no relevant conflicts of interest.

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