2. Background

Before diving into our study, we explain the relevant prior work as it relates to the biological and genomic pathogenesis, clinical characteristics, and classification of B-NHLs.

2.1. Biological and Genomic Pathogenesis of B-NHLs

2.1.1. B-Cell lymphomagenesis occurs in germinal centres where transcriptional changes regulate B-cell development

The majority of B cell lymphomas originate in the Germinal Centres (GCs). The GCs are histological structures whose goal is to proliferate naïve B cells and enable their differentiation into Memory B cells and Plasma Cells [16–18](Figure 2a). Functionally, the Germinal Centre reaction takes three steps[19]. First, naïve B cells become activated upon encounter with an antigen and interaction with $CD4^+$ T cells in T cell-rich areas of secondary lymphoid organs. They subsequently aggregate into follicles to form GCs. In the dark zone of the GC, B cells proliferate rapidly, and use immunoglobulin somatic hypermutation to produce a high diversity of antibodies. Second, B cells move into the light zone where they are selected on the basis of antigen affinity. Finally, B cells either differentiate into Memory B cells, differentiate into a Plasma Cells, re-enter the dark zone, or undergo apoptosis.

Crucially, the GC reaction is regulated by a complex transcriptional network whose dysregulation produces various lymphomas [20,21](Figure 2b). The first phase of the GC reaction—initiation, B cell proliferation, and somatic hypermutation—is regulated by three major transcriptional events. First, the *MYC* gene is induced to initiate dark zone formation and encourage B cell proliferation. Although the exact molecular mechanisms are unknown, *MYC* generally stimulates proliferation by increasing DNA replication, metabolism, and telomerase activity[22]. Second, *BCL6* is induced as the master regulator of GC maintenance and formation (Figure 2c). *BCL6* encourages somatic hypermutation of immunoglobulin loci by inhibiting differentiation, B cell activation, and the DNA damage response[23–26]. Third, *EZH2*-mediated epigenetic silencing occurs to further promote proliferation and prevent differentiation[27].

The second phase and third phases of the GC reaction – selection for high affinity antigens; and differentiation, dark zone re-entry, or apoptosis – are regulated by four transcriptional events. First, the induction of *MYC* allows for dark zone re-entry[25,26]. Second,

the activation of *NF-KB* promotes selection of high affinity antibodies and differentiation of corresponding B cells[28–33]. Third, the downregulation of *BCL6* leads to GC exit and differentiation[34]. Finally, the induction of *PRDM1* allows plasma cell differentiation[35–38].

2.1.2. Dysregulation of the GC Reaction defines the characteristic genomic alterations of B-NHLs

Dysregulation of the GC reaction described above is the source of the majority of B-NHLs. Indeed, BL, FL, and DLBCL jointly comprise 80% of B-NHLs and result from dysregulation of different steps of the GC reaction[39]. These B-NHLs contain mutations standard to most tumours: deletions, amplifications, and nonsynonymous point mutations with loss-of-function or gain-of-function. More importantly, B-NHLs share a series of characteristic genomic alterations stemming from GC dysregulation. Owing to the immunoglobulin remodelling function of the GC, B-NHLs carry lesions from aberrant somatic hyper mutation and chromosomal translocation that are less common in other cancers. Moreover, translocations in B-NHLs generally pair the coding element of a gene with a heterologous promoter, leading to dysregulated expression of an oncogene[19]. By contrast, translocations in other cancers, like Acute Leukemia, generally result in fusion genes and chimeric proteins. Translocations in B-NHLs can be grouped into three categories based on the source of the error. First, translocations such as t(14;18) involving *IGH* and *BCL2* in FL result from mistakes in the RAG-mediated V(D)J recombination process. Second, translocations such as immunoglobulin-*MYC* translocations in sporadic BL result from mistakes in the AID-dependent class switch recombination process. Third, translocations such as immunoglobulin-*MYC* translocations in endemic BL result from errors in the AID-mediated somatic hypermutation mechanism which may lead to DNA breaks[19].

In addition to these characteristic genomic alterations, each B-NHL has a set of uniquely defining genetic characteristics (Figure 2a).

2.1.2.1. BL is defined by *MYC* translocation, mutations in *TCF3* and *ID3*

BL samples have gene expression patterns similar to dark zone B cells and represent aggressive malignancies[40,41]. Three main genetic changes characterize BL. Occurring in 100% of cases, the hallmark genetic lesion of BL is *MYC* translocation into the immunoglobulin locus[42,43]. Translocation causes ectopic *MYC* expression which promotes replication, causing replication stress in proliferative dark zone B cells and thus lymphomagenesis[26,40,44]. Second, 70% of BL mutations have mutations of *TCF3* or *ID3*

which promote "tonic" BCR signalling to occur in an antigen-independent way. By contrast, cells without this mutation, for example ABC-DLBCL samples (described below), rely on chronic activation of BCR[45]. Third, the Ga13-dependent pathway is dysregulated, thus causing GC B cell migration and preventing confinement[46]. This mutation also occurs in GCB-DLBCL (described below).

2.1.2.2. FL is defined by t(14;18) translocation and *KMT2D* inactivation

FL results from the clonal expansion of follicles containing GCs with high SHM activity[47]. These samples often have gene expression patterns similar to B cells arrested in the light zone[40,48]. Though an indolent disease, FL can transform into DLBCL[49,50]. Two main genetic events distinguish FL. First, 80% of FL samples have a t(14; 18) translocation, juxtaposing the *BCL2* gene with the *IGH* locus and causing ectopic expression[51,52]. The dysregulation of *BCL2* leads to an anti-apoptosis response. Second, >80% of FL cases exhibit the genetic inactivation of *KMT2D*[53,54]. The exact consequences of this inactivation are currently unknown.

2.1.2.3. DLBCL is defined by *BCL6* dysregulation, inactivation of chromatin modifiers (*EP300, CREBBP, KMT2D*), and disruption of immune surveillance

Comprising 40% of all B-NHL, DLBCL represents the most common form of B-NHL lymphoma. While some DLBCL cases arise de novo, other cases arise from transformation of less aggressive B-NHLs (chronic lymphocytic leukaemia and FL)[50,55]. DLBCL samples have gene expression profiles that map into two broad categories: activated B cell-like DLBCL (ABC-DLBCL) and GC B cell-like DLBCL (GCB-DLBCL). GCB-DLBCL samples' gene expression profiles match those of light zone B cells[40,48]. ABC-DLBCL samples' gene expression profiles match those of GC cells arrested during early stages of post-GC plasma cell differentiation (plasmablasts)[40,48]. While some mutations occur across DLBCL subtypes, each DLBCL subtype (ABC-DLBCL or GCB-DLBCL) has specific genomic lesions characterizing it.

Three broad types of genomic lesions are shared across DLBCL subtypes. First, many DLBCL patients have inactivation of *EP300* or *CREBBP* (40%) and/or *KMT2D* (30%), chromatin modifiers crucial to epigenetic regulation[53,54,56,57]. Second, 30% of DLBCL cases and 15% of FL cases exhibit *BCL6* dysregulation, thereby suppressing the DNA damage response and inhibiting differentiation[58]. *BCL6* dysregulation can occur either via disruption of *BCL6*'s autoinhibitory circuit or through chromosomal translocations with promoters of

other genes or the IGH locus[59–61]. Finally, >60% of DLBCL cases exhibit immune escape through various mechanisms. Diminished expression of MHC-I allows DLBCL cells to evade cytotoxic T lymphocytes. CD58 Inactivation or disrupted transport similarly allows evasion of natural killer cells[61]. Combined, these mutations allow DLBCL samples to evade both cytotoxic T lymphocytes and natural killer cells.

2.1.2.3.1. GCB-DLBCL, the first DLBCL subtype, is characterized by *EZH2* activation and altered GC B cell migration

GCB-DLBCL samples share some genetic overlap with BL and FL. In particular, 10% of GCB-DLBCL samples exhibit *MYC* translocation; 40% exhibit *BCL2* translocation; and samples exhibiting both (i.e. double hit cases) show worse clinical outcomes[63,64].

Beyond the similarities, two additional genetic alterations characterize GCB-DLBCL. First, 21% of GCB-DLBCL cases have a gain of function mutation in *EZH2*, thereby promoting GC proliferation and inhibiting post-GC differentiation[65,66]. Second, 30% of GCB-DLBCL cases and 15% of BL cases exhibit mutations in *S1PR2, GNA13, ARHGEF1*, or *PR2Y8* which disrupt the Ga13-dependent pathway, thus allowing B cells to migrate from the GC into lymph and blood circulation[67]. In spite of knowledge of these alterations, the precise pathogenesis of GCB-DLBCL is not well understood.

2.1.2.3.2. ABC-DLBCL, the second DLBCL subtype, is characterized by constitutive NF-KB signalling and inhibition of terminal differentiation

Two main genetic alterations characterize ABC-DLBCL. First, NF-KB is constitutively activated. Such activation can occur through multiple mechanisms. In 20% of cases, *CD79A* and/or *CD79B* mutations generate chronic BCR signalling[68]. In 10% of cases, *CARD11* activating mutations constitutively activate NF-KB. In 35% of cases, *MYD88* mutations constitutively activate MYD88 and affect JAK/STAT3 signalling[69]. In 30% of cases, *TNFAIP3* inactivating mutations inhibit the stoppage of NF-KB responses[70]. Finally, antigens or autoantigens can chronically stimulate BCR. Second, the negative regulation of *PRDM1*, the plasma cell master regulator, blocks terminal differentiation to plasma cells. This negative regulation occurs through either bi-allelic activation of *PRDM1* (30% of cases), *SPIB* gain of function which increases inhibition of *PRDM1* transcription (25% of cases), or *BCL6* translocations, which cause constitutive repression of *PRDM1*[71–75]. Combined, these genomic lesions grant ABC-DLBCL a worse clinical course and outcome than GCB-DLBCL[48,76].
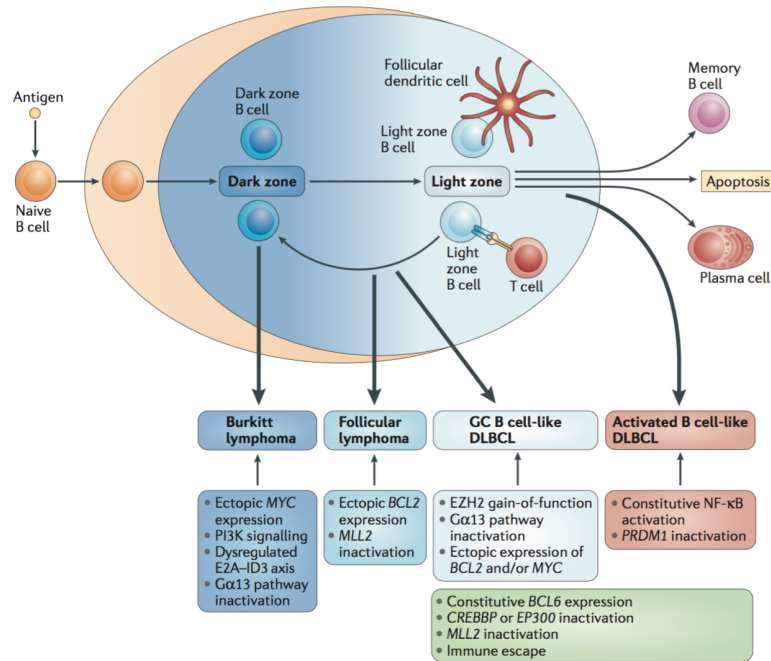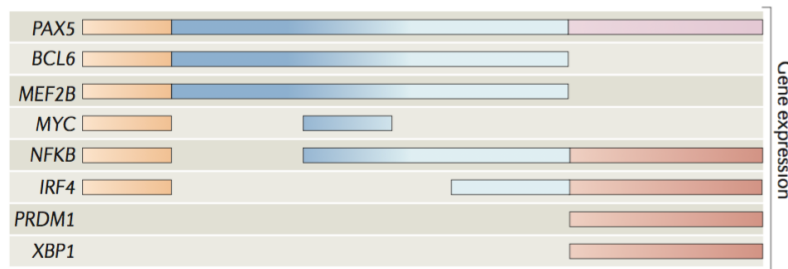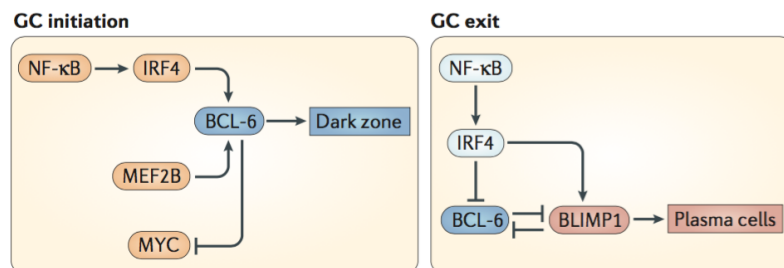
Figure 2 B-Cell Lymphomagenesis originates in the Germinal Centres. (a) B-NHLs correspond to dysregulation of different stages of B-Cell development. Each carry hallmark mutations disrupting a specific transition. **(b)** Transcriptional activity drives normal B cell development with gene expression driving transitions between stages. **(c)** Transcriptional networks work jointly to create major transitions such as GC initiation and GC exit, with *BCL6* as a master regulator. Adapted from Basso et al. 2015.

2.2. Clinical Characteristics of B-NHLs

B-NHLs share a set of clinical characteristics although they differ in their distinctive features, all reviewed below. Survival outcomes vary, but in all cases, prognostication and treatment lag behind recently acquired understanding of the molecular and genetic heterogeneity of B-NHLs. All citations from this section are taken from *Pathophysiology of Blood Disorders, Volume 2* by H. Franklin Bunn and Jon Aster[77].

2.2.1. B-NHLs share symptoms of immune dysregulation and are measured by a common staging system

Generally, B-NHLs share a set of common clinical features. B-NHLs usually present as a mass in the lymph nodes or secondary lymphoid tissues, though they can also present in virtually any organ in the body. Once presented, symptoms associated with immune dysregulation result, namely: B symptoms (weight loss, night sweats, fever), immunosuppression, and breakdown of immune tolerance. Additionally, B-NHLs generally have infectious agents as cofactors in development (*Helicobacter pylori*, HTLV-1, HHV-8, HIV, and EBV).

All B-NHLs share the same staging system: the Ann Arbor Staging for Lymphomas. Four stages exist consistent with increasing progression of the disease that are based on the number and location of nodes. Stage I corresponds to the involvement of a single lymph node group (I) or a single extralymphatic organ or site (IE). Stage II corresponds to the involvement of two or more lymph node groups on the same side of the diaphragm without (II) or with localized involvement of an extralymphatic organ or site (IIE). Stage III corresponds to the involvement of lymph node groups on both sides of the diaphragm without (III) or with localized involvement of an extralymphatic organ or site (IIIE). Stage IV corresponds to the extensive involvement of one or more extralymphatic organs or sites (i.e. bone marrow) with or without lymphatic involvement. In spite of the consistent staging system, however, the clinical course for each B-NHL is distinct: FL is indolent, DLBCL is aggressive, and BL is very aggressive. Moreover, this staging system lacks the resolution necessary to account for patient heterogeneity, particularly in DLBCL.

2.2.2. FL is an indolent lymphoma with a passive clinical course

FL is the most common indolent lymphoma, representing 20,000 new cases per year in the US. Upon presentation, FL is usually asymptomatic with painless lymphadenopathy. Patients are diagnosed via a biopsy of the lymph node and fall into two categories. The first

category of patient (20%) have spontaneous and transient remissions. The second category of patients have local symptoms due to FL progression: cytopenias from bone marrow involvement, hypersplenism, B symptoms, symptomatic extranodal disease (i.e. pleural effusions), and/or compromised organ function. Stage I patients generally are cured by local radiation. Patients in more advanced stages receive chemotherapy and rituximab whereupon >90% show excellent responses over five years with resistance being common afterwards. Finally, 2% of patients transform to more aggressive lymphomas per year. Overall, FL now shows a median survival of >10 years.

2.2.3. BL is a rare but highly aggressive lymphoma

BL is a highly aggressive lymphoma accounting for less than 2% of adult lymphomas. BL occurs in three clinical settings: (1) in subequatorial Africa where BL is latently infected with EBV and/or malaria as a cofactor, (2) in the US where BL presents in a sporadic form and 30% of cases occur with EBV, and (3) in patients with immunodeficiency, often resulting from HIV and/or EBV. Regardless of the clinical setting, BL arises in extranodal sites often in the abdomen as a rapidly growing tumour mass with a "starry sky" appearance. Immunohistochemistry shows pan-B-cell (i.e. CD20) and GC B-cell (i.e. CD10 and BCL6) markers but no BCL2. Additionally Ki-67 is seen as a marker of active growth and *MYC* rearrangements are common.

The prognosis and treatment of BL depend on stage, gender, age, and clinical setting. Endemic BL is localized and responds to chemotherapy. Sporadic and HIV-associated BL generally spreads to the Central Nervous System, thus requiring prophylactic treatment. Sporadic BL is treated with intensive combination therapies and rituximab regimens coupled with intrathecal therapy to prevent disease in the CNS.

2.2.4. DLBCL is a common and aggressive lymphoma in which 30% of patients are not cured by first line treatment

DLBCL is the most common lymphoma, accounting for 30,000 new cases per year in the US. Most DLBCL presents in older adults with a median presentation age of 65. Most DLBCL presents in lymph nodes (2/3) though some (1/3) presents in extranodal sites, generally in the gastrointestinal tract. Almost any organ can be involved in DLBCL. Regardless of subtype, DLBCL is a rapidly expanding mass with B symptoms that mark it as an aggressive disease. Diagnosis is made by tissue biopsy and immunophenotyping which reveal pan-B-cells markers (i.e. CD20), BCL6 expression, and variable expression of CD10,

BCL2, and surface immunoglobulins. Additionally, serum lactate dehydrogenase (LDH) levels are elevated in over half of DLBCL patients unlike in indolent lymphomas.

DLBCL patients are prognosticated based on the Revised International Prognostic Index (R-IPI). The R-IPI considers negative prognostic factors at the time of diagnosis (stage III/IV of the disease, age > 60 years, elevated lactate dehydrogenase (LDH) levels, Eastern Cooperative Oncology Group (ECOG) performance status >=2, and >1 extranodal sites of disease) to sort patients into three risk categories. Patients with zero risk factors have >90% chance of 4-year progression-free survival. Patients with 1 or 2 risk factors have an 80% chance of 4-year progression-free survival. Finally, patients with 3, 4, or 5 risk factors have a 50% chance of 4-year progression-free survival[78].

Regardless of prognostication, all patients today are treated with R-CHOP. Up to one-third of patients do not achieve a cure with their initial therapy. Relapse and non-responsive patients have a poor prognosis. These patients may undergo more aggressive therapies such as stem cell transplantation; however, only 25% of such patients survive > 5 years. Today, there are no effective methods to distinguish up-front which patients will not be cured by first-line chemotherapeutic treatment. Identifying these patients up-front would allow doctors to move them toward more aggressive clinical regimens sooner or potentially toward experimental therapies.

2.3. Classification of B-NHLs

Although recent studies have uncovered the genetic heterogeneity inherent to DLBCL, current classification schemes have not yet fully incorporated this heterogeneity. Similarly, these classifications have done little to change clinical practice: the same frontline treatment is given to all patients although 30% of patients are not cured by R-CHOP. Our primary goal, therefore, is to improve upon known classification systems with the hope of discovering distinctive pathogenic and clinical characteristics that can guide treatments.

The primary goals of any classification system are three-fold. First, to delineate subcategories of the disease with interpretable differences that generate biological insights related to pathogenesis. Second, harness those insights to create targeted therapies for each class. Third, to then administer the optimal treatments for patients based upon which class of the disease they express.

Consistent with these goals, classification system schemes have been increasingly shifting towards molecular and genetic classification. As an example, some high grade B-cell lymphomas are now defined on the basis of whether they exhibit *MYC* and *BCL2* and/or *BCL6* rearrrangements.

Below, we describe the three current classification systems for DLBCL and B-NHLs: the WHO classification, cell-of-origin classification, and consensus clustering.

2.3.1. WHO Classification relies primarily on morphologic, biologic, immunophenotypic, and clinical parameters

The primary classification for lymphoid neoplasms including DLBCL is the WHO classification. The WHO classification primarily uses morphologic, biologic, immunophenotypic, and clinical parameters to separate lymphoid neoplasms into subgroups. Each subtype, described below, carries unique characteristics that often translate into distinct clinical courses.

2.3.1.1. DLBCL NOS

Accounting for 25-30% of NHL, DLBCL NOS is the most common WHO subtype. Crucially, DLBCL NOS is primarily an exclusion category: rather than having positive defining characteristics, DLBCL NOS samples are defined by not fitting the characteristics of other categories.

DLBCL NOS can originate de novo or as a result of transformation from FL or CLL. The most common genetic aberrations of DLBCL NOS include *BCL6* mutations (30% of cases[29]), *MYC* translocations (10% of cases[79]), and *BCL2* mutations in GCB-DLBCL.

Historically, DLBCL was resolved on the basis of morphological features. In particular, the recognition of centroblastic, immunoblastic, and anaplastic subtypes enabled classification and corresponded with clinical differences: centroblast tumours exhibited better prognostic outcomes than immunoblast tumours[1]. Major issues exist with this approach however. First, reproducibility of clinical differences is poor. Additionally, relatively small numbers of patients show immunoblastic morphology (only 7.4% of nearly 1000 patients in a clinical trial) showing that such morphological based classification had limited clinical applicability[80]. More recently, resolution of DLBCL NOS subgroups has been accomplished through gene expression studies delineating the cell of origin, described above.

2.3.1.2. DLBCL in specific subtypes

Other subtypes of DLBCL affect specific sites of the body: intravascular large B-cell lymphoma (IV-LBCL), primary cutaneous DLBCL, leg-type, and primary CNS DLBCL. Of those subtypes, only IV-LBCL was present within our study. IV-LBCL is rare and characterized by large B-cells occurring in the lumen of small blood vessels. The majority of IV-LBCL shows a gene-expression profile consistent with ABC-DLBCL and expresses the CD5 surface marker[81]. However in the absence of definitive radiological or clinical evidence and diverse symptoms, the disease is rarely diagnosed until autopsy.

2.3.1.3. High Grade B-Cell Lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements

This category includes all large B cell lymphomas with *MYC* and *BCL2* and/or *BCL6* rearrangements except those that fulfil criteria corresponding to follicular or lymphoblastic lymphoma[82]. These double hit and triple hit lymphomas correspond to a set of very aggressive tumours that generally exhibit chemoimmunotherapy refractoriness and high relapse rates. Substantial research is now being conducted to improve treatment for these patients[83,84].

2.3.1.4. B-Cell Lymphoma, unclassifiable with features intermediate between DLCBL and Hodgkin Lymphoma

This category, also known as grey zone lymphoma (GZL) contains samples intermediate between classical Hodgkin's lymphoma (cHL) and DLBCL (especially PMBL) in terms of clinical, morphologic, and immunophenotypic characteristics. Defining characteristics of GZL include: mediastinal involvement[85], diversity in cytologic appearance[85], and more cytogenetic aberrations than cHL, PMBL, and GZL[85,87,88]. The gene expression profile of this subcategory has not been examined. Additionally, the optimal treatment is unknown and cHL and NHL treatments have both been ineffective[89–91]. A more refined genetic profile and understanding of the pathogenesis of GZL could therefore inform treatment approaches.

For consistency with figures, we have used BCL, Int. as the abbreviation for this class.

2.3.1.5. T-Cell/Histiocyte Rich Large B-Cell Lymphoma

THR-LBCL is characterized by tumour cells high in reactive T cell or histiocyte content. THR-LBCL has distinct clinical features from other DLBCL subtypes: it presents predominantly in males in their fourth decade; includes spleen, liver, and bone marrow involvement; and follows an aggressive clinical course[92–94]. Generally, THR-LBCL is closely pathologically related to lymphocyte predominant Hodgkin lymphoma but differs in a few respects: the absence of small B-cells, the lack of a follicular structure, and the absence of T-cell rosettes around atypical B-cells.

2.3.1.6. Plasmablastic lymphoma

PB-LBCL results when immune surveillance declines due to advanced age and/or iatrogenic immunosuppression[95]. PB-LBCL occurs primarily in males with a median age of 50, with most cases being EBV-positive. Additionally, PB-LBCL patients generally have *MYC* translocations[89,96]. In terms of treatment, PB-LCBL show early responses to therapy but a poor overall prognosis including high likelihood of relapse[90].

2.3.1.7. Additional WHO subtypes not included within our study

In addition to the subtypes in our study, described above, additional subtypes exist and are discussed in the corresponding references. Two subtypes of DLBCL relate to the presence of EBV. First, EBV[+] DLBCL, NOS has an aggressive clinical course[97]. Second, DLBCL associated with chronic inflammation[98] primarily presents in males between age 65 and 70 with an aggressive clinical course[99,100]. An additional three subtypes of DLBCL

exhibit a plasmablastic phenotype (i.e. acquisition of plasma cell markers like CD38/CD138 with loss of or weak B-cell markers and MUM-1 positivity: ALK$^+$ large B-cell lymphoma[101–106], plasmablastic lymphoma[89–91,95,96,107–109], and primary effusion lymphoma[110–117]). For additional rare subtypes, we refer to the official WHO classification[82].

2.3.1.8. Follicular Lymphoma, Large Cell

In addition to the WHO classification presented above, one additional subtype (Follicular Lymphoma Large Cell or FL-LC) was present within our study. Generally, FL-LC is a subset of FL that is distinct from indolent follicular lymphomas. FL-LC is an aggressive lymphoma that presents with favourable prognostic features compared to FL. Both the clinical features and treatment response in FL-LC are similar to those in DLBCL[118].

2.3.1.9. Splenic Marginal Zone Lymphoma

While we didn't have any samples explicitly diagnosed as SMZL cases, our later classification analysis uncovered patients with genetic profiles consistent with SMZL. Clinically, SMZL is a low grade B-cell lymphoma showing splenomegaly, moderate lymphocytosis, and autoimmune thrombocytopaenia or anemia[119–121]. The immunophenotype of SMZL is similar to splenic marginal zone B-cells (CD27+, IgM+, IgD+[119,120,122]), however, the cell of origin is ultimately unknown. Indeed, ~90% of cases include multiple somatic mutations at variable degrees, suggesting the possibility for multiple cells of origin.

Genetically, SMZL manifests mutations in various pathways, all affecting marginal zone B-cell development: *KLF2* (20-42%), *NOTCH2* (6.5-25%), *NF-KB* (*CARD11* ~7%, *IKBKB* ~7%, *TNFAIP3* ~7-13%, *TRAF3* ~5%, *BIRC3* 6.3%). Marginal zone B-cell development, however, is not broadly well understood making the exact pathogenesis of SMZL unclear. Additionally, most SMZL shows recurrent gains and losses (7q32 deletion in 18-44% of cases) and translocations resulting in somatic hypermutation (*IGHV1-2* in 90% of cases[123–128]). Overall, the most common changes in SMZL are 7q deletion, *KLF2* mutation, *NOTCH2* mutation, and *IGHV1-2* usage[129]. The presence of these together implies that oncogenic cooperation may occur. For example, *KLF2* and *TRAF3* mutations may work together to activate the NF-KB pathway.

2.3.2. Gene expression profiling has classified DLBCL on the basis of cell of origin, yet issues remain

More recently, DLBCL categorization has moved toward the identification of distinct genetic and epigenetic changes. Gene expression profiling has resolved the DLBCL NOS group of the WHO classification into two subcategories: ABC-DLCBL and GCB-DLBCL. These subgroups, whose genomic and pathogenetic differences are described above, are based upon a "cell of origin" interpretation of DLBCL. Additionally, ABC-DLBCL and GCB-DLBCL have been shown to follow distinct pathways toward transformation and oncogenesis.

Consistent with this, targeted therapies affecting pathways responsible in the pathogenesis of only one subtype have helped patients primarily of that subtype. As an example, Bortezomib, a protease inhibitor blocking NF-KB signalling improves survival for ABC-DLBCL but not GCB-DLBCL patients.[77] Other studies have specifically suggested downregulating the BCR pathway through inhibition of BTK, PI3K, STK, MTOR, and SRC kinases in order to improve ABC-DLBCL survival.[77]

Issues exist with the gene expression profiling based classification, however. Gene expression profiling is technically difficult to perform and has limited availability in laboratory settings. As a result, immunohistochemistry has been proposed as an alternative way to identify ABC-DLBCL and GCB-DLBCL subtypes. Immunohistochemistry, however, (1) does not correspond directly to ABC-DLBCL and GCB-DLBCL distinctions although correlations exist, (2) produces unclassifiable cases, (3) uses the Hans algorithm which shows reproducibility and reliability issues.[77] If an alternative and more reliable way to identify cell of origin could be created, for example through the identification of specific mutations that correlate with these outcomes, the ABC-DLBCL and GCB-DLBCL classification would gain substantial clinical impact. Such a question could potentially be answered by a follow-up to our present study including gene expression data.

2.3.3. Alternatively, consensus clustering strives to classify DLBCL on the basis of metabolic pathway regulation

Finally, an independent classification has arisen based on consensus clustering which separates DLCBL samples by the up and down regulation of metabolic pathways.[130] The first cluster, the OxPhos consensus cluster, expresses genes important to mitochondrial metabolism and oxidative phosphorylation. The second cluster, the BCR consensus cluster, expresses genes critical to B-cell receptor signalling, regulation of the cell cycle, DNA repair, and B-cell transcription factors. The final cluster, the host response consensus cluster, expresses genes involved in the immune inflammatory response, the classic component

pathway, and the T-cell mediated immune response. Overall, OxPhos clustering has little overlap with the gene-expression cluster subtypes (ABC-GLBCL, GBC-DLBCL) and WHO classification above. As such, it is difficult to compare with the prior classification schemes and lies largely tangential to the classification presented in this paper.