Genomics of aggressive B-cell lymphoma

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The growing body of genomic information collected and applied to mature aggressive B-cell lymphoma diagnosis and management has exploded over the last few years due to improved technologies with high-throughput capacity, suitable for use on routine formalin-fixed, paraffin-embedded tissue biopsies, and decreasing costs. These techniques have made evaluation of complete DNA sequences, RNA-expression patterns, translocations, copy-number alterations, loss of heterozygosity, and DNA-methylation patterns possible on a genome-wide level. This chapter will present a case of aggressive B-cell lymphoma and discuss the most important genomic abnormalities that characterize this group of entities in the recent update to the fourth edition of the World Health Organization (WHO) lymphoma classification system. Genomic abnormalities discussed will include those necessary for certain diagnoses such as translocations of *MYC*, *BCL2*, or *BCL6*; gene-expression-profiling categorization; the newly defined Burkitt-like lymphoma with 11q abnormalities; prognostic and predictive mutations, as well as tumor heterogeneity. Finally, our current practices for clinical triage of specimens with a potential diagnosis of aggressive B-cell lymphomas are also described. Options for treatment at relapse, in light of these genomic features, will be discussed in the third presentation from this session.

Learning Objectives

- Explain the current diagnostic approach to classification of aggressive B-cell lymphomas including the current approach to testing for diffuse large B-cell lymphoma (DLBCL), high-grade lymphoma, and Burkitt lymphoma
- Describe some of the major differences in activated B-cell and germinal center B-cell subtypes of DLBCL
- Perceive the complex mutational spectrum and clonal heterogeneity in DLBCL, and how these may affect precision medicine

Patient case

A 74-year-old female patient presenting with fatigue, intermittent night sweats, and weight loss underwent imaging of the abdomen and pelvis revealing retroperitoneal lymphadenopathy. A computed tomography (CT)-guided fine-needle-aspiration biopsy was nondiagnostic. Positron emission tomography (PET)/CT showed nonbulky enlarged fluorodeoxyglucose-avid lymph nodes above and below the diaphragm with standardized uptake value ranging from 2.5 to 12. A second biopsy including 6 needle core biopsies of the most PET-avid lymph node resulted in a diagnosis of diffuse large B-cell lymphoma (DLBCL) with typical centroblastic cytology. In addition to B-cell markers, there was expression of B-cell lymphoma 2 (BCL2) and multiple myeloma oncogene 1 (MUM1) by immunohistochemistry (IHC). CD10, BCL6, Epstein-Barr virus–encoded small RNA, and MYC were negative by IHC. Fluorescence in situ hybridization (FISH) studies demonstrated rearrangement of *MYC* but not *BCL2* or *BCL6*. Cell-of-origin (COO) assignment by the molecular COO, Lymph2Cx, assay demonstrated an "activated B-cell" (ABC) molecular subtype. Her bone marrow biopsy was negative. Both her complete blood count and lactate dehydrogenase were normal at baseline. Her revised International Prognostic Index was 2.

Disease-defining abnormalities

Disease-defining abnormalities include DLBCL, high-grade (HG) B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements (also known as double hit or triple hit, abbreviated as HG-DH or HG-TH), and HG B-cell lymphoma–not otherwise specified (HG-NOS).

DLBCL, by far the most common aggressive B-cell lymphoma, with a heterogeneous outcome, has been intensely studied to subclassify this broad disease into more specific diagnostic categories with stratification by phenotypes, genotypes, and outcomes. It has been long recognized that cases with the morphology of DLBCL can have a *MYC* translocation similar to the translocations detected in Burkitt lymphoma (BL). However, the prognostic significance of the *MYC* translocation in DLBCL has been debated. In recent years, the so-called DH or TH lymphomas have been defined to contain a *MYC* translocation (occurring with any partner gene) in combination with a translocation at *BCL2* or *BCL6* (DH) or both (TH[1]). All 3 translocations are reported to occur in 8% of all cases with DLBCL cytologic features.[1] Some studies suggest that DH and TH lymphomas may have a worse prognosis than DLBCL with no *MYC* translocation or a *MYC* translocation alone, however, further research is needed.[2] Whether the *MYC* gene fusion partner impacts the clinical significance of the *MYC* translocation is of some debate, however, this question may be complicated by variations in probe performance

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Table 1. Summary of known genomic and mutational data in aggressive B-cell lymphomas

Category	Associated genotype	Most frequent mutations	
GCB-DLBCL	*BCL2* or *MYC* translocations, DH or TH, complex karyotype, *PTEN* deletion	*EZH2*, *GNA13*, *BCL6*, *TNFSR14*, *FOXO1*, *ACTB*, *SOCS1*, *BCL2*, *SGK1*	*KMT2D*, *CREBBP*, *EP300*, *TP53*, *HST1H1E/C*, *B2M*
ABC-DLBCL	*BCL2* amplifications, *CDKN2A/B* deletion, complex karyotype, rare DH or TH	*MYD88*, *CD79A/B*, *CARD11*, *TNFAIP3*, *PIM1*, *NOTCH1*, *SPIB*, *PRDM1* (mutually exclusive with *BCL6* rearrangements)	MHC class I, *TP53*, *MEF2B*, *NOTCH 2*, *BTG1/2*, *SOCS1*, *DTX*, *SPEN*, *TNFAIP3*, *TMSB4X*
UNC-DLBCL	Complex karyotype, *NOTCH2* amplification, BCL6 translocations, rare *MYC* with *BCL6* DH	*NOTCH2*	
HG-DH/TH	DH or TH of *MYC* and *BCL2* and/or *BCL6*		
HGBL, NOS	No DH nor TH		
BL	*MYC* rearrangement, 1q gains, no DH or TH, low complexity karyotype	*TCF3*, *ID3*	
BLL with 11q alteration	11q alterations, no *MYC* rearrangement, no 1q gains		

by various commercial vendors.[3,4] Altogether, there is sufficient evidence that B-cell lymphomas containing a DH or TH have a worse prognosis with standard therapy, earning them a separate diagnostic category in the World Health Organization (WHO) 2017 classification as "high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements" (HGBL-DH/TH). This category includes cases with the morphologic appearance of DLBCL (centroblastic, immunoblastic, and other rare variants) as well as cases with cytologic features intermediate between BL and DLBCL (also termed "Burkitt-like" lymphoma [BLL]) or resembling lymphoblasts (also termed "blastoid").[5] Thus, this diagnostic category uses gene-rearrangement information to define the disease along with loosely defined morphologic features. As such, cases must contain a DH or TH alongside a diffuse histology with medium- to large-sized cells and cannot meet criteria for a specific diagnosis such as follicular lymphoma or lymphoblastic lymphoma/leukemia. Cases with HG cytology (BLL or blastoid) and often a "starry sky" appearance, frequent mitoses, and prominent apoptosis, without a DH or TH genotype, and lacking features diagnostic of BL, are included in the new diagnostic category HG-NOS. In terms of frequency of occurrence, *MYC*/*BCL2* HG-DH cases are the most common, followed by *MYC*/*BCL2*/*BCL6* HG-TH, and then *MYC*/*BCL6* HG-DH translocations.[6] The *MYC*/*BCL6* HG-DH translocated cases may have a propensity for extranodal sites, older patients, and are evenly divided between the ABC and germinal center B-cell (GCB) subtypes of DLBCL (discussed below in "RNA expression patterns in DLBCL molecular subtypes"), and have outcomes intermediate between HGBL-DH with *MYC* and *BCL2* translocations and DLBCL without *MYC* rearrangement; however, these correlations are based on low numbers of cases and further confirmation is needed.[6] The genetic features of DLBCL are summarized in Table 1. This patient's case, with typical centroblastic DLBCL cytology and a single *MYC* hit, diagnostically remained in the DLBCL category with a notation of the *MYC* translocation and high proliferation rate.

MYC and *BCL2* amplifications and DLBCL with "double expression"

Cases lacking *MYC* translocations but harboring extra copies or amplification of *MYC* genes currently do not fit the criteria for a HG-DH/TH. Similarly, cases with a *MYC* translocation with extra copies of *BCL2* and/or *BCL6* do not qualify as HG-DH/TH.[5] However, this is an area of ongoing investigation with conflicting studies, possibly due to different definitions of copy-number gains or amplifications.[7-9] Because the cytogenetic or FISH studies needed to detect these HG lymphomas can be expensive and are not readily available at some centers, and the fact that there are tissue limitations from small biopsies, expression of the MYC and BCL2 proteins has been proposed as a surrogate or even superior marker to define high-risk groups.[10-12] In an early paper, IHC cutoffs of 40% for MYC and 50% for BCL2 were used, however, subsequent work suggests that higher cutpoints may be more specific. Tumors that express both MYC and BCL2 protein by IHC, described as DLBCL double expressers (DLBCL-DEs), may contain FISH evidence of *MYC* and *BCL2* translocations and/or amplifications or neither. DLBCL-DE is more common than HG-DH/TH lymphomas, accounting for almost 30% and 10% of all DLBCL cases, respectively.[13] Coexpression of BCL6 with MYC or BCL2 at the protein level is not considered evidence of a DLBCL-DE because BCL6 protein by IHC is merely considered a marker of germinal center differentiation. A current matter of discussion is whether IHC can be used as a screening tool to identify cases that should undergo FISH testing for *MYC* translocations. Although appealing from a cost-containment point of view, there is no consensus on this approach because, as noted, some cases with MYC and BCL2 protein expression do not harbor the translocations and a small number of cases negative for MYC by IHC will have a positive FISH study.[1] Differences in tissue preservation, antibody performance, and interpretation make standardization of IHC somewhat difficult in routine practice. In the current case, the patient's tumor was one such exception with evidence of a *MYC* translocation by FISH but lacking sufficient MYC protein expression by IHC to be considered a DLBCL-DE.

RNA-expression patterns in DLBCL molecular subtypes

DLBCL was one of the first lymphoid tumors studied by gene-expression profiling (GEP), first using competitive microarrays, then oligonucleotide arrays, and more recently with digital arrays.[14-16] The earliest report described the presence of 2 different COO GEP for DLBCL called the ABC and GCB cell types. A follow-up paper described the same 2 categories and a third unclassifiable (UNC) category with intermediate features of ABC and GCB.[17,18] The ABC-DLBCL subtype evidently arises from post–germinal center, early plasmablastic B cells, has a worse prognosis than the GCB-DLBCL subtype, and different genomic alterations such as more frequent *BCL2* amplifications as well as a different mutational profile that can be therapeutically exploited.[19,20] In contrast, the GCB-DLBCL

subtype arises from germinal center B cells and has a better prognosis. Furthermore, the MYC/BCL2 DH and TH cases are almost exclusively GCB-DLBCL.[1] Within each COO subgroup, *BCL2* genetic alterations, amplifications in ABC-DLBCL, and translocations in GCB-DLBCL, identify high-risk patients.[1] In other studies, increased *MYC* and *BCL2* gene expression appears to override the prognostic significance of COO; however, this could be related to selection bias toward healthier patients being enrolled into clinical trials as recently described.[10,21] Recently, digital-expression profiling has been used to develop a promising assay to diagnose ABC-DLBCL, GCB-DLBCL, and UNC cases that will soon be undergoing US Food and Drug Administration (FDA) review for clinical use.[14] This patient's tumor underwent testing using this assay, known under the research name Lymph2Cx and commercial name LymphMark with results indicating that her tumor was the ABC-DLBCL type.

BLL with 11q abnormality: alternative mechanisms to obtain an "*MYC*-like" phenotype

Genomic alterations identified through array chromosomal genomic hybridization (array CGH) have also been incorporated into the new classification scheme. Similar to classical cytogenetics, array CGH evaluates copy-number aberrations across the entire genome of test samples using a normal diploid genome for comparison. Array CGH has a technical advantage in that it can detect alterations in smaller regions of the genome (kilobase resolution) compared with karyotyping, which has a resolution of megabases. Thus, array CGH allows detection of smaller structural aberrations that would be difficult to impossible to resolve using karyotyping. Overall, BL has a low karyotypic complexity with an average of only 1.7 chromosomal abnormalities in addition to the characteristic *MYC* translocation as compared with 3.3 abnormalities in unclassified B-cell lymphoma and 21.6 in DLBCL.[22] A recent cytogenetic and array CGH study of cases with a BL GEP but no *MYC* translocation (so-called "molecular Burkitt") identified a region of chromosome 11q with proximal gains and telomeric losses, the absence of 1q gains frequently seen in BL, and a more complex karyotype than typical BL.[23] These cases exhibited classical BL morphology or slightly more pleomorphism, were occasionally nodular in appearance, possessed the classic BL immunophenotype ($CD20^+$, $CD10^+$, $BCL6^+$, $BCL2^-$, Ki67 100%), and were found to occur predominantly in children and young adults. In light of these unique features, "Burkitt-like lymphoma with 11q aberrations" is a new diagnostic entity in the update to the fourth edition of the WHO classification, compared with other aggressive B-cell lymphomas in Table 1.[5] However, a recent paper identified lymphomas with the 11q abnormality that also had *MYC* translocations and typical cytology of true BL as well as blastoid cytology typical of HG-NOS.[24] Other authors have identified microRNA alterations in a limited number of otherwise classical BL cases lacking *MYC* translocations.[25]

Altogether, these studies serve to highlight that, just as with *MYC* and *BCL2* in HG-DH/TH and DE-DLBCL, there is not a single genetic mechanism that accounts for all phenotypic BL cases.

Prognostic and predictive mutations in DLBCL

Mutational profiling of aggressive B-cell lymphomas has identified numerous genes that are involved in but not exclusive to, certain diagnostic categories. DLBCL has an approximate median of 5 somatic mutations per megabase, ranking it as the ninth highest mutation rate of 27 tested types of malignancies.[26] Distribution charts of DLBCL identify ~15 mutations present in at least 10% of DLBCL, with a long tail of infrequent mutations. Recently and in agreement with prior studies, a large targeted sequencing study indicated that the 4 most frequently mutated genes in DLBCL included *KMT2D*, *MYD88*, *CREBBP*, and *TP53*, each found in >15% of DLBCL.[27] Other papers identified similar recurrent mutations that also included *BCL2*, *HST1H1E*, and *PIM1*.[28] Mutated genes include those that are relatively specific to B cells such as those involving B-cell receptor (BCR)-signaling pathways as well as genes related more generally to cell function.[27,29] As might be expected for B-cell lymphomas, some of the mutated genes carry variants typical of aberrant somatic hypermutation due to increased activity of the activation-induced cytidine deaminase enzyme (termed AID or AICDA) normally used by B cells in the antibody diversification process. Activation-induced cytidine deaminase enzyme–targeted genes include *BCL6*, *IRF4*, *IRF8*, *CIITA*, *PIM1*, *MYC*, *SOCS1*, and *BCL7A*. Other mutated genes common to both ABC-DLBCL and GCB-DLBCL include histone modification genes such as *CREBBP* and *EP300* (acetyltransferases) and *KMT2D/MLL2* methyltransferase. Translocations involving *BCL6*, as well as mutations in the β2-microglobulin (*B2M*) and *PRDM1* genes, the latter 2 related to loss of immune surveillance, have also been described.[30,31] Altogether, there are currently 9 genes considered actionable (*CDK6*, *TP53*, *CDKN2A*, *PTEN*, *MYC*, *ARID1A* and *CD79B*, *EZH2* and *NOTCH1*) as potential therapeutic targets of drugs in early clinical trials.[27]

TP53 is a tumor suppressor gene and 1 of the most commonly deleted or mutated genes in all cancer types. TP53, known as the "guardian of the genome," is a critical regulator of cell-cycle arrest, DNA repair, and apoptosis.[32] Recently, *TP53*-truncating and missense mutations within the DNA-binding domain were found to be enriched in ABC-DLBCL, whereas other *TP53* mutations can be present in both ABC- and GCB-DLBCL.[33] Seventy-four percent of *TP53* mutations were associated with 17p loss or copy-number neutral loss of heterozygosity. Overall, both mutations and copy-number loss are more common in ABC-DLBCL and are statistically significant independent predictors of poor progression-free and overall survival in a multivariate analysis (validated in an independent cohort).[27] Other types of *TP53* mutations, not involving truncating or missense mutations in the DNA-binding domain, did not have the same clinical significance.[33] With TP53 abnormalities playing such a central role in the persistence of lymphoma, efforts to target this pathway are ongoing.

ABC-DLBCLs have many distinctive features including messenger RNA patterns, phenotype, and genotype. *BCL2* amplifications are more frequent whereas *MYC/BCL2* DH translocations are rare in ABC-DLBCL.[33] The mutational profile in ABC-DLBCL is also different from the other types of aggressive B-cell lymphomas with frequent mutations in the BCR-signaling pathway. Some of the earliest sequencing studies in DLBCL demonstrated mutations in *CD79A* and *CARD11* contributing to enhanced NF-κB activity through chronic activation of BCR signaling.[34,35] Mutations in *MYD88* L265P (the same mutation found in lymphoplasmacytic lymphoma but infrequently in other low-grade lymphomas) has been shown to enhance NF-κB and JAK-STAT signaling via Toll-like receptor signaling.[36] Mutations in genes associated with terminal differentiated B cells such as *PRDM1/BLIMP1* are found in 25% of ABC-DLBCL and appear to be mutually exclusive with *BCL6* gene rearrangements, suggesting that these genes have complementary roles in blocking differentiation.[37-39] *PIM1* kinase mutations are also more frequent in ABC-DLBCL. Altogether, these findings imply that ABC-DLBCL may be susceptible to targeted therapies aimed at these pathways.[40,41]

GCB-DLBCLs also have a distinctive mutational profile. Although GCB-DLBCLs have an overall better outcome compared with ABC-DLBCL, the HG-DH/TH lymphomas are found almost exclusively in GCB-DLBCL.[13] A large-scale genomic change in GCB-DLBCL includes deletions of the *PTEN* tumor suppressor gene leading to activation of the phosphatidylinositol 3-kinase–signaling pathway.[42] On an individual gene level, 22% of GCB-DLBCL cases have mutations in the *EZH2* polycomb group of oncogenes involved in DNA methylation.[43] These mutations are of particular interest due to the clinical development of EZH2 and phosphatidylinositol 3-kinase inhibitors.[44,45] The *GNA13* and *GNA12* genes encoding small GTPases that regulate B-cell homing to the germinal center are also mutated in 20% of GCB-DLBCL.[46,47] Other mutations more commonly found in GCB-DLBCL include *KMT2D*, *CREBBP*, *TNFSR14* (adhesion molecules), *B2M* (antigen presentation), *FOXO1* (transcription factor), *ACTB* (cytoskeleton), *SOCS1* (suppressor of cytokine signaling).[27] The different mutations found in aggressive B-cell lymphomas are summarized in Table 1.

Genetic subtypes of DLBCL

Recently, a combined study of DNA mutation analysis, RNA sequencing, copy-number variation, and gene-expression profiling of 574 DLBCL revealed 4 different genetic subtypes termed MCD (with cooccurrence of *MYD88* and *CD79B* mutations), BN2 (*BCL6* fusions and *NOTCH2* mutations), N1 (*NOTCH1* mutations), and EZB (based on *EZH2* mutations and *BCL2* translocations). Interestingly, MCD and N1 subtypes were mainly ABC-DLBCL, EZB included mostly GCB-DLBCL, and BN2 included ABC-DLBCL, GCB-DLBCL, and UNC-DLBCL. This is the first study to demonstrate a possible genetic basis, BN2, for the UNC-DLBCL group. The BN2 and EZB subtypes had favorable survival compared with the other groups.[43] Other authors classified all of their 304 patients into 6 subgroups. with cluster 5 corresponding to MCD, cluster 1 to BN2, and cluster 3 to EZB. Additional clusters were cluster 2 (showing mutations and deletions of *TP53* and genomic instability), cluster 4 (primarily GCB-DLBCL and distinct from cluster 3), and cluster 0 (small group lacking driver mutations).[48] The power of genetic classification is readily evident in that these 2 completely independent studies overlapped so well. Altogether, these findings may have impact on the selection of subgroup-specific targeted therapies given the distinct difference in clinical outcomes after rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP), though larger studies with particular attention to genetic subtypes and associated treatment response are needed to make further conclusions.

Clonal heterogeneity

Sequencing depth or coverage refers to the number of times a nucleotide is sequenced by different "reads" where the greater the depth, the more confident one can be in correctly calling the DNA sequence. In deep sequencing, a genomic region is read hundreds, possibly thousands, of times in order to confidently call the genomic sequence in that region, which in turn allows identification of mutations and allele frequencies in a given malignancy. Indeed, neoplastic transformation is a multistep process in which cells acquire various attributes including mutations as they evolve.[49] The general consensus regarding the mutagenic evolution of a tumor is that transformation is initially driven by the acquisition of early "driver" mutations in a progenitor cell. Subclones emerge as a result of branched evolution wherein additional mutations, both driver and passenger, are subsequently acquired at different times as the cells proliferate giving rise to a tumor mass composed of genomically distinct subpopulations (subclones) stemming from a common ancestral progenitor cell.[50,51] The malignant phenotype of a given subclone is dependent, in part, on the interplay of its mutational landscape, to which passenger mutations are not believed to significantly contribute. The patterns of both driver and passenger mutations can be used to construct a tumor's mutational hierarchy. In a study of relapsed DLBCL, there was evidence of clonal expansion (increased variant allele frequency) and clonal selection (varying proportions of unique mutations) after selective pressure from RCHOP treatment. These authors identified several genes involved in treatment resistance including *TP53*, *FOXO1*, *KMT2C*/*MLL3*, *CCND3*, *NFKB1Z*, and *STAT6*. Deep sequencing demonstrated that these clones were present in the original tumor although sometimes at lower allele frequency than in the relapsed sample.[52] The clear implication is that these minor subclones may be important to consider in developing new therapeutic strategies.

Circulating tumor DNA

Circulating tumor DNA (ctDNA), also known as cell-free DNA, found in the plasma, is a promising and minimally invasive method for the detection of tumor-associated genomic alterations at diagnosis and relapse. Plasma ctDNA may include DNA from subclones at different anatomical sites with the hope that it will better reflect overall clonal heterogeneity. The earliest of these studies in DLBCL used clonotypic immunoglobulin rearrangements to detect tumor DNA in the blood.[53,54] The amount of clonotypic immunoglobulin circulating in the plasma has been correlated with clinical indices such as tumor burden and patient outcome. More recently, DLBCL-focused panels of DLBCL-associated mutations, indels, and *BCL2*/*BCL6*/*MYC*/*IgH* breakpoints have been detected in ctDNA and found to be concordant with the abnormalities detected in a simultaneous tissue biopsy.[55,56] Circulating cell-free DNA analysis is a promising new technology that may be helpful in in determining DLBCL COO, identifying actionable mutations, and detecting early relapse.[55,56]

In our current practice, after initial morphologic review, either through frozen section, touch preparation, or histology, aggressive B-cell lymphomas undergo immunophenotyping by flow cytometry (if a diagnosis of lymphoma was anticipated ahead of time and fresh material is available) or by IHC (if only paraffin-embedded tissue is available). Immunophenotyping includes lineage-specific and/or targetable surface proteins such as CD19, CD20, CD22, and CD30, CD21 to assess for follicular dendritic cells and evaluate architecture, CD5 and cyclin D1 to exclude $CD5^+$ DLBCL or mantle cell lymphoma, CD34 and terminal deoxynucleotidyl transferase to exclude lymphoblastic lymphoma/leukemia, and Epstein-Barr virus–encoded small RNAs, to fit the case into a current WHO 2017 diagnostic category.

Additional stains may be performed based on the morphologic features such as to rule out plasmacytic differentiation or other diagnosis. In cases with DLBCL cytology, IHC for the prognostic proteins MYC and BCL2 are evaluated using cutpoints of 40% and 50%, respectively.[11] After phenotyping, all cases with DLBCL, HG, or Burkitt cytology are sent for FISH for *MYC* rearrangement (break-apart and fusion probes) with reflex testing for *BCL2* and *BCL6* if a *MYC* rearrangement is detected, and for COO testing by the Lymph2Cx assay (or IHC using the Hans algorithm).[14,57] To facilitate these multiple demands, we instituted a procedure to up front separate needle cores into separate blocks, in the tissue-grossing room, so as not to exhaust any 1 block. Using this procedure, we have had few cases with insufficient material as long as multiple true

needle core biopsies (not fine-needle aspirations) or excisional biopsies are obtained. In this case, all testing was performed on the available 6 needle core biopsies that were split: 1 core used for flow cytometry; 3 cores into the first formalin-fixed, paraffin-embedded block for histology, IHC, and Lymph2Cx; and the final 2 cores into a second formalin-fixed, paraffin-embedded block for histology and FISH studies.

Patient case continued

The patient received 3 cycles of RCHOP chemotherapy but an interim PET/CT scan showed progression of her lymphoma and she was classified as primary refractory. Treatment was switched to rituximab plus gemcitabine, dexamethasone, and cisplatin (carboplatin substituted for cisplatin). A PET/CT scan after 2 cycles showed no response to treatment. With a suboptimal response to chemotherapy, this patient was not felt to be a candidate for an autologous stem cell transplant. Additional options were considered, taking underlying disease biology into account (ABC-DLBCL subtype and *MYC* rearrangement detected by FISH). Her third line of treatment was lenalidomide with rituximab, which also yielded no significant response though it was well tolerated, and she was switched to ibrutinib to which she has had the most measurable response to date. After 6 months on therapy, she developed difficult to manage atrial fibrillation and treatment was discontinued. A repeat biopsy with mutational profiling was offered but was cost-prohibitive and the patient declined.

With good organ function, an Eastern Cooperative Oncology Group (ECOG) performance status of 1, and good marrow function, she is currently enrolled in a clinical trial using a novel Bruton tyrosine kinase inhibitor for patients with relapsed/refractory DLBCL based on preferential efficacy in ABC-DLBCL.

Summary

Our knowledge of the molecular features of aggressive B-cell lymphomas is speedily evolving. Additional information on the genetic alterations, mutations, clonal heterogeneity, and ctDNA is rapidly being combined into novel treatments, prognostic, and predictive assays. Specific therapies under development for relapsed or refractory patients are the subject of the next chapter.

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