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The Genetic Landscape of Diffuse Large B Cell Lymphoma

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Abstract

Diffuse large B cell lymphoma (DLBCL), the most common lymphoid malignancy in the western world, is an aggressive disease that remains incurable in approximately 30% of patients. Over the past decade, the rapid expansion of sequencing technologies allowing the genome-wide assessment of genomic and transcriptional changes has revolutionized our understanding of the genetic basis of DLBCL by providing a comprehensive and unbiased view of the genes/pathways that are disrupted by genetic alterations in this disease, and may contribute to tumor initiation and expansion. These studies uncovered the existence of several previously unappreciated alterations in key cellular pathways that may also influence treatment outcome. Indeed, a number of newly identified genetic lesions are currently being explored as markers for improved diagnosis and risk stratification, or are entering clinical trials as promising therapeutic targets.

This review focuses on recent advances in the genomic characterization of DLBCL and discusses how information gained from these efforts has provided new insights into its biology, uncovering potential targets of prognostic and therapeutic relevance.

INTRODUCTION

Diffuse large B cell lymphoma (DLBCL) is the most prevalent B cell non-Hodgkin lymphoma (B-NHL) in the adult, comprising 30-40% of all new diagnoses and including cases that arise *de novo* and cases that result from the histologic transformation of various, less aggressive B-NHL types (i.e., follicular lymphoma and chronic lymphocytic leukemia)¹. Although curable in a substantial proportion of patients by contemporary R-CHOP chemo-immunotherapy, as many as 40% of cases do not achieve durable remissions and will

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succumb to their disease. It has become clear that one of the reasons for such lack of success is the remarkable heterogeneity of this malignancy, which encompasses multiple distinct subgroups reflecting the origin from B cells at various developmental stages or the coordinated expression of comprehensive consensus clusters. These molecular subgroups differ not only in the expression of specific gene signatures, but also in the oncogenic pathways that drive tumor development, often predicting discrete overall survival rates. Thus, a more precise definition of the genetic changes that are associated with DLBCL is fundamental to improve our understanding of the disease, identify new therapeutic targets and develop stratified approaches to treatment.

Here we review current knowledge about the molecular pathogenesis of DLBCL, with emphasis on major biological programs/pathways that are dysregulated by genetic lesions in the two main subtypes of the disease, as revealed by recent genomic profiling efforts.

CELLULAR ORIGIN OF DLBCL

The germinal center reaction

Analogous to most B-NHL, DLBCL arises from the clonal expansion of B cells in the GC, a specialized microenvironment that forms in secondary lymphoid organs upon encounter of a naïve B cell with its cognate antigen, in the context of T-cell dependent co-stimulation². GCs are highly dynamic structures where mature B cells undergo rapid proliferation (<12 hours doubling time) and iterative rounds of somatic hypermutation (SHM), affinity maturation and clonal selection, as well as class switch recombination (CSR), with the aim of favoring the emergence of cells that produce antibodies with increased affinity for the antigen and capable of distinct effector functions³.

These processes are compartmentalized within two anatomically distinct areas where B cells recirculate bidirectionally: the dark zone (DZ), populated by rapidly dividing centroblasts, and the light zone (LZ), which is composed of smaller non-dividing lymphocytes admixed with a reticulum of follicular dendritic cells (Figure 1). DZ and LZ B cells are characterized by unique biological programs that are executed by a network of transcription factors required for orderly GC development and whose deregulated expression is implicated in lymphomagenesis. The initiation of the GC reaction, i.e. the formation of the DZ, is orchestrated by a transitory peak in the expression of NF- κ B, IRF4 and MYC by a few GC founder cells, followed by their downregulation in the overall DZ population^{3,4}. In particular, MYC transcription is directly silenced by the GC master regulator BCL6⁵, a potent transcriptional repressor that, in the B cell lineage, is expressed specifically during the GC reaction. BCL6 enables the DZ phenotype by modulating the activity of a broad set of genes involved in multiple signaling pathways (Suppl Data I), and is thought to sustain the proliferative status of GC cells while allowing the execution of DNA remodeling events required for SHM and CSR, without eliciting DNA damage responses; additionally, BCL6 prevents the premature activation and differentiation of GC B cells prior to the selection for the survival of high-affinity clones⁶. Additional transcription factors that are required for GC formation and are relevant for lymphomagenesis include TCF3(E2A), which enforces tonic BCR signaling in DZ B cells by regulating the expression of downstream effectors; and

EZH2, a histone methyltransferase that helps establish bivalent chromatin domains at key regulatory loci, transiently suppressing terminal differentiation^{7,8}.

Upon completion of this proliferative expansion in the DZ, B cells migrate to the LZ, where a variety of signals, including engagement of the BCR by the antigen, activation of the CD40 receptor by CD40 ligand, and stimulation of the BAFF and Toll-like receptors (TLR) activate downstream signaling cascades including PI3K, MEK, and NF- κ B. One consequence of this reaction and particularly of NF- κ B activation is the re-expression of IRF4, which binds to the BCL6 promoter and turns off its transcription⁹, thus relieving the expression of another master regulator of plasma cell differentiation, BLIMP1 (Supplemental Data I). Downregulation of BCL6 will thus restore the ability of the B cell to become activated and differentiate into an antibody-secreting plasma cell.

The identification of the molecular circuits that regulate the transition between these two phases is central to the understanding of lymphomagenesis, as these transcriptional programs can be recognized to some extent in the two major subtypes of systemic DLBCL based on cell of origin (see below); moreover, the same circuits are recurrent targets of genetic lesions, indicating that tumor cells exploit the unique properties of GC cells for their own selective advantage.

DLBCL subtypes derive from distinct phases of B cell differentiation

The clinical and biological heterogeneity of DLBCL has been known to pathologists and clinicians for decades; however, it was the introduction of GEP technologies that allowed the formal recognition of multiple distinct subtypes, reflecting either the derivation from discrete B cell differentiation stages or the coordinated expression of specific transcriptional signatures. According to the current taxonomy for DLBCL and based on resemblance to the transcriptional profiles of their presumed cell of origin (COO), at least three molecular subgroups have been recognized within this diagnostic entity: GC B cell-like (GCB) DLBCL, activated B cell-like (ABC) DLBCL, and primary mediastinal large B-cell lymphoma (PMBCL), while an additional 15-30% of cases remain unclassified¹⁰⁻¹². Both GCB and ABC-DLBCL appear more related to light-zone B cells¹³; however, GCB-DLBCLs are defined by the elevated expression of BCL6 and CD10, the absence of post-GC markers (e.g., IRF4 and BLIMP1), and highly mutated immunoglobulin genes with ongoing SHM¹⁴. Conversely, the GEP of ABC-DLBCL resembles that of BCR-activated B cells or plasmablastic B cells, suggesting that its putative normal counterpart has received signals to downregulate the GC specific program and is poised to terminal B cell differentiation. ABC-DLBCLs do not show evidence of ongoing SHM, consistent with their late GC origin^{14,15}.

PMBCL, a tumor of the mediastinum that shares significant similarities with Hodgkin lymphoma, is thought to develop from a thymic post-GC B cell or from a GC B cell migrated to the thymus^{11,12}. This lymphoma is recognized as a separate clinical-pathological entity from systemic DLBCL in the revised 2008 WHO classification of lymphoid malignancies¹ and has been extensively reviewed recently; thus, it will not be discussed¹⁶.

Although imperfectly reproduced by immunohistochemistry¹⁷, risk stratification based on the COO classification holds prognostic significance, since patients diagnosed with GCB-DLBCL have superior overall survival compared to those presenting with ABC-DLBCL¹⁸.

A distinct classification scheme reflecting the coordinated transcription of comprehensive consensus clusters (CCC) identified three robust signatures defined by the expression of genes involved in oxidative phosphorylation, B-cell receptor/proliferation, and tumor microenvironment/host inflammatory response¹⁹. Both COO and CCC classifications are highly reproducible, yet they do not overlap, indicating that they capture different aspects of DLBCL biology, and further underscoring the complexity of this disease.

MECHANISMS OF GENETIC LESION IN DLBCL

SHM and CSR are essential for the execution of effective immune responses^{20,21}; yet, because of their ability to introduce DNA breaks, they expose the genome of GC B cells to a constant risk. Moreover, these reactions take place in an environment –the GC– wherein B cells replicate at remarkably fast rates and DNA damage checkpoints are silenced as the result of BCL6 transrepressive activity. Accordingly, many of the structural alterations that are implicated in DLBCL development –namely, chromosomal translocations and aberrant somatic hypermutation (ASHM)– derive from errors occurring during one of these two reactions²². Formal proof to this model came from the demonstration that ablation of AID, the enzyme required for both SHM and CSR, in lymphoma-prone mouse models was able to prevent the formation of MYC-IgH rearrangements and the development of DLBCL^{23,24}.

ASHM is a mechanism of genomic instability that targets the 5' sequences of actively transcribed genes as the result of a malfunction in the physiologic SHM process²⁵. In GC B cells, SHM is restricted to a few genes, including the immunoglobulin genes and *BCL6*^{26,27}, because although AID can bind to multiple DNA sequences²⁸, mutations at off-target genes are normally repaired with high accuracy²⁹, preventing widespread mutational activity. On the contrary, nearly half of DLBCL cases display multiple somatic mutations in a large number of actively transcribed genes, including the proto-oncogenes *PIMI* and *MYC*^{25,30}. ASHM-generated lesions are typically distributed within promoter-proximal sequences and, depending on the genomic configuration of the target gene, may affect untranslated as well as coding regions. As such, ASHM has the capability to either alter gene transcriptional regulation or modify key structural/functional properties²⁵. While a comprehensive characterization of the potentially extensive genetic damage caused by ASHM is still missing, this mechanism likely contributes to the heterogeneity of DLBCL via the alteration of diverse cellular pathways in different cases.

Analogous to other cancers, DLBCL also harbors genomic deletions, amplifications and point mutations that lead to oncogenic activation or to inactivation of tumor suppressor genes.

THE GENOMIC LANDSCAPE OF DLBCL

In recent years, the development of powerful sequencing technologies has offered an unprecedented opportunity to interrogate the cancer genome in a comprehensive and

unbiased manner. The integration of whole-genome, whole-exome and RNA sequencing approaches has significantly improved our understanding of the genetic landscape of DLBCL by defining its degree of complexity and by revealing previously unrecognized genes/pathways that may have contributed to its clonal expansion, including many that impact on remodeling the epigenome. While, for some such candidates, a detailed functional characterization is still lacking, these studies have provided an incremental gain in our knowledge of the pathogenesis of DLBCL. Most importantly, they identified dependencies of the tumor cells on specific molecules/circuits, which represent attractive targets for therapeutic intervention.

Compared to other B cell malignancies such as chronic lymphocytic leukemia and acute leukemias, the coding genome of DLBCL is relatively complex, with an average of 50-100 lesions/case and great variability across different patients³¹⁻³⁴. Nonetheless, the collection of genetic changes observed in each patient often converge on common cellular pathways, suggesting critical roles in DLBCL pathogenesis. It is important to note that, because whole-exome sequencing approaches do not examine non-coding portions of the genome (i.e., sterile transcripts, micro RNAs and 5' sequences, the latter representing the target domain of ASHM), the overall mutation load of the DLBCL genome is expected to be even higher.

DYSREGULATED CELLULAR PATHWAYS

The molecular complexity of DLBCL can be recapitulated by genetic lesions that are shared across different COO-defined phenotypic subtypes, and alterations that preferentially or even exclusively segregate with GCB- and ABC-DLBCL, suggesting the addiction to distinct oncogenic pathways (Figure 1). The following sections will focus on the most relevant genes/functional programs that are derailed in DLBCL, selected based on historical relevance, functional characterization, recurrence in the disease, and evidence for specific targeting by genetic alterations (e.g., genes encompassed by focal lesions).

Genetic lesions common to GCB- and ABC-DLBCL

Alterations of histone modification genes—One commonly disrupted program only recently appreciated in DLBCL is represented by epigenetic remodeling.

Up to 30% of cases, with some preference for GCB-DLBCL, harbor mutations and/or deletions inactivating *CREBBP* and, more rarely, *EP300*, two ubiquitously expressed acetyltransferases that modify lysine residues on both histone and non-histone nuclear proteins, modulating the activity of a large number of DNA-binding transcription factors³⁵. *CREBBP* mutations include truncating events that remove the C-terminal HAT domain and amino acid changes that impair its affinity for Acetyl-CoA, severely reducing its enzymatic activity. Except for few cases, they are observed in heterozygosis and are accompanied by expression of the residual wild-type allele, suggesting a role as haploinsufficient tumor suppressors. Indeed, loss of a single *CREBBP* (or *EP300*) allele is the cause of a rare congenital disorder (Rubinstein-Taybi syndrome) associated with developmental defects and tumor predisposition, providing evidence for a dose-dependent pathogenic effect of these genes³⁵. While additional efforts will be needed to comprehensively define the transcriptional network regulated by *CREBBP* in GC B cells and disrupted in DLBCL

patients carrying *CREBBP* mutations, one mode by which these alterations contribute to lymphomagenesis is through impaired acetylation of its substrates BCL6 and p53, which leads to constitutive activation of the oncoprotein and decreased function of the tumor suppressor^{36,37}. The balanced activity of these two proteins is key for regulating DNA damage responses during immunoglobulin remodeling in the GC³⁸; thus, one consequence of BCL6 activity overriding p53 would be an increased tolerance for genomic instability in the context of impaired apoptotic responses. The discovery of mutations in *CREBBP* and *EP300* has therapeutic implications because of the availability of drugs that inhibit deacetylation mechanisms and could provide therapeutic benefits by re-establishing physiologic acetylation levels in these patients.

At least one third of DLBCLs feature mutations in the mixed lineage lymphoma/leukemia 2 (*MLL2*) gene. *MLL2* encodes for a methyltransferase that controls epigenetic transcriptional regulation by mono-, di- and trimethylating the lysine 4 position of histone 3 (H3K4). While the consequences of *MLL2* mutations in DLBCL have not been elucidated yet, most events are predicted to generate severely truncated proteins lacking the catalytic SET domain, which is required for its methyltransferase activity. Thus, *MLL2* mutations are likely to have a broad effect on chromatin regulation, which may contribute to lymphomagenesis by reprogramming the epigenome of the precursor cancer cell. Interestingly, inactivating mutations of both *CREBBP* and *MLL2* are also observed in FL cases (40% and ~89% respectively)^{33,34,37}, emerging as one of the most common genetic alterations reported in B-NHL to date. Moreover, recent studies reconstructing the history of clonal evolution during FL transformation to DLBCL suggest that *MLL2* and *CREBBP* mutations represent early events introduced in a common mutated ancestral clone before divergent evolution to FL/tFL^{39,40}. Alterations in epigenetic modifiers may thus facilitate the initial stages of transformation, by creating a permissive environment for the proliferation and survival of the cancer clone.

Alterations deregulating BCL6—Deregulation of BCL6 activity represents a key mechanism of transformation in DLBCL, achieved via multiple direct and indirect modalities (Figure 2). Chromosomal rearrangements of the *BCL6* locus characterize as many as 35% of DLBCL patients, although with two- to three-fold higher frequencies in ABC-DLBCL^{41,42}. These balanced, reciprocal recombination events juxtapose the coding domain of *BCL6* downstream to heterologous promoters derived from alternative chromosomal partners, leading to deregulated expression of an intact protein, in part by preventing its downregulation during post-GC differentiation. It remains to be studied whether the consequences of BCL6 translocations are different in ABC- and GCB-DLBCL, since BCL6 controls functionally separate biological programs depending on the interaction with distinct co-repressor molecules⁴³. The BCL6 5' sequences are also targeted by multiple point mutations in >70% of cases^{26,27}; while these events largely reflect the physiologic activity of SHM in the GC, a subset of mutations clustering in the first *BCL6* noncoding exon are exquisitely restricted to lymphoma, where they deregulate BCL6 expression by at least two modalities: i) they disrupt a negative autoregulatory loop by which the BCL6 protein controls its own transcription^{44,45}; ii) they prevent IRF4 binding and transcriptional repression following CD40 signaling⁹. Intriguingly, BCL6 deregulating

mutations are only found in GCB-DLBCL, suggesting that tumor cells exploit different mechanisms depending on their functional state. Since the full extent of *BCL6* mutations with documented functional consequences has not been characterized, the exact percentage of cases carrying *BCL6* genetic abnormalities cannot be determined.

In addition to genetic lesions directly affecting the *BCL6* gene, DLBCL have devised a number of ways to deregulate the *BCL6* function indirectly. Besides *CREBBP/EP300* loss, which impairs acetylation-mediated inactivation of *BCL6*, 10-15% of patients harbor gain-of-function somatic mutations in the *MEF2B* transcription factor, a protein highly expressed in the GC and involved in *BCL6* transcriptional activation^{33,46}. These lesions promote the activity of *MEF2B* by at least two mechanisms, entailing enhanced transcriptional activation (mutations in the N-terminal MEF/MAD domain) or the loss of phosphorylation- and sumoylation-mediated negative regulatory motifs⁴⁶. In 5% of cases, loss-of-function mutations/deletions of *FBXO11* impair proteasomal-mediated degradation of the *BCL6* protein, which is controlled by this E3 ubiquitin ligase⁴⁷. Consistent with a prominent oncogenic role in lymphomagenesis, deregulated *BCL6* expression in a mouse model mimicking the DLBCL-associated translocation leads to the development of clonal lymphoproliferative disorders recapitulating features of the human disease⁴⁸.

Loss of immune surveillance mechanisms—A large proportion of DLBCLs have evolved mechanisms to escape both CTL-mediated and NK-cell mediated immune surveillance. In 29% of cases, the beta-2-microglobulin (*B2M*) gene is lost because of structurally disruptive mutations and/or deletions, and another 30% of cases lack *B2M* expression in the absence of genetic lesions, suggesting the existence of additional genetic or epigenetic mechanisms of inactivation⁴⁹. *B2M* encodes for an invariant subunit of the HLA class I (HLA-I) complex, which is expressed on the surface of all nucleated cells and is required for recognition by cytotoxic T lymphocytes⁵⁰. As a result, over 60% of DLBCL lack surface HLA-I expression, which in turn may favor lymphomagenesis by allowing evasion from immune surveillance.

Other lesions—Mutations and deletions of *TP53* remain an important pathogenic lesions in ~20% of all DLBCL^{34,51}, including those derived from FL transformation³⁹; recent studies also suggest that *TP53* mutations affecting its DNA binding site are most important from a prognostic standpoint. Also shared across both DLBCL subtypes are mutations of the **FOXO1** transcription factor. These events cluster around a phosphorylation site required for AKT-mediated nuclear-cytoplasmic translocation and inactivation of FOXO1, and were suggested to enhance its activity by preventing its nuclear export following PI3K signaling⁵²; however, a systematic examination of the effects of these mutations in the context of B cells is still lacking, warranting further studies. FOXO1 mutations are significantly enriched in patients with aggressive disease, suggesting a role for prognostication and risk stratification⁵².

GCB-DLBCL

Until recently, only a few lesions had been found preferentially associated with GCB-DLBCL, including chromosomal translocations of *BCL2* (35-45% of cases) and *MYC*

(10-14% of cases), which both lead to ectopic expression of the involved protein, in part by allowing escape from BCL6-mediated transcriptional repression^{5,53}; deletions of the tumor suppressor *PTEN* (~10% of cases)⁵⁴; and, less frequently, amplifications of the region encompassing the mir-17-92 microRNA cluster, also a negative regulator of *PTEN*⁵⁴. This picture changed significantly after genomic profiling studies uncovered recurrent mutations in several previously unrecognized genes specifically in this subtype. The following paragraphs will cover two novel mutation targets with well-defined functional roles; other lesions that, although frequent, are less characterized will not be discussed.

Mutations of EZH2—Heterozygous somatic mutations of the polycomb-group oncogene *EZH2* have been reported in ~22% of GCB-DLBCL patients⁵⁵. With few exceptions, *EZH2* mutations result in the replacement of a single evolutionary conserved residue (Tyr641) within the protein SET domain, leading to enhanced catalytic specificity and increased levels of H3K27me3⁵⁶. In line with this, conditional expression of mutant *EZH2* alleles in mice promotes GC hyperplasia and cooperates with *BCL2* in inducing DLBCL⁷. Notably, small molecule *EZH2* inhibitors have just entered clinical trials for the treatment of patients with NHL, with promising results⁵⁷.

Mutations in the Gα13 pathway—Approximately 20% of GCB-DLBCLs are characterized by structurally damaging mutations in various components of a G-protein coupled inhibitory circuit that regulates the growth and local confinement of GC B cells (namely *GNAI3*, *SIPR2* and, more rarely, *ARHGEF1* and *P2RY8*)(Figure 1)⁵⁸. Loss of these genes in the mouse was associated with increased GC B cell survival and dissemination to the lymph and bone marrow, ultimately leading to lymphoma development⁵⁸.

ABC-DLBCL

Of the three main subtypes of DLBCL, the genomic landscape of ABC-DLBCL is the best characterized, being associated with a constellation of genetic abnormalities that converge on two signaling pathways: activation of NF-κB and block in terminal B cell differentiation. Additional recurrent lesions include amplifications of the *BCL2* locus and deletions or lack of expression of the *CDKN2A/2B* tumor suppressor genes⁵⁴.

Genetic lesions leading to constitutive activation of the NF-κB transcription factor—A prominent feature of ABC-DLBCL is the constitutive activation of the NF-κB signaling pathway, first evidenced by the enriched expression of NF-κB target genes and the requirement of NF-κB for the proliferation and survival of ABC-, but not GCB-DLBCL cell lines⁶⁰. A number of studies have subsequently provided direct genetic evidence for the presence of genetic alterations in molecules whose common denominator is the ability to induce activation of NF-κB.

Mutations activating the BCR signaling pathway: Mature B cells require “tonic” signaling from the BCR in order to survive; however, ABC-DLBCL cells were found to display a chronic, active form of BCR signaling, which requires CARD11 and is sustained by the presence of genetic alterations in proximal members of the pathway⁶¹.

Over 20% of patients harbor somatic mutations in the Ig superfamily members **CD79B** and, at lower frequencies, **CD79A** (Figure 3)⁶¹. In most cases, the mutations replace the first tyrosine residue (Y196) in the cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs). These events are thought to circumvent negative feedback circuits that attenuate BCR signaling, thus maintaining it chronically active. Consistently, knockdown of several BCR proximal and distal subunits is specifically toxic to ABC-DLBCL, supporting its direct involvement in the pathogenesis of this disease and providing the basis for the development of targeted therapies directed against this pathway.

In ~9% of ABC-DLBCL (and a smaller subset of GCB-DLBCL), activation of BCR and NF- κ B is sustained by oncogenic mutations of the **CARD11** gene. CARD11 is a major component of the “signalosome” complex, the coordinated recruitment of which is required for proper transduction of BCR signaling (Figure 3)⁶². These events cluster in the exons encoding for the protein coiled-coil domain and enhance the ability of CARD11 to transactivate NF- κ B target genes⁶³.

Since activation of the BCR can trigger multiple downstream signaling cascades, besides canonical NF- κ B (i.e. PI3K, ERK/MAP kinase and NF-AT), it is expected that additional programs contribute to the neoplastic transformation of ABC-cells, as also suggested by initial experiments demonstrating the cooperative toxicity of NF- κ B and PI3K inhibitors in ABC-DLBCL cell lines⁶³.

Mutations activating the TLR pathway: Oncogenically active **MYD88** mutations are found in one third of ABC-DLBCLs, where they target an evolutionarily invariant residue within the TIR (Toll/IL1 receptor) domain, leading to a L265P substitution⁶⁴. This mutation induces IRAK4 kinase activity and phosphorylation through the spontaneous assembly of a protein complex containing IRAK1 and IRAK4, which in turn can activate NF- κ B as well JAK/STAT3 transcriptional responses, also a phenotypic trait of ABC-DLBCL and a requirement for their survival. Other mutations in the MYD88 TIR domain were observed in both ABC- and GCB-DLBCL, but their significance remains to be established.

Mutations inactivating negative regulators of NF- κ B: Almost one third of ABC-DLBCL and fewer GCB-DLBCL harbor biallelic **TNFAIP3** truncating mutations and/or deletions^{65,66}. **TNFAIP3** encodes for a dual function ubiquitin-modification enzyme (A20) involved in the termination of NF- κ B responses triggered by TLR and BCR stimulation. A20 regulates these functions through the post translational modification of several substrates, where it first removes K63-linked regulatory ubiquitines via its OTU domain, and subsequently conjugates K48-linked ubiquitines via its zinc finger domains, targeting them for proteasome-mediated degradation. By removing these domains, A20 mutations are thought to induce inappropriately prolonged NF- κ B responses^{65,66}. Consistently, A20 knock-out mice display an inflammatory phenotype reflecting overactive NF- κ B and TLR responses⁶⁷.

In summary, multiple alterations dysregulate the NF- κ B cascade at different levels in ABC-DLBCL. These vulnerabilities offer a unique opportunity for the development of tailored therapeutic strategies; indeed, the Bruton Tyrosine Kinase (BTK) inhibitor Ibrutinib is

rapidly emerging as a novel paradigm for the treatment of ABC-DLBCL, where it should be effective in those cases harboring mutations in the BCR signaling cascade upstream of BTK (Figure 3)⁵⁷.

Genetic lesions preventing terminal differentiation—The differentiation of GC B cells into plasma cells requires PRDM1, a sequence-specific transcriptional repressor that is upregulated in a subset of LZ B-cells poised to undergo plasma cell differentiation and in all plasma cells⁶⁸. However, ~25% of ABC-DLBCLs have lost the *PRDM1* gene owing to truncating mutations, missense mutations, and/or genomic deletions⁶⁹⁻⁷¹, while an additional sizeable fraction of patients lacks the PRDM1 protein due to transcriptional repression by constitutively active, translocated *BCL6* alleles⁶⁹. Rearrangements of *BCL6* and alterations inactivating *PRDM1* are mutually exclusive, supporting a complementary role in promoting lymphomagenesis by blocking terminal differentiation. In line with this model, conditional deletion of *PRDM1* in GC B cells *in vivo* leads to human-like ABC-DLBCL^{69,72}.

PERSPECTIVE

Over the past decade, targeted resequencing and genomic profiling have led to the discovery of recurrent, previously unappreciated genetic lesions, revealing the involvement of biological programs and signaling pathways that are central to DLBCL pathogenesis and identifying, in some cases, important new regulators of GC development and therefore humoral immunity. These pathways represent vulnerabilities of the lymphoma cell that could be exploited for improved diagnosis, prognostication and therapeutic intervention. Notably, a number of drugs have been either newly developed or “repositioned” to target genetically disrupted programs in DLBCL (Figure 3). While these drugs are expected to impact the standard of care for this malignancy, the complexity of the involved pathways and the overall heterogeneity of the disease suggest that precise patient stratification will be necessary in order to identify sensitive and resistant cases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Germinal center and DLBCL pathogenesis
Schematics of the GC reaction and its relationship with major molecular subtypes of DLBCL. The most common shared and subtype-specific genetic alterations are shown, with color codes indicating the involved biological pathway. Blue, loss of function; red, gain of function.

Figure 2. Deregulation of BCL6 activity by genetic lesions in DLBCL

Recurrent genetic alterations deregulating BCL6 function in DLBCL, either directly or indirectly. Representative biological programs modulated by BCL6 in the GC, and disrupted as a consequence of these lesions are shown at the bottom.

Figure 3. Disrupted pathways in ABC-DLBCL

Key signaling cascades engaged in LZ B cells and disrupted by genetic lesions at multiple levels in ABC-DLBCL. Bolts, gain-of-function mutations; crosses, loss-of-function mutations.