Promising Personalized Therapeutic Options for Diffuse Large B-cell Lymphoma Subtypes with Oncogene Addictions

James J. Steinhardt¹ and Ronald B. Gartenhaus¹,²

Abstract
Currently, two major classification systems segregate diffuse large B-cell lymphoma (DLBCL) into subtypes based on gene expression profiles and provide great insights about the oncogenic mechanisms that may be crucial for lymphomagenesis as well as prognostic information regarding response to current therapies. However, these current classification systems primarily look at expression and not dependency and are thus limited to inductive or probabilistic reasoning when evaluating alternative therapeutic options. The development of a deductive classification system that identifies subtypes in which all patients with a given phenotype require the same oncogenic drivers, and would therefore have a similar response to a rational therapy targeting the essential drivers, would significantly advance the treatment of DLBCL. This review highlights the putative drivers identified as well as the work done to identify potentially dependent populations. These studies integrated genomic analysis and functional screens to provide a rationale for targeted therapies within defined populations. Personalizing treatments by identifying patients with oncogenic dependencies via genotyping and specifically targeting the responsible drivers may constitute a novel approach for the treatment of DLBCL. Clin Cancer Res; 18(17); 4538–48. ©2012 AACR.

Introduction
Diffuse large B-cell lymphoma (DLBCL) is a highly malignant form of non-Hodgkin's lymphoma (NHL) that constitutes about 40% of all lymphoma diagnoses (1). In 1966, the chemotherapy regimen of cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (CHOP) was introduced to manage lymphoma. This regimen improved the response rates previously achieved by single chemotherapeutic regimens from 50% to 69% as well as provided superior survival (2–6). Numerous efforts were made over prior decades to improve on the results obtained with CHOP, including complicated multidrug regimens such as MACOP-B and COPLAM, unfortunately with no success. It was not until 1997 that CHOP was modified with the addition of chimeric anti-CD20 monoclonal antibody rituximab, resulting in significantly improved cure rates (7, 8). However, with the exception of rituximab, no other targeted therapy has been used extensively as a front line therapy for DLBCL. This is felt to be in large part due to the heterogeneity of DLBCL and the inability to identify the oncogenic drivers responsible for a patient's disease.

The phenomenon of oncogene "addiction" has been defined as a tumor requirement for constitutive expression and activity of a single aberrant gene, regardless of other tumor-related alterations (9). BCR-ABL–expressing chronic myelogenous leukemia (CML) exemplifies this phenomenon as the direct targeting of BCR-ABL with the specific kinase inhibitor imatinib results in a 95% response rate despite the presence of other genetic lesions. This strong clinical evidence for oncogene addiction is further supported by the BCR-ABL reactivation that occurs in patients who develop resistance to imatinib with the most common mechanism of resistance being a de novo T315I mutation in the ATP-binding pocket (10–12).

Unlike CML, other malignancies have poorly defined addictions to oncogenic drivers. This is primarily due to the fact that BCR-ABL is a unique fusion protein that is exclusive to CML, whereas many other malignancies are dependent on the overexpression of oncogenic signaling cascades that are not cancer specific. Conversely, it has been shown that the overexpression of an oncogene does not necessarily predict for an oncogenic dependency. A recent study sought to stratify human cancer cell lines with tumor-related alterations (9). BCR-ABL–expressing chronic myelogenous leukemia (CML) exemplifies this phenomenon as the direct targeting of BCR-ABL with the specific kinase inhibitor imatinib results in a 95% response rate despite the presence of other genetic lesions. This strong clinical evidence for oncogene addiction is further supported by the BCR-ABL reactivation that occurs in patients who develop resistance to imatinib with the most common mechanism of resistance being a de novo T315I mutation in the ATP-binding pocket (10–12).

Unlike CML, other malignancies have poorly defined addictions to oncogenic drivers. This is primarily due to the fact that BCR-ABL is a unique fusion protein that is exclusive to CML, whereas many other malignancies are dependent on the overexpression of oncogenic signaling cascades that are not cancer specific. Conversely, it has been shown that the overexpression of an oncogene does not necessarily predict for an oncogenic dependency. A recent study sought to stratify human cancer cell lines with tumor-related alterations (9). BCR-ABL–expressing chronic myelogenous leukemia (CML) exemplifies this phenomenon as the direct targeting of BCR-ABL with the specific kinase inhibitor imatinib results in a 95% response rate despite the presence of other genetic lesions. This strong clinical evidence for oncogene addiction is further supported by the BCR-ABL reactivation that occurs in patients who develop resistance to imatinib with the most common mechanism of resistance being a de novo T315I mutation in the ATP-binding pocket (10–12).
This is a particularly attractive concept in the treatment of cancer, as targeting a gene that is related to a recurrent cancer mutation should selectively kill malignant cells only. This concept is nicely reviewed by Kaelin (14). One such synthetic lethality screen in DLBCL identified CARD11 as crucial mediator between BCR and NF-kB signaling in a subtype of DLBCL (15).

B-Cell Maturation, Lymphomagenesis, and Subtype Classification

B-cells primarily originate from common lymphoid progenitor cells in the bone marrow and undergo recombination-activating gene (RAG1 and RAG2)–mediated gene rearrangement during B-cell receptor (BCR) development (16). During this process, double-strand breaks (DSB) are introduced into the V, D, and J gene segments to facilitate recombination and formation of immunoglobulin heavy-chain (IgH) and light chain (IgL) genes (17). The DSBs introduced are repaired by homologous recombination but have been shown to contribute to chromosomal translocations involving immunoglobulin loci (18).

Until B cells encounter an antigen, they are considered mature naïve B cells. Upon antigen-induced B-cell activation, B cells proliferate and can differentiate into centroblasts or plasma cells via the follicular or extrafollicular pathway, respectively (19). The centroblasts go through the dark zone of the germinal center (GC) where they rapidly proliferate, differentiate, and revise their antigen receptors via immunoglobulin somatic hypermutation (SHM) of the immunoglobulin heavy chain variable region (IgHV) genes as well as undergo class switch recombination (CSR; refs. 20–23). Both of these processes are mediated by activation-induced cytidine deaminase (AID), which deamidates cytosine to uracil (24, 25). SHM modulates the affinity of the antibodies to a specific antigen and it is believed that the mistargeting of SHM can also result in the translocation of oncogenes. Mouse models have firmly established the cause and effect link between AID enzymatic activity and IgH-cMyC translocations (26). Mouse models have also showed that AID is necessary for BCL6-mediated lymphomagenesis in some DLBCL populations (27).

GC B-cell lymphomas (GCB-DLBCL) are DLBCLs that resemble GC B cells (28). They generally have switched IgH classes and continue to undergo somatic hypermutation. Translocations of BCL-2 and Myc with IGHV are commonly observed in this population (29, 30). These translocations may lead to a malignant transformation by preventing apoptosis or blocking terminal differentiation by placing these genes under the IGHV promoter, which results in their constitutive expression. Usually normal GC B cells are primed for apoptosis as they lowly express the anti-apoptotic protein BCL2, and require selection to progress and further differentiate (31). The t(14;18) translocation results in an increased expression of BCL2 and provides a mechanism for GCB-DLBCL to evade apoptosis. The differentiation of GC B cells can be inhibited by BCL6 proteins, which work along with other transcription factors such as B-lymphocyte–induced maturation protein (BLIMP1), PAX4, and XBP1, to regulate and coordinate GC B-cell to plasma cell differentiation (32, 33).

Activated peripheral blood B-cell (ABC-DLBCL) origination is less understood but this subtype is characterized by a gene expression pattern similar to that of normal plasma cells including the expression of the transcription factor XBP1, which regulates immunoglobulin secretion (34, 35). The ABC subtype is also characterized by constitutive NF-kB activity, which induces IRF4 expression (36). Generally, this would drive B cells towards plasmacytic differentiation, but some data suggest that inactivation of BLIMP-1 in IgM-positive post-GC memory cells inhibits plasmacytic differentiation and may be crucial for lymphomagenesis in the ABC subtype (37). Other data suggest that ABC-DLBCL may develop from extrafollicular B cells with high levels of AID (38). Regardless of its origin, this subtype accumulates chromosomal translocations involving the IgH switch regions and may be associated with high levels of AID generating DSB.

Gene expression signatures have been used to identify relevant DLBCL subsets. The cell of origin (COO) classification system is a developmentally defined system that divides DLBCL into two main subsets based on how closely the tumor resembles either a GCB-DLBCL or an ABC-DLBCL with tumors that do not resemble either being left unassigned or referred to as type III (28). The Hans classification system, albeit imperfect, is a widely accepted method used for research studies to distinguish the ABC versus GCB-DLBCL subtypes, which are not yet recognized by the WHO 2008 classification, but are distinct lymphomas in regards to their biologic and clinical presentation (39). The method uses immunoperoxidase tissue microarrays (TMA) and classifies CD10-positive and BCL6-positive/MUM1-negative tumors as GCB tumors. Both CD10 and BCL6 are GC markers, whereas MUM1 is expressed by plasma cells and later stage B cells (40, 41). The remaining BCL6-negative and BCL6-positive/MUM1-positive tumors are then classified as non-GC. One group observed that DLBCLs harboring the t(3;14) translocation between BCL-6 with IGHV are predominantly MUM1-positive and therefore classified as non-GC (42). This classification system is quite useful in understanding the mechanisms for lymphomagenesis in DLBCL, as many B-cell lymphomas hijack regulatory processes during B-cell development and are functionally defined by their differentiation state. One of the caveats to this approach is its limited accuracy. Antibodies that are more specific to GCB cells are now being used in an immunohistochemical algorithm to increase the 80% accuracy of the Hans classification system to 93% (43). Shakhnovich and colleagues, using a DNA methylation signature of 16 genes, had a 98% accuracy in predicting ABC versus GCB subtypes from the same cohort of DLBCL samples that were previously classified by the Staudt group using gene expression profiling (44, 45).

Recently, a consensus clustering system has been used to classify DLBCL subsets in an unbiased manner. This highly reproducible method has identified B-cell receptor/proliferation (BCR), oxidative phosphorylation (OxPhos), and
host response (HR) populations (46). The BCR subtype has higher expression of BCR signaling components such as CD19, IgH, CD79a, BLK, SYK, PLC\(\gamma\)2, and MAP4K and higher expression of cell-cycle regulatory genes and DNA repair genes such as CDK2, H2AX, and p53. The BCR subtype also displays a higher expression of PAX5, OBF-1, E2A, STAT6, Myc, and BCL6, which have been implicated in neoplastic transformation and tumorigenesis. The OxPhos phenotype has increased expression of mitochondrial-associated proteins, such as NADH complex, cytochrome c, cytochrome c oxidase (COX), and ATP synthase components as well as the anti-apoptotic protein BFL-1. Finally, their high levels of CD2\(^+\)/CD3\(^+\) infiltrating lymphocytes and CD1a\(^+\)/CD123\(^+\) dendritic cells as well as their lower expression of genetic aberrations generally characterize the HR tumors.

**BCL6**

The most commonly involved oncogene in B-cell lymphomagenesis is BCL6. Its gene rearrangements at 3q27 have been reported in 30% to 40% of DLBCL cases with a higher percentage being observed in the ABC subtype (47). Point mutations in the 5\(^{\prime}\) noncoding region occur independently of the chromosome translocation in roughly 75% of DLBCL cases with a higher frequency in patients with GCB (48).

BCL6 is a bric-a-brac, tramtrack, broad complex/Pox virus zinc finger (BTB/POZ) transcriptional repressor. Under normal conditions, B cells express BCL6 exclusively during GC differentiation, as B cells require BCL6 expression for GC and immunoglobulin affinity development (49). BCL6 regulates survival and differentiation via distinct corepressor complexes (50). Survival is regulated through both the SMRT and NCoR corepressors, which are found in large multi-protein histone deacetylase (HDAC)-containing complexes. Both corepressors contain a highly conserved 17-residue BCL6-binding domain (BBD) that binds to the homodimeric BCL6 BTB domain (51). The complexes mediate survival by repressing transcription of ATR, TP53, and CDKN1A, which are involved in DNA damage and cell

---

**Figure 1.** An illustration of putative oncogene dependencies in DLBCL. A, BCL2 dependency is observed within t(14;18) positive populations, which are generally described as GCB-DLBCL, and is generally attributed to aberrant SHM. B, BCL6 dependency is observed within the BCR-DLBCL population and may be the result of aberrant V(D)J rearrangement, SHM, or other mechanisms. C, NF-\(\kappa\)B dependency is primarily observed within the ABC-DLBCL population that is believed to be the result of NF-\(\kappa\)B activating mutations along with BLIMP1 inhibition, thus preventing plasmacytic differentiation. D, ALK dependency is very rare and the mechanism for acquiring the t(2;17) translocation generally observed within this population is poorly understood.
cycle regulation (52, 53). Differentiation is regulated through BCL6 interactions with the MTA3/NuRD corepressor complex, which represses a regulator of plasmacytic differentiation, BLIMP1 (54).

BCL6 is believed to contribute to lymphomagenesis when its downregulation, which usually occurs after affinity maturation, is disrupted. One proposed mechanism for BCL6 downregulation is the loss of IRF4-binding sites of the BCL6 gene. IRF4 expression is induced by sustained CD40 stimulation of the NF-kB pathway in GC cells. IRF4 usually binds to exon 1 and intron 1 of the BCL6 gene and represses BCL6 expression but chromosome translocations or point mutations introduced during SHM, which commonly targets the 5’ noncoding promoter region of BCL6, may prevent this repressive effect (55). BCL6 promoter binding and gene repression has also been shown to vary between normal and malignant cells. The repression of genes implicated in malignant transformation such as BCL2 and Myc is lost in DLBCL, showing the role of deregulation of the BCL6 transcriptional network in lymphomagenesis (56). For a more comprehensive review of BCL6 and lymphomagenesis, please refer to the work of Basso and Dalla-Favera (57).

BCL6 dependency has no correlation to the COO classification system as dependency occurs in both ABC and GCB cell lines. Also, response to BCL6 inhibition is independent of the mechanism for BCL6 deregulation. Recently, a study showed the differential regulation of BCL6 target genes in BCL6-dependent versus BCL6-independent cell lines. The transcriptional profiles of cell lines were used to identify BCR and OxPhos representative cell lines according to the comprehensive clustering method previously established. Chromatin immunoprecipitation-on-chip analysis identified BCL6 target genes. Gene set enrichment analysis was then used to determine the differentially expressed BCL6 target genes using the cell line gene list (58).

Subsequent experiments showed that treatment with a BCL6 peptide inhibitor (BPI) selectively increased the expression of the BCL6 target genes in BCR cell lines but not OxPhos cell lines. The data suggest that BCL6 represses target genes in BCR DLBCLs. BPI treatment also inhibited growth of BCR cells in vitro and in vivo. These findings showed a correlation between BCL6-dependent cell lines and the BCR subtype of DLBCL.

Targeting the BTB domain of BCL6 by mimicking the structure of SMRT BBD appears to be a rational and effective approach in preventing BCL6-dependent DLBCL survival (59). The cell-penetrating BPI is highly selective in that DLBCLs are killed in vitro and in vivo without any negative side effects including inflammatory disease that could potentially result from BCL6 depletion (60). The use of this specific peptide interference to identify the BCL6 transcriptional network in BCL6-dependent populations also identified HDAC and Hsp90 as synergistic targets. These findings have significant translational implications, as small inhibitory molecules targeting HDAC and Hsp90 are already approved for clinical use or in clinical trials (61). Additional therapies including peptide aptamers and small-molecule inhibitors that antagonize BCL6 function also show promise for the treatment of BCL6-dependent DLBCLs (62, 63).

BCL2

BCL2 (B-cell lymphoma 2) was initially described by the t(14;18) chromosome translocation observed in 90% of follicular lymphomas (64–66). In roughly 20% of DLBCL cases BCL2 is overexpressed because of the BCL2/IgH t(14;18) chromosome translocation, which constitutively activates the antiapoptotic protein by placing it under the immunoglobulin heavy chain gene transcriptional elements (67, 29). BCL2 gene amplifications at 18q21 have also been observed in 10% of GCB-DLBCLs as well as 34% of ABC DLBCLs irrespective of t(14;18) status. B cells without a higher affinity to an antigen would generally not be selected out by follicular dendritic cells and T cells for further differentiation into plasma cells and memory cells. Usually the bulk of the GC B cells undergo apoptosis when they acquire somatic mutations that reduce antigen binding, unless the mutation provides a mechanism to evade this process (68).

The BCL2 family of apoptosis regulating proteins contains key regulators of the mitochondrial-dependent (intrinsic) apoptosis pathway (69). There are 3 subgroups of BCL2 proteins: multidomain antiapoptotic, multidomain proapoptotic, and BH3-only proapoptotic, which are segregated by both their function as well as their regions of BCL2 homology (BH) domains (70). The BH3-only proapoptotic proteins can respond to transcriptional or post-translational modifications and become activators initiating the oligomerization of BAX and/or BAD (71, 72). This rapid oligomerization process leads to mitochondrial outer membrane permeabilization (MOMP), which allows for the release of proapoptotic proteins including cytochrome c (73). Once released into the cytoplasm, cytochrome c can bind to APAF-1 and unfold it forming a heteroheptameric complex called the apoptosome (74). APAF-1 is then able to activate widespread proteolysis through the activation of cysteine proteases, known as caspas. This induces cellular dysfunction and the apoptotic cell is tagged for phagocytosis.

Multiple mechanisms can be used by malignant cells to evade programmed cell death. Reduction or elimination of BH3-only proapoptotic proteins through gene deletion or inactivation results in a class A block (75). An apoptotic block may be used by tumors with inactivated tumor suppressor p53, which regulates both the PUMA and NOXA BH3-only proapoptotic proteins (76, 77). Downregulation of multidomain proapoptotic proteins such as BAX and BAK provides an alternative mechanism for defying apoptosis and is referred to as a class B block (78). Finally, a class C block can occur through increased expression of multidomain antiapoptotic proteins such as BCL2 and BCL-XL (79).

Multidomain antiapoptotic proteins such as BCL2, BCL-XL, BCL-w, and BFL1 have sequence homology in 4 α-helical BH regions, BH1-4; MCL-1 is the exception, sharing
homology in only BH1-3 (80–83). The BH1-3 domains in the hydrophobic groove of these anti-apoptotic proteins can bind the hydrophobic α-helical BH3 region of proapoptotic proteins. This interaction provides an antiapoptotic mechanism by binding and sequestering activator BH3-only proapoptotic proteins or by binding and preventing oligomerization of multidomain proapoptotic proteins and subsequent MOMP.

Many DLBCLs may be dependent on BCL2, as BCL2 provides an advantageous class C block, but the mechanisms that result in this survival advantage also produce many confounding factors. This has made elucidating the mechanism for dependency as well as describing the BCL2-dependent phenotype quite difficult. BCL6 suppresses BCL2 expression by interacting with the transcriptional activator Miz1, which inhibits Miz1-induced expression of BCL2 (84). Both chromosome translocations and amplifications can evade the tumor suppressor function of BCL6, which explains how high levels of both BCL2 and BCL6 can be observed in some cases of DLBCL. BCL2 can also be upregulated by constitutive NF-κB activation as BCL2 is a target gene of NF-κB (85).

BCL2 has been specifically targeted with the antisense DNA agent, oblimersen, as well as the small-molecule inhibitors ABT-263 and ABT-737. A phase II clinical trial for oblimersen, in combination with rituximab, showed an overall response rate of 42% in relapsed/refractory B-NHL, but oblimersen has yet to fulfill its early promise (86). On the other hand ABT-263, the orally available form of ABT-737, has been tested in clinical trials for small cell lung cancer, chronic lymphocytic leukemia, and lymphoma (87). This drug was designed to bind the binding pockets of BCL2 and BCL-XL by using a strategy called structure–activity relationships by nuclear magnetic resonance (NMR; SAR by NMR). Although these 3 multidomain antiapoptotic proteins are inhibited by ABT-263 with subnanomolar affinity, it only shows a low affinity for MCL1 and BFL1. Acquired resistance to ABT-737 has been shown in lymphoma cells that upregulate MCL1 and BFL1 and is one of the main mechanisms for intrinsic resistance (88).

To determine the dependency on BCL2, one group used BH3 profiling to detect the class of apoptotic block in a panel of 18 DLBCL cell lines (89). Briefly, BH3 profiling tests a panel of sensitizer peptides for their ability to induce MOMP from mitochondria isolated from the lymphoma cell lines. MOMP is determined by measuring cytochrome c release with ELIZA. The group identified that 9 out of 10 DLBCLs harboring the t(14;18) translocation generally have a "primed" phenotype with a class C block. Their study also showed that t(14;18) is positively correlated with sensitivity to ABT-737. Interestingly, BFL1 expression in a t(14;18) positive cell line conferred resistance to ABT-737 treatment, likely by providing an alternative mechanism for evading apoptosis (89).

NF-κB

The NF-κB system was first linked to lymphomagenesis in an avian reticuloendotheliosis viral study. The v-Rel oncoprotein, which is a member of the Rel/NF-κB transcription factor family, induced rapidly fatal hematologic malignancies in birds with numerous studies thereafter making similar observations in human cells (90). Gene signature analysis has shown increased expression of NF-κB target genes such as BCL2 family proteins, IRF-4, c-FLIP, and cyclin D2 in ABC-DLBCL (36). Constitutive NF-κB activity characterized by high NF-κB DNA–binding activity, IKK activity, and IκB degradation have also been observed exclusively in ABC-DLBCL and has become the hallmark of this subtype (36). It is interesting to note that the IκB gene is rarely mutated in DLBCL (91).

The NF-κB protein family shares an N-terminal Rel homology domain, which appears to form DNA-binding complexes. The class I NF-κB proteins are NF-κB1/p105 and NF-κB2/p100 and undergo ubiquitin/proteasome–mediated partial degradation of their C-terminus to produce active p50 and p52 subunits, respectively (92, 93). Class II NF-κB proteins include RelA/p65, RelB and c-Rel which all have transactivation domains in their C-terminus and generally form heterodimers with class I NF-κB proteins to initiate transcription activation (94–96). NF-κB activity is regulated by the IκB family of inhibitors, which bind to NF-κB dimers and sequester them in the cytoplasm (97). IκB phosphorylation by the IκB kinase (IKK) complex results in IκB ubiquitination and proteasomal degradation, thus releasing the NF-κB dimer for nuclear translocation and gene transcription (98). This mechanism makes NF-κB a rapidly acting primary transcription factor, as it does not require new protein synthesis for activation.

In normal B cells, NF-κB signaling is activated upon antigen binding to the BCR and is involved in numerous functions such as proliferation, isotype switching, cytokine production, and mature B-cell maintenance (99–101). The BCR complex is composed of a ligand-binding moiety, which is a transmembrane form of immunoglobulin and a signal transduction moiety composed of the Ig-α/α-β (CD79a/b) heterodimer. The cytoplasmic tails of the CD79a/b heterodimer contain immunoreceptor tyrosine–based activation motifs (ITAM), which recruit Src homology 2 (SH2)-containing proteins upon tyrosine phosphorylation. Upon antigen binding and BCR oligomerization, Lyn or Fyn tyrosine kinases phosphorylate the ITAMs and initiate downstream signaling (102). Spleen tyrosine kinase (Syk) and B-cell linker protein (BLNK) are recruited to the ITAMs via their SH2 domains. Phosphorylation of BLNK by Syk recruits phospholipase C (PLCγ2), which is also phosphorylated by Syk, but not fully activated until phosphorylation by Bruton’s tyrosine kinase (BTK). PLCγ2 cleavesPIP2 into IP3 and DAG, which ultimately activates protein kinase C (PKCβ). One of the substrates of PKCβ is CARD11, which functions upstream of the IKK complex (103).

In addition to BCR signaling, both Toll-like receptors (TLR), an ancient and highly conserved component of the immune system found in vertebrates and invertebrates, and interleukin-1 receptor (IL-1R) family members can activate NF-κB signaling in B cells. This signal is transduced by a myeloid differentiation primary response gene 88
ALK

protein (118). Approximately, 50 cases of this rare and feature a t(2;17) resulting in a clathrin (CLTC)-ALK fusion proteins were first described in 1997 and generally within the ABC-DLBCL subtype. Normal B-cell homeostasis strongly suggests a dependency through the hijacking of signaling pathways essential for signaling (114). The recurrence of aberrant NF-κB signaling, occur in 39% of ABC-DLBCL and also activates NF-κB dependent population rather difficult. Upstream activation of IKKβ via CARD11-, BCL10-, and MALT-dependent mechanisms, which form the CBM complex, are commonly observed in ABC-DLBCL. Activating missense mutations in CARD11 have been observed in 10% of ABC-DLBCLs, whereas many ABC-DLBCL tumors without CARD11 mutations display chronic activation of BCR signaling which activates the CBM complex (108, 109). Amplification of c-Rel at 2p13 has also been observed in roughly 25% of DLBCL (110, 111). Recent studies also found that 30% of ABC-DLBCLs have biallelic inactivation of the A20 deubiquitinase, which negatively regulates NF-κB, suggesting another mechanism for constitutive NF-κB expression (112, 113). Mutations in oncogenic MyD88, an adaptor protein that mediates toll and interleukin receptor signaling, occur in 39% of ABC-DLBCL and also activates NF-κB signaling (114). The recurrence of aberrant NF-κB signaling through the hijacking of signaling pathways essential for normal B-cell homeostasis strongly suggests a dependency within the ABC-DLBCL subtype.

The NF-κB pathway has been targeted through IKK-specific kinase inhibitors, Hsp90 inhibitors, IκB super-repressor molecules, and proteasome inhibitors. The small molecular IKK inhibitors PS-1145 and MLX015 are highly specific kinase inhibitors that were found to be very effective at inducing cell death in ABC-DLBCL cells after reducing NF-κB target genes and inducing activation of proapoptotic caspases (115). The Hsp90 inhibitor geldanamycin (GA) has also shown efficacy by disrupting the formation of the Hsp90/IκK complex (116). The use of the super-repressor IκBα (S32G/S36A) results in selective killing of ABC-DLBCL cell lines (36). The super-repressor cannot be phosphorylated by IKK, preventing its degradation and the activation of NF-κB, which is critical for ABC-DLBCL survival. Indirect inhibition of NF-κB has been accomplished through the use of the proteasome inhibitor bortezomib, which prevents IκBα degradation. Phase II clinical trials for bortezomib in combination with chemotherapy showed response rates of 85% in refractory ABC-DLBCL, but only 13% in GCB-DLBCL, again showing a NF-κB dependency in the ABC subtype (117).

**ALK**

DLBCLs positive for ALK (anaplastic lymphoma kinase) fusion proteins were first described in 1997 and generally feature a t(2;17) resulting in a clathrin (CLTC)-ALK fusion protein (118). Approximately, 50 cases of this rare and distinct form of DLBCL have been reported, and a poor prognosis with a 5-year survival rate of only 25% has been observed in these patients (119, 120). Studies indicate that virtually all patients belonging to this subgroup are CD20-negative and R-CHOP is unlikely to significantly improve survival.

ALK is an orphan receptor tyrosine kinase in the insulin receptor family and is commonly activated in cancer through the generation of ALK fusion proteins. Fusion proteins resulting in the constitutive activation of the ALK kinase domain have an oncogenic potential by enhancing cell proliferation and survival via numerous redundant and interconnected pathways. This subtype’s immunophenotype has been characterized by the positive expression of plasma cell associated antigens CD38 and CD138, epithelial membrane antigen (EMA), and immunoglobulin light chain κ or λ and most closely resemble post GC B-cell lymphomas. ALK-DLBCL also lacks T-cell–related antigen (CD2, CD3, CD5, CD43, and CD8), B-cell–related antigen (CD20 and CD79a), and CD30 expression (121, 122). Although the reported incidence of ALK-DLBCL is very low, this may be due to being misdiagnosed as ALCL which is typically CD30 positive, or plasmablastic lymphoma, plasmablastic myeloma, immunoblastic DLBCL, and anaplastic carcinoma which are all generally ALK negative.

One group recently developed the first CLTC-ALK–positive DLBCL cell line and evaluated its dependency on ALK kinase function (123). The selective ALK inhibitor TAE-684 showed a reduction in the activation of downstream pathways associated with lymphomagenesis such as constitutive JAK-STAT3, ERK, and PI3K-AKT activation (124–126). This inhibitor also induced cell death in vitro as well as in complete regression in xenograft models. The dual c-Met/ALK inhibitor crizotinib (PF-02341066), which is structurally unrelated to TAE-684, has also been explored as a therapeutic option for ALK-dependent malignancies.

**Future Directions**

The COO classification system and subsequent functional studies have defined the ABC subtype as highly dependent on NF-κB signaling. Unfortunately, at each differentiation state, there are multiple mechanisms for lymphomagenesis as illustrated in Figure 1 and identifying the dependency in the GCB subtypes can be limited using this method. In addition, multiple lesions can lead to NF-κB activation but differential dependencies on these lesions result in variable responses to targeted therapies. This is exemplified by the documented resistance to both BTK and PKC inhibitors in ABC-DLBCL cell lines harboring CARD11 or A20 mutations (127). Consensus clustering and subsequent functional studies have identified the BCR subtype as a superb candidate for BCL6-targeting therapies. At the same time, much work still remains in determining a dependency and therapy for both the OxPhos and HR subtypes. The HR subtype is characterized by a heterogeneous population with significant T-cell infiltration, which poses additional complications for analysis, as the tumor is...
not readily recapitulated for in vitro studies. FISH can also identify DLBCLs harboring t(14;18) and t(2;17) translocations as BCL2- and ALK-dependent populations, respectively. The subtypes identified from classification systems and FISH can be merged to stratify DLBCL subtypes by oncogenic dependencies. This is essential for effectively designing and interpreting clinical trials exploring novel therapeutic options selectively targeting the responsible drivers. Even after merging these classification systems, there remain large gaps in information vis-à-vis defining the epidemiology of both the subtypes with known dependencies and those that remain undefined.

Other mechanisms, which may include putative drivers through direct or indirect mechanisms, are being explored and may address some of the current gaps in the field. Recently, coding genome analysis by next-generation sequencing and copy number analysis revealed a role of aberrant epigenetic regulation in lymphomagenesis (128). Specifically, alterations in histone-lysine N-methyltransferase (MLL2), CREB-binding protein (CREBBP), E1A-binding protein p300 (EP300), and other chromatin modifying enzymes have been observed in over one-third of DLBCL cases, regardless of COO status (128–130). EP300 has recently been proposed as a mechanism for BCL6 dependency as BCL6 represses EP300 expression and lymphomas insensitive to BCL6 inhibitors often have p300 mutations (61). In addition, p300 has been shown to be necessary for BCL6 acetylation and recruitment of HDACs (131). This BCL6-p300 axis as well as the newly identified lesions suggests that HDAC inhibitors may be rational therapies for discreet populations of DLBCL. Characterization of populations dependent on these mechanisms versus populations where these abnormalities are passenger mutations may further elucidate their role in lymphomagenesis.

Some of these epigenetic alterations may lead to the disruption of tumor suppressor genes and contribute to lymphomagenesis. One study recently observed the inactivation of CD58 in 21% of DLBCL cases which serves as a means for evading T- and natural killer (NK) cell-mediated cytolyis (132–134). In the same study, B2M mutations or deletions were also observed in 29% of DLBCL cases, thus preventing surface expression of human leukocyte antigen (HLA) class I molecule, which is essential for CD8+ cytotoxic T-cell (CTL) recognition and cytolysis (135). Roughly, 61% of DLBCL cases lack both CD58 and B2M expression on the plasma membrane, which likely results in tumor evasion of both CTL and NK cells (132). The high incidence of DLBCL tumors coselecting these complementary mechanisms suggests an essential role of immune evasion in DLBCL development and/or progression stressing the need for further characterization and development of treatment options targeting these mechanisms.

In addition, the emergence of microRNA profiling will almost certainly reveal additional oncogenic mechanisms thereby promoting additional stratification of heterogeneous populations (136). MicroRNAs have a repertoire of mechanisms to regulate both gene transcription and translation and the concept of onco-miRs has been recently proposed (137, 138). Specifically, the oncogenic nature of miR-155 has been implicated in DLBCL by the Aguiar group, which observed the segregation of miR-155 expression with NF-kB activity in DLBCL cell lines as well as increased expression in primary samples defined as ABC-DLBCL (139). Such miRs often regulate a cluster of functionally related genes and may hold great potential as therapeutic targets (140).

It is expected that even after drivers are identified and targeted, responses will be variable as de novo mutations, oncogene switching, and inactivation of tumor suppressors will likely seem providing resistance mechanisms to therapies. The revelation of these secondary events may identify additional tractable and rational targets that could greatly improve the survival of discrete populations where they are essential for tumor survival. Defining dependent phenotypes in DLBCL will ultimately benefit patients by personalizing treatments through the specific targeting of the responsible drivers and has the potential to significantly advance the treatment of DLBCL.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: J. Steinhardt, R.B. Gartenhaus
Development of methodology: J. Steinhardt
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Steinhardt
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Steinhardt
Writing, review, and/or revision of the manuscript: J. Steinhardt, R.B. Gartenhaus
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Steinhardt
Study supervision: J. Steinhardt

Acknowledgments
We thank Dr. Martin Fajnik for helpful and insightful discussion. We apologize to those authors whose original articles could not be cited due to space constraints.

Grant Support
This work was supported in part by an R01AA017972 from the NIH (to R.B. Gartenhaus) and a Merit Review Award from the Department of Veterans Affairs (to R.B. Gartenhaus).

Received January 25, 2012; revised May 8, 2012; accepted June 18, 2012; published OnlineFirst June 27, 2012.

References

Identifying and Targeting Oncogenic Drivers in DLBCL


Promising Personalized Therapeutic Options for Diffuse Large B-cell Lymphoma Subtypes with Oncogene Addictions

James J. Steinhardt and Ronald B. Gartenhaus


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-0217

Cited articles
This article cites 139 articles, 53 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/18/17/4538.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/18/17/4538.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.